THE UNIVERSITY OF MICHIGAN

INDUSTRY PROGRAM OF THE COLLEGE OF ENGINEERING

SEPARATION OF BACTERIA BY ADSORPTION ONTO ION EXCHANGE RESINS

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in the
University of Michigan
Department of Chemical and Metallurgical Engineering
1967

April, 1967

IP-778

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To my wife Carol Yvonne, for her infinite support and patience, and to my parents, for my development and being, this thesis is gratefully dedicated.

Stacy L. Daniels
Ann Arbor, Michigan
April, 1967

"Our doubts are traitors, and make us lose the good we oft might win by fearing to attempt."

- Measure for Measure
Act I, Scene V

ACKNOWLEDGMENTS

My sincerest appreciation is extended to the Chairman of my Doctoral Committee, Dr. Lloyd L. Kempe. His inspiration, counsel, and encouragement were inestimable in nurturing this work to its completion. I would also like to thank the members of my Doctoral Committee for their continued interest and suggestions.

The part-time employment provided me by the U. S. Bureau of Commercial Fisheries aided greatly in making this thesis possible. The interests of my many esteemed friends from this organization were truely appreciated.

The friendly discussions with my fellow graduate students, Dr. Gary F. Bennett, Mr. Daniel Bull, and Mr. Jens Pedersen, augmented our common orientation in Biochemical Engineering. The supporting research by Messrs. Charles E. Bell, John C. Reilly, Charles J. Sawyer, and Dick Weber, is hereby acknowledged.

The materials kindly contributed by George S. Fearnehough, Chief Technologist of the Department of Microbiology, were invaluable in the classification of the bacterial species for this thesis. Many of the ion exchange resins used in this study were obtained with the assistance of Dr. Robert Wheaton of the Dow Chemical Company, Midland, Michigan.

I further extend my gratitude to the Faculty, office staff, and shop personnel of the Department of Chemical and Metallurgical Engineering for the many untold suggestions and assistances during my years as a student. The personnel of the Engineering College-Industry Program contributed a definite and much appreciated part in the preparation of this manuscript.

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ABSTRACT

Bacterial cells behave as macroscopic "ions" and quantitatively adsorb onto and desorb from the surfaces of ion exchange resins. Various features of this phenomenon are discussed in a survey of the related literature. Particles of either anion or cation exchange resins were contacted with agitated suspensions containing bacterial cells. The species of bacterium undergoing exchange adsorption and the type of resin promoting this process are most important; the pH and the concentrations of salt ions are also important.

Exchange of anionic bacterial cells at pH values above their isoelectric points is postulated to involve negatively charged carboxyl groups on the surfaces of the cells. These groups are capable of interacting with the quaternary ammonium groups of a positively charged anion exchange resin. Conversely, exchange of cationic bacterial cells at pH values below their isoelectric points is postulated to involve positively charged amino groups on the surfaces of the cells. These groups are capable of interacting with the sulfonic groups of a negatively charged cation exchange resin. Both anion and cation exchange processes were found to be reversible. The cells were desorbed from the resin surfaces by changing the pH or the salt content of the suspending medium. Rates of adsorption and desorption were studied. Both quantities are composite functions of the cumulative time and the square root of the cumulative time of agitated contact between bacterial cells and resin particles. Optical techniques were used to monitor the rate of disappearance

of adsorbing cells or the rate of appearance of desorbing cells. Simultaneous exchanges of counter ions were observed through pH changes.

The exchange of bacterial cells was assumed to be a diffusion-controlled reaction. A mathematical model was developed by extending the classical colloid coagulation theory of Smoluchowski. Diffusion coefficients of the order of 10^{-7} cm^2/sec were determined for the adsorption of Bacillus subtilis cells onto the anion exchange resin, Dowex 1 x 8, 100/200 mesh, chloride form. Saturation values of approximately 10^{10} cells per gram of resin were determined. A monolayer of cells was apparently adsorbed onto the available surface area of the resin. The adsorbed cells were shown in photomicrographs to be attached to the resin surfaces in "pincushion" patterns.

The variables of cell concentration, resin concentration, resin particle size, pH, salt concentration, agitation, time of contact, and temperature were evaluated. The bacteria used in the experimental work included Pseudomonas ovalis, Escherichia coli, Proteus vulgaris, Staphlococcus aureus, Bacillus cereus, and Bacillus subtilis. The ion exchange resins used were the anion exchange resin, Dowex 1 and Dowex 2, and the cation exchange resin, Dowex 50W.

Mixed suspensions of cells of two bacterial species were effectively resolved into two fractions, each containing the cells of one of the component species. Two resolution types were defined: Type I, selective adsorption of the cells of one species, and Type II, nonselective adsorption of the cells of both species followed by the sequential desorption of the cells of each species. Five equally-proportioned binary mixtures were resolved by these techniques: E. coli - B. subtilis,

E. coli - *S. aureus*, *E. coli* - *P. ovalis*, *S. aureus* - *B. subtilis*, and *B. subtilis* - *P. vulgaris*. Efficiencies of resolution of greater than 90% were achieved. The relative ease of resolution of several other binary mixtures are predicted. A scheme is also proposed for the resolution of a mixture containing the cells of four different species.

The phenomena investigated in this study have potential application in the purification, concentration, or resolution of bacterial cells suspended in aqueous media. They could have practical value in fermentation technology, waste and water treatment, and in clinical microbiology.

I. INTRODUCTION

A. General Considerations

The resolution of mixtures into their component parts has been the subject of much research since the beginnings of the chemical sciences. This was first accomplished by physically separating discrete phases, such as removing a solid from a liquid through filtration, or expelling a liquid from a solid by drying. Greater selectivities for the components present in a mixture are shown by other separation techniques, such as crystallization and distillation, which are based upon more subtle differences among the individual chemical species. Such techniques have been utilized on a rudimentary level since the times of the alchemists.

These methods of separation and others have been refined to a considerable extent by modern technology. Practical applications appear in many areas of the chemical industry. Requirements for separation, however, have become progressively more stringent as the mixtures to be resolved have increased in their complexity. The parallel development of improved, or completely new, separation techniques are continually necessitated by the development of new products, or new applications for old ones.

Several specific problems are presented, for example, in the separation of liquid mixtures of biochemical origin. Certain restrictions are imposed upon any separation technique to be applied due to the high degrees of chemical complexity, the extremely dilute concentrations, and the physico-chemical labilities often encountered. The process of ion exchange is particularly adaptable to biochemical separations.

Ion exchange can be defined as the selective absorption of ions from solution into a dispersed solid and the simultaneous replacement of these ions by other ions originally present in the solid exchanger. Recovery of the absorbed chemical species is then accomplished by subsequent desorption with a selected eluting agent. Adsorption of suspended particles on to the surface of an ion exchanger may also be considered to be an ion exchange process if such a transfer of ions occurs. The primary advantages of ion exchange processes are: the mildness of the reaction conditions and the high degree of specificity found in most situations. The dominant driving force is ionic in character. Many dilute mixtures can be resolved by ion exchange techniques.

Biochemical applications of ion exchange in liquid systems are prevalent in the food and pharmaceutical industries, and elsewhere in the chemical industry as a whole. The major industrial uses of adsorption and ion exchange as unit operations include: desalting, ion exchange, removal of contaminants, pH control, and separation of products from dilute process streams. Typical examples are: deionization of organic acids, water softening, elimination of taste- and odor-producing substances from drinking water, neutralization of acids and bases, and the isolation of antibiotics, alkaloids, and other pharmaceutical products from dilute solutions. Industrial applications and process design procedures involving adsorption and ion exchange are discussed by Hiester, Vermeulin, and Klein (1963), Kunin (1958), Webb (1964), and in a booklet by the Dow Chemical Company (1964).

Many areas of analysis, synthesis, and purification have been encompassed in the applications of ion exchange on a laboratory scale.

Determination of trace components, such as pesticides in water, can often be improved by the concentration of such components using ion exchange resins. Analyses of complex mixtures of proteins, amino acids, sugars, nucleotides, or other compounds of biological significance, are simplified by chromatography. Separation of low-yield products from organic syntheses can be facilitated by ion exchange. Alternatively, contaminating substances present in a reaction mixture can be preferentially removed. Excellent references are available concerning analytical (Samuelson, 1963), medical (Martin, 1955), and general biochemical (Calmon and Kressman, 1957; Morris and Morris, 1963) applications of ion exchange techniques.

The applicability of ion exchange techniques to biological systems is quite general. Many classes of chemical compounds to be found in diverse areas of biological, medical, chemical, and engineering research and development have been treated. Most applications have been limited to the exchange of soluble species in the areas of chemical analysis, biochemical preparation, or water purification. One specific area that has not received much attention is the adsorption of intact living cells onto ion exchange media. Scattered reviews of activity in this specialized area include those dealing with cells and tissues (Rothstein, 1957), bacteria (Harris, 1957; Martin, 1955; and Rotman, 1960), and viruses (Muller, 1957).

Direct contact of cell suspensions with ion exchangers has not been extensively considered in microbiology as a means of isolating and purifying mixtures. The applications of adsorptive phenomena in microbiology have developed through a diversity of interrelated investigations. These have been concerned with attractive-repulsive forces interacting

between living cells and substances in their environment. The intertwining complexities rampant in studies of microbial adsorption are stated very well by Lerche (1953, p.2). "The literature on surface problems in bacteriology is already very extensive but also so heterogeneous that it is difficult to coordinate the many results published."

Studies on microbial adsorption can be separated into three areas of interest: (1) sorption to the microorganism, i.e. the absorption of a dissolved ion, dye, surface-active agent, or hydrophilic colloid, (2) sorption by the microorganisma themselves, i.e. the adsorption of cells to surfaces or to other cells, and (3) charge phenomena of a nonsorptive nature, e.g. electrokinetic behavior, motion in fluids, and liquid-liquid and gas-liquid partition.

The connotations of these phenomenological classifications are not without considerable overlap. They are elaborated in the subsequent sections of this introduction and supported by abstracts of selected references. This discussion is not meant to be all inclusive. Its main purpose is to introduce the great variety of recognized surface charge phenomena relevant to microbiology and to establish a foundation for a more specific discussion of ion-cell combinations to be presented in later sections of this report.

B. Sorption to Microorganisms

The interactions between microorganisms and their environment include situations in which various chemical species are attracted to or released from the surfaces of individual cells. These transfers of material may be essential, detrimental, or inconsequential for normal

growth and development of the organism. Flocculation, plasmolysis, bacteriostasis, or complete sterilization are results of these interactions. Extracellular interactions between charged species existing on the cell surface and those present in the bulk solution can be arbitrarily subdivided into four groups: ions, dyes, surface-active agents, and hydrophilic colloids. The overlaps of these divisional stata are obvious.

1. Dissolved ions

Dissolved ions are usually directly associated with the normal growth and developmental processes of the cell. Inorganic ions can contribute to the establishment of both the pH and the ionic strength of a given system. Larger organic ions of amino acids, exoenzymes, and other compounds can either stimulate or inhibit microbial activity by affecting respiration or synthesis of cellular structures.

Although the overall metabolic functioning of these ions can be quite complex, certain features are easily distinguished. Their ability to permeate the cell wall, regulate osmotic pressure, facilitate transport, and alter electrical charges, are observed microscopically through changes in the shape and size of individual cells, or by interaction with other cells and surfaces. Changes in turbidity or gross flocculation can be observed macroscopically. The sorption of metabolically active compounds can often be observed by monitoring changes in the pH or the concentration of the entire population.

The ability of dissolved ions to flocculate bacterial cells has been demonstrated by several investigators. Northrup and DeKruif

(1922a) found that the agglutination of bacterial cells was promoted by the addition of electrolytes. Eggerth (1923) studied flocculation as a function of pH in various buffer solutions. Absorbance maxima occurring at specific pH values were established by Lasseur, Dupaix-Lasseur, and Renaux (1934). Ryan and Kolin (1964) described a technique for the continuous turbidimetric analysis of coalescence processes of biological systems. Nakamura (1961) surveyed the flocculent action of various metal salts upon bacteria.

Turbidity changes of bacterial suspensions occurring after the addition of salts and other compounds were observed by Mager et al. (1956). Abram and Gibbons (1960) confirmed similar behavior by halophilic bacteria. The work of Bernheim (1963b) included the optical effects of other ions and buffer systems. These effects are discussed in more detail in Appendix A of this report.

The permeability of bacterial spores to various solutes is described in a series of papers by Black and Gerhardt (1961a, 1961b). The extent of penetration depended upon at least three molecular properties: (1) dissolution of electrolytes, resulting in high or low uptake which was predicted on the basis of charge, (2) lipid insolubility, restricting permeation of small molecules, and (3) increasing molecular weight, leading to decreasing permeation and eventual exclusion. Bovell, Packer, and Helgerson (1963) studied the permeability of bacterial cells to organic compounds and inorganic salts by measuring the amount of light scattered by suspensions following the introduction of these compounds into suspensions. The active transport of inorganic ions through bacterial cell walls is also discussed by Kepes and Cohen (1962). The field of biological transport is surveyed in a book by Christensen (1962).

Certain compounds can modify the surface charge of bacteria. Haydon (1961b) observed that extremely small concentrations of dissolved cations were often effective in reducing the surface charge of bacterial cells. This effect was greater with cations of higher valence. Little effect was produced by anions. Ives (1959) considered the importance of surface charge in the flocculation of algae in water treatment. Gittens and James (1963b) employed such agents as diazomethane and methanolic hydrochloric acid to chemically modify cell surfaces. The extent of these modifications, presumably of carboxyl groups, was studied by electrophoresis. Cohen (1945) also used electrophoretic techniques to follow the substitution of imidazole and amino groups on the surfaces of bacterial cells that had been treated with benzene sulfonyl chloride.

The true adsorptive exchange of ions by bacteria was first demonstrated by McCalla (1940a, 1941b). Bacteria were found to be capable of absorbing hydrogen ions which could then be replaced by other monovalent and divalent cations. Brown (1964) considered many aspects of the response of bacteria to their ionic environment. The principal mode of action of salts upon bacterial cells was considered to involve an electrostatic process in which cations associate with carboxyl groups situated on the surfaces of the cells. Anderson, Thompson and Snell (1964) found that the ability of bacterial spores to resist heat could be extended by replacing hydrogen ions on the spore surface with calcium ions.

The absorption of polymyxin E, a cationic polypeptide antibiotic, by bacterial cells was studied by Few and Schulman (1953). The shape of the resulting absorption isotherm and the amount of antibiotic

absorbed were both functions of the species of bacteria under study. Newton (1954) observed a competition between polymyxin and certain other cations for anionic sites on the surface of bacterial cells. The activity of subtilin A, another surface-active polypeptide antibiotic, was strongly affected by the pH of the bacterial suspension according to Sacks and Pence (1958). Cooper (1955) studied the properties of the penicillin-binding component of bacterial cells in order to determine the site of action. The adsorption of the antibiotic, vancomycin, onto the cell walls of bacteria was studied by Best and Durham (1965). This adsorption was considered to result from an ionic binding between basic groups on the antibiotic molecule and acid groups on the bacterial cell wall. Magnesium and other cations were in competition with vancomycin for binding sites.

Goldstein (1949) prepared an extensive review of the interactions which can occur between drugs and proteins. The specific chemical combinations of antimetabolities and drugs with bacterial cells was reviewed by Woolley (1959). Davies and Feingold (1962) also reviewed the use of antimicrobial agents and their mechanism of action. The syntheses of protein molecules and the subsequent formation of cell walls and cell membranes can be seriously affected by these agents.

Dissolved ions can therefore significantly affect cellular behavior in many ways. Flocculation can be initiated and changes in turbidity can be produced in suspensions. Permeability and surface charge can be modified. Combination of extracellular ions with microorganisms is generally considered to involve a true adsorptive exchange with electrostatic binding of specific surface groups being the principal mechanism.

2. Dyes

The chemical combination of colored compounds with specific units of a microbial cell has two desirable features. Differential staining of certain cellular structures is very useful in microscopic observation. Secondly, the adsorption of dyes by cells is easy to follow by visual or optical means. The reaction of acidic and basic dyes with cells, and the mode of action of the differential Gram stain, have been intensively investigated. The separate classification of dyes in this report does not necessarily imply unique biological activity. Dyes are considered in this separate catagory since much attention has been directed toward staining as a phenomenon that can be influenced by surface charges present on the cells.

Stearn and Stearn (1928) reviewed the physico-chemical behavior of suspensions of bacterial cells that behaved as an equilibrated system of amphoteric particles. Cations were observed to combine with negatively charged cells at pH values basic to the apparent isoelectric points of the cells; conversely, anions combined with positively charged cells at pH values below the apparent isoelectric points of the cells. Bacteria were differentiated according to their ability to adsorb acid or basic dyes by Tolstoouhov (1929). The extent of dye combination was a function of the isoelectric points of the bacterial species. Lasseur and Dupaix-Lasseur (1934) studied the adsorption of dye as a function of pH and buffer strength.

McCalla (1941a) attempted to resolve the controversy as to whether dye adsorption is a physical or a chemical affinity between a stain and a bacterial cell. He concluded that staining is an adsorptive

exchange process, chemical in nature, and reaching stoichiometrical proportions. Basic stains such as methylene blue replaced magnesium, or hydrogen ions on the cellular surface. In a subsequent reference McCalla and Clark (1941) observed adsorption of acidic or basic dyes when the bacterial cell was, respectively, positively or negatively charged.

Harris (1949) studied the adsorption of fluorane derivative dyes as a function of pH. He later (1951) reduced the negative charge of the bacterial cells by one of three methods: lowering the pH of the suspending medium, adding readily adsorbable cations, or increasing the ionic concentration of the medium. The adsorption of acidic dyes of the sulfonaphthalein type was greater by the more negatively charged cells.

The Gram stain is a differential technique for classifying microorganisms on the basis of their ability to sequentially retain acidic or basic dyes. A fixation step using an iodine-potassium iodide solution followed by a washing with a mixture of acetone and ethyl alcohol is intermediate between the attempts to stain the cells with acidic or basic dyes. The actual mechanism of this process is still subject to controversy. Bartholomew and Mittwer (1952) extensively reviewed the possible mechanisms involved in Gram stain. They classed the proposed theories into three groups: (1) chemical theories attributing Gram-positivity to specific combination with substances or complexes, (2) isoelectric point theories which attribute a greater degree of retention to Gram-positive organisms because of their supposed lower isoelectric points, and (2) permeability theories which explain Gram-positivity on the basis of permeability characteristics peculiar to the cell wall or cell membrane. The currently held theory of Gram staining ability is discussed by

Salton (1963; 1964, p 29). He attributes the specificity in the staining of Gram-negative and Gram-positive cells to a difference in the permeability of the cell walls of these organisms to the crystal violet-iodine complex following the application of organic solvents.

The physical affinities of bacterial cells for dyes were studied in detail by Yuri (1928a, 1928b). The combinations of dyes with cells were controlled largely by the pH of the suspending fluid. The adsorption of these dyes could be described by Freundlich isotherms. An electrostatic affinity was assumed to be operative. Borzani and Vairo (1959) reported that Gram-negative and Gram-positive organisms were fundamentally different with respect to their abilities to adsorb crystal violet. They found that the adsorption of this dye by Gram-positive organisms could be described by Freundlich or Langmuir isotherms; no correlation was found for the adsorption of this dye by Gram-negative organisms. Vairo and Borzani (1960), however, reported that the adsorption of dyes by Gram-negative bacteria could be described by the same types of isotherms. Finkelstein and Bartholomew (1960) also studied the binding of crystal violet by both Gram-negative and Gram-positive organisms. They interpreted the adsorption of dye by both organisms as obeying the Freundlich and Langmuir adsorption laws. Leman (1964) investigated the absorption of dye by yeast cells and found it to be dependent upon the pH of the suspension. Giles and McKay (1965) summarized various factors that influence the adsorption of cationic dyes by fixed yeast cells. Isotherms were determined and the results were consistent with an ion exchange mechanism. Phosphate or some other strongly acidic group was considered to be the most important site for adsorption.

It can be concluded from a review of the literature that the binding of dyes by microorganisms is a true adsorption which can often be described by isotherms of the Freundlich or Langmuir type. The combination of dyes and cells are apparently stoichiometric in many cases and involve the exchange of dye ions with positively or negatively charged groups on the cellular surface.

3. Surface-active agents

Several types of compounds, which are not necessarily required for normal growth by microorganisms, are included in this classification. They may even interfere with life processes or cause noticable effects upon the surfaces of cells vis-a-vis similarly acting compounds which permeate into the interiors of cells. These surface-active compounds can alter the environment surrounding a cell by reducing surface tension, increasing viscosity, etc., which in some instances can lead to disruption of the cell.

Glassman and Putnam (1947) and Glassman (1948) have extensively reviewed the actions of surface-active agents upon bacterial cells and the applications of these compounds in bacteriology. The precipitation, complexation, and denaturation of cellular proteins by surface-active agents were emphasized. Nakamura (1961) studied the coagulation and precipitation of bacterial cells by surface-active electrolytes of large molecular size. Typical compounds that were evaluated included: alkyl pyridinium salts, and amine salts.

Baker, Harrison, and M iller (1941a, 1941b, 1941c) studied the action of synthetic detergents upon the metabolism of bacterial cells.

Metabolism by the cells of both Gram-positive and Gram-negative species was effectively inhibited by cationic detergents. The matabolism of the cells of the Gram-positive species was selectively inhibited by anionic detergents. Ordal and Borg (1942) found that the oxidation of lactate by bacteria was inhibited by surface-active agents.

The antibacterial action of surface-active cations of the quaternary ammonium type was considered by Valko and Dubois (1944). This action can be classed as an ion exchange phenomenon. The bacterial cells function as cation exchangers because of their acidic surface groups. Other cations may exert a protective action by competitive adsorption. The process may be reversed by the presence of other cations and anions, and is dependent upon the pH of the system. In a later paper (1945) the same authors correlated antibacterial action with chemical structure of the surface-active agents.

Kivela, Mallman, and Churchill (1948) studied the reversibility of the physical action of surface-active cations upon bacteria. They postulated that the destruction of vegetative cells might be explained in terms of the high osmotic pressure exerted on the bacterial cell by the adsorbed cations. Shafa and Salton (1960) completely disaggregated the cell walls of Gram-negative bacteria using anionic surface-active compounds such as sodium dodecyl sulface (SDS). They suggested that the mechanism involved an interaction between SDS and the lipid, lipoprotein, and lipopolysaccharide parts of the cell walls. The dissaggregation, or dissolution, of the cell walls was attributed to the action of the anionic compounds on weak molecular forces involved in lipid-protein associations. Bernheim (1963a) studied the effects of various surface-active compounds

upon the assimilations of ions by bacterial cells. Eagon and Carson (1965) observed lysis of isolated cell walls and intact cells of bacteria which had been contacted with lysozyme or with ethylenediamine tetraacetic acid (EDTA). The effects were noted upon measuring the reduction in the amount of light absorbed by a suspension of bacterial cells.

Dyar and Ordal (1946) studied the effects of surface-active agents upon the electrophoretic mobilities of bacterial cells. Little or no increase in the cellular mobilities were produced upon increasing the concentrations of the anionic surface-active agent, sodium tetradecyl sulfate. A general pattern of decrease of charge, reversal of charge, and ultimately, stabilization of charge was observed upon increasing the concentration of the cationic surface-active agent, cetyl pyridinium chloride. The extent of these changes and the concentrations at which they occurred were found to vary widely among the bacterial species studied. The changes in mobility must therefore depend upon the chemical natures of the surface-active agent and the bacterial cell surface, as well as upon the pH of the suspending medium. Dyar (1948) continued the investigation of surface lipids, amphoteric materials, and other surface properties of bacterial cells.

Surface-active agents can be instrumental in the flocculation of bacterial cells. They can also exert a bacteriostatic effect upon growing cells leading to a complete disaggregation of the cell walls. The surface charges of bacteria are strongly altered by cationic or anionic agents. This latter condition can be easily observed by electrophoretic techniques. The combination of surface-active compounds with bacterial cells is considered to be an ion exchange phenomenon.

4. Hydrophilic colloids

Hydrophilic colloids are at least an order of magnitude larger in size than the molecular species whose interactions with microbial cells were considered in the earlier sections of this report. These colloids may be natural proteins, synthetic polyelectrolytes, etc. The most important characteristic that can be used to identify this class of compounds is their ability to flocculate cells of microorganisms. Such flocculation can often occur in a very specific manner. Flocculation can often occur in a very specific manner. Flocculation can result from a reduction of the net surface charge of the cells to zero upon direct adsorption of the colloidal particles, or from the formation of multivalent ion bridges by the colloids between the cells.

Eggerth and Bellows (1922) flocculated suspensions containing bacterial cells by adding various proteins, such as gelatin, albumin, etc. Maximum flocculation occurred near the isoelectric points of the added proteins. Northrup and DeKruif (1922) stated that the addition of proteins or serum to bacterial suspensions at different pH values caused a broadening of the zone of acid agglutionation and shifted the isoelectric point of the cells to that of the added substance. Mudd, Nugent, and Bullock (1932) reviewed the chemistry of bacterial agglutination and its relationship to colloidal theory. Chesbro and Hedrick (1957) evaluated the effects of buffer type, salt concentration, and pH upon the stabilities of some yeast and bacterial suspensions. Jansen (1958) described the flocculation of suspended yeast cells. Mill (1964a, 1964b) investigated the effects of nitrogenous impurities and metal ions upon the flocculation of yeast cells.

The flocculation of bacteria by hydrophilic colloids as a method of concentrating cells from dilute suspensions was studied by Hodge and Metcalfe (1958). The efficiency of the process and the optimum concentration of colloid required to achieve it were found to vary with the species of microorganism. The colloids apparently functioned by adsorbing onto the hydrated surfaces of the cells. A bridging process thus promoted between the cells was then followed by extensive flocculation. Klein, Metcalfe, and Lincoln (1958) determined the effects of the settling velocity, the degree of vibration, and the angle of the settling vessel upon the flocculation.

Terayama (1954) titrated suspensions of bacterial cells with a positive polyelectrolyte, macramine (N-polymethylated chitosin), and a negative polyelectrolyte, polyvinyl alcohol sulfate potassium salt (PVS-K). The remarkable electronegativity of the bacterial surface was exhibited by the inability of the cells to combine with PVS-K and a very noticable ability to combine with micramine. The degree of macramine combination was much greater at alkaline pH values. This reaction was stated to have application in the determination of isoelectric points, ion combining capacities, and enumeration of bacterial populations. The high degree of positive-negative specificity of bacterial cells was further observed by Ambrose, Easty, and Jones (1958). Differences were found between the surfaces of tumor cells and their analogous normal cells in their ability to adsorb positively charged polymers. One polymer, polyethylenimine, was specific in reducing the surface charge of tumor cells.

McKinney (1956) proposed a theory for the bioflocculation of bacterial cells under natural conditions. Tenny and Stumm (1965) demonsteated that chemical flocculation of bacteria can be achieved by hydrolyzed metal ions and by synthetic organic polyelectrolytes. Busch (1966) extended this work to include anionic and nonionic polymers and clarified the importance of specific chemical interactions between polymers and biocolloids.

The antibody-antigen reaction is an example of a highly specific flocculation. Cohen (1945) found little change in antibody-combining capacities by organisms treated with benzene sulfonyl chloride although changes in sensitivity to agglutination were observed. Bacterial cells that have lost the ability to agglutinate are also less susceptible to adsorption by aluminum phosphate and, consequently, are less precipitable according to Unger and Muggleton (1949). Isliker (1953) recovered specific antibodies of high purity by combining them with their corresponding antigens that had been previously linked with ion exchange resins. Curtain (1954) promoted the adsorption of influenza virus particles onto powdered cellulose by coupling haemagglutination inhibitors onto the surfaces of this adsorbent. The antigenic analysis of cell structure was elaborated in a review by Lennox (1960). Kelen and Labzoffsky (1960) studied the agglutination of leptospires with latex particles. Diena, Wallace, and Greenberg (1963) used sensitized particles of bentonite in a flocculation test that was specific for typhoid organisms. Galvez (1966) studied the adsorption of viruses by antibodies coupled to a solid matrix.

Two hypotheses, advanced to explain the antibody-antigen reaction, have been summarized by Lamanna and Mallette (1959, p.270ff) and by Wilson and Miles (1964, p.264ff). In the "two-stage hypothesis, an antigen is assumed to combine with a monovalent antibody and the complex then flocculates in the presence of an electrolyte. In the "lattice" hypothesis, both the antigen and the antibody are multivalent entities which flocculate as a continuous mass.

The flocculation of bacteria by hydrophilic colloids can be a highly specific interaction. Such specificity is observed in the combination of bacterial cells with various natural and synthetic polymeric compounds. These combinations can be particularly useful in the determination of the isoelectric points and ion-combining capacities of microbial cells. In addition, bacterial populations can be enumerated and dilute suspensions can be concentrated by flocculation techniques. The similarities between the flocculation of bacterial cells and the more general reactions considered in colloidal theory will be more evident in the subsequent discussion of this report.

C. Sorption by Microorganisms

The adsorptions of microorganisms onto particulate material or extended surfaces involve the same forces of attraction which cause the suspended cells themselves to absorb or adsorb dissolved ions or small particles. The movement of microorganisms through soil strata, filter beds, and capillary materials are restricted by the various forces of adsorption. The cells of two completely dissimilar species can also interact through the mechanisms of adsorption.

1. Surfaces

Reactions between microorganisms and charged surfaces are often accompanied by significant effects in cellular movement. Organisms traveling through the interstices of soil strata, filter media, mats of plant fibers, etc., are influenced by the electrical charges existing on the surfaces adjacent to the flowing fluid. The metabolic rates of microorganisms adsorbed on surfaces can also be greatly affected. Both nutrients and cells can be adsorbed and concentrated at surfaces. The metabolic rates of the cells can increase because of improved availability of growth factors, or decrease because of the proximity of toxic materials. No general rule can be made because each cell-surface system is peculiar unto itself.

Two viewpoints of the adsorption of microorganisms onto surfaces can be considered. Several particles of dimensions smaller than the microorganism can adhere to a single cell. Conversely, a great number of cells can become attached to an extended surface. Regardless of the viewpoint chosen, the only assumption made is the existence of surface charges and liquid media surrounding the contacted surfaces.

Friedberger (1919) and his student Putter (1920) showed that bacteria were capable of rising to different levels in strips of filter paper dipped into cell suspensions. The capillary action was retarded for those species of bacteria exhibiting a stronger adsorption affinity for the filter paper. Frei and Erismann (1922) studied several of the variables involved in the filtration of bacteria through layers of sand. Aside from the purely hydraulic factors of particle size and fluid velocity, the filtration was favorably influenced by the addition of

electrolytes, notably divalent cations. The addition of electrolytes also affected the capillary rising ability of bacteria in filter paper.

According to Porter (1946, pp.214-6), filtration of micro-organisms is not limited solely to a mechanical sieving action. Other chemical and physical factors which must be considered are: surface adsorption, substrate character, and the pressure and temperature of filtration. Most bacterial filters are negatively charged and thus can adsorb positively charged materials from the suspension being filtered. Particles can therefore be filtered from bacteriological suspensions with greater ease by negatively charged filters if the pH of the suspensions are acidic relative to the isoelectric points of the particles to be removed.

Waksman (1938, pp.626-631) listed the colloidal properties of soil that are of particular importance to microbial growth. These are: the adsorption and concentration of soluble substances at surfaces, the absorption of large amounts of water, and the phenomena of flocculation and deflocculation. The physico-chemical behavior of soil bacteria in relation to soil colloids was discussed by McCalla (1940b). Bacterial growth was stimulated by colloidal clay, possibly by the exchange of essential nutrients and ions. Zobel (1943) found that bacterial activity was enhanced at solid surfaces where the organic matter present in dilute nutrient solutions could be concentrated. The diffusion of exoenzymes and other compounds away from the cells was retarded by adsorption of the cells onto the surfaces. Laren and Dimmick (1964) showed that the attachment of bacteria onto surfaces of continuous culture vessels was an important factor in establishing the kinetics of their growth.

Estermann and McLaren (1959) found that the proteolytic action of bacteria was stimulated by adsorption onto particulate matter. They concluded that substrates and exoenzymes were concentrated at the surface of the adsorbent and therefore became more accessible to bacterial action. According to Lahav (1962), the electrophoretic mobilities of bacterial cells were also influenced by the adsorption of small particles onto the cells. The resulting influences of these small adsorbed particles were greatest at low pH values and high ionic strengths.

The activity of microorganisms can therefore be influenced in several ways by their adsorption onto suspended material or macroscopic surfaces. The freedom of movement of adsorbed cells can be greatly restricted. Growth of adsorbed cells may be promoted by the concentration of nutrients or suppressed by the accumulation of toxic products. The surface charges of bacterial cells may be altered by the attachment of small particles.

2. Cells

Interactions between microbial cells having surfaces of opposing or complementing charge can be considered to be of three types. Cells that are radically different, such as bacteria and viruses, can interact because of gross differences in their surface characteristics. The cells of different species of bacteria may have either positive or negative surface charges depending upon the pH of the suspending medium and the isoelectric points of the cells of the respective species. Conceivably, variant cell forms within a single bacterial species, such as smooth and rough forms, could interact.

The sorption of bacteriophages onto living and dead cells of susceptible bacterial species was studied under equilibrium conditions by Krueger (1931). Adsorption isotherms were established but no attempt was made to elucidate the mechanism of combination. Schlesinger (1932) also investigated the binding of bacteriophages by homologous bacteria. A monomolecular reaction rate was proposed and the reversibility of this reaction was also discussed.

The attachment of viruses onto host cells was discussed at considerable length in a series of papers by T. T. Puck and his associates. Puck, Garen, and Cline (1951) found that in a mixed suspension the attachment of bacteriophages onto host cells was enhanced by the addition of certain cations. A two step reaction was proposed by Garen and Puck (1951) to account for the observed behaviors of bacteriophages and bacterial cells. The first step was considered to be reversible, independent of temperature, and to involve strong electrostatic interactions between specific charged groups present on the surfaces of the bacteriophages and the bacteria. The second step was considered to be irreversible, dependent upon temperature, and to involve linkages of a covalent nature. Garen (1954) later conducted thermodynamic and kinetic studies of this phenomenon.

Tolmach and Puck (1952) concluded from their studies of the pH dependence of the attachment of bacteriophages onto bacterial cells that ionizations of amino and carboxyl groups on both reactants were involved. A mechanism of attachment that accounted for an electrostatic bonding between amino and carboxyl groups was definitely established in a later study by the same authors (1954).

Puck and Sagik (1953) found that bacteriophages attached onto the surfaces of anion exchange resins when suspended in either distilled water or in salt solutions, but attached onto the surfaces of cation exchange resins only when suspended in salt solutions. The host bacterial cells were observed to attach onto anion exchange resins but not onto cation exchange resins even when suspended in salt solutions. Zago (1956) also used ion exchange resins in his study of the mechanism of adsorption of neurotropic viruses.

The first steps of virus invasion were concisely reviewed by Puck (1953). Goldfarb (1958) summarized the salient features of the adsorption of bacteriophages onto bacterial cells. A brief review by Sagik (1959) also contained a discussion of the bacterial cell surface and its relationship to the attachment of viruses. Various theoretical models have been proposed to account for the adsorption of viruses onto bacterial cells. These models will be discussed in detail in Section III of this report.

D. Nonsorptive Charge Phenomena

Certain phenomena that are associated with the surface charges of microorganisms can be considered to be of a nonsorptive nature. The transport of dissolved or suspended material to or from the cells, and the movement of the cells themselves to a well-defined surface are processes which are not directly involved in phenomena of this type. Examples of nonsorptive charge phenomena are: the transfer of cells between two liquid phases, the transfer of cells between a gas and a liquid phase, and the influences of applied electric forces upon cells suspended in a

conducting medium. These nonsorptive processes can operate simultaneously with those processes involving true sorption which were listed in previous sections of this report.

1. Electrokinetic phenomena

Electrostatically charged particles that are under the influence of an electric field will migrate with respect to the suspending fluid toward the electrode which has the opposite sign of the charge on the particles. This phenomenon is called electrophoresis. A related phenomenon, electroosmosis, involves the migration of the fluid relative to a solid surface. Two other electrokinetic phenomena are inversely related to electrophoresis and electroosmosis. These are, respectively, the sedimentation potential, produced by movement of the particles, and the streaming potential, produced by movement of the fluid. All of these processes have been outlined by Abramson (1934, pp.283-309), by Porter (1946, pp.57-8), and by Overbeek (1952).

Of the four phenomena mentioned above, electrophoresis is the most important in biological research. Electrophoresis is an extremely useful quantitative tool for separating difficult or complex mixtures. Mixtures of particles or cells with well-defined surface charges can be differentiated on the basis of variations in their mean mobilities. Electrophoretic measurements, to a great extent, are dependent upon the magnitude of the applied electric field, the pH and ionic strength of the suspending fluid, and the size and sign of the charges of the migrating particles. Lerche (1953) has presented an excellent review of electrophoresis. Abramson, Moyer, and Gorin (1942) have considered the electrophoresis of proteins in detail.

Dyar and Ordal (1946) and Dyar (1948) studied the effects of surface-active agents upon the electrophoretic mobilities of bacteria. The mobilities of the bacterial cells were slightly increased by an anionic agent and considerably reduced by a cationic agent. Harris (1953) found that significant decreases in the electrophoretic mobilities of Gram-positive and Gram-negative bacteria resulted upon treating the cells of both types with ribonuclease.

The interactions occurring between the molecules of certain drugs and the surface components of bacterial cells were easily followed by electrophoretic techniques by Lowick and James (1955, 1957). Douglas (1957) observed pronounced effects upon the electrophoretic mobilities of resting spores and vegetative cells of bacteria following enzymatic treatment. Kolin (1955) discussed the significances of isoelectric points and electrophoretic mobilities of charged particles in various separations.

2. Movements of fluids

Microorganisms can travel through a gas or a liquid by a random "molecular" diffusion or kinetic movement known as Brownian motion. Movement of the cells in a suspension may also occur simultaneously as a result of mechanical agitation, convection currents, or self-propulsion. Cellular movement can also be influenced by attractive or repulsive forces that are associated with other suspended particles, extended surfaces, or by electrical or magnetic forces that are produced by externally applied fields.

Weibull (1960) reviewed the theoretical aspects of the self-propulsion of bacteria, and the various hydrodynamic, thermokinetic, and electrokinetic theories of cellular movement. According to Mitchell (1956)

an uneven gradient in electrical potential is produced across a bacterial cell by the secretion of positive ions at one end and the adsorption of these same ions at the other end. The bacterial cells and its adjacent fluid medium move in opposite directions as a result of this electrical force. Harris and Kline (1956) found considerable variation in the electrical potential required to counteract the movement of motile and non-motile cells.

The interactions which occur between bacterial cells and bacteriophages were interpreted by Boisot (1955) to be due to Brownian motion. The interactions of virus particles with rigid surfaces and with larger suspended particles were a result of Brownian motion according to Valentine and Allison (1959). These interactions were strongly affected by mechanical agitation. The rate of attachment of bacteriophages onto bacterial cells and the mechanisms of collision were discussed by Lamanna and Mallette (1959, pp.560-573).

Bacterial aerosols are particularly important from the standpoint of the transmission of air-borne diseases. The nature and composition of some bacterial aerosols were discussed by Kethley, Cown, and Fincher (1957). Hammond (1958) used ammonium alginate wool to filter microorganisms from large volumes of air. Vlodavets (1959) studied the electrical charge of particles and droplets contained in a bacterial aerosol. He also investigated the influence of this charge upon the processes of sedimentation and coagulation. The species of bacteria frequently encountered in the atmosphere of a room were often found to carry a positive charge; molds and certain other bacterial species often carried a negative charge. Fulwyler (1965) used the Coulter counter to electronically monitor the volumes of suspended cells.

Albertsson (1960) separated cell particles and macromolecules by partioning them between two liquid phases containing water-soluble polymers. Distribution potentials can arise from solubility differences between positive and negative electrolytes present in a two-phase system. Phase separations of mixtures containing ionic polymers occur only at pH values at which one phase is positively-charged and the other is negatively charged. Selective migration of charged cells between two liquid phases may depend upon several factors: the potential difference between the phases, the pH and ionic strength values of the phases, and the net surface charges of the cells.

If a stream of gas is passed through a solution containing several components, the more surface-active materials will be preferentially removed in the resulting foam. Dognon (1941a, 1941b) found that tubercle bacilli were easily removed from suspension by foaming. Electrolytes were required for the removal of the cells of three other microbial species from suspension by the same process. Hopper and McCowen (1952) used a foaming technique to purify surface waters of most of their solid particles and microorganisms. Bikerman (1953) discussed the separation of several labile materials using a foaming process.

Boyles and Lincoln (1958) separated and concentrated both bacterial spores and vegetative cells from liquid media by foam flotation. Gaudin, Mular, and O'Connor (1960a, 1960b) separated bacterial spores from cellular debris by a selective flotation. Gaudin, Davis, and Bangs (1962a, 1962b) later studied the flotation of vegetative cells of bacteria from a suspension to which inorganic salts had been added to facilitate flotation.

Levin et al. (1962) harvested algae by froth flotation. Golueke and Oswald (1965) discussed several methods, including foaming, of harvesting algae which had been cultured in sewage. Rubin et al. (1965) described a microflotation technique for separating bacteria and algae from suspension. Bretz, Wang, and Grieves (1966) also developed methods based on the foaming phenomenon for separating bacterial cells from suspension. Grieves and Bhattacharyya (1965) and Grieves and Wang (1966) developed a model of the foam separation process to be used in waste treatment applications. Freeman (1964) reviewed the aspects of flotation as a specific method for removing bacteria from liquid media and for separating spores from vegetative cells.

E. Statement of Intent

The distribution of microbial cells in a mixed suspension containing several species of microorganisms, as well as dissolved and particulate matter, can be altered in three ways: (a) purification of the cells by removal of the dissolved or suspended contaminants with no concentration of the cells, (b) nonspecific concentration of the cells with or without attendent purification, and (c) resolution of the mixed cell suspension into fractions containing the cells of the component species. All three of these processes may be operating separately, or simultaneously, in any given system depending upon the separation technique applied.

The separation of microbial cells from liquid media can be accomplished by a number of different techniques. Freeman (1964) reviewed the following eight techniques: centrifugation, filtration, freeze drying, spray drying, froth flotation, electrophoresis, flocculation, and ion exchange. The first four techniques are relatively nonspecific and the

cells are subjected to rather severe chemical or physical treatment. Considerable time may also be required and the resulting packed cell preparations are not always most suitable for subsequent use.

Phenomena associated with surface charge effects are primarily involved in applications of the latter four of the eight techniques listed above. The effects of surface charges may also be manifest, but to a lesser degree, in applications involving the first four techniques. Flotation, or gas-liquid partition, and a closely related process, liquid-liquid partition, are both interfacial phenomena that are being investigated more intensely in recent years with respect to their applications to biological systems. Gaudin (1957) has reviewed flotation from a general standpoint; Albertsson (1960) has authored a text dealing with the liquid-liquid partition of cell particles and macromolecules. The electrophoresis of viruses, bacteria, and other types of cells has been reviewed by Brinton and Lauffer (1959).

Flocculation and ion exchange are related phenomena. They potentially are the most specific and versatile of the above mentioned techniques of separation. Flocculation can be considered to be a reaction of suspended cells with dissolved or colloidal substances that ultimately results in an aggregation or coagulation complex that can be separated from the bulk of the liquid phase. In comparison, an actual transfer of ions between the cells and an insoluble material is usually considered to occur in ion exchange. The exchange medium, and the absorbed material associated with it, can be separated from the bulk liquid. The overlap in the definitions of flocculation and ion exchange is apparent. This is particularly evident in the case of soluble and

insoluble surface-active agents which can interact with living cells. The following distinction will therefore be made in this discussion. The process of flocculation will be assumed to involve a combination of cells with suspended particles.

Flocculation has been reviewed by Nakamura (1961). The flocculation of the cells of a number of yeasts, bacteria, and algae upon the addition of various metal salts, quaternary ammonium salts, and polymeric materials were investigated. Tenny and Stumm (1965) and Busch (1966) also demonstrated that chemical flocculation of bacteria could be achieved by hydrolyzed metal ions and by synthetic polyelectrolytes. The specificities which can occur in the chemical interactions between polyelectrolytes and biocolloids were stressed.

As a method of recovering microbial cells from dilute suspensions, flocculation methods have the disadvantage of producing an intimate complex of the cells with the molecules of the flocculating agent which often cannot be easily broken apart after the complex has been separated from the bulk of the suspending fluid. The additional settling and filtration steps that are required of a flocculation process can also be unacceptable in a practical procedure for the separation of cells from suspension. The applicability of flocculation techniques should not be entirely discounted, however, since the features mentioned above are not disadvantageous in certain cases, such as the removal of suspended particles from liquid wastes, or the precipitation of bacteria by a specific antibody.

The general approach in the past in applying ion exchange techniques in separations of microbial systems has been summarized by

Rotman (1960). He stated that the viability of the microorganisms should not be affected by their contact with the exchanger. In addition, the microorganisms themselves should not adsorb appreciably onto the exchanger. Furthermore, the exclusion of contaminants from a suspension should be accomplished with a minimum number of operations.

Two alternative approaches to the application of ion exchange to cellular suspensions must therefore be distinguished. The dissolved ions, be they nutrients, metabolic products, buffers, etc., can be selectively adsorbed leaving the cells freely dispersed in the suspension; or the cells themselves can be adsorbed and the soluble material left in solution. The former procedure has been the usual approach. Although cell suspensions can be purified to some degree by this process, the cells are not concentrated to any extent. Adsorption of the suspended cells themselves by ion exchange materials, however, is a relatively new concept. Both purification and concentration can be achieved since large volumes of suspension can be contacted with the exchange medium in a relatively short period of time using a columnar technique. The adsorbed cells can then be subsequently recovered by desorbing them with suitable regenerants.

The resolution of a mixed suspension of microorganisms into fractions containing the cells of the component species is a more difficult task. The identification of individual species of microorganisms present in a mixed suspension has been attempted by a great variety of techniques. Wilson and Miles (1964, p.480ff) list eight properties that can be used for identification purposes: morphology, staining reactions, cultural reactions, resistance to inimical conditions, metabolism,

fermentative and other biochemical reactions, antigenic structure, and pathogenicity. A pure culture is a requisite for these tests. Certain solvent extracts, dried films, or pyrolysis products derived from cellular preparations can be instrumentally analyzed by infrared spectrophotometry according to Greenstreet and Norris (1957) or by gas chromatography according to Henis, Gould, and Alexander (1966). Suitable reference standards obtained under carefully controlled conditions are required by both of these techniques.

Limitations of the previously listed techniques for obtaining resolution or identification of the components present in a cellular mixtures can be summarized: (a) lack of sufficient specificity, (b) destruction of the cells, (c) irreversible reaction or combination, or (d) fractionation only of cellular fragments. One or more of these limitations cannot be tolerated in applications in which a highly specific, nondestructive method of resolution is required to recover intact cells of a given microorganism from suspension. Considerations of this type can be very important from the standpoints of product recovery, contaminant removal, or cell enumeration and identification, when applied to the areas of industrial fermentation, waste water treatment and examination, and clinical microbiology.

The four techniques most applicable to the resolution of mixed cell suspensions are probably ultracentrifugation, electrophoresis, liquid-liquid partition, and ion exchange chromatography. Charlwood (1966) resolved mixtures containing different species of viruses, and other mixtures of cell particles, nucleic acids, and proteins, by using the techniques of ultracentrifugation. Kolin (1955) used electrophoretic

techniques to resolve mixtures containing algae, and other mixtures containing different species of viruses. Strickler, Kaplan, and Vigh (1966) used electrophoretic techniques to fractionate mixtures containing the cells of several bacterial species. Isolation of bacteria by application of a magnetic field to a suspension has also been mentioned by Knoll and Tresselt (1965).

The counter-current distribution, or partition, or cells between two immisible liquid phases can also be a very specific resolution technique. Particles of several viruses were partitioned in a liquid two-phase system by Albertsson and Frick (1960). Cells of related species of bacteria, as well as cells of algae and a yeast, were also resolved by this technique by Albertsson and Baird (1962). Dognon (1941a, 1941b) indicated that a gas-liquid partition technique could be specific for certain bacteria. Adsorption chromatography, with ion exchange resin as the adsorbent, has been used to resolve liquid suspensions containing mixed populations of microbial cells having different surface charge characteristics. Various combinations of a bacterial species, an algal species, and several yeasts were partially or completely resolved by this technique by Kurozumi, Itoh, and Shibata (1965). The resolutions of several microbial mixtures by selective adsorption techniques are compiled in Table XV in Section II of this report.

Specific, gentle, and efficient techniques are necessary prerequisites for the resolution of mixed suspensions because of the high degree of chemical complexity, physico-chemical lability, and dilute concentrations of the microorganisms often encountered in practice. Of the four techniques described in the two preceeding paragraphs, no one

technique is distinctly superior to the other in resolving cellular mixtures. Ion exchange techniques, however, are relatively simple to apply, require a minimum of equipment, and can be conducted in a short time. In addition, the migration of living cells to a selective solid adsorbent is a process that is similar to many natural phenomena exhibited by microorganisms, such as attraction of nutrients to suspended cells, attachment of cells onto soil particles, interaction of cells with viruses, etc.

The primary goals of the research reported in this manuscript are to provide explanations for the phenomena involved in the adsorption of microorganisms onto ion exchange materials and to delineate the features of engineering design that could be applied to the development of practical separation processes based upon these phenomena. These objectives are outlined in the following paragraphs.

1. Establishment of Adsorption

Interaction of bacterial cells with ion exchange resins can be determined by microscopic techniques to be a surface phenomenon (adsorption). Two features are evident: the disappearance of freely dispersed cells from the bulk suspension, and the concentration of these cells upon the surfaces of the exchange resin particles which in turn flocculate upon contact with the cells. Rate or equilibrium data can be obtained for adsorptions or desorptions conducted on a batch or on a columnar basis.

2. Determination of Variables

A reproducible system is determined by separating the dependent variables into three categories: (1) those variables associated with the

organism to be exchanged, (2) those variables associated with the absorbent producing the exchange, and (3) those variables associated with the chemical environment of the exchange. The exchange adsorption, being a surface charge phenomenon, is influenced by factors which establish the magnitude and orientation of this charge. The species of bacteria and the functional exchange groups of the adsorbent are the most distinguishing variables. The salt content and pH of the medium are variables common to all ion exchange reactions.

3. Elucidation of Mechanism and Model

The surface chemistry of both reactants should be of prime consideration in formulating a mechanism to adequately explain the adsorptive interactions which can occur between bacterial cells and particles of ion exchange resin. The nature of the possible bonding forces should also be considered. A mathematical model can be derived to attempt to predict the kinetic behavior of the exchange from consideration of the analogous phenomena of colloid coagulation and bimolecular reactions in solution.

4. Development of Separation Techniques

The contact between a suspension of bacterial cells and the particles of an ion exchange resin can result in the selective removal of exchangeable impurities, the concentration of the cells, and/or the selective isolation of the cells of one species. The specificity of these processes are largely governed by the surface characteristics of the cells of the species undergoing adsorption or desorption, and the nature of the adsorbing medium. The principal features required for

applications of these processes are: a distinct and reversible capacity of the cells for adsorption and desorption, and an order of selectivity by the adsorbent.

II. LITERATURE SURVEY

A. General Approach

A survey of the voluminous literature of the diverse fields of microbiology, geology, chemistry, engineering, and medicine, that is associated with the general subject of surface phenomena exhibited by microorganisms was reviewed in the previous section of this manuscript. The discussion contained in the present section will be restricted by choice and by necessity to the adsorption of microorganisms onto solid surfaces.

The more important references dealing with the adsorption of microorganisms other than bacteria are first summarized in chronological order. These and other references are then elaborated in a series of abstracts. The literature dealing with the adsorption of bacteria is compiled in a similar chronological outline followed by a series of abstracts. Certain features of a general nature are prevalent throughout the literature dealing with the adsorption of microorganisms. Those features, which are associated with the microorganism, the adsorbent, and the environment, are discussed in a phenomenological order. Listings of the many microorganisms and adsorbents reported in the literature are compiled at the end of this section.

B. Historical Chronology

1. Adsorption of microorganisms onto solid surfaces

The present discussion dealing with the adsorptive behavior of microorganisms other than bacteria onto surfaces is a preamble to the subsequent, more exhaustive compendium of the adsorptive behavior of bacteria. The bulk of the related literature is devoted to the adsorption

of viruses onto solid surfaces for purposes of purification, fractionation, etc., of suspensions. A smaller number of reports have been published dealing with the adsorption of yeasts and algae from suspensions onto surfaces, and only scattered references are available dealing with the adsorption of molds, protozoa, and rickettsiae from suspensions onto surfaces.

Among the first investigators to observe the adsorption of viruses were Pyl (1931), who used charcoal, kaolin, and aluminum hydroxide as absorbents and Sabin (1932), who used alumina gel as an adsorbent. Cultures of viruses were purified by ion exchange resins by Muller (1950) and LoGrippo (1950). Riley (1950a) adsorbed viruses onto diatomaceous silica; Shepard and Woodend (1951) used several inorganic materials for the same purpose. Virus and host cell interactions with ion exchangers were studied by Puck and Sagik (1953). Ion exchange resins were also used by Kelly (1953), Takemoto (1954), and Martin (1955) to adsorb viruses. Curtain (1954) prepared specific adsorbents for suspended virus particles by coupling haemagglutination inhibitors onto powdered cellulose. The adsorption of viruses onto an anion exchange cellulose was considered by Hoyer et al. (1958). Zago (1956) reviewed the principles underlying the mechanisms of the adsorption of viruses onto host cells and onto ion exchange resins.

Drescher (1957) described the adsorption of virus particles onto aluminum oxide in terms of various isotherms. Youngner and Noll (1958) discussed the role of ionic and nonionic binding of viruses to lipids. A mathematical model for the attachment of viruses to nonbiological surfaces was proposed by Valentine and Allison (1959). Several

strains of the same virus could be fractionated by adsorption onto an anion exchange resin according to Matheka and Armbruster (1958). Much of the earlier work was also reviewed by these authors. Techniques for the purification of cultures of viruses by adsorption were developed by Frommhagen and Knight (1959), Horodniceanu et al. (1962), and Nicoli, Betail, and Colobert (1964). Bengtsson et al. (1964) observed differences between the particles of two strains of virus in regard to their adsorption onto polyions. The specific adsorptions of certain plant viruses by antibodies coupled to a solid matrix were studied by Galvez (1966). The kinetics and mechanisms associated with the adsorption of virus particles onto activated carbon were discussed by Cookson (1966).

The adsorption of the cells of several species of yeasts onto various naturally occurring materials were studied by Oksentian (1940). Bair and Stannard (1955) treated irradiated yeast cells with a cation exchange resin. Kalyuzhnii (1957) observed the adsorption of yeast cells onto cellulose. Anion and cation exchange resins were used by Zvyagintsev (1962) and by Gillissen, Schatz, and Dehnert (1961) to adsorb yeast cells. Kurozumi, Itoh, and Shibata (1965) fractionated the cells of different species of yeasts from each other, and from cells of algae and bacteria. Algal cells were chromatogrammed on columns containing calcium phosphate by Albertsson (1956). Ives (1959) suggested that an adsorptive mechanism was involved in the flocculation of algae during water treatment. Kurozumi et al. (1965) observed that algal cells were strongly adsorbed onto an anion exchange resin.

Novogrudskii (1936b) studied the adsorption of the spores of several species of fungii onto a variety of soils. The adsorption of

protozoa onto soil particles was studied by Cutler (1919). Rickettsiae were found to adsorb onto anion exchange resins by Hoyer et al. (1958). Hara (1958) and Yamamoto et al. (1958) used cation exchange resins for the same purpose.

Details reported in the references cited in the above summary and in other supplementary references will now be presented in a series of abstracts.

Pyl (1931) studied the adsorption of the virus of foot-and-mouth disease by charcoal, aluminum hydroxide, and kaolin. The adsorptions of this virus by the latter two adsorbents were dependent upon the pH of the suspensions; the adsorption of this virus by charcoal was independent of the pH. Adsorbed virus particles were eluted from all three adsorbents, however, by changing the pH. The stability of the virus was not materially reduced by contact with the adsorbent. Virus particles could be concentrated by adsorption from large volumnes of fluid. Sabin (1932) purified cultures of poliovirus by adsorbing the viruses onto alumina gel contained in a column. The virus particles were eluted from the gel by contact with a phosphate buffer. Schaffer and Brebner (1933) also used aluminum hydroxide to adsorb polioviruses from tissue suspensions.

Ion exchange resins were used by Muller (1950) to purify suspensions of neurotropic viruses. Nitrogenous impurities were removed from crude extracts containing virus particles by a cation exchange resin. Muller and Rose (1952) concentrated influenza viruses by adsorbing them onto cation exchange resins and eluting them with salt solutions.

The partial purification of suspensions containing viruses using an anion exchange resin accomplished by LoGrippo (1950). His procedure

differed from the procedure used by Muller (1950) in that both viruses and nitrogenous imputities were initially adsorbed; the viruses were subsequently eluted from the resin. LoGrippo and Berger (1952) expanded this procedure by using both anion and cation resins for the purification of suspensions of viruses. The advantages accruing from treating large volumes of solution in a short time with a minimum of equipment were mentioned.

Riley (1950a, 1950b, 1950c) separated virus particles from chicken tumors using a chromatographic column containing diatomaceous silica. The effects of salt concentration upon adsorption and elution of the virus particles from this adsorbent were studied. The adsorption of T_2 bacteriophage onto several inorganic substances was investigated by Shepard and Woodend (1951). A simple, rapid, and comparatively safe method for detecting Coxsakie viruses in sewage was developed by Kelly (1953). She found that the virus particles could be adsorbed onto anion exchange resins and then eluted from the resin by salt solutions. Gilcreas and Kelly (1955) and later Sanderson and Kelly (1964) discussed the removal of viruses from waste water.

The interactions between viruses and host cells were studied by Puck and Sagik (1953) with the aid of ion exchange resins. The presence of salt was found necessary in order for certain bacteriophages to attach to cation exchangers; salt was not necessary for the attachment of bacteriophages to anion exchangers. Particles of influenze virus behaved in a similar manner. Both T_1 bacteriophage and influenza virus particles were readily eluted from cation exchangers; T_2 bacteriophages were desorbed in two separate fractions. The affinity of cation exchange resins for the influenza virus was great enough to almost completely

remove particles of this virus from their previous attachment to fowl erythrocytes.

Takemoto (1954) isolated influenza virus from various suspensions using a cation exchange resin. The virus particles were eluted from the resin by a salt solution. Curtain (1954) prepared adsorbents that were specific for influenza viruses by coupling haemagglutination inhibitors to powdered cellulose. Several species of influenza viruses were resolved by chromatography of mixed suspensions using this selective adsorbent. Martin (1955) speculated that ion exchange resin and adsorbents had certain medical applications. Viruses could be rendered avirulent by adsorption onto resins.

An extensive thesis was developed by Zago (1956) concerning the mechanism by which neurotropic viruses adsorbed onto particles of ion exchange resins which served as "synthetic" host cells. The adsorption of the viruses by cation exchange resins was improved by the addition of inorganic cations to the virus-resin suspension. Anion exchange was inhibited by the addition of inorganic anions. A two-step mechanism was proposed to explain the mechanism of adsorption of viruses onto ion exchange resins. The literature dealing with the adsorption of viruses was also reviewed.

Creaser and Taussig (1957) described a method for purifying suspensions of bacteriophage by selective adsorption and desorption using an anion exchange cellulose. Rubin, Franklin, and Baluda (1957) determined rate constants for the adsorption of the virus of Newcastle disease onto its host cell. In a series of papers Drescher (1957, 1959a, 1959b, 1960) reported his findings concerning the adsorption of viruses

onto aluminum oxide. The adsorptions were strongly dependent upon both the pH values and the salt contents of the media. Freundlich isotherms were described. The application of adsorption as a means of separating different viruses was also considered by Drescher (1959b).

Cochran, Chidester, and Stocks (1957) used a column containing carboxymethylcellulose to chromatogram tobacco mosaic viruses. The conductivity, pH, optical density, and virulence of the effluent from this column were monitored. Possible methods of isolating viruses were proposed. Commoner et al. (1956) fractionated the components of tobacco mosaic virus using an anion exchange cellulose. The ion exchange chromatography of tobacco mosaic virus and of potato virus was studied by Levine (1958). Particles of the first virus were fractionated into various subgrouping by step-wise elution from an anion exchange cellulose. Hoyer et al. (1958) used columns containing an ion exchange cellulose to purify tissue cultures of poliomyelitis viruses. Excellent recoveries of the adsorbed viruses elution with salt solution were obtained. Appreciable purification of the suspensions were demonstrated as measured in terms of the removal of phosporus and protein.

Chang et al. (1958a, 1958b) studied the removal of Coxsackie and bacterial viruses from water by flocculation with inorganic iron and aluminum salts. They also used the ion exchange technique of Kelly (1953) to separate viruses from water. Removal of viruses from suspension by chemical flocculation was apparently a result of the nonspecific formation and aggregation of metal-virus complexes. Clake and Chang (1959) reviewed methods of removing viruses from water that included flocculation, adsorption, and filtration.

Suspensions of a number of different viruses could be purified by adsorption of the viruses onto cholesterol according to Youngner and Noll (1958). The nature of the bonds between the virus and cholesterol surfaces was independent of the ionic environment. The observed interaction between viruses and cholesterol were concluded, therefore, to be due to van der Waals' forces rather than to hydrogen bonding or electrostatic forces. This method of purification can be applied in studies of virus-host cell combinations, in fractionation of virus particles into smaller components, and immunological studies. In a second paper, Noll and Youngner (1959) considered as either lipophilic or hydrophilic. Details of the kinetics of adsorption, the efficiency of adsorption, and the reversibilities of the bonding of T_1 bacteriophages to water-insoluble polar lipids were provided by Zelkowitz and Noll (1959).

The adsorption of viruses onto nonbiological surfaces, such as glass plates and latex spheres, was reported in a series of papers by Valentine and Allison (1959) and Allison and Valentine (1960a, 1960b). A theoretical model based upon the mechanics of diffusion was developed so that rates of adsorption could be predicted. The rates of adsorption of the viruses were found to increase if the concentration of cations in the suspensions were decreased. On the basis of these studies, the main groups interacting in virus-host cell combinations were concluded to be: amino groups on the surfaces of the viruses and phosphate groups on the surfaces of the host cells.

Matheka and Armbruster (1958) obtained several fractions of three strains of tobacco mosaic virus by adsorbing the viruses onto an anion exchange resin and subsequently eluting them with a salt solution.

Comparisons were made between those fractions obtained by ion exchange chromatography and others obtained by adsorption on and elution from chicken erythrocytes. A brief review of earlier work was also included. A column containing anion exchange resin was used by Taylor and Graham (1958) to purify suspensions containing polioviruses labelled with radiophosphorus. Frommhagen and Knight (1959) purified suspensions containing influenze viruses by adsorption of the viruses onto aluminum phosphate. The advantages of this method were stated to be: convenience and rapidity, higher infectivities and haemagglutination activity, lower temperatures, no contamination of products with organic materials, and yield of a sedimentable host material as a byproduct.

Polioviruses were concentrated and partially purified using a cation exchange resin by Horodniceanu et al. (1962). The viruses were first adsorbed onto a zinc hydroxide gel that was separated from the suspension and subsequently dissociated. The free zinc ions were then removed by cation exchange. Suspensions of polyoma T virus were purified by adsorption of the virus onto an anion exchange resin according to Sheinin (1962). The adsorbed virus particles were then eluted from the resin by salt and purified further by ultracentrifugation. Nicoli et al. (1964) compared several mechanisms proposed to explain the adsorption of particles of Myxovirus parainfluenzae onto substituted celluloses. A number of non-specific sites on the adsorbent may be involved in the electrostatic binding of the virus to the adsorbent. Other more specific sites located on the adsorbent may also be involved. Desorption of the particles of this virus from the different adsorbents could be induced by increasing the temperature or the concentration of salt.

Bengtsson et al. (1964) noted differences between the m^+ and m^- strains of poliovirus when interacted with polyions. Differences between these viruses when adsorbed onto anion and onto cation exchangers were correlated with the isoelectric points of the two strains. Adsorption of these viruses was influenced by both the type and concentration of the buffer salts present in the respective suspensions. Taniguchi (1964) separated various components of tobacco mosaic virus using a cation exchange resin and an anion exchange cellulose. Dunn and Hitchborn (1965) used bentonite to adsorb a wide variety of plant viruses from suspensions. Galvez (1964) observed that viruses could be filtered from suspension by beds of charcoal. In a later paper, Galvez (1966) prepared solid adsorbents were seriologically specific for certain plant viruses. Particles of tobacco mosaic virus were separated from mixed suspensions that also contained particles of southern bean mosaic virus using chromatographic columns packed with the immuno-adsorbent. Carlson, Woodward and Sproul (1966) found that the adsorption of viruses by several types of clays could be improved by the addition of sodium or calcium ions to the suspensions. The formulation of a clay-cation-virus bridge was proposed. Adsorption of viruses onto caly particles was not directly related to the base-exchange capacities of the clays but rather to the number of surface exchange sites per unit of surface area of the clays.

The kinetics and mechanisms of the adsorption of T_4 bacteriophages onto activated carbon were investigated by Cookson (1966). The kinetics of this process were described in terms of a reversible second-order equation. Adsorption of T_4 bacteriophages onto activated carbon could be described by a Langmuir isotherm. The reaction was considered to

be diffusion-limited and thermodynamically reversible. Attraction between the virus and the activated carbon was assumed to be due to electrostatic forces.

Oksentian (1940) found that the cells of Saccharomyces ellipsoideus could be adsorbed by charcoal, kaolin, and talc. The variabilities of the cells were not affected by adsorption. The effects of X-irradiation upon the growth of yeast calls were modified by starvation of the cells, treatment of the cells with cation exchange resin, or both, according to Bair and Stannard (1955). The effects of radiation upon yeast cells are: suppression of both colony formation and growth in liquid media. These effects are enhanced by starvation induced by aeration of yeast cells suspended in distilled water. Colony formation and growth in liquid media were increased by the addition of the cation exchange resin to irradiated suspensions. The two permanent effects of irradiation on growth, inactivation and delay, could be distinguished on the basis of these contrasting observations.

Kalyuzhnii (1957) divided certain species of alcohol-producing yeast into two classes: those species that adsorb or do not adsorb onto fibers of non-bleached cellulose. Both multiplication and fermentation by the adsorbable yeasts were stimulated by adsorption. The productions of biomass and alcohol by the adsorbed cells were greater and more efficient when compared to non-adsorbed yeast cells suspended in a homogeneous medium. Kalyuzhnii (1964) and Kalyuzhnii and Petrushko (1965) explained certain differences observed between the cells of flocculating and non-flocculation strains of Saccharomyces and Torula yeasts in terms of their content of free and bound water, electrical charge, and polysaccharide

content. Cells of the non-flocculating species were characterized by a resilient surface layer of water, a high electrical charge, and a low polysaccharide content. The nonflocculating cells could be converted to the flocculating type in the presence of multivalent cations.

Gillisson et al. (1961) found that spores of baker's yeast could be removed from aqueous suspensions by an anion exchanger but not by a cation exchanger. Resin could be reused after regeneration with salt. Cells of Saccharomyces cerevisiae were adsorbed by a cation exchange resin according to Zvyagintsev (1962). Partial removal of the adsorbed yeast cells was then obtained by washing the resin with water. Complete removal of cells from the resin was easily achieved by the addition of a weakly alkaline solution. Bacteria were found to adsorb in much greater numbers than the yeasts onto the particles of the same type of anion exchange resin.

Several different species of yeasts were found by Kurozumi et al. (1965) to be capable of adsorbing onto an anion exchange resin and then being eluted by salt solutions. Cells of baker's yeast were completely separated from cells of Escherichia coli or Chlorella ellipsoidea. Resolution of a mixture containing cells of two species of yeast was less satisfactory. The viabilities of the chromatogrammed yeast cells were identical to the controls.

Cells of Chlorella pyrenoidosa were chromatogrammed on a column containing calcium phosphate by Albertsson (1956). The adsorbed cells were eluted with phosphate buffers. Cells of the same algal species were adsorbed both in a batch contactor and in a moving bed column. Ives (1959) suggested that an adsorptive mechanism was involved in the

flocculation of algae by coagulating agents such as iron and aluminum hydroxides. The positively charged hydroxides and the negatively charged algal cells were mutually attracted and their respective charges neutralized. Kurozumi et al. (1965) studied the adsorption of cells of Chlorella ellipsoidea onto columns of an anion exchange resin. The cells of this algal species were eluted by various salt solutions. The algal cells were very strongly adsorbed. A mixed suspension containing both algal and yeast cells was resolved by selective adsorption and desorption of the algal cells.

Golueke and Oswald (1965) and Golueke, Gotaas, and Oswald (1957) observed the removal of algal cells from suspension using columns containing strong or weak cation exchange resins. The negative charges of the algal cells were first neutralized by the adsorption of an excess of hydrogen ions onto the cells from the acidified medium. The cationic cells were then removed by a cation exchange process. Algal cells could not be precipitated by sodium, calcium, or magnesium ions but cation exchange of algae was reduced in the presence of these cations. Harvesting of algae by ion exchange was considered to be uneconomical but the technique can aid in the study of the surface properties and the principles of flocculation of algal cells.

The adsorption of the spores of various species of fungii by soils was studied by Novogrudskii (1936b). Considerable variation in the degree of adsorption shown by the cells of different species was observed. The degree of adsorption was a function of the type of soil and the pH of the suspending medium. The greatest selectivity in adsorption of fungal spores was exhibited by an average podzol (acid soil).

Spores of Aspergillus niger and Aspergillus glaucus were only slightly adsorbed; spores of Penicillium glaucum and Mucor mucedo were adsorbed to an intermediate degree; spores of Fusarium sp. and Botrytis cinerea were very strongly adsorbed. Adsorptions of the spores of these same species by voronezh chernozem (alkaline soil) were uniformly high.

The factors governing the adsorption of protozoa by soil particles are those of surface "action" according to Cutler (1919). The capacities of various substances, such as sand, soil, and clay for retaining the cells of various species of amoebae and flagellates were specific and constant. The degree of adsorption was greatest for those substances containing the finest particles. Adsorption was independent of the concentration of the cells in suspension and the time of contact.

Hoyer et al. (1958) found that suspended Q fever or epidemic typhus rickettsiae could be purified by adsorption onto and selective elution from cellulose anion exchangers. Yamamoto et al. (1958) purified separate suspensions of three species of rickettsiae using a cation exchange resin. Hara (1958) discussed the purification of suspensions of Rickettsia mooseri using the same cation exchange resin. Emphasis was placed upon the superiority of cation exchange over other methods of purifying suspensions of rickettsiae. The purified rickettsial suspensions retained infectivity and maintained their antigenicity.

2. Adsorption of Bacteria onto Solid Surfaces

The periodic advances in the understanding of the adsorption of bacteria are interesting to note. Only a few scattered appear prior to World War I. The work of Krüger (1889) is most signigicant during this

period. Beginning in 1915, considerable interest was generated with regard to the adsorption of microorganisms by various soils and other naturally occurring compounds. The early German and Russian literature is particularly important.

Differences between the adsorptive behavior of the cells of Salmonella typhosa and those of Escherichia coli onto a variety of solid substances were observed by a number of investigators, notably Kuhn (1915) and Michaelis (1918). The adsorption of the cells of other bacterial species were studied by Salus (1917), Eisenberg (1918) and Bechhold (1918). Friedberger (1919) and Putter (1920) studied the capillary rise of bacterial cells in filter paper. Frei and Erismann (1922) studied the filtration of bacteria by sand.

A number of researchers, particularly Dianova and Voroschilova (1925), Khudiakov (1926a), Karpinskaya (1926), and later Minenkov (1929), Lasseur, Dombray, and Palgren (1934), Rubentschik, Rosin, and Bieljansky (1934, 1936), and Novogrudskii (1936a, 1936b), elaborated upon the subject of the adsorption of bacteria by soils, sediments, and other materials. Several of these authors also studied the metabolism of adsorbed versus unadsorbed cells.

The hydrogen ion concentration and the type of electrolytes present in a suspension containing bacterial cells undergoing adsorption were most important according to Peele (1936). Results of a similar nature were reported by Gunnison and Marshall (1937), Waksman and Vartiovaara (1938), and Oksentian (1940). Dikusar (1940) reviewed many of the early works (pre-World War II) dealing with the adsorption of microorganisms by solid surfaces. Ham and Barnes (1947a) were probably the first to use

synthetic ion exchange resins to remove bacterial cells from aqueous suspensions.

Investigation of the phenomenon of the adsorption of bacteria was resumed following World War II. Kunin (1947) indicated that bacteria could be adsorbed onto anion exchange resins and eluted with salt solutions. Tschapek and Garbosky (1950b) made a serious study of the effects of pH, salt content, agitation, and the concentration of both reactants upon the interaction between bacterial cells and sand particles. Debusmann (1950) and Weiss (1951) contributed their findings concerning the adsorption of bacteria by soils. Puck and Sagik (1953) used synthetic ion exchange resins to study the interactions between bacteriophages and host bacteris. Martin (1955) attempted to quantitate the adsorption of bacteria onto ion exchange resins.

About 1959, at the time the subject of this thesis germinated in an undergraduate course in microbiology, research dealing with the adsorption of bacteria was accelerated. Several references worthy of special mention are those of Zvyagintsev (1959b), Adamov (1959), and Gillissen (1960). The adsorptions of the cells of several bacterial species onto both anion and cation exchange resins were considered by these investigators. The metabolic activities of the cells of various bacterial species adsorbed onto the surfaces of soil and resin particles were considered by Estermann and McLaren (1959) and by Hattori and Furusaka (1959a). Rotman (1960) reviewed some of the applications of ion exchange technology to microbiology.

The applicability of ion exchange as a selective process for the separation of different types of cells was considered by Helmstetter and

Cummings (1963). Saprophytic organisms could be removed from mixed suspensions also containing pathogenic organisms according to Adamov (1959). Schwartz and Mayer (1963) used an ion exchange resin to concentrate cells of Mycobacterium tuberculosis from a mixed suspension of unspecified bacteria. Kurozumi et al. (1965) separated the cells of several species of yeasts, an algal species, and a bacterial species by ion exchange chromatography.

Details of the references cited in the preceeding summary, as well as those of other supplementary references, will now be reported in a series of abstracts. The literature of the fifty-year period, 1915-1965, has been surveyed in a comprehensive fashion. The contents of reports pertinent to a study of the phenomenon of the adsorption of bacteria onto solid surfaces are discussed.

One of the earliest documentations of the adsorption of bacteria onto surfaces is that of Krüger (1889). The physical effects of sediments upon water-borne microorganisms were studied. Reductions in the number of bacteria present in aqueous suspensions were observed upon the addition of clay, brick-dust, charcoal, kieselguhr, calcium carbonate, and other adsorbents. A greater adsorptive action was exerted by substances of low specific gravity, e.g. chalk or megnesium oxide, than those of high specific gravity, e.g. coke or sand. It was concluded that bacteria were "grasped" within the pores of sedimentable substances as a result of the combined action of chemical and physical forces.

Geronne and Lenz (1915), and Kalberlah (1915) studied the combination of charcoal granules and typhoid bacilli as a possible therapeutic method to reduce the severity of the disease. Strell (1915)

described a chemical process for removing bacteria from surface water. The bacterial cells were first adsorbed onto fine particles of humic substances that were added to the wast water. The complex of bacterial cells and humic substances was then flocculated upon the addition of aluminum sulfate. The flocculated complex was allowed to settle and the supernatant liquid was filtered to produce a sterile effluent.

Kuhn (1915) observed that bacteria were strongly adsorbed by charcoal. The degree of adsorption was found to be stronger for the cells of Salmonella typhosa and Salmonella paratyphi than for those of Escherichia coli. Based upon this selective adsorption behavior, a procedure was developed to detect typhoid bacteria in feces and urine. Kuhn and Heck (1916) showed that both charcoal and clay were selective in their adsorption of typhoid bacteria. According to Salus (1917), the selective adsorption of bacteria by solid adsorbents is dependent upon the magnitude of the electrical charges of each species.

Eisenberg (1918) discussed the specific adsorption of bacteria by many adsorbents. His report is the most significant of the early literature. Cells of four Gram-positive and four Gram-negative bacteria were adsorbed to various degrees by some fifty water-insoluble organic and inorganic substances. Removal of bacterial cells from suspension was stated to be due more to the adhesiveness of the bacteria and to the surface qualities of the adsorbents than to the chemical natures of the adsorbents. Gram-positive bacteria were more strongly adsorbed to some adsorbents than were Gram-negative bacteria. The cells of certain species were found to be selectively adsorbed from suspensions containing cells of two bacterial species. Cells of the preferred species were concentrated

upon the surface of an adsorbent immersed in the suspension. Differentiation of the cells of pathogenic species was attempted based upon the selective adsorption of the cells of individual bacterial species.

Michaelis (1918) also used selective adsorption as a technique to isolate typhoid bacteria. In contrast to the findings of Kuhn (1915), who used charcoal and clay, Michaelis found that the cells of Escherichia coli were preferentially adsorbed by kaolin from a mixture also containing cells of Salmonella typhosa. The selective adsorptions of these bacteria were tentatively postulated to be due to differences in "mechanical stickiness" of the bacterial cells rather than to any definable characteristics of the adsorbent. A good review of other early reports of bacterial adsorption with special mention of the work of Eisenberg (1918), was included in this report.

Gutfield (1919) found no noteworthy differences in the adsorptive capacities of kaolin, boluphen (sic), and sea sand for the cells of Escherichia coli and Salmonella typhosa grown as pure cultures or obtained from stools of typhoid patients. The behavior of these adsorbents were not uniform at all times and no selectivities for the cells of either species were observed. The results of Kuhn (1915) also could not be confirmed by Reiter and Meyer (1921). They attributed any differences in the adsorptive behaviors of the cells Escherichia coli and Salmonella typhosa to an antagonistic action between the species that was enhanced by the intimate contact occurring during adsorption. Little practical value was given to adsorption by kaolin as a technique for isolating bacterial cells from suspension or from each other.

The adsorption of bacteria by a number of powders and fibrous materials was studied by Bechhold (1918). Staphlococcus aureus and Escherichia coli, organisms of opposing Gram reaction, were selected for the adsorption tests. The adsorption of bacterial cells was related to the analgous adsorption of dyes. The ability of fuller's earth and charcoal to adsorb bacteria surpassed that of all other powders investigated. The importance of the extent of surface development was recognized; the finer the adsorbent, the greater the adsorption. Differences in the chemical natures of the adsorbents and the adsorbing bacteria could not be correlated.

Friedberger (1919) reviewed several of the earlier reports dealing with the adsorption of bacteria and added his own contribution. He studied the capillary rise exhibited by bacteria in strips of filter paper partially immersed in suspensions containing bacterial cells. Differences were observed in the relative heights of rise attained by the cells of various bacterial species. Putter (1920), a student of Friedberger, continued the investigation of the ability of bacteria to rise by capillary action in strips of filter paper. The relative heights attained by the cells of various bacterial species was governed by their adsorptive behavior, which in turn was dependent upon their Gram-staining behavior. Gram-positive bacteria were more strongly adsorbed and did not rise as high as Gram-negative bacteria. The adsorption of bacteria was considered to be a mechanical rather than an electrochemical phenomenon. Adsorption was very rapid and terminal heights were reached within five minutes. A discussion of the separation of bacterial mixtures by paper chromatography was also included in this early work.

Supfle and Muller (1920) investigated the mechanism by which bacteria were killed by toxic sublimates of heavy metals. A large portion of the bacteria and most of the sublimate present in a given suspension were removed by adsorption onto charcoal. The unadsorbed cells remained viable.

Frei and Erismann (1922) made an excellent contribution to the theory of the filtration of bacterial cells by sand and by paper. They found an increase in the filtering ability of sand beds as the depth was increased or the particle size was decreased. The filtration was affected by the concentration of various electrolytes and by those variables of a purely hydraulic nature. The capillary rising abilities of bacterial cells in filter paper were also retarded by the addition of electrolytes to the cell suspensions. Adsorption of the bacterial cells onto the paper was consequently favored. The adsorption was improved to a limited extent by increased agitation.

Dianova and Voroschilova (1925) conditionally used the term "adsorption" to describe the ability of soil particles to combine with an enormous number of bacteria. Some direct microscopic observations and good quantitative data dealing with this phenomenon were included in their study. The adsorptions were affected by the concentrations of bacteria and soil. The degree of adsorption was greatest for highly dispersed particles (clay) and least for gross particles (sand). The chemical activities of the bacteria present in soil were influenced by the degree of their adsorption. Adsorbed cells ferment (sic) more weakly than those in a free state. The difference in metabolic rates observed between adsorbed and unadsorbed cells was greatest for those species exhibiting the greatest degree of adsorption.

Khudiakov (1926a, 1926b), under whose direction the previous work was done, termed the attraction of soil for bacteria "adsorption." The extent of adsorption of bacteria onto soil particles was found to be a function of the bacterial species. Several colored drawings of bacterial cells adhering to soil particles were made on the basis of microscopic observations. The metabolic activities of both adsorbed and unadsorbed cells were measured. The amount of carbon dioxide liberated was greater for the adsorbed cells. The significance of the adsorption of bacteria with respect to various microbial processes in the soil was discussed at length.

Karpinskaya (1926) continued the earlier work of Khudiakov discussed in the above paragraph. The adsorption of bacteria was again found to be greatest for silt and least for sand. A preferential adsorption by silt of the cells of certain bacterial species was observed. The adsorption of bacteria was promoted by the agglomeration of the soil particles. Bacterial cells and soil colloids were also considered to combine by a process of adsorption. Surface effects as well as electrochemical effects were considered in the explanation of the adsorption of bacteria. A singular depression of bacterial vitality was observed upon adsorption and partial death was probably promoted. No differences were observed, however, between the adsorption of living and of dead bacterial cells.

Minenkov (1929), who also worked under the direction of Khudiakov, investigated the adsorptive behavior of various soils toward bacteria. The extent of the adsorption depended upon the particular soil-bacteria combination under consideration. A relationship between adsorption and

the "mechanical composition" of the soils was distinctly noted from the results. The conclusion that the extent of adsorption of bacterial cells was greatest for silts and least for sands was reiterated.

Rubentschik et al. (1934, 1936) studied the adsorption of bacteria by the sediments of limans (salt lakes). Differences were noted in the adsorptive capacities of various sediments for cells of the same bacterial species, and in the degree of adsorption of the cells of different bacterial species for the same sediment. Adsorbed bacterial cells could be partially desorbed into sterilized water. Preferential adsorption and displacement occurred with mixtures containing cells of different bacterial species. Adsorption was considered to play some part in the naturally occurring differentiation between planktonic and benthic bacteria in the limans. Adsorption was also considered to be a strong factor affecting the life processes of all living forms in an ecological system. Living bacteria were adsorbed to a greater extent than those killed by heat.

The adsorptions of bacteria onto paper and onto activated carbon were investigated by Lasseur, Dombray, and Palgen (1934). The nature of the adsorbent and the species of bacteria were reported as being the most important variables. No relation was found between the particle size of the activated carbon and its capacity for adsorption of bacterial cells. Several photomicrographs of bacterial cells adhering to various types of paper surfaces were included.

Novogrudskii (1936a, 1936b) studied the adsorption of the cells of eight bacterial species onto three types of soil. The adsorption of fungal spores was also investigated. The intensity of adsorption of bacteria depended upon the mechanical composition of the soils, i.e.

greater adsorption of bacterial cells by fine silt than by coarse sand once again was observed. The intensity of adsorption of bacterial cells by the particles of a given soil was not the same for each bacterial species. This was assumed due to the surface properties of the cells of the particular species and not due to either the size or the shape of the cells. In general, Gram-positive bacteria were adsorbed more strongly than Gram-negative ones. The degree of adsorption was primarily determined by the pH of the medium; adsorption could be reversed by altering the pH. Novogrudskii (1937) found seasonal variations in the degree of adsorption of bacteria by podzolized soil. Maximum adsorption was observed during the summer months. The ability of soil of adsorb bacteria was dependent upon two independent factors: the mechanical composition of the soil and the degree of saturation of the soil with microorganisms. The seasonal variation in the degree of adsorption of bacteria by a soil was ascribed to the variations in the numbers of microorganisms populating the soil.

The experiments conducted by Peele (1936) were designed to obtain additional information concerning the type of attraction occurring between bacterial cells and soil particles. Adsorption was considered by Peele to be due to the mutual attraction of unlike electrical charges. Negatively charged bacteria were removed from suspension by attachment to positively charged particles of loam. This behavior was markedly influenced by the type of base predominating in the soil exchange complex. The specificity of a soil for the cells of different bacterial species may be determined by this latter factor.

Soils saturated with trivalent aluminum or iron cations were found to adsorb the cells of Azotobacter chroococcum more strongly than did either divalent or monvalent ion-saturated soils. These observations were offered in explanation of the strong adsorption of negatively charged bacteria exhibited by iron and aluminum hydrogels. The efficiency of rapid sand filters in municipal water treatment was explained to be a result of the bacteria being adsorbed as well as filtered from the water. The biochemical activity of bacteria, as measured by the amount of carbon dioxide evolved, was markedly decreased upon adsorption of the cells onto soil particles. A direct correlation existed between the percentage of adsorbed cells and the retardation of carbon dioxide evolution.

Gunnison and Marshall (1937) found an apparent adsorption of the cells of several species of bacteria in vitro by inert particulate reagents. They did not consider the removal of cells from suspension to be a "true" adsorption since the process did not occur in proportion to the charge on the adsorbents and was not particularly influenced by the type of adsorbent. The species of bacteria appeared to be the most important variable of the adsorption although no correlation was found with respect to Gram-staining behavior. A somewhat greater degree of adsorption occurred at lower values of the pH of the suspension but the range studied was not great. The degree of adsorption was not greatly influenced by either the time of contact of the cells with the adsorbent or by the relative concentration of the bacterial cells initially in suspension.

Waksman and Vartiovaara (1938) observed that marine mud was capable of adsorbing bacterial cells from sea water suspensions. Sand

had very little capacity for adsorption. The adsorption of cells of a mixed bacterial population by mud was soon followed by a rapid increase in the number of adsorbed bacteria produced at the expense of the organic matter contained in the bottom material. A large number of cells initially could be adsorbed, removed, or otherwise inactivated by mud but a permanent paralysis (sic) of their growth or metabolism was not found.

Dikusar (1940) critically reviewed much of the earlier literature. He conditionally used the term "adsorption" to mean "a concentration of microorganisms on the surface of the solid phase." The effects of purely mechanical forces, such as surface tension, were discounted as having little significance in the process of adsorption. Electrical forces were considered to be the prime factors contributing to the attraction between cells and adsorbents. A concentration of microorganisms upon a solid surface was assumed to occur only under conditions where a large potential difference existed between the adsorbent and the microorganism. This behavior was also related to the isoelectric states of both reactants. These electrical forces were not considered to have broad significance is soils since both soils and bacteria are negatively charged under natural conditions. An interesting feature that was determined upon comparison of data collected for the adsorption of motile and non-motile bacteria, was cited in the above reference. The motile forms apparently were capable of counteracting the forces of mechanical settling (gravity), and could even push aside the much larger soil particles. It was therefore concluded that adsorption was a function of the motility of the microorganism.

Dikusar (1941) made further distinctions between mechanical adsorption and orthokinetic coagulation as the two processes by which

bacteria can become concentrated upon the surface of soil particles. The process of mechanical adsorption was stated to be independent of the surface charges of the soil particles. This process was also considered to be insignificant since the adsorbed bacteria were easily washed off the soil. Orthokinetic coagulation was presumed to be initiated by the presence of certain electrolytes, such as calcium and magnesium in the soil. A decrease of the electrical potential or either the soil particles or the bacterial cells which are both negatively charged was initiated by these cations. The degree of adsorption was found to be independent of the concentration of microorganisms. No exchange adsorption of cells of different bacterial species was observed but non motile forms initially were more strongly adsorbed. The evolution of carbon dioxide by the adsorbed cells of various species were unrelated to their degree of adsorption. The rates of multiplication of the cells of the same species, however, were greater in a medium of high capacity for adsorption than in one of low capacity for adsorption.

Oksentian (1940) tested charcoal, kaolin, and talc for their capacities to adsorb cells of lactic acid bacteria and yeasts from aqueous suspensions. She recognized the importance of the electrokinetic potentials of the adsorbents, the interaction of electrical charges, and the role of surface energy upon the adsorption of microbial cells. An increase in the degree of adsorption was observed upon decreasing the negative charge of the adsorbent. Adsorption of cells onto particles did not affect their multiplication, but did sharply reduce their physiological functioning. The bacteria were also coagulated in definite stoichiometric ratios by charge interaction with amphoteric proteins.

Ham and Barnes (1947a, 1947b) and Barnes and Ham (1942) succeeded in removing bacteria from fluids by contacting cell suspensions with an anion-active polymer with guanidinyl groupings that was formed by a formaldehyde condensation. The attraction between this resin and the negatively charged bacterial cells was increased by application of a high voltage across the resin bed. Somewhat later Barnes (1952a, 1952b) used beds of the same resin to remove cells from suspension without application of the high voltage. Suspensions of cells were also sterilized by the oligodynamic action of silver ions precipitated on and bound with the resin.

Some unpublished work concerning the adsorption of bacteria onto ion exchange resins was cited by Kunin (1947 p.134, 1964). He disclosed that strongly basic anion exchange resins in chloride form were capable of adsorbing bacterial cells from suspension. The adsorbed cells were considered to have isoelectric points above pH 5. Regeneration of the resin without death of the cells was considered feasible by elution with a brine solution. The bacterial cells were assumed to behave as macro-ions and adsorb at surfaces; the uncharged spores did not adsorb. Separation of cells and spores was suggested as a possible application of ion exchange.

The principles underlying the adsorption of the cells of an unspecified Azotobacter onto sand were reported in an interesting series of papers by Tschapek and Garbosky (1950a, 1950b, 1951). The adsorption was studied as a function of the time of agitated contact between the cells and the sand particles. Concentrations of bacteria, adsorbent, and various electrolytes were also varied, as was the pH of the solution.

Adsorption was reversible and primarily a function of the pH. An inverse relationship was found between the viability of the bacteria and their coagulation by electrolytes. The concentration of electrolyte required for coagulation was an inverse function of the valence of the added cation.

Debusmann (1950) considered the comparative physical and bacteriological effects of pectin-containing compounds, that were combined with other agents, as remedies for infant dyspepsia and toxic intestinal disturbances. Several of the pectin-containing compounds had considerable capacity for the adsorption of dyes, alkaloids, toxins, and bacteria.

Weiss (1951) noted that cells of Escherichia coli were adsorbed onto the particulate matter suspended in naturally turbid waters. He questioned the general use of the term "adsorption" but did use it himself to describe the attraction existing between bacterial cells and various types of particulate metter. The degree of adsorption of bacterial cells was a function of the type and size of the silt particles. The flocculation of silt was greater in salt water but the capacity of silt to adsorb bacteria was reduced and desorption of cells was observed in some waters of high salt content. The rate of sedimentation of bacterial cells was increased upon their adsorption onto soil particles.

Puck and Sagik (1953) found in their studies of virus and host cell interactions that the cells of Escherichia coli were easily removed from suspension by an anion exchange resin but that a cation exchange resin had relatively little effect. The cells of this species did not appreciably adsorb onto the cation exchange resin even in the presence of sodium chloride. Anion exchange of cells was evident in distilled water or in salt solutions. The attraction between a negatively charged

bacterial cell and a positively charged resin particle was confirmed by these observations.

Martin (1955) attempted to obtain quantitative adsorption of the cells of several species of bacteria onto a weakly basic anion exchange resin and onto several other adsorbing substances. These studies were developed from a related investigation concerning the association of certain microorganisms with gastrointestinal disturbances. Only a small fraction of the cells suspended in their growth medium were observed to adsorb to the resin. Comparable quantities of sodium-aluminum-silicate and aluminum hydroxide were much more effective adsorbents than the ion exchange resin.

Barr and Arnista (1957a, 1957b) studied the adsorption of two alkaloids and diptheria toxin by activated attapulgite, halloysite, and kaolin. Barr (1957) developed a method for the quantitative determination of the adsorption of bacteria by the same clays. The cells of four bacterial species, all causitive agents for enteric infections, were contacted with the clays in aqueous suspensions. Cells of Staphlococcus aureus were almost completely adsorbed onto all three adsorbents, but cells of Proteus vulgaris, Salmonella enteritides, and Shigella paradysenteriae were not adsorbed to any appreciable extent.

According to Kuwajima, Matsui, and Kishigami (1957) growth of the cells of Haemophilus pertussis, which usually require an extended surface such as carbon or starch, could be supported by anion exchange resins. Partial or complete destruction of the ion exchanging capacities of the resins resulted in reduction or failure of cellular growth. The growth-supporting effect was observed only for anion exchange resins.

The negatively charged bacterial cells, therefore, were considered to behave as large anions in suspension. The microorganisms and the various required nutritional materials were assumed to be distributed around the resin particles in the most appropriate density of growth. The maximum degree of adsorption of cells was observed for resins of low crosslinkage; the greatest extent of growth was observed for the highly crosslinked resins.

Rotman (1956) found no adsorption of the cells of Escherichia coli strain K-12 by either an anion or a cation exchange resin. This result is in apparent contradiction with the results obtained earlier by Puck and Sagik (1953), who used the cells of Escherichia coli strain B. Rotman (1960) later explained this apparent contradiction to be due to the use of different ionic forms of the anion exchange resins in the separate investigations. Partial explanation was also attributed the structural configuration and distribution of the surface charges on the cells of the two strains of bacteria. Rotman (1960) also considered the general applications of ion exchange resins in the field of microbiology. The separation of microorganisms from extracellular material by ion exchange was compared to the standard method of washing cells by centrifugation. The distinction was made, however, that the adsorption should be of the extracellular ions to be excluded, and not of the microorganisms themselves.

Zvyagintsev (1959a) reported that adsorption of bacteria onto glass slides varied considerably with the species of bacteria. No direct correlation was evident between the adsorption and either taxonomic or Gram-staining characteristics of the bacterial cells. In a related work

Zvyagintsev (1959b) conducted some very informative research dealing with the activities of microorganisms adsorbed onto soil particles and other agents. He distinguished between adsorbents having relatively inert surfaces containing no biologically active compounds, and those adsorbents containing such active compounds. No significant influence upon either the lag phase or the rate of oxygen consumption was observed for bacterial cells adsorbed onto adsorbents of the inert type when compared with the same parameters for freely suspended cells. Greatly altered lag phases and oxygen consumption rates were observed for bacterial cells adsorbed onto adsorbents of the active type.

In a later work Zvyagintsev (1962) described some of the regularities of the adsorption of microorganisms onto both anion and cation exchange resins. The degree of adsorption varied with the species of microorganism. Adsorption of cells onto cation exchange resins greatly depended upon the nature of the cells and the exchangeable cations of the resin. Exchange adsorption, i.e. the cells of one species displacing those of another from the resin, was noted in rare instances involving weak adsorption. Photomicrographs of bacterial cells adsorbed onto the surfaces of resin particles were also included in this report.

Hattori and Furusaka (1959a) studied the adsorption of the cells of Escherichia coli onto an anion exchange resin. The rates of oxidation of various substrates by adsorbed cells and by freely suspended cells were compared. An electronphotomicrograph of the adsorbed cells was also published (1959b). Additional studies of the mechanism and physiological significance of differences in the pH of optimum activity, lag time, etc., between adsorbed and unadsorbed cells of Escherichia coli (1960)

and Azotobacter agile (1961) were also reported. The rates of oxidation of glucose and succinate were slower for the adsorbed cells of both bacterial species.

Stimulation of the proteolytic activity of bacteria by adsorbents was reported by Estermann and McLaren (1959). The rate of ammonia production from protein by bacteria was enhanced by adsorption of the protein onto kaolinite. This beneficial effect was observed in cases involving both adsorbed and unadsorbed organisms but disappeared in the case of a nonadsorbable substrate. It was concluded that the adsorbent acts as a concentrating surface for the adsorbable substrates and exoenzymes. A more rapid breakdown of the substrates is thereby promoted. An excellent review of the literature is included in the above report. A more comprehensive review is included in the doctoral thesis of Estermann (1957).

In an excellent reference, Adamov (1959) suggested that selective adsorption could be used as a technique for the rapid removal of the cells of saprophytic organisms from mixed suspensions also containing pathogenic organisms. Data for fifteen adsorbents interacting with the cells of eight bacterial species were presented. The most selective adsorbent was tricalcium phosphate. The cells of certain Gram-negative saprophytes and Gram-positive coccoids were strongly adsorbed while the cells of various species of Salmonella were only slightly adsorbed by this compound. The relationship between selectivity and chemical structure was further emphasized by the failure of monocalcium phosphate to show any preference for the cells of the same species.

Adamov (1964) continued his earlier work by presenting results of the effects of contact time, particle size, quantity of adsorbent, pH

of the medium, and conditions of culture, upon the adsorption of the cells of saprophytic and pathogenic bacteria by tricalcium phosphate. The degree of adsorption was increased by increasing the quantity of adsorbent or by decreasing the particle size of the adsorbent. Bacterial cells grown on dried agar surfaces adsorbed more intensely onto tricalcium phosphate than those cells grown on moist agar. Adamov and Noskov (1962) passed suspensions containing cells of Salmonella typhosa and Escherichia coli through columns of an anion exchange resin and obtained almost complete removal of all cells. A similar high degree of removal of the cells of both species was obtained using a cation exchange resin.

Gillissen (1960) found that the cells of Escherichia coli could be removed from aqueous suspensions by an anion exchange resin but not by a cation exchange resin. The anion exchange resin was regenerated by the addition of dilute sodium hydroxide. The capacity of this resin for bacterial cells was reduced by repeated regeneration. The degree of adsorption was also significatnly reduced by loading the resin column with sodium chloride or sodium hypochlorite. In a second report Gillissen et al. (1961) described the removal of yeasts, bacteria, and bacterial spores from aqueous suspensions by anion exchange. Cation exchange resins, however, had little capacity for removal of these organisms. The efficiency of removal of microorganisms by anion exchange resins was a function of the species to be adsorbed and the concentration of cells in suspension.

Lahav (1962) investigated the influence of particles of sodium bentonite, which were smaller than bacterial cells, upon the electrophoretic mobilities of cells of Bacillus subtilis. The adsorption of these

particles of bentonite onto the bacterial cells was found to be reversible. The electrophoretic mobilities of the cells were functions of the pH, ionic strength, and the electrolyte in solution. The change in the mean mobility was found to be higher at lower pH values and at higher ionic strengths. Three hypothetical mechanisms of clay adsorption onto bacterial cells were proposed. Depending upon the orientation of the clay particles in relation to the bacterial surfaces, this adsorption could occur with respect to plane surfaces, edges, or a combination of both plane surfaces and edges of the clay particles.

Helmstetter and Cummings (1963) developed a technique for obtaining synchronous growth of cells of Escherichia coli. They first assumed that bacteria will bind to a variety of surfaces and retain their ability to divide while bound to these surfaces. Differences in the binding strength of the cells were then assumed to exist at different stages of growth. Synchronous growth was successfully achieved by continuously eluting daughter cells from a growing culture of mother cells that were adsorbed onto an anion exchanger cellulose. Other binding materials, including ordinary filter paper, glass and rubber tubing, and various ion exchange resins, were also somewhat selective in their adsorption of bacterial cells of different ages. Much lower yields of daughter cells were obtained using these materials, however, compared to the cellulose.

Martinez (1963) purified suspensions containing the flagella of Spirillum serpens, Proteus vulgaris, and Bacillus subtilis using an ion exchange technique. The whole cells were first homogenized and them removed by centrifugation. The isolated flagella were subsequently

centrifuged, resuspended in a buffer solution, and then adsorbed onto an anion exchange cellulose. The flagella were eluted from the adsorbent by a salt solution.

The applications of ion exchange to the concentration of the cells of Mycobacterium tuberculosis from aqueous suspensions were considered by Schwartz and Mayer (1963). Adsorption of the cells of this species was observed by both anion and cation exchange resins. Cells of Mycobacterium tuberculosis was separated by selective adsorption from a mixture also containing the cells of four other bacterial species. The cells of the former species were adsorbed onto an ion exchange resin independent of the pH and subsequently desorbed by the addition of acid or base. The cells of the four latter species passed through the column without adsorbing to any appreciable extent, although the degree of their adsorption was a function of the pH. The capacity of the ion exchange resin for bacterial cells was not reduced as much by repeated regeneration as by sterilization in an autoclave.

Geller, Khariton, and Dobrotvorskaya (1963) observed that bacteria could be adsorbed onto the roots of plants. A higher capacity for bacterial cells was noted for certain plants. The degree of adsorption depended a great deal upon the initial concentration of bacteria in the suspension contacting the plant roots. More intense adsorption of cells onto plant roots occurred in an acid medium then in either a neutral or weakly alkaline medium.

According to Marazzi-Uberti et al. (1964), the salts of methylene bisphenolic acids, in particular mono-dihydroxy-aluminum pamoate, were capable of adsorbing bacterial cells. Cells of both Shigella flexneri

and an unspecified Staphlococcus were adsorbed to greater extents by these compounds than by either charcoal or kaolin. Methylene blue dye was also strongly adsorbed.

Kurozumi et al. (1965) described a technique for separating different species of cells using chromatographic columns containing anion exchange resin. Five species of cells were examined, including Escherichia coli, three species of yeast, and a unicellular alga. Cells of mixtures of different species were separated by adsorption onto resin columns followed by selective desorption with salt solutions. Complete resolutions of mixtures containing cells of widely different size and character, such as yeasts and bacteria, and yeast and algae were achieved. Mixtures of various yeasts were only partially resolved. An excellent discussion discussion of the various factors which may influence cellular chromatography was included in this reference.

C. Phenomenological Classification

Many of the conclusions regarding the adsorption of bacteria onto solid surfaces put forth in the early literature remain irrefutable to the present day. Other hypotheses have required continuous revision to account for new data obtained using better adsorbents and more refined techniques. Considerable controversy has also periodically erupted concerning certain issues. What is the proper definition of the process by which bacteria are concentrated at the surface of a solid? Can this process be predicted upon the basis of viability, motility, surface charge, Gram-staining ability, etc., of a particular microorganism?

A gamut of other factors related to the adsorption of cells onto surfaces are also evident in surveying the literature. These factors are best discussed as separate entities dependent upon the organism, the adsorbent, and the environment. The practical applications of the phenomenon of the adsorption of bacteria onto solid surfaces should also be considered. These have included: studies of the metabolism of bacterial in the adsorbed and free states, investigations of phage-host cell interactions, removal of pathogens from mixed suspensions, and chromatography of mixed suspensions.

1. Mechanism

The concentration of bacteria at the surface of a solid phase is a complex phenomenon. It has endured throughout the progression of literature under a variety of labels. Krüger (1889) concluded that bacteria were "grasped" within the pores of a contacting solid. Eisenberg (1918) discussed cell "adhesiveness" but entitles his report, "The specific adsorption of bacteria." Michaelis (1918) considered the interaction of bacterial cells and solids to be due to a "mechanical stickiness" but labelled it adsorption.

The terminology of "adsorption," as applied to bacteria, originally came into widespread usage as a natural carryover from studies of similar phenomena involving surface concentration of dissolved or colloidal material. Some reluctance remained, however, toward complete acceptance of this term to include suspended particles of bacterial size. Dianova and Voroschilova (1925) and Khudiakov (1926b) conditionally used the term "adsorption" to describe the ability of bacteria to combine with soil particles. Rubentschik et al. (1936) provisionally used the term

"adsorption" and concluded that in most cases it was accompanied and occasionally disguised by other attendant phenomena.

Gunnison and Marshall (1937) qualified their definition of the adsorption of bacteria by inert particulate reagents as "apparent" adsorption. They frankly doubted that the observed phenomenon was "true" adsorption, directly dependent upon the electrical charges of the reagent surfaces, the pH, the opportunity for "collision" between a bacterial cell and the surface of a particle, and similar factors. They stated that, "If, as may happen in the few extreme examples observed, bacteria do come into contact with inert particles, attempt to wet them, and become fixed around such particles, perhaps by electrical attraction, it would still tax the imagination to call the phenomenon 'adsorption.'"

Reservations toward acceptance of the term "adsorption" have persisted into the current literature. Tschapek and Garbosky (1950b) suggested that from the standpoint of bacterial size it may be more correct to consider the interaction of bacterial cells with adsorbents as "adhesion" and reserve the term "adsorption" "to describe the attraction between bacteria and various types of particulate matter, although the action may not be necessarily analgous to true adsorption."

Estermann and McLaren (1959) retained the term "adsorbent" for its convenience, but called the process "adhesion" because the mechanisms were not well delineated. Lahav (1962) strongly urged the use of the term "adsorption", viv-a-vis "adhesion", and proposed several hypothetical mechanisms. Helmstetter and Cummings (1963) discussed the "binding" of bacterial cells to surfaces.

"Adsorption" has been reported in most of the recent studies of the interaction occurring between bacterial cells and ion exchange resins. The definition of this interaction as "adsorption" has been fully qualified by the many verifications that an actual transfer and accumulation of cells at the surface of a solid has occurred. Evidence supporting this conclusion are: the observed dependence of this process upon both the pH and the concentration of electrolyte in the suspension, and the vivid photomicrographs in which bacterial cells can be seen firmly attached to the surfaces of solid particles of various adsorbents.

The family terminology of adsorption, adsorbent, and adsorbate, to mean, respectively, the process by which bacteria are concentrated at a surface, the material instigating this process, and the material undergoing this process, are no longer simple descriptive conveniences but valid representations.

The actual mechanism by which bacteria become adsorbed onto surfaces has been reported under a great many guises. Krüger (1889) suggested that the increase in the number of bacteria present in a suspension shaken with various powdery materials was due to mechanical settling of the cells with the solid particles. Salus (1917) was of the opinion that the adsorption of the cells of different bacterial species was a function of the magnitude of their electrical charges. Eisenberg (1918) considered the differences in the adsorption of the cells of various bacterial species to be due to the physical nature and not the chemical nature of the adsorbent.

Bechhold (1918) did not observe any marked differences between the chemical natures of iron oxide and silica gel. Michaelis (1918) felt

that the basis for the phenomenon of the adsorption of bacteria was the purely mechanical adhesion of the bacteria to the adsorbent and was relutant to accept the presence of a supporting electrical charge effect. Friedberger (1919) discussed the relative merits of electrochemical versus mechanical adsorption. His student Putter (1920) considered the adsorption to be more mechanical. Dianova and Voroschilova (1925), Karpinskaya (1926), and Khudiakov (1926b) agreed that surface effects were paramount in the interactions between bacteria and soil particles.

Rubentschik et al. (1936) considered the dependence of adsorption upon electrical effects to be a justified conclusion but also felt that additional surface factors contributed to the total phenomenon. Peele (1936) stated that the adsorption of bacteria could be due to a reaction of unlike electrical charges, i.e. between a negatively charged bacterial cell and a positively charged adsorbent. Gunnison and Marshall (1937) seriously doubted, however, that the adsorption was directly dependent upon the electrical charge of the adsorbent surface and they suggested that other factors must be operative.

Oksentian (1940) explained the variation in the degree of adsorption exhibited by various materials as due to different in the magnitude of their negative charges. She correlated electrokinetic potentials of several adsorbents with their abilities to adsorb bacteria. Dikusar (1940) stated that mechanical forces associated with the surface of an adsorbent are dominant. Electrical forces are an additional contribution to adsorption but do not have broad significance at the physiological pH values at which both soils and bacteria are negatively charged. Chemical effects may often be masked by physical effects.

Weiss (1951) and Barr (1957) considered the difference in the degree of adsorption of bacteria by sediments and by clays, respectively, to be governed by the quantitative nature of the electrical charge difference between these adsorbents. Estermann and McLaren (1959) stated that the adsorption of bacteria onto soil particles was dependent upon the electrokinetic potentials of both the cells and the particles. In reports dealing with the adsorption of bacteria onto ion exchange resins which were published in the recent literature the authors generally agree that cells can behave as macro-ions and electrostatically react with charged surfaces (Kunin, 1947 p.134; Puck and Sagik, 1953; Kuwajima et al., 1957; Zvyagintsev, 1959b; Rotman, 1960; and Schwartz and Mayer, 1963).

The reversibility of the adsorption process has also been recognized by many authors. Frei and Erismann (1922) observed a deterioration of the adsorption of bacterial cells by sand particles suspended in an agitated system. Dianova and Voroschilova (1925) noted a partial desorption of bacteria from soil suspended in sterile water. Rubentschik et al. (1936) desorbed cells of Serratia marcescens from mud by washing it with water. Novogrudskii (1936b) desorbed bacterial cells from soil by altering the pH of the suspension.

Kunin (1947, p.134) indicated that bacteria adsorbed onto anion exchange resins could be regenerated with brine. Gillissen et al. (1961) observed the adsorption of bacteria onto anion exchange resins that had been repeatedly regenerated but did not give details. Lahav (1962) found that the adsorption of the cells of Bacillus subtilis onto sodium bentonite was a reversible process. The equilibrium of this process could be displaced by changes in the concentrations of the reacting species.

Schwartz and Mayer (1963) used basic and acidic solutions, respectively, to recover cells of Mycobacterium tuberculosis that had previously been adsorbed onto anion or cation exchange resins. Zvyagintsev (1962) recovered bacterial cells from anion and from cation exchange resins by contacting the cell-resin complexes with weakly alkaline solutions.

The microscopic analysis of the adsorption of bacterial cells onto colloidal particles as pointed out by Estermann and McLaren (1959) can be most ambiguous because of the possibilities of introducing artifacts due to drying, staining, convection currents in hanging drops, and adsorption onto glass surfaces. The streaming chamber developed by Kalyuzhnii (1957) for studying the adsorption of yeast cells onto cellulose fibers may be applicable to similar studies of bacteria. Microscopic evidence of the adsorption of bacteria has been presented in a number of reverences.

Khudiakov (1926a, 1926b) and Karpinskaya (1926) presented several excellent illustrations of the cells of three bacterial species adsorbed onto the surfaces of various soil fractions. Lasseur, Dombray, and Palgren (1934) presented pictures of filter paper impregnated with dyes and with bacterial cells. Peele (1936) showed several photomicrographs of bacterial cells adsorbed onto soil particles. Beutelspacher (1955) also published pictures of the adsorption of bacteria. Zvyagintsev (1959b) determined the number of bacterial cells adsorbed onto a particle of adsorbent by fluorescent microscopy. He later (1962) published some photomicrographs of the cells of several bacterial species adsorbed onto an anion exchange resin. Bacterial cells that were adsorbed onto and anion exchange resin were also evident in an electronphotomicrograph obtained by Hattori and Furusaka (1959b).

2. Microorganism

The extent and the specificity of the adsorption of bacteria onto solid surfaces are most strongly influenced by the species of bacteria undergoing the adsorption. References to the following eight genera have appeared most frequently in the literature: Azotobacter, Escherichia, Serratia, Salmonella, Staphlococcus, Sarcina, Bacillus, and Pseudomonas. Sixteen other genera have been mentioned to somewhat less degrees. The most thoroughly investigated bacterial species are Bacillus subtilis and Escherichia coli. This probably has come about because of their wide distribution, ease of culture, opposing Gram behavior, and low pathogenicity. References to the adsorption of the cells of microbial species selected from the literature are tabulated in Tables I-X.

Several criteria have been applied to explain apparent variations in the character of the adsorption of the cells of individual bacterial species. The conditions of culture and the methods of preparation of a cell suspension prior to the study of the adsorption have certainly not been standardized. Differences in adsorption between species, not directly associated with the techniques, have been correlated with staining, viability, and motility of the cells.

Attempts to correlate the adsorptive behavior of the cells of a particular bacterial species with their Gram-staining behavior have led to conflicting results. The question as to whether Gram-positive (G+) or Gram-negative (G-) species are preferentially adsorbed as separate groups by a given adsorbent is still open to debate.

Eisenberg (1918) found in general that the cells of Staphlococcus aureus (G+), Mycococcus albus (G+), Bacillus megaterium (G+) and Sarcina

lutea (G+), were more strongly adsorbed by charcoal, clay, fluorspar, and kieselguhr than the cells of Salmonella typhosa (G-), Escherichia coli (G-), Pseudomonas aeruginosa (G-), and Vibrio comma (G-). Bechhold (1918) found a selectivity by iron oxide for the cells of Staphlococcus sp. (G+) over those of Escherichia coli (G-) but the reverse order for silica gel. Friedberger (1919) and Putter (1920) concluded from their studies of the capillary rising ability of the cells of some thirty-four bacterial forms that the cells of Gram-positive species adsorbed more strongly and consequently did not rise as high in the capillaries of the filter paper as did the cells of Gram-negative species.

The cells of Bacillus cereus (G+) were more strongly adsorbed by silt than those of Serratia marcescens (G-) according to Karpinskaya (1926). Minenkov (1929) reached the same conclusion using cells of the same species and twelve different soils. Novogrudskii (1936b) concluded from his data collected for the adsorption of the cells of ten bacterial species onto three different soils that equal or stronger adsorption was evident for the cells of the Gram-positive species when compared to the cells of the Gram-negative species.

The cells of Gram-positive species were more strongly adsorbed than those of Gram-negative species by kaolin and charcoal, but about equally by calcium carbonate, aluminum hydroxide, and barium sulfate, according to data collected by Gunnison and Marshall (1937). These authors were adamant, however, in concluding that the hypothesis of a greater degree of adsorption of the cells of Gram-positive bacteria advanced by Eisenberg (1918) could not be substantiated by their data. Some selectivity by both an anion exchange resin and sodium-aluminum-silicate for the cells of Staphlococcus aureus (G+) over those of several

Gram-negative species was noted by Martin (1955). Differences in the concentrations of cells in suspension make comparison of his data difficult. Barr (1957) also found that the cells of Staphlococcus aureus (G+) were significantly adsorbed by clay while the cells of three Gram-negative species were not adsorbed at all.

Adamov (1959) in a very comprehensive survey studied the adsorption of the cells of eight bacterial species by fifteen adsorbents. All adsorbents except charcoal, kaolin, and monocalcium phosphate, were preferential in their adsorption of the cells of Gram-positive species compared to those of Gram-negative species. The excepted adsorbents were all relatively nonselective in their adsorption of the cells of the eight bacterial species. The following approximate order was followed by the remaining twelve adsorbents: cells of Staphlococcus aureus (G+) and Sarcina lutea (G+) were strongly adsorbed, cells of Paracolobactrum sp. (G-) were adsorbed only slightly less than the two Gram-positive species, cells of Escherichia coli (G-) and Proteus sp. (G-) were adsorbed to an intermediate extent, and cells of Salmonella typhosa (G-) and Salmonella paratyphi (G-) were very weakly adsorbed.

Estermann and McLaren (1959) did not offer an opinion with regard to Gram-staining character and adsorbability of the cells of a particular bacterial species. They did discuss the relative significance of explanations presented elsewhere in the literature. According to their own data the cells of Flavobacterium sp. (G-) and Bacillus subtilis (G+) were both almost completely adsorbed by kaolinite while the cells of Pseudomonas sp. (G-) were only slightly adsorbed. Schwartz and Mayer (1963) observed strong adsorption of the cells of Mycobacterium tuberculosis (G^+) by several anion and cation exchange resins. The cells of

nine other bacterial species (4 G+, 5 G-) did not appear to adsorb onto either type of ion exchange resin to any significant extent. These results are in direct opposition to those obtained by other researchers with regard to the adsorption of the cells of the other species. This discrepancy may be partially explained on the basis of the criterion used by Schwartz and Mayer to establish adsorption. Tubes containing liquid media were inoculated with samples from three sources: the initial suspension, the filtrate from the columns of ion exchange resin, and the fluid eluted from the column during regeneration. Adsorption was assumed to occur in those cases in which no growth was observed for the filtrate; desorption was verified by growth in the regenerated fluid. Resistance of the cells of a species toward desorption or loss of viability upon adsorption or desorption can be sufficient reasons for conflicting results.

The possibility of differences existing between the adsorption of living and of dead bacteria has also been considered. Karpinskaya (1926) observed equal adsorption of living and dead bacteria by various soils. Rubentschik et al. (1936), however, found a greater degree of adsorption for living bacteria than for heat-killed cells by the sediments of a liman (salt lake).

The adsorption of the cells of a particular bacterial species may also be dependent upon the extent of their motility. Friedberger (1919) concluded that the capillary rising abilities of the cells of several bacterial species in filter paper were independent of their motility. Cells of a naturally motile and those of a nonmotile species of Sarcina rose to equal heights; this was also found to be the case for motile and nonmotile cells of Salmonella paratyphi. The capillary rising

ability of bacterial cells was not affected by eliminating their motility by artificial means. These viewpoints were also supported by Putter (1920).

Khudiakov (1926b) and Karpinskaya (1926), contrary to the conclusions reached by the previous authors, presented some evidence that actively motile bacteria were more weakly adsorbed by soils than were nonmotile bacterial Dikusar (1940) suggested that motile microbes can counteract the settling action of the soil particles. The immotile cells settle with the soil particles. The immotile cells settle with the soil particles and the motile ones remain in suspension. Gunnison and Marshall (1937) did not find any dependency of adsorption upon motility. This dependency can be supported to some degree by the data of Martin (1955). The data of Adamov (1959) are consistent with the conclusion that motile bacterial cells are adsorbed onto solid surfaces to a lesser extent than are nonmotile bacterial cells.

The effect of the initial concentration of bacterial cells in suspension upon the extent of adsorption of the cells must also be considered. The total number of bacteria adsorbed will increase with increasing initial concentration but the percentage of adsorbed cells will decrease (Eisenberg, 1918; Dianova and Voroschilova, 1925). Little variation in selectivity was found over a wide range of concentrations of two bacterial species in a binary mixture according to the data of Michaelis (1918). Gunnison and Marshall (1937) did not observe any change in the degree of adsorption upon varying the concentrations of cells over a wide range.

Saturation of an adsorbent with bacteria was actually observed for the case of the adsorption of cells of Azotobacter sp. onto sand by Tschapek and Garbosky (1950a) and later by Zvyagintsev (1962), who tested

other bacteria-adsorbent combinations. Schwartz and Mayer (1963) found that the concentration of cells in suspension could be a deciding influence upon the degree of saturation of ion exchange columns by the cells. Weiss (1951) used a fixed quantity of adsorbent and found adsorption of bacterial cells to be most complete at low concentrations of cells. Gillissen (1960) noted a dependency of adsorption upon the initial concentration of bacterial cells. Geller (1963) also found that the adsorption of bacteria was dependent upon their concentration in suspension.

3. Adsorbent

The second most important variable involved in the adsorption of bacteria onto a solid surface is the type of adsorbent in contact with the cell suspension. The early investigators were limited to the use of poorly defined adsorbents which were low capacity and which had little selectivity. Much of the early work was done using soils and sediments as adsorbents. Common adsorbents, such as carbon, kaolin, filter paper, calcium carbonate, and barium sulfate, were also tested, primarily because of their insolubility in water and their general availability. Synthetic ion exchange resins have been used in recent investigations to obtain more meaningful results.

A list of substances which have been found to be capable of adsorbing bacteria is given in Table XI. More specific listings of anion and cation exchange resins are presented, respectively, in Tables XII and XIII. Manufacturers of some of the ion exchange resins cited in these tables are listed in Table XIV. The discussion included in the following paragraphs has been divided into three categories: undefined soils, inorganic adsorbents, and ion exchangers.

Krüger (1889) studied the adsorption of bacteria by clay and found a definite concentration of cells in the settled solid phase after agitation was halted. The concentrating effect exhibited by sand was very slight in comparison. Clay was also used to adsorb bacteria by Kuhn and Heck (1916) and by Bechhold (1918). Dianova and Voroschilova (1925) observed that large numbers of bacteria were adsorbed by various soils. This work was elaborated by Karpinskaya (1926), Khudiakov (1926b), and Minenkov (1929). The concensus was that soils with a high silt content were the best adsorbents for bacterial cells.

Rubentschik et al. (1936) found that various types of sediment differ in their adsorptive capacity for cells of the same bacterial species. Peele (1936) made a penetrating study into the chemistry of the adsorption of bacteria by two loamy soils. Novogrudskii (1936b) observed a concentration of microorganisms upon the surfaces of an average podzol (acid soil) and veronozem chernozem (alkaline soil). He supported the conclusions reached by Khudiakov (1926b) and others. Waksman and Vartiovaara (1938) found that marine mud was a very strong adsorbent while sand was a poor adsorbent for bacterial cells.

Tschapek and Garbosky (1950b), contrary to other investigators, found a substantial adsorption of bacteria by dispersed sand. Weiss (1951) found that the degree of adsorption of the cells of Escherichia coli onto river and estuarine silts was apparently characteristic of the particular silt. Zvyagintsev (1959b) elaborated concerning the adsorption of bacteria by sand, podzol, and chernozem.

Krüger (1889) treated suspensions containing bacterial cells with calcium carbonate, kieselguhr, aluminum oxide, charcoal, coke,

magnesium oxide, and chalk. Kuhn (1915) and Kuhn and Heck (1916) separated mixtures containing cells of Salmonella typhosa and Escherichia coli using charcoal as a selective adsorbent. Salus (1917) removed bacteria from drinking water by adsorption onto charcoal. The specific adsorption of bacteria by fifty water-insoluble inorganic and organic substances was reported in the monumental work of Eisenberg (1918). Three of the more important adsorbents studied were charcoal, clay, and kieselguhr.

Michaelis (1918) studied the action of kaolin as a selective adsorbent for the cells of Salmonella typhosa in mixed suspensions that also contained cells of Escherichia coli. Bechhold (1918) investigated the removal of bacteria from suspension by barium sulfate, calcium oxalate, iron oxide, charcoal, silica gel, and fuller's earth. Friedberger (1919), Putter (1920), and Frei and Erismann (1922) adsorbed bacteria onto filter paper. Sand was also reported in the latter reference to have adsorbent characteristics.

Lasseur, Dombray, and Palgren (1934) used filter paper and activated carbon as adsorbents for bacterial cells. Peele (1936) observed that cells of Azotobacter chroococcum were adsorbed by aluminum and iron hydroxides. Kaolin, charcoal, calcium carbonate, aluminum hydroxide, and barium hydroxide were tested as adsorbents by Gunnison and Marshall (1937). Oksentian (1940) found a considerable adsorption of bacteria by charcoal, kaolin, and talc. Debusmann (1950) adsorbed bacteria onto various pectin-containing substances. Martin (1955) found that bacteria were adsorbed by sodium-aluminum-silicate and aluminum hydroxide.

Barr (1957) compared the capacities of three well-defined clays for adsorption of bacterial cells. Bacteria were adsorbed by kaolinite

according to Estermann and McLaren (1959). Adamov (1959) determined the capacities and selectivities of fifteen organic and inorganic adsorbents for the cells of eight bacterial species. These adsorbents included charcoal, kaolin, aluminum oxide, calcium phosphates, and several others. The adsorption of sodium bentonite particles onto cells of Bacillus subtilis was studied by Lahav (1962).

The first reference made to the application of synthetic ion exchange resins as a means of removing bacterial cells from suspension is attributed to Ham and Barnes (1947a, 1947b). They observed the adsorption of bacterial cells by an anion exchange resin. Kunin (1947, p.134) also mentioned the possibilities of using an anion exchange resin to remove bacterial cells from suspension. Puck and Sagik (1953) studied the adsorption of the cells of Escherichia coli by both anion and cation exchange resins. No adsorption of the cells of this species was observed for the latter. Martin (1955) found that cells of Staphlococcus aureus (G^+) and several Gram-negative species were removed from suspension by an anion exchange resin.

Kuwajima et al. (1957) observed growth-supporting effects in cultures of cells of Haemophilus pertussis upon the addition of anion exchange resins. No such effects were noted for cation exchange resins. Zvyagintsev (1962) observed wholesale adsorption of bacteria onto an anion exchange resin but only selective adsorption onto a cation exchange resin. Rotman (1960) noted considerable variation in the adsorption of the cells of Escherichia coli onto various anion exchange resins. Hattori and Furusaka (1960, 1961) adsorbed the cells of Escherichia coli and those of Azobacter agile onto anion exchange resins and studied the resulting chemical activities of the adsorbed cells.

Gillissen (1960) removed cells of Escherichia coli from a suspension by an anion exchange resin but not by a cation exchange resin. In a later reference Gillissen et al. (1961) extended these findings to include other species. Schwartz and Mayer (1963) found a selective adsorption of the cells of Mycobacterium tuberculosis by both anion and cation exchange resins but no appreciable adsorption of the cells of ten other bacterial species. Kurozumi et al. (1965) separated the cells of several widely different microbial species using an anion exchange resin.

The concentration of the adsorbent added to a cell suspension can be very important. Krüger (1889) and Eisenberg (1918) observed improvements in the degree of adsorption with increases in the quantities of adsorbent added to several different cell suspensions. Greater degrees of adsorption occurred with increasing concentration of adsorbent up to an ultimate equilibrium according to Dianova and Voroschilova (1925). Rubentschik et al. (1936) noted an increase in the percentage of adsorbed cells with an increase in the quantity of adsorbent. Gunnison and Marshall (1937) observed no effect of the concentration of the adsorbent on the basis of rather limited data. Waksman and Vartiovaara (1938) found a definite increase in the degree of adsorption of bacteria using increased amounts of adsorbent. Tschapek and Garbosky (1950b) noted an increase in the degree of adsorption of bacteria using increased concentrations of adsorbent. Martin (1955) indicated that a greater degree of adsorption was possible with more adsorbent present. Barr (1957) also observed an approach to an equilibrium adsorption upon increasing the amount of adsorbent.

The particle size of the adsorbent can also be critical. Bechhold (1919) determined the average diameters of several particulate adsorbents. The degree of adsorption of bacterial cells was dependent upon the extent of "outer surface development" (external surface). Large particles of charcoal and fuller's earth also adsorbed bacteria, presumably because of extensive "inner surface development" (pores). Frei and Erismann (1922) noted high degrees of adsorption of bacterial cells by sand of small particle size. Dianova and Voroschilova (1925) found that bacteria were adsorbed weakly by large sand particles but very strongly by fine salt particles. Karpinskaya (1926) found that the capacity of an adsorbent for bacterial cells increased with decreased particle size up to a limit. Khudiakov (1926b) supported these conclusions. Minenkov (1929) also observed the effect of particle size upon adsorption and correlated it with particle size measurements. Novogrudskii (1936b) supported the earlier conclusions regarding increased adsorption with decreased particle size. The particle size of the adsorbent has not been considered to be an important variable in the recent studies of adsorption of bacterial cells by ion exchange resins.

Different electrolytes can be bound to the soil adsorption complex. Frei and Erismann (1922) noted a strong influence by electrolytes on the filtration of bacteria through sand and filter paper. This process was enhanced more by divalent cations than by monovalent cations. Lithium ion produced a swelling of the cellulose filter paper and a correspondingly greater degree of filtration. Peele (1936) reiterated the prime importance of the ionic form of the adsorbent. Trivalent cations were more effective than divalent cations which in turn were more effective

than monovalent cations at the same concentration. Tschapek and Garbosky (1950a), however, found that cells of Azotobacter sp. were adsorbed to a greater extent onto sand particles in the presence of a divalent cation than in the presence of a monovalent cation.

The selectivity of adsorption exhibited by an ion exchange resin is a function of the type of exchangable counter ion. Kunin (1947, p.134) stated that the chloride form of a strongly basic anion exchange resin was capable of adsorbing bacterial cells. Puck and Sagik (1953) converted a similar resin to its phosphate form before successfully adsorbing cells of Escherichia coli strain B. Kuwajima et al. (1957) used both hydroxide and chloride forms of several anion exchange resins to support the growth of cells of Haemophilus pertussis. The cells of this species did not grow in the presence of sodium or hydrogen form cation exchange resins. Rotman (1960) indicated that the cells of Escherichia coli strain K-12 did not adsorb onto the hydroxide form of a strongly basic anion exchange resin but did adsorb onto the chloride form of a similar anion exchange resin. Hattori and Furusaka (1960, 1961) also used a chloride form anion exchange resin to adsorb cells of Escherichia coli and Azotobacter agile. Gillissen (1960) regenerated an anion exchange resin with a weakly basic solution and a cation exchange resin with acidic or salt solutions. Cells of Escherichia coli adsorbed only onto the anion exchange resin.

Zvyagintsev (1962) conducted an extensive series of tests using many ionic forms of ion exchange resins. He used only the chloride form of an anion exchange resin but tested ten different ionic forms of a cation exchange resin. The degree of adsorption of bacteria onto the cation exchange resin was very dependent upon the ionic form. A definite

specificity for the cells of different bacterial species was noted among different ionic forms of the cation exchange resin. It was suggested that the cells of different species of bacteria could be separated by selective adsorption. Schwartz and Mayer (1963) used sodium hydroxide and hydrochloric acid, respectively, to regenerate anion and cation exchange resins that had previously been saturated with bacteria. Cells of several different microorganisms were nonselectively adsorbed by the acetate form of a strongly basic anion exchange resin according to Kurozumi et al. (1965). The adsorbed cells were then selectively eluted from the resin by sodium acetate, sodium chloride, sodium phosphate, or potassium iodide in solutions of various concentrations. The extent of separation of the cells of the different species depended upon the type and concentration of the eluting electrolyte.

The degree of crosslinkage present in certain copolymeric anion exchange resins can have a decided influence upon the adsorption of bacterial cells. Kuwajima et al. (1957) noted a relationship between the extent of growth shown by cells Haemophilus pertussis and the degree of crosslinkage of an anion exchange resin added to the suspension. The degree of adsorption of the cells was increased upon decreasing the crosslinkage of the resin but the extent of growth was correspondingly decreased. Kurozumi et al. (1965) also observed an increase in the degree of adsorption with decreasing crosslinkage.

4. Environment

The interaction between bacterial cells and adsorbent particles suspended in a fluid is very dependent upon the hydrogen ion concentration

of the suspension. Novogrudskii (1936b) stated that the pH of the medium was one of the most important factors which determine the degree of adsorption of bacteria by soils. The adsorptions of the cells of Bacillus cereus and Escherichia coli by podzol were found to be very strong at low pH values, approach minima slightly below neutral pH values, and increase once more at higher pH values. These adsorptions could also be reversed by changes in the pH of the suspensions.

Peele (1936) noted differences in the degree of adsorption of the cells of Azotobacter chroococcum by iron and aluminum hydroxides, and by silt loam as a function of the pH of the suspension. These differences were small in the case of aluminum hydroxide but were quite considerable for iron hydroxide. This latter adsorbent was tested at or near its isoelectric point. Gunnison and Marshall (1937) observed equal or greater adsorption of bacteria at pH 5.6 as compared to pH 7.2 for almost all adsorbents and bacterial species tested. Dikusar (1940) discussed the possibility of a charge reversal of bacterial cells with changing pH.

Kunin (1947, p.134) mentioned that bacterial cells which adsorbed onto anion exchange resins undoubtly had isoelectric points about pH 5. Tschapek and Garbosky (1950a, 1950b) observed a rapid decrease in the adsorption of the cells of Azotobacter sp. by sand from a maximum near pH 4 down to practically zero above pH 7. Barr (1957) recognized the effect of pH upon the electrical charges of the bacterial surface. Estermann and McLaren (1959) obtained a better adhesion of the cells of three bacterial species to kaolinite at low pH values than at high pH values.

Hattori and Furusaka (1960, 1961) observed differences in the chemical activity of adsorbed and freely suspended cells as a function of the pH. Bacterial cells were more intensely adsorbed onto plant roots contained in an acid medium than in either a neutral or weakly alkaline medium according to Geller (1963). Adamov (1964) studied the effect of pH upon the adsorption of bacteria by tricalcium phosphate. The mean electrophoretic mobility of cells of Bacillus subtilis while adsorbed onto sodium bentonite was a function of the pH of the suspending medium according to Lahav (1962). The change in mean mobility was found to be higher at lower values of the pH. Schwartz and Mayer (1963) observed some effects of pH upon the adsorption of bacteria by a weak base anion exchange resin and by a cation exchange resin. The adsorptions of bacteria by two strongly basic anion exchange resins and three other cation exchange resins were independent of the pH.

The introduction of electrolytes into a suspension containing both bacterial cells and adsorbent particles can influence their combination. Putter (1920) used trivalent cations to reverse the charge of bacteria suspended in an acid medium. Frei and Erismann (1922) found that the filtration and adsorption of bacteria by sand and filter paper can be significantly improved by the addition of divalent cations. Peele (1936) found that greater numbers of bacterial cells were adsorbed by soil-exchange complexes previously saturated with multivalent cations. Tschapek and Garbosky (1950a) noted an increase in the adsorption of the cells of Azotobacter sp. by sand as the concentration of electrolyte was increased. Contrary to many of the previous findings, a divalent cation was much less effective in improving adsorption than a monovalent cation associated

with the same anion. Weiss (1951) observed that the cells of Escherichia coli were adsorbed to a lesser degree by silt particles that were suspended in higher concentrations of sodium chloride.

Puck and Sagik (1953) found no adsorption of the cells of Escherichia coli strain B by a cation exchange resin regardless of the salt concentration of the suspending medium. The cells of this bacterial species were adsorbed onto an anion exchange resin suspended in either distilled water or in a salt solution. Gillissen et al. (1961) mentioned that the capacity of an anion exchange resin to adsorb bacterial cells could be lost by treatment with salt but did not elaborate. Lahav (1962) found that by increasing the ionic strength of a cell suspension he could increase the electrophoretic mobility of cells of Bacillus subtilis having particles of bentonite clay adsorbed on their surfaces. Kurozumi et al. (1965) found some variation in the effectiveness of various anions used to elute adsorbed cells of several microbial species from an anion exchange resin.

Studies of the effects of the duration and intensity of agitation upon the adsorption of bacteria onto solid surfaces have been rather limited. Most researchers terminated their experiments at arbitrary values of time after which no further adsorption of cells was assumed to occur. The mechanics of the agitation have been equally ill-defined. Michaelis (1918) decided upon an adsorption time of five minutes after observing that the extent of adsorption was not improved using longer periods of time. Frei and Erismann (1922) found that the adsorption of bacteria onto sand particles was promoted by agitation which increased the number of contacts between cells and particles. A deterioration in adsorption (desorption) eventually developed upon extended agitation.

Gunnison and Marshall (1937) "gently agitated" a test tube containing bacterial cells and the selected adsorbent. Equilibrium was observed within fifteen minutes; no further change was evident after three hours. The "violence" of agitation had no effect upon the rate of adsorption. The degree of adsorption of bacteria by marine mud was improved by agitation according to the data of Waksman and Vartiovaara (1938). Tschapek and Garbosky (1950a) observed an equilibrium adsorption of the cells of Azotobacter sp. onto quartz sand after one hour of agitation. Approximately two-thirds of the total adsorption was complete within the first five minutes of agitation. Adamov (1964) also studied the effects of agitation upon the adsorption of bacterial cells onto tricalcium phosphate.

The conditions of growth probably have a significant effect upon the subsequent adsorption of bacterial cells. These conditions have not been investigated in detail. Cells of Azotobacter chroococcum that had been cultivated on alkaline media were adsorbed by silt loam to a lesser extent than the cells of same species cultivated on slightly acidic media according to Peele (1936). Similar results were obtained using the cells of Bacillus mycoides (cereus). Kuwajima et al. (1957) found that the pH of a growth medium containing cells of Haemophilus pertussis could be altered by the addition of the hydroxide form of an anion exchange resin. Adamov (1964) found that bacteria grown on dried agar were adsorbed more intensely by tricalcium phosphate than those grown on moist agar.

The capacity of an adsorbent for bacterial cells can be greatly altered by chemical and physical means. Heating of a soil increased its capacity for adsorption according to Novogrudskii (1936b). Rubentschik

et al. (1936) observed no changes, however, in the adsorptive capacity of mud for bacterial cells after subjecting the mud to chemical oxidation or to heat sterilization in an autoclave. The capacity of the mud for bacterial cells was sharply reduced, however, upon treatment of the mud with an acid solution.

Changes in the adsorption capacity of ion exchange resins have also been observed. Kuwajima et al. (1957) reduced the exchange capacity of several anion exchange resins by heating them in an autoclave. Complete or partial destruction of the exchange capacity was also achieved by treating the resins with hydrogen peroxide. Gillissen et al. (1961) inactivated an anion exchange resin by an antistatic treatment and also by contact of the resin with salt. They do not elaborate regarding their techniques. No appearance of fatigue was observed, however, after repeated regenerations of the resins with weakly basic solutions. Schwartz and Mayer (1963) also found that regenerations of the resins with weakly basic solutions. Schwartz and Mayer (1963) also found that regeneration of ion exchange resins did not reduce their capacity for adsorbing bacteria as much as did sterilization in an autoclave.

5. Applications

Several practical applications can be based upon the phenomenon of the adsorption of bacterial cells onto the surfaces of solids. A study of the metabolic rates and pathways of adsorbed cells compared to freely suspended cells can be of value in explaining natural processes in the soil or in implementing specific fermentations. The interactions between host cell bacteria and their infective bacteriophage particles can be explored. The determination of specific surface groups can be of value in

designing specific antibiotics. Certain pathogenic microorganisms can be selectively removed from dilute suspensions. Mixed suspensions can be resolved into their component species.

Considerable interest has been generated toward gaining a more complete understanding of the differences observed between the metabolic functioning of bacterial cells adsorbed onto a solid surface and those existing in a freely suspended state. Dikusar (1940) has reviewed the early literature (58 references). Estermann and McLaren (1959) have published a similar review for a later period (63 references). Only the most significant references of the early literature and several of the most recent investigations dealing with the metabolism of adsorbed and free cells are abstracted in the following discussion.

The early investigations of Dianova and Voroschilova (1925) and Karpinskaya (1926) have been summarized by Khudiakov (1926b). The chemical activities of the cells of a particular bacterial species adsorbed onto a given soil fraction, as measured by the liberation of carbon dioxide, is reduced in direct proportion to the intensity of adsorption. Rubentschik et al. (1936) found that the rate of nitrification was lowered but that the rate of sulfate reduction was increased upon the adsorption of the responsible bacteria.

Peele (1936) also found a decrease in the amount of carbon dioxide evolved from a suspension of bacteria cells adsorbed onto soil particles. This decrease was proportional to the extent of adsorption of the bacteria by the soil. The extent of physiological functioning of a bacterial population can be controlled by the degree of adsorption of the individual cells onto mud particles but no permanent paralysis of growth or metabolism

was indicated according to Waksman and Vartiovaara (1938). Oksentian (1940) observed that the physiological functioning of bacterial cells were sharply reduced by adsorption of the cells but their reproduction capabilities were unaffected. Tschapek and Garbosky (1951) observed that a coagulation of bacterial cells by inorganic salts resulted in a lowering of the vitality and motility of the cells. Nitrogen fixation by the cells ceased above a critical concentration of salt. This ability to fix nitrogen could be restored by transferring the coagulation cells into a fresh culture medium.

Kuwajima et al. (1957) found that the growth of cells of the parasitic species, Haemophilus pertussis, was supported by anion exchange resins but not by cation exchange resins. Activated carbon or soluble starch is usually required as an essential growth factor by the cells of this species. The adsorption of the bacterial cells onto particles of anion exchange resin placed them in close proximity to adsorbed nutritional materials. No growth of cells occurred if the exchange capacity of the resin was destroyed by chemical means or by heat treatment.

Zvyagintsev (1959b) reviewed much of the previous literature dealing with the metabolism of adsorbed microbial cells. He stated that, "Adsorption may in some cases affect microorganisms greatly, but ordinarily does not result in inhibition of their activity and sometimes even stimulates processes which they bring about." He also indicated that no significant influence upon either the lag phase or the rate of oxygen consumption resulted from the binding of bacterial cells to adsorbents having relatively inert surfaces and containing no biologically active compounds. The length of the lag phase was greatly altered, however, by the presence

of biologically active materials on the adsorbents. The rate of oxygen consumption was increased or decreased in accordance with the properties of both the adsorbent and the species of microorganism.

Hattori and Furusaka (1960) studied the chemical activities of cells of Escherichia coli adsorbed onto an anion exchange resin. They observed much lower oxidation rates of succinate, glucose, and other media by the adsorbed cells compared to freely suspended cells. Plots of relative activity as a function of pH were similar in shape for both the adsorbed and the free cells. An increase of about one pH unit in the maximum of the former was explained on the basis of a cationic layer forming outside an anionic layer on the surface of the resin. Curves of activity versus pH of succinate oxidation by cells desorbed from the resin and subsequently resuspended in distilled water were similar to those of the adsorbed cells. Certain compounds were apparently removed from suspensions of metabolizing cells by the resin. These studies were later extended by Hattori and Furusaka (1961) to include Azotobacter agile. Glucose and succinate oxidation rates by the adsorbed cells of this species were also found to be slower than the corresponding rates exhibited by the free cells. The curves of activity versus pH for Azotobacter agile were similar to those of Escherichia coli. A lag time for succinate oxidation with glucose-growth freely suspended cells of Azotobacter agile disappeared when the cells were adsorbed onto a resin. It was again concluded that inhibitory material was removed from solution by the resin.

The interactions of viruses and host bacteria have been investigated using ion exchange techniques. Puck and Sagik (1953) found that adsorption of both host cell bacteria and bacteriophage onto anion exchange resins was inhibited by salt. Salt was required for phage to bind to cation exchange resin, however. Zago (1956) used ion exchange resins as a synthetic system to evaluate the role of physical forces upon the adsorption of viruses. Adsorption of viruses onto ion exchange resins was dependent upon both the pH and the concentration of electrolyte in the suspending medium.

The distribution of microbial cells in a mixed suspension containing several species of microorganisms, as well as dissolved and particulate matter, can be altered in three ways: (a) nonspecific concentration of the cells with no purification, (b) purification of the cells by removal of the dissolved or suspended contaminants with no concentraton effected, and (c) resolution of the mixed cell suspension into its componenet species. All three of these processes may operate separately or simultaneously depending upon the type of separation technique applied.

The literature dealing with the adsorption of bacteria includes some references of the nonspecific removal of bacterial cells from suspensions. Few references are available, however, regarding the removal of contaminants from suspensions rather than the cells themselves. A great deal of literature is available dealing with the purification of viruses by selective adsorption of the contaminating materials. A variety of references dealing with the selective adsorption of certain bacterial species is also to be found in the literature. This latter catagory can be further subdivided into three groups: the resolution of grossly different species, the selection of pathogens, and the resolution of mixtures.

The nonselective adsorptions and concentrations of bacterial cells onto the surfaces of solids immersed into suspensions were reported by Krüger (1889), Khudiakov (1926b), Dikusar (1940), and Rotman (1960). Rubentschik et al. (1936), Waksman and Vartiovaara (1938), and Weiss (1951) investigated the adsorption of bacteria by various soils and sediments and discussed its possible significance in the purification of natural waters. Debusmann (1950), Martin (1955), and Barr (1957) considered the therapeutic aspects of the adsorption of toxic materials and bacteria from the gastrointestinal tract. Ham and Barnes (1942a, 1942b), Barnes and (1952a, 1952b) purified liquids of suspended bacterial cells by ion exchange. Gillissen (1960) and Gillissen et al. (1961) removed yeast, bacteria, and bacterial spores from water with anion exchange resins. Adamov and Noskov (1964) obtained almost complete removal of the cells of two bacterial species from water suspensions also using an anion exchange resin.

The removal of pathogenic microorganisms from dilute suspensions in the analysis of contaminated water supplies has been studied by several researchers. Certain pathogenic bacteria were observed to adsorb more strongly than saprophytic bacteria according to Kuhn (1915) and Kuhn and Heck (1916) who separated cells of Salmonella typhosa from those of Escherichia coli with charcoal and kaolin. Michaelis (1918) also separated the cells of these two bacterial species by adsorption onto kaolin but observed the reverse order of selectivity of adsorption. Gutfield (1919) and Reiter and Meyer (1920) seriously disagreed with these results. They could find no selectivity by kaolin for the cells of either species.

Freidberger (1919) and Putter (1920) explained selective differences in the capillary rising abilities of the cells of various bacterial species in terms of the degree of adsorption of these cells. Cells of Escherichia coli were observed to rise in filter paper capillaries to a lesser extent than those of Salmonella typhosa and therefore were considered to be adsorbed to a greater extent. Bechhold (1918) observed some selectivity by iron oxide and silica gel for the cells of Escherichia coli and Staphlococcus aureus. The cells of several saprophytic species were preferentially removed from mixtures also containing the cells of both Salmonella typhosa and Salmonella paratyphi by selective adsorbents according to Adamov (1959) Schwartz and Mayer (1963) used anion and cation exchange resin to adsorb the cells of Mycobacterium tuberculosis from a mixture containing cells of other bacterial species. The cells of ten other bacterial species did not significantly adsorb onto either of these resins. The cells of Mycobacterium tuberculosis could be eluted from the resins by weakly acidic or basic solutions.

Resolutions of mixed suspensions into their component species by techniques involving selective adsorption have been considered by several investigators. A representative cross-section of the variety of actual and possible resolutions reported in the literature is given in Table XV. These examples range from the resolution of grossly different cells, such as algae and yeasts, to similar cells, such as Gram-positive and Gram-negative bacteria. Very specific resolutions are also possible, such as the separation of smooth and rough forms of a single species.

The most complete resolutions have been obtained for alga-yeast and yeast-bacterium mixtures by Kurozumi et al. (1965). The separation

of bacteriophage-bacterium mixtures may also be possible according to the data of Puck and Sagik (1953). Other systems studied include fungus-fungus (Novogrudskii, 1936b), yeast-yeast (Kurozumi et al. 1965), and virus-virus (Curtain, 1954).

One of the first generalizations to be made regarding the adsorption of bacteria was the conclusion that Gram-positive bacteria were adsorbed more strongly than Gram-negative bacteria. This conclusion was first extended by Eisenberg (1918) based on extensive data collected for eight bacterial species and over fifty adsorbents. Freidberger (1919) and Putter (1920) supported the conclusion of Eisenberg with data from their studies of the capillary rising abilities of bacteria in filter paper. Further support can be garnered from the data of Novogrudskii (1936b), Gunnison and Marshall (1937), Martin (1955), Barr (1957), and Adamov (1959). Most of the latter authors were relutant to accede to the same conclusion regarding the correlation of Gram character and adsorption intensity of bacterial cells. No extensive study has been made of a number of Gram-positive and Gram-negative organisms cultures and adsorbed under similar conditions of adsorbent, agitation, pH, salt content, etc.

Selective adsorption as a means of preferentially removing certain organisms from a mixture has not reached its full potential. Indications of favorable results have been reported, however, in several references. Barr (1957) observed strong adsorption of Staphlococcus aureus by activated attapulgite and little adsorption by three other bacterial species. Zvyagintsev (1962) found considerable variation in the affinities of the cells of several bacterial species for a cation exchange resin. Adamov (1959) actually separated the cells of certain saprophytic species from

those of certain pathogenic species by selective adsorption of the former onto tricalcium phosphate. More specific applications of selective adsorption have included the separations of: cells from spores (Kunin, 1947 p.134), smooth and rough cell forms of Salmonella typhosa (Adamov, 1959), and mother and daughter cells of Escherichia coli (Helmstetter and Cummings, 1963).

D. Tabulation of Microbial Adsorption

Almost four hundred separate citations of the adsorption of microorganisms onto solid surfaces have been reviewed in this report. Bacteria are mentioned in slightly over half of these citations. Nine of the thirteen families of the Order, Eubacteriales, and three families of other orders are represented. The most frequently encountered genera (eight or more citations) are: Azotobacter (10), Escherichia (43*), Serratia (12), Salmonella (29), Staphlococcus (26*), Sarcina (14), Bacillus (40*), and Pseudomonas (25*). Four of these genera (*) are represented in the test organisms selected for this research.

A listing of organisms found to adsorb onto solid surfaces is given in Table I. A more specific listing for bacteria is given in Table II. Citations of individual bacterial genera are contained in Tables III through X. The variety of substances showing adsorptive affinity toward bacteria are listed in Table XI. Specific citations of bacterial adsorption by anion and cation exchange resins, respectively, are contained in Tables XII and XIII. The manufacturers of these resins are listed in Table XIV. Examples of the resolution of microbial mixtures are given in Table XV.

TABLE I

ADSORPTION OF MICROORGANISMS ONTO SOLID SURFACES

Microorganism	Reference
Algae	
Asterionella sp.	Ives (1959)
Chlorella ellipsoida	Kurozumi *et al.* (1965)
" *pyrenoidosa*	Albertsson (1956)
" *vulgaris*	Ives (1959)
" sp.	Golueke *et al.* (1958)
Scenedesmus sp.	"
Tribonema sp.	
Bacteria	
Fifty-four species	See TABLE II
Bacteriophages	
Nine types	See Viruses (Bacterial)
Molds	
Aspergillis niger	Novogrudskii (1936b)
" *glaucus*	"
Botrytis cinerea	"
Fusarium sp.	"
Mucor mucedo	"
Penicillium glaucum	"
Protozoa	
Amoebae glebae	Cutler (1919)
" *lawesiana*	"
" sp.	"
Bodo sp.	"
Colpoda cucullus	"
Monas termo	"
Oicomonas sp.	"
Trichomonas vaginalis	Eugere (1956)
Rickettsiae	
Coxiella burnetii (Q fever)	Hoyer *et al.* (1958)
Rickettsia typhi (Typhus)	"
" " (*R. mooseri*)	Yamamoto *et al.* (1958)
" " " "	Hara (1958)
" *orientalis*	Yamamoto *et al.* (1958)
" *tamigai*	"

TABLE I (cont'd)

Microorganism	Reference
Viruses (Animal)	
Adenovirus (Types 1,3,8)	Haruna et al. (1961)
" " 2,5	Klemperer & Pareira (1959)
" " 2	Philipson (1960)
Chicken tumor virus	Lewis & Andervont (1927)
" " "	Riley (1948,1950a,1950b,1950c)
" " "	Oker-Blom & Nikkilä (1958)
Colorado tick virus	Hoyer et al. (1958)
Coxsackie virus	Kelly (1953)
" " (Type 5)	Gilcreas & Kelly (1955)
" " " 2	Stevenson et al. (1956)
" "	Chang et al. (1958a,1958b)
" " " A9	Hoyer et al. (1958)
" " " A10,B1	Oker-Blom & Nikkilä (1958)
" "	Taverne et al. (1958)
" " " A9	Noll & Youngner (1959)
" " " A3,A9,B4,B5	Sanderson & Kelly (1964)
" " " B2	Cliver (1965)
" " " A9	Phillips & Grim (1965)
Eastern equine encephalomyelitis virus	Muller (1950)
" " " "	Zago (1956)
Enteric cytopathogenic human orphan v.	Hoyer et al. (1958)
" " " " "	Noll & Youngner (1959)
" " " " "	Gravelle & Chin (1961)
" " " " "	Phillips & Grim (1965)
" " " " "	Wallis & Melnick (1966)
Fowl plague virus	Kraus & Barbara (1915)
" " "	Lewis & Andervont (1927)
" " "	Kligler & Olitzki (1931)
" " "	Elford et al. (1948)
" " "	Allison & Valentine (1959)
Foot-and-mouth disease virus	Poppe & Bush (1930)
" " " " "	Pyl (1931)
" " " " "	Brown & Cartwright (1959)
" " " " "	Matheka (1961)
Herpes B virus	Noll & Youngner (1959)
" simplex virus	" "
" " "	Roizman & Roane (1963)
" " "	Wallis & Melnick (1966)

TABLE I (cont'd)

Microorganism				Reference
Viruses (Animal) (cont'd)				
Infectious bovine rhinotracheitis virus				Newton & Bevis (1959)
Infectious canine hepatitis virus				Noll & Youngner (1959)
Influenza virus	(Type		A)	Salk (1941)
"	"	"	A	Stanley (1945)
"	"			Dawson & Elford (1949)
"	"			Voorspuij (1949)
"	"	"	A	Davenport & Horsfall (1950)
"	"	"	A	Muller & Rose (1952)
"	"	"	A	Isliker (1953)
"	"	"	A	Puck & Sagik (1953)
"	"	"	A,B	Curtain (1954)
"	"	"	A	Nakagawa & Schlesinger (1955)
"	"	"	A	Takemoto (1954)
"	"	"	A,B	Miller & Schlesinger (1955)
"	"	"	A	Tagaya (1955)
"	"	"	A	Burke et al. (1957)
"	"	"	A	Drescher (1957)
"	"	"	A,B	Matheka & Armbruster (1958)
"	"	"	A	Taverne et al. (1958)
"	"	"	A,B	Youngner & Noll (1958)
"	"	"	A,B	Frommhagen & Knight (1959)
"	"	"	A,A',B,B'	Noll & Youngner (1959)
"	"	"	A	Nicoli et al. (1964)
"	"			Menyshikh et al. (1965)
"	"	"	A,B	Warren et al. (1966)
"	"	"	A	Pepper (1967)
Japanese encephalitis virus				Muller (1950)
Measles virus				Wallis & Melnick (1966)
Mouse melanomas virus				Riley et al. (1949)
Mouse pneumonia virus				Davenport & Horsfall (1950)
Mumps virus				Elford et al. (1948)
"	"			Soule et al. (1959)
Murine encephalomyocarditis virus				Taverne et al. (1958)
"		"	"	Kaighn et al. (1964)
Newcastle disease virus				Elford et al. (1948)
"	"	"		Youngner & Noll (1958)
"	"	"		Noll & Youngner (1959)
"	"	"		Wilson (1962)

TABLE I (cont'd)

Microorganism		Reference
Viruses (Animal) (cont'd)		
Novy rate virus		Zago (1956)
Poliovirus		Rhoads (1931)
"		Sabin (1932)
"		Schaeffer & Brebner (1933)
"		Carlson et al. (1942)
"	(Type 2)	Wenner (1945)
"	" 2	LoGrippo (1950)
"	" 2	LoGrippo & Berger (1952)
"	" 1,2,3	Hoyer et al. (1958)
"	" 2	Oker-Blom & Nikkilä (1958)
"	" 1	Taylor & Graham (1958)
"	" 3	Taverne et al. (1958)
"	" 1	Youngner & Noll (1958)
"	" 1,2	Noll & Youngner (1959)
"	" 1	Levintow & Darnell (1960)
"	" 1,3	Gravelle & Chin (1961)
"	" 1	May & Shu (1961)
"	" 3	Charney (1962)
"	" 1,3	Horodniceanu et al. (1962)
"	" 1	Boeye (1963)
"	" 1,2,3	Koza (1963)
"	" 1	Bengtsson et al. (1964)
"	" 1	Cliver (1965)
"	" 1	Phillips & Grim (1965)
"	" 1	Carlson et al. (1966)
"	" 3	Cookson (1966)
"		Wallis & Melnick (1966)
"	" 3	Cookson & North (1967)
Polyoma T virus		Sheinin (1962)
Rabies virus		Kraus et al. (1909)
"	"	Kraus & Barbara (1915)
"	"	Muller (1950)
"	"	Oker-Blom & Nikkilä (1958)
"	"	Thomas et al. (1963)
Rhinovirus		Phillips & Grim (1965)
Semliki forest virus		Taverne et al. (1958)
Sinbis virus		Pferrerkorn & Hunter (1963)
Theiler virus		Leyon (1949)
"	"	LoGrippo (1950)
"	"	Kelly (1953)
"	"	Gilcreas & Kelly (1955)

TABLE I (cont'd)

Microorganism				Reference
Viruses (Animal) (cont'd)				
Vaccinia virus				Kraus & Barbara (1915)
"	"			Lewis & Andervont (1927)
"	"			Taverne et al. (1958)
"	"			Youngner & Noll (1958)
"	"			McCrea & O'Loughlin (1959)
"	"			Noll & Youngner (1959)
"	"			Valentine & Allison (1959)
"	"			Allison & Valentine (1959,1960)
Vesicular exanthema virus				Newton & Bevis (1959)
Vesicular stomatitis virus				Newton & Bevis (1959)
Western equine encephalomyelitis virus				Muller (1950)
Viruses (Bacterial)				
Escherichia	coli	bacteriophage	T_1,T_4	Puck et al. (1951)
"	"	"	T_2	Shepard & Woodend (1951)
"	"	"		Kelly (1953)
"	"	"	T_1,T_2	Puck & Sagik (1953)
"	"	"	T_{4r}	Gilcreas & Kelly (1955)
"	"	"	T_{2r},T_{2r+}	Creaser & Taussig (1957)
"	"	"	T_3	Drescher & Raettig (1957)
"	"	"	T_1	Sproul (1957)
"	"	"	T_{2r}	Youngner & Noll (1958)
"	"	"	T_1	Noll & Youngner (1959)
"	"	"	T_1	Zelkowitz & Noll (1959)
"	"	"	T_2	Hjerten (1962)
"	"	"	T_2	Carlson et al. (1966)
"	"	"	T_4	Cookson (1966)
"	"	"	T_1,T_2,N_4	Schito (1966)
"	"	"	T_4	Cookson & North (1967)
Staphlococcus	sp.	bacteriophage		Callow (1927)
"	albus	"		Dieterich (1953)
"	"	"		Chang et al. (1958a,1958b)
"	"	"		Ryckman (1959)
Bacteriophage				Gildemeister & Herzberg (1924)
"				Kligler & Olitzki (1931)

TABLE I (cont'd)

Microorganism	Reference
Viruses (Plant)	
Bean pod mottle virus	Dunn & Hitchborn (1965)
Broad bean virus	Dunn & Hitchborn (1965)
Brome mosaic virus	Galvez (1964)
Carnation mottle virus	Dunn & Hitchborn (1965)
Cocoa yellow virus	Dunn & Hitchborn (1965)
Potato virus X	Levin (1958)
" " X	Corbett (1961)
" " X,Y	Venekamp & Mosch (1964)
" " X	Dunn & Hitchborn (1965)
" stem mottle virus	Venekamp & Mosch (1964)
Prune dwarf virus	Fulton (1959)
Sour cherry necrotic ringspot virus	Fulton (1959)
Southern bean mosaic virus	Markhan (1959)
" " " "	Galvez (1964)
" " " "	Galvez (1966)
Sowbane mosaic virus	Dunn & Hitchborn (1965)
Soybean mosaic virus	Galvez (1966)
Tobacco mosaic virus	Tiselius (1954)
" " "	Commoner et al. (1956)
" " "	Cochran et al. (1957)
" " "	Levin (1958)
" " "	Kammen et al. (1961)
" " "	Galvez (1964)
" " "	Taniguchi (1964)
" " "	Venekamp & Mosch (1964)
" " "	Dunn & Hitchborn (1965)
" " "	Galvez (1966)
" " "	Taniguchi (1966a,1966b)
" necrosis virus	Dunn & Hitchborn (1965)
" rattle virus	Dunn & Hitchborn (1965)
Tomato bushy stunt virus	Dunn & Hitchborn (1965)
" spotted wilt virus	Martin (1964)

TABLE I (cont'd)

Microorganism	Reference
Viruses (Plant) (cont'd)	
Turnip crinkle virus	Dunn & Hitchborn (1965)
" yellow mosaic virus	Dunn & Hitchborn (1965)
" " " "	Hitchborn & Dunn (1965)
Wild cucumber mosaic virus	Dunn & Hitchborn (1965)
" " " "	Hitchborn & Dunn (1965)
Yeasts	
Saccharomyces cerevisiae (Baker's yeast)	Bair & Stannard (1955)
" "	Zvyagintsev (1959a)
" " " "	Gillissen *et al.* (1961)
" "	Zvyagintsev (1962)
" " " "	Kurozumi *et al.* (1965)
" " var. *ellipsoides*	Oksentian (1940)
" spp.	Kalyuzhnii (1957,1964,1965)
Schizosaccharomyces pombe	Dikusar (1941)
Torula spp.	Kalyuzhnii (1957,1964,1965)
Trigonopsis variabilis	Kurozumi *et al.* (1965)
Wine yeast	Kurozumi *et al.* (1965)

TABLE II

ADSORPTION OF BACTERIA ONTO SOLID SURFACES

Preferred Classification	Reference Classification[1]	Page[2]	Entries	References
Order: Eubacteriales			218	
Family: Azotobacteraceae		283	10	
Azotobacter agilis		284	1	See TABLE III
" *chroococcum*		283	3	" " "
" spp.		-	6	" " "
Family: Rhizobiaceae		285	7	
Rhizobium leguminosarum		286	1	Novogrudskii (1936b)
" *lupini*		287	1	Zvyagintsev (1959a)
" *melitoti*		288	1	Peele (1936)
Agrobacterium radiobacter		291	1	Stotzky & Rem (1966)
Chromobacterium violaceum	*Bact. violaceum*	294	1	Dianova & Voroschilova (1925)
" "	*Pseudomonas violacea*	"	1	Zvyagintsev (1959a)
" "	*Pseudomonas violacea*	"	1	Zvyagintsev (1959b)
Family: Achromobacteraceae		296	5	
Alcaligenes faecalis	*B. faecalis alcaligenes*	297	1	Salus (1917)
Achromobacter sp.		300	1	Stotzky & Rem (1966)
Flavobacterium sp.		309	1	Estermann (1957)
"		"	1	Estermann & McLaren (1959)
"		"	1	Stotzky & Rem (1966)

[1] Species name as abbreviated and spelled in reference cited if different than preferred classification.

[2] See designated pagination in "Bergey's Manual of Determinative Bacteriology," 7th Ed. (Breed, Murray, & Smith, 1957)

TABLE II (cont'd)

Preferred Classification	Reference Classification	Page	Entries	Reference
Family: Enterobacteriaceae		334	102	
Tribe: Escherichiae		334	48	
Escherichia coli		336	42	See TABLE IV
" *intermedia*		340	1	" " "
Aerobacter aerogenes	*Lactis aerogenes*	342	1	Putter (1920)
Klebsiella pneumoniae	*Bac. pneumoniae Friedlander*	344	1	Bleyer (1922)
" "		"	1	Schwartz & Mayer (1963)
Paracolobactrum sp.	*Paracolobactrum*	346	2	Adamov (1959,1964)
Tribe: Serratieae		359	12	
Serratia marcescens		361	12	See TABLE V
Tribe: Proteeae		364	8	
Proteus vulgaris		365	1	Putter (1920)
" "	*P. vulgaris*	"	1	Barr (1957)
" "	*Bact. proteus*	"	1	Zvyagintsev (1959a)
" "		"	1	Martinez (1963)
" "	*Prot. vulg.*	"	1	Schwartz & Mayer (1963)
" "		"	1	Stotzky & Rem (1966)
" sp.	*Proteus* OX 19	-	2	Adamov (1959,1964)
Tribe: Salmonelleae		368	34	
Salmonella enteritidis		371	1	See TABLE VI
" *paratyphi*		373	6	" " "
" *schottmuelleri*		373	6	" " "
" *typhosa*		372	16	" " "

TABLE II (cont'd)

Preferred Classification	Reference Classification	Page	Entries	Reference
Family: Enterobacteriaceae (cont'd)				
Tribe: Salmonelleae (cont'd)				
Shigella dysenteriae	*B. dysenteriae*	384	1	Eisenberg (1918)
" "	*Dysenteriae*	"	1	Putter (1920)
" "	*S. dysenteriae*	"	1	Martin (1955)
" *flexneri*	*S. paradysenteriae*	387	1	Barr (1957)
" "		"	1	Marazzi-Uberti *et al.* (1964)
Family: Brucellaceae		394	2	
Bordetella pertussis	*Haemophilis pertussis*	402	1	Unger & Muggleton (1949)
" "	*Haemophilis pertussis*	"	1	Kuwajima *et al.* (1957)
Family: Micrococcaceae		454	41	
Micrococcus agilis	*Staphlococcus agilis*	462	1	Karpinskaya (1926)
Staphlococcus aureus		465	24	See TABLE VII
" spp.		-	2	" " "
Sarcina flava		471	1	See TABLE VIII
" *lutea*		470	8	" " "
" *ureae*		472	3	" " "
" spp.		-	2	" " "
Family: Lactobacillaceae		505	5	
Tribe: Streptococceae		506	3	
Diplococcus pneumoniae	*Dipl. pneum.*	507	1	Schwartz & Mayer (1963)
Streptococcus pyogenes	*Strept. haem.*	512	1	Schwartz & Mayer (1963)
" *lactis*		525	1	Oksentian (1940)

TABLE II (cont'd)

Preferred Classification	Reference Classification	Page	Entries	Reference
Family: Lactobacillaceae (cont'd)				
Tribe: Lactobacilleae		541	2	
Lactobacillus acidophilis		545	1	Gunnison & Marshall (1937)
" helveticus		"	1	Oksentian (1940)
Family: Corynebacteriaceae		578	4	
Corynebacterium diphtheriae		581	1	Eisenberg (1918)
" "	Diphtheriae	"	1	Putter (1920)
" pseudodiphtheriticus	Corynebact. pseudodiphtheriae	588	1	Eisenberg (1918)
Arthrobacter globiformis		607	1	Stotzky & Rem (1966)
Family: Bacillaceae		613	42	
Bacillus anthracis		618	4	See TABLE IX
" cereus		617	12	" " "
" megaterium		616	6	" " "
" subtilis		620	18	" " "
Clostridium perfringens	Clostridium welchii	666	1	Gunnison & Marshall (1937)
" sp.	Granulob. putreficas	-	1	Eisenberg (1918)
Order: Pseudomonadales		35	28	
Family: Pseudomonadaceae		88	25	
Pseudomonas aeruginosa		99	8	See TABLE X
" chlororaphis		103	1	" " "
" fluorescens		105	7	" " "
" putida		107	1	" " "
" solanacearum		144	1	" " "
" striata		107	1	" " "
" stutzeri		115	1	" " "
" spp.		-	5	" " "

TABLE II (cont'd)

Preferred Classification	Reference Classification	Page	Entries	Reference
Family: Spirillaceae		228	3	
Vibrio comma	V. cholerae	231	1	Eisenberg (1918)
" "	Cholera	"	1	Putter (1920)
Spirillum serpens		254	1	Martinez (1963)
Order: Actinomycetales		694	7	
Family: Mycobacteriaceae		695	7	
Mycobacterium phlei		697	1	Stotzky & Rem (1966)
" tuberculosis		701	2	Schwartz & Mayer (1963)
" spp.		-	2	Zvyagintsev (1959a)
Micrococcus albus	M. candicans	708	1	Eisenberg (1918)
" rhodochrous		712	1	Stotzky & Rem (1966)

TABLE III

ADSORPTION OF THE GENUS *Azotobacter*

Preferred Classification	Reference Classification	Reference
Azotobacter agilis	*Azotobacter agile*	Hattori & Furusaka (1961)
Azotobacter chroococcum	*Azotobacter chroococcum*	Novogrudskii (1936b)
" "	*Azotobacter chroococcum*	Peele (1936)
" "	*Azotobacter chroococcum*	Zvyagintsev (1959b)
Azotobacter sp.	*Azotobacter*	Tschapek & Garbosky (1950a)
" "	*Azotobacter*	Tschapek & Garbosky (1950b)
" "	*Azotobacter*	Tschapek & Garbosky (1951)
" "	*Azotobacter vinelandii* O	Rotman (1958)
" "	*Azotobacter vinelandii* O	Rotman (1960)
" "	*Azotobacter*	Geller *et al.* (1963)

TABLE IV

ADSORPTION OF THE GENUS *Escherichia*

Preferred Classification	Reference Classification	Reference
Escherichia coli	*Coli*	Kuhn (1915)
" "	*Coli*	Kuhn & Heck (1916)
" "	*B. coli*	Salus (1917)
" "	*Bacterium coli*	Bechhold (1918)
" "	*B. coli*	Eisenberg (1918)
" "	*Bact. coli*	Michaelis (1918)
" "	*Coli*	Friedberger (1919)
" "	*Coli*	Gutfeld (1919)
" "	*Coli*	Putter (1920)
" "	*Coli*	Reiter & Meyer (1921)
" "	*Bacterium coli commune*	Frei & Erismann (1922)
" "	*Bact. coli communis*	Dianova & Voroschilova (1925)
" "	*Bact. coli commune*	Khudiakov (1926)
" "	*Bacterium coli commune*	Karpinskaya (1926)
" "	*Bact. coli*	Bechhold & Keiner (1927)
" "	*Bact. coli commune*	Rubentschik *et al.* (1934)
" "	*Escherichia coli*	Gunnison & Marshall (1937)
" "	*Bact. coli*	Dikusar (1941)
" "	*B. coli*	Barnes (1952a)
" "	*B. coli*	Barnes (1952b)
" "	*Coli*	Debusmann (1950)
" "	*E. coli*	Weiss (1951)
" "	*E. coli* B	Puck & Sagik (1953)
" "	*E. coli* B	Bush & Rainey (1954)
" "	*Esch. coli*	Martin (1955)
" "	*Escherichia coli* K 12	Rotman (1958)
" "	*E. coli* (commune)	Adamov (1959)
" "	*E. coli* (aerobacter)	Adamov (1959)
" "	*E. coli* (citrobacter)	Adamov (1959)
" "	*E. coli*	Hattori & Furusaka (1959a)
" "	*E. coli*	Hattori & Furusaka (1959b)
" "	*Bact. coli*	Zvyagintsev (1959b)
" "	*Coli*	Gillissen (1960)
" "	*E. coli*	Hattori & Furusaka (1960)
" "	*Escherichia coli* K 12	Rotman (1960)
" "	*Escherichia coli*	Adamov & Noskov (1962)
" "	*Escherichia coli*	Hjerten (1962)
" "	*E. coli*	Schwartz & Mayer (1963)
" "	*E. coli haemolyt.*	Schwartz & Mayer (1963)
" "	*Bact. coli commune*	Adamov (1964)

TABLE IV (cont'd)

Preferred Classification	Reference Classification	Reference
Escherichia coli	Escherichia coli	Kurozumi et al. (1965)
" "	Escherichia coli	Stotzky & Rem (1966)
Escherichia intermedia	Escherichia intermedia	Stotzky & Rem (1966)

TABLE V

ADSORPTION OF THE GENUS *Serratia*

Preferred Classification		Reference Classification	Reference
Serratia	*marcescens*	*B. prodigiosum*	Eisenberg (1918)
"	"	*Prodigiosis*	Putter (1920)
"	"	*Bac. prodigiosus*	Dianova & Voroschilova (1925)
"	"	*Bact. prodigiosus*	Khudiakov (1926b)
"	"	*Bacterium prodigiosum*	Karpinskaya (1926)
"	"	*Bact. prodigiosum*	Minenkov (1929)
"	"	*Bact. prodigiosum*	Rubentschik *et al.* (1934)
"	"	*Serratia marcescens*	Rubentschik *et al.* (1936)
"	"	*Bact. prodigiosum*	Dikusar (1941)
"	"	*Bact. prodigiosum*	Zvyagintsev (1959a)
"	"	*Bact. prodigiosum*	Zvyagintsev (1959b)
"	"	*B. prodigiosus*	Zvyagintsev (1960)

TABLE VI

ADSORPTION OF THE GENUS *Salmonella*

Preferred Classification	Reference Classification	Reference
Salmonella enteritides	*S. enteritides*	Barr (1957)
Salmonella paratyphi	*B. paratyphi* A	Eisenberg (1918)
" "	*Paratyphus* A	Putter (1920)
" "	*Paratyphus* A	Bleyer (1922)
" "	*Paratyphus*	Bechhold & Keiner (1927)
" "	*Salm. paratyphi* A	Adamov (1959)
" "	*S. paratyphi* A	Adamov (1964)
Salmonella typhosa	*Typhus*	Kuhn (1915)
" "	*Typhus*	Geronne & Lenz (1915)
" "	*Typhus*	Kalberlah (1915)
" "	*Typhus*	Kuhn & Heck (1916)
" "	*B. typhi*	Salus (1917)
" "	*B. typhi*	Eisenberg (1918)
" "	*Typhus*	Michaelis (1918)
" "	*Typhus*	Friedberger (1919)
" "	Typhoid bacteria	Gutfeld (1919)
" "	*Typhus*	Putter (1920)
" "	Typhoid bacteria	Reiter & Meyer (1921)
" "	*B. typhosus*	Salk (1938)
" "	*S. typhosa*	Martin (1955)
" "	*Salm. typhosa*	Adamov (1959)
" "	Typhoid fever bacteria	Adamov & Noskov (1962)
" "	*S. typhi*	Adamov (1964)
Salmonella schottmuelleri	*Paratyphus* B	Kuhn (1915)
" "	*B. paratyphi* B	Eisenberg (1918)
" "	*Paratyphus* B	Putter (1920)
" "	*S. schottmuelleri*	Martin (1955)
" "	*Salm. paratyphi* B	Adamov (1959)
" "	*S. paratyphi* B	Adamov (1964)

TABLE VII

ADSORPTION OF THE GENUS *Staphlococcus*

Preferred Classification	Reference Classification	Reference
Staphlococcus aureus	*Staphlococcus*	Salus (1917)
" "	*Staphlococcus*	Bechhold (1918)
" "	*M. pyogenes*	Eisenberg (1918)
" "	*Staphlococc. alb.*	Putter (1920)
" "	*Staphlococc. aureus*	Putter (1920)
" "	*Staphlococcus pyogenes*	Bleyer (1922)
" "	*Staphlococcus pyogenes albus*	Dianova & Voroschilova (1925)
" "	*Staphlococcus pyogenes albus*	Khudiakov (1926b)
" "	*Staphlococcus pyogenes albus*	Karpinskaya (1926)
" "	*Staphlococci*	Bechhold & Keiner (1927)
" "	*Staphlococcus aureus*	Gunnison & Marshall (1937)
" "	*S. aureus*	Salk (1938)
" "	*Staph. aureus*	Martin (1955)
" "	*S. aureus*	Barr (1957)
" "	*Staph. aureus*	Adamov (1959)
" "	*Staph. albus*	Adamov (1959)
" "	*Staphlococcus aureus*	Zvyagintsev (1959a)
" "	*Staphlococcus aureus*	Zvyagintsev (1959b)
" "	*M. pyogenes aureus* Sg 511	Gillissen *et al.* (1961)
" "	*Staph. albus*	Gillissen *et al.* (1961)
" "	*Staph. pyog. aur.*	Schwartz & Mayer (1963)
" "	*Staph. albus*	Schwartz & Mayer (1963)
" "	*Staphlococcus*	Marazzi-Uberti *et al.* (1964)
" "	*Staphlococcus aureus*	Adamov (1964)
Staphlococcus sp.	*Staphlococcus* sp.	Süpfle & Müller (1920)
" "	*Staphlococcus* sp.	Zvyagintsev (1959a)

TABLE VIII

ADSORPTION OF THE GENUS *Sarcina*

Preferred Classification	Reference Classification	Reference
Sarcina flava	*Sarcina flava*	Putter (1920)
Sarcina lutea	*Sarc. lutea*	Eisenberg (1918)
" "	*Sarcina lutea*	Putter (1920)
" "	*Sarcina lutea*	Rubentschik *et al.* (1934)
" "	*Sarcina lutea*	Rubentschik *et al.* (1936)
" "	*Sarcina lutea*	Gunnison & Marshall (1937)
" "	*Sarcina lutea*	Adamov (1959)
" "	*Sarcina lutea*	Adamov (1964)
" "	*Sarcina lutea*	Stotzky & Rem (1966)
Sarcina ureae	*Sarcina agilis*	Putter (1920)
" "	*Planosarcina urea*	Dianova & Voroschilova (1925)
" "	*Sarcina ureae*	Novogrudskii (1936b)
Sarcina sp.	*Sarcina* sp.	Zvyagintsev (1959a)
" "	*Sarcina* sp.	Zvyagintsev (1959b)

TABLE IX

ADSORPTION OF THE GENUS *Bacillus*

Preferred Classification	Reference Classification	Reference
Bacillus anthracis	*Bacillus anthracis*	Eisenberg (1918)
" "	Anthrax	Putter (1920)
" "	Anthrax spores	Supfle & Müller (1920)
" "	*Bac. anthracis* spores	Gillissen *et al.* (1961)
Bacillus cereus	*B. mycoides*	Dianova & Voroschilova (1925)
" "	*Bac. Ellenbachensis*	Dianova & Voroschilova (1925)
" "	*Bac. mycoides*	Khudiakov (1926b)
" "	*Bacillus mycoides*	Karpinskaya (1926)
" "	*Bac. mycoides*	Minenkov (1929)
" "	*Bac. mycoides*	Rubentschik *et al.* (1934)
" "	*B. mycoides*	Novogrudskii (1936b)
" "	*Bacillus mycoides*	Peele (1936)
" "	*B. mycoides*	Rubentschik *et al.* (1936)
" "	*Bac. mycoides*	Dikusar (1941)
" "	*Bac. mycoides*	Zvyagintsev (1959b)
" "	*Bacillus mycoides*	Zvyagintsev (1960)
Bacillus megaterium	*Bacillus tumescens*	Eisenberg (1918)
" "	Megatherium	Putter (1920)
" "	*Bact. megatherium*	Khudiakov (1926b)
" "	*B. megatherium*	Novogrudskii (1936b)
" "	*Bac. megaterium phosph.*	Geller *et al.* (1963)
" "	*Bacillus megaterium*	Stotzky & Rem (1966)
Bacillus subtilis	*Bac. subtilis*	Eisenberg (1918)
" "	Subtilis	Putter (1920)
" "	Mesenthericus	Putter (1920)
" "	*Bac. mesenthericus fuscus*	Dianova & Voroschilova (1925)
" "	*Bac. mesenthericus vulgatus*	Rubentschik *et al.* (1934)
" "	*B. mesentericus*	Lasseur, Dombray & Palgen (1934)
" "	*B. mesentericus vulgatis*	Novogrudskii (1936b)
" "	*B. vulgatis*	Rubentschik *et al.* (1936)
" "	*Bacillus subtilis*	Gunnison & Marshall (1937)
" "	*Bac. mesentericus*	Dikusar (1941)
" "	*B. subtilis* Moran	Estermann (1957)
" "	*B. subtilis* Moran	Estermann & McLaren (1959)
" "	*Bac. subtilis* spores	Gillissen *et al.* (1961)
" "	*Bacillus subtilis*	Lahav (1962)
" "	*Bac. mesentericus*	Geller *et al.* (1963)
" "	*Bacillus subtilis*	Martinez (1963)
" "	*Bac. subtilis*	Schwartz & Mayer (1963)
" "	*Bacillus subtilis*	Stotzky & Rem (1966)

TABLE X

ADSORPTION OF THE GENUS *Pseudomonas*

Preferred Classification	Reference Classification	Reference
Pseudomonas aeruginosa	*B. pyocyaneum*	Eisenberg (1918)
" "	*Pyocyaneus*	Putter (1920)
" "	*Pyocyaneus*	Ham & Barnes (1947a)
" "	*Pyocyaneus*	Ham & Barnes (1947b)
" "	*Pseudomonas pyocyanea*	Zvyagintsev (1959a)
" "	*Pseudomonas pyocyanea*	Zvyagintsev (1959b)
" "	*Pseudomonas aerug.*	Schwartz & Mayer (1963)
" "	*Pseudomonas aeruginosa*	Stotzky & Rem (1966)
Pseudomonas chlororaphis	*B. chlororaphis*	Lasseur, Dombray & Palgen (1934)
Pseudomonas fluorescens	*B. fluorescens*	Eisenberg (1918)
" "	*Fluorescens*	Putter (1920)
" "	*Bac. fluorescens liquefaciens*	Dianova & Voroschilova (1925)
" "	*Bact. fluorescens liquefaciens*	Rubentschik *et al.* (1934)
" "	*Bact. fluorescens*	Novogrudskii (1936b)
" "	*Bact. fluorescens liquefaciens*	Zvyagintsev (1959a)
" "	*Pseudomonas fluorescens*	Gillissen *et al.* (1961)
Pseudomonas putida	*B. putidum*	Eisenberg (1918)
Pseudomonas solanacearum	*Pseudomonas solanacearum*	Stotzky & Rem (1966)
Pseudomonas striata	*Pseudomonas striata*	Stotzky & Rem (1966)
Pseudomonas stutzeri	*Bact. stutzeri*	Dikusar (1941)
Pseudomonas sp.	*Pseudomonas sp.*	Estermann (1957)
" "	*Pseudomonas sp.*	Estermann & McLaren (1959)
" "	*Pseudomonas*	Geller *et al.* (1963)
" "	*Pseudomonas sp.* B-47	Stotzky & Rem (1966)
" "	*Pseudomonas sp.* B-1	Stotzky & Rem (1966)

TABLE XI

SOLID SURFACES CAPABLE OF ADSORBING BACTERIA[1]

Inorganic Compounds

Aluminum hydroxide
Aluminum oxide
Aluminum phosphate
Aluminum sulfate
Antimony pentasulfide
Barium sulfate
Bismuth nitrate
Calcium carbonate
Calcium oxalate
Calcium phosphate (mono)
Calcium phosphate (tri)
Ferric hydroxide
Ferric oxide (magnetic)
Magnesium hydroxide
Magnesium oxide
Magnesium pyrophosphate
Sodium aluminum silicate
Zinc hydroxide

Organic Compounds

Anion exchange resins
(See TABLE XII)

Cation exchange resins
(See TABLE XIII)

Amonium alginate
Anthracene
-amino anthraquinone
Cetyl alcohol
Cholesterol
Dimethyl-p-amino diazobenzene
m-dinitrobenzene
2,4-dinitrochlorobenzene
8-hydroxyquinoline
Methylene bisphenolic acids
-napthol salicilic acid
o-nitroaniline
p-nitrophenol
Pectin

Soils and Minerals

Alumina
Asbestos
Attapulgite
Bentonite
Chalk
Cinnabar
Diatomaceous earth
Emery
Fuller's earth
Hornblende
Halloysite
Hydroxylapatite
Illite
Kaolinite
Kieselguhr
Meerschaum
Montmorillonite
Sand
Silica gel
Talc
Various clays, earths
soils, and sediments

Miscellaneous

Ash
Brick dust
Cellulose
Cellulose (anionic)
Cellulose (cationic)
Charcoal
Coke
Cuttlefish bone
Filter paper
Gamboge
Glass
Mastic
Plant roots
Rubber
Various natural gums
and resins

[1] No specific literature citations are given because of the great intertwinement of the types of materials evaluated. The compounds listed in this table have been reported to possess various degrees of affinity for adsorption of bacterial cells.

TABLE XII

ANION EXCHANGE RESINS ADSORBING BACTERIA

Trade Name[1]	Ion Form	Mesh Size	Remarks	Reference
Strong base quaternary ammonium Type I, $-N(alkyl)_3^+$				
Dowex 1	$C_2H_3O_2$	50/100	1, 4, 16% crosslinkage	Kurozumi et al. (1965)
"	n.s.[2]	n.s.	4% crosslinkage	Schwartz & Mayer (1963)
"	Cl	100/200	4% crosslinkage	Hattori & Furusaka (1961)
"	Cl	100/200	4% crosslinkage	Hattori & Furusaka (1960)
"	Cl	200		Rotman (1960)
"	OH	n.s.		Rotman (1958)
"	PO_4	n.s.		Puck & Sagik (1953)
"	OH, Cl	40/60	1, 2, 4, 8% crosslinkage	Kuwajima et al. (1957)
MSB	n.s.	n.s.		Schwartz & Mayer (1963)
"	n.s.	0.4/1.0 mm		Gillissen et al. (1961)
"	n.s.	0.4/1.0 mm		Gillissen (1960)
Duolite A 42	OH, Cl	40/60		Kuwajima et al. (1957)
Daiya-ion SA	OH, Cl	40/60		Kuwajima et al. (1957)

TABLE XII (cont'd)

Trade Name	Ion Form	Mesh Size	Remarks	Reference
n.s.	Cl	n.s.	Probably Amberlite resin	Kunin & Meyers (1947)
Strong base quaternary ammonium Type II, $-N(alkylol)(alkyl)_2^+$				
Dowex 2	n.s.	n.s.	4% crosslinkage	Schwartz & Mayer (1963)
"	OH, Cl	40/60		Kuwajima et al. (1957)
IRA 410	Cl	16/50		Rotman (1958)
Duolite A 40	OH, Cl	40/60	Also Duolite A 40L	Kuwajima et al. (1957)
Weak base aliphatic amine				
Dowex 3	Free base	(20/50)	Only mesh size available	Schwartz & Mayer (1963)
SCB	n.s.	n.s.		Gillissen (1960)
IR 4 B	OH, Cl	40/60		Kuwajima et al. (1957)
Duolite A 2	OH, Cl	40/60	Also A 4, A 6, A 70	Kuwajima et al. (1957)
n.s.	n.s.	n.s.		Martin (1955)
Multifunctional quaternary ammonium-polyamine				
Duolite A 41	OH, Cl	40/60		Kuwajima et al. (1957)

TABLE XII (cont'd)

Trade Name	Ion Form	Mesh Size	Remarks	Reference
Intermediate base epoxypolyamine				
Duolite A 30	OH, Cl	40/60		Kuwajima et al. (1957)
Unclassified				
Duolite ES 102	OH, Cl	40/60		Kuwajima et al. (1957)
Anionite AN-2F	Cl	n.s.		Zvyagintsev (1962)
EDE 10 F	n.s.	n.s.		Adamov & Noskov (1962)

[1] For a comparison of equivalent resins see: Helferrich (1962, pp.574-584), Samuelson (1963, pp.432-434), or Calmon & Kressman (1957, pp.116-129).

[2] n.s. = not specified

TABLE XIII

CATION EXCHANGE RESINS ADSORBING BACTERIA

Trade Name[1]	Ion Form	Mesh Size	Remarks	Reference
Sulfonic acid, $-SO_3^-$				
Dowex 50	Na	n.s.[2]	Referred to as Nalcite	Rotman (1958)
Duolite C 20	H, Na	n.s.	Also C 25	Kuwajima et al. (1957)
Wofatit KPS-200	n.s.	(0.3/7.5 mm)	Only available mesh size	Schwartz & Mayer (1963)
STS	n.s.	0.4/1.0 mm		Gillissen et al. (1961)
STS	n.s.	0.4/1.0 mm		Gillissen (1960)
Phosphinic, $-HPO_2^-$				
Duolite C 62	H, Na	n.s.		Kuwajima et al. (1957)
Acrylic carboxylic, $-COO^-$				
Duolite CS 101	H, Na	n.s.		Kuwajima et al. (1957)
Unclassified				
FN	n.s.	n.s.		Schwartz & Mayer (1963)
N-Wofatit	n.s.	n.s.		Schwartz & Mayer (1963)
Escarbo	n.s.	n.s.		Schwartz & Mayer (1963)

TABLE XIII (cont'd)

Trade Name	Ion Form	Mesh Size	Remarks	Reference
Cationite CDB 3	H, NH_4, Na		Also Fe,Al,Cu,Ca,Mg,Mn,K	Zvyagintsev (1962)
Daiya-ion K	n.s.	n.s.	Also Daiya-ion BK	Kuwajima et al. (1957)
KU-1	n.s.	n.s.		Adamov & Noskov (1962)

[1] For a comparison of equivalent resins see: Helfferich (1962, pp.574-584), Samuelson (1963, pp.432-434), or Calmon & Kressman (1957, pp.116-129).

[2] n.s. = not specified

TABLE XIV

MANUFACTURERS OF ION EXCHANGE RESINS

Trade Name	Manufacturer
Dowex	Dow Chemical Co., Midland, Mich.
Duolite	Chemical Process Co., Redwood City, Calif.
Amberlite	Rohm and Haas Co., Philadelphia Pa.
Wolfatit	Veb. Farbenfab. Wolfen, Wolfen, Kr., Bitterfeld, Germany
Daiya-ion	Mitsubishi-kasei, Toyko, Japan

TABLE XV

RESOLUTION OF MICROBIAL MIXTURES BY SELECTIVE ADSORPTION[1]

Type of Mixture	Species 1	Species 2	Type of Adsorbent	Reference[2]
Alga-Yeast	Chlorella ellipsoidea	Saccharomyces cerevisiae	Anion exchange resin	Kurozumi et al. (1965)
Yeast-Bacterium	Saccharomyces cerevisiae	Escherichia coli	Anion exchange resin	Kurozumi et al. (1965)
Virus-Bacterium	E. coli bacteriophage T_2	Escherichia coli	Cation exchange resin	*Puck & Sagik (1953)
	Escherichia coli	E. coli bacteriophage T_{4r}	Filter paper pulp	Bush & Rainey (1954)
Fungus-Fungus	Fusarium sp.	Aspergillus glaucus	Podzol (acid soil)	*Novogrudskii (1936b)
Yeast-Yeast	Trigonopsis variabilis	Saccharomyces cerevisiae	Anion exchange resin	Kurozumi et al. (1965)
Virus-Virus	Influenza A, strain MIL.B	Influenza A, strain WSE	Cellulose-haemagglutination inhibitor complex	Curtain (1954)
	Influenza A, strain PR8	Influenza B, strain Lee	Aluminum phosphate	Miller & Schlesinger (1955)
	Lipophilic types	Hydrophilic types	Water-insoluble polar lipids	*Noll & Youngner (1959)
	Poliovirus, Type 1, m^-	Poliovirus, Type 1, m^-	Dextran sulfate	Bengtsson et al. (1964)
Bacterium-Bacterium	Gram-positive species	Gram-negative-species	Many	Eisenberg (1918)
	" " "	" " "	Filter paper	*Friedberger (1919)
	" " "	" " "	Filter paper	*Putter (1920)
	" " "	" " "	Podzol (acid soil)	*Novogrudskii (1936b)
	" " "	" " "	Kaolin, charcoal	*Gunnison & Marshall (1937)
	" " "	" " "	Several	*Adamov (1959)

[1] The adsorption(s) of species 1 is greater than the adsorption(s) of species 2 by the adsorbents indicated.

[2] Resolution of species reported in references marked (*) may be possible on the basis of the data presented; resolution of species reported in unmarked references were actually confirmed by the authors themselves.

TABLE XV (cont'd)

Type of Mixture	Species 1	Species 2	Type of Adsorbent	Reference
Bacterium-Bacterium	Salmonella typhosa	Escherichia coli	Charcoal, clay	Kuhn & Heck (1916)
	Escherichia coli	Salmonella typhosa	Kaolin	Michaelis (1918)
	Sarcina lutea	Salmonella schottmuelleri	Filter paper	*Putter (1920)
	Pseudomonas chlororaphis Bacillus caryocyaneus Staphlococcus aureus	Serratia marcescens	Filter paper	Lasseur, Marchal & Maguitot (1934)
	Staphlococcus aureus	Proteus vulgaris Salmonella enteritides Shigella paradysenteriae	Activated attapulgite	*Barr (1957)
	Escherichia coli Paracolobactrum sp. Staphlococcus aureus Sarcina lutea	Salmonella typhosa Salmonella paratyphi Salmonella schottmuelleri	Tricalcium phosphate	Adamov (1959)
	Sarcina sp.	Azotobacter chroococcum	Cation exchange resin	*Zvyagintsev (1962)
	Mycobacterium tuberculosis	Staphlococcus aureus Escherichia coli Pseudomonas aeruginosa Proteus vulgaris	Anion and cation exchange resins	Schwartz & Mayer (1962)
	Bacterial cells	Bacterial spores	Anion exchange resin	Kunin (1947, p.134)
	Salmonella typhosa S form	Salmonella typhosa R form	Tricalcium phosphate	Adamov (1959)
	Escherichia coli mother cells	Escherichia coli daughter cells	Anion exchange celluloses	Helmstetter & Cummings (1963)

III. THEORETICAL CONSIDERATIONS

Several factors have been considered in investigating the adsorption of bacterial cells onto ion exchange resins. The nature of the chemical reaction, the types of materials involved, and the variables controlling the process of ion exchange are outlined in the present section. The bacterial cell surface is represented as a large, multivalent "ion" that is capable of combining with other ions of opposite charge. The variables affecting the magnitude and orientation of the cellular surface charge are defined. A possible mechanism is proposed to explain the reversible interaction between bacterial cells and ion exchange resins.

A mathematical model is developed to aid in predicting the rates of adsorption. The diffusion of bacterial cells to the surfaces of individual resin particles suspended in a well-stirred liquid of finite volume is considered to be a process analogous to the coagulation phenomena exhibited by colloidal particles. The rate constants and the diffusion coefficients of this adsorption are determined for various cell and resin concentrations and for a variety of conditions of pH, ionic strength, agitation, and temperature.

A. Chemical Representation of Bacteria

1. Ion-protein interactions

A more complete understanding of the behavior of bacterial cells that are suspended in aqueous media can be obtained through a brief discussion of the behavior of proteins existing under similar conditions. Three important physico-chemical changes that a typical

protein can undergo in aqueous solution are: denaturation, precipitation, and complexation. The actual mechanisms involved in these changes can be expected to overlap depending upon the definitions selected.

Denaturation is a physical or intramolecular rearrangement, rather than a chemical alteration. A change in specific spatial configuration can occur without hydrolysis of primary covalent bonds. The protein molecule may uncoil and assume an elongated form. This change can be promoted by various factors such as: changes in temperature, pH, or pressure, and by the presence of organic and inorganic solvents or solutes, electrolytes, and proteclytic enzymes. Denaturation of a protein may or may not be completely reversible depending upon the severity of the attact by the denaturing agent. In any event, marked changes are noted in the chemical and physical properties of the affected protein when compared to those exhibited by the same protein in its free state. This subject has been comprehensively reviewed by Putnam (1953).

Precipitation and complexation of proteins that have been promoted by purely chemical actions are difficult to differentiate. Precipitation can be considered to involve definite removal of the affected protein from suspension or solution; a phase separation may not necessarily by involved in complexation phenomena. Acid-base titrations or proteins generally result in precipitations which are reversible, irreversible, or intermediate between these two extremes. The formation of insoluble complexes, in comparison, has been the classical method of analysis of antibody-antigen combinations. A good deal of useful information concerning complex stoichiometry and the estimation of the nature of charged chemical groups has been obtained by precipitation techniques.

The electrical charge of a protein is a function of the pH and ionic strength of the containing solution. A protein may be considered to be a dipolar ion, or zwitterion, capable of assuming either a net negative or a net positive electrical charge. This feature is illustrated in Figure 1. The pH at which the number of protons dissociated from the acidic groups is equal to the number of protons associated with the basic groups is called the isoionic point. The average net electrical charge at this point is zero. The isoionic point is not necessarily the point of minimum electric charge. There may be more charged groups present at this point than at more acidic or basic pH values.

The isolectric point (pI), in comparison, is defined as the pH at which a dipolar ion does not migrate in an electric field. The isoelectric point is usually not identical to the isoionic point since electrophoretic measurements must be made in solutions of appreciable ionic strength. The isoionic point, therefore, is the limiting value of the isoelectric point determined for a salt-free solution. Both pH and ionic strength should be specified when reporting the isoelectric point of a particular material.

The presence of various ionizable groups on a given protein can be determined by titration with acid or base. The more important ionizable groups of biological significance and their approximate dissociation constants (pK') in aqueous solution are presented in Table XVI. Values of pI and pK' of several specific amino acids found in the bacterial cell wall are given in Table XVII.

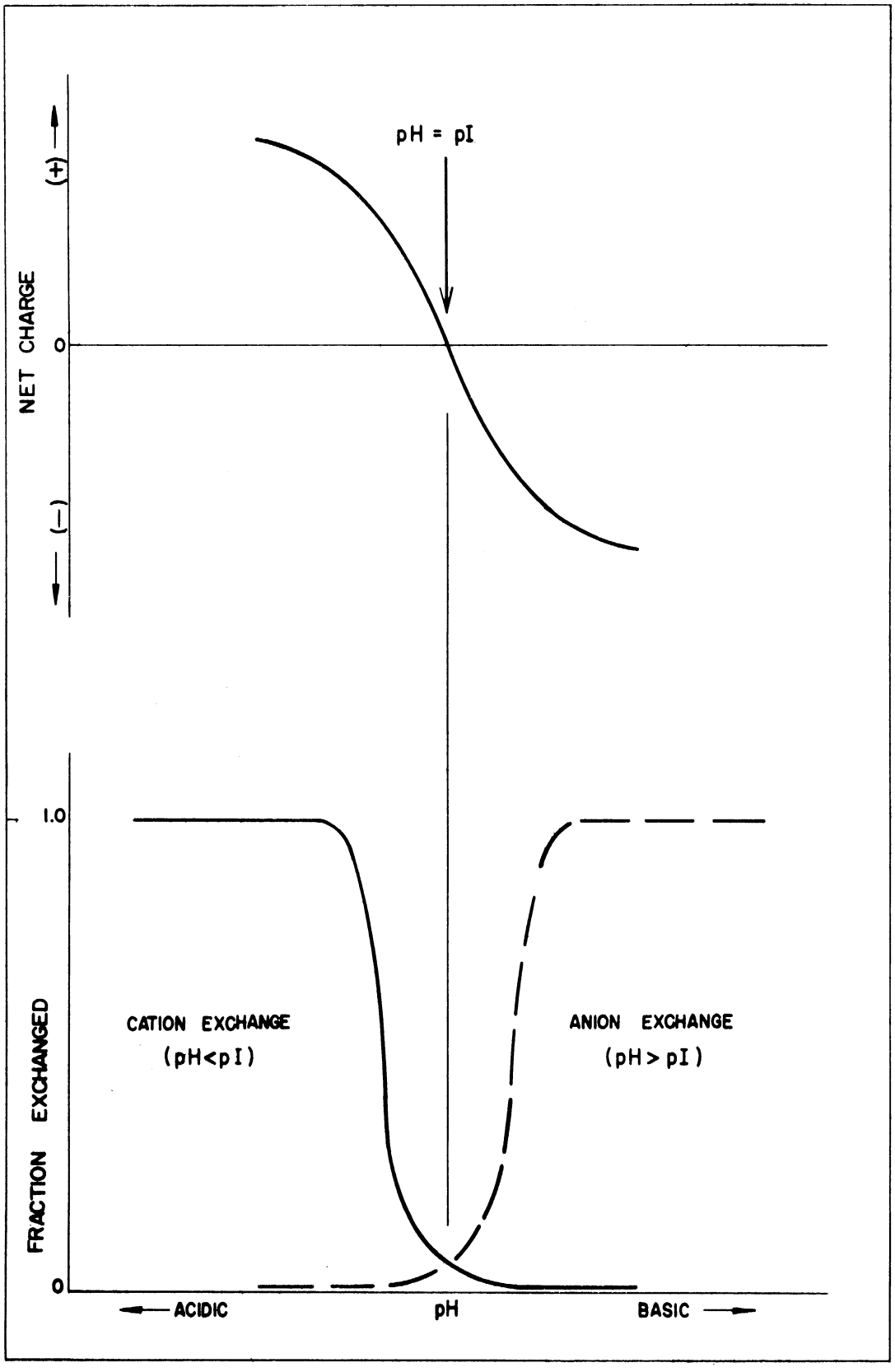

Figure 1. Prevailing Surface Charge and Exchange Behavior of a Dipolar Ion as a Function of pH.

TABLE XVI

IONIZABLE CHEMICAL GROUPS ON PROTEINS[1]

Chemical Group	pK'
Phosphatidic	1.8
Carboxyl (α)	3.0-3.2
Carboxyl (aspartyl)	3.0-4.7
Carboxyl (glutamyl)	ca. 4.4
Imidazolium (histidine)	5.6-7.0
Phenoxyl (diiodotyrosine)	6.5
Ammonium (α)	7.6-8.4
Ammonium (α , cystine)	6.5-8.5
Ammonium (ε , lysine)	9.4-10.6
Sulfhydryl	9.1-10.8
Phenoxyl (tyrosine)	9.8-10.4
Guanidinium (arginine)	11.6-12.6

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[1] All pK' values selected from Cohn and Edsall (1943, p 445) except the phosphatidic pK' which was taken from Kruyt (1949, p 192).

TABLE XVII

DISSOCIATION CONSTANTS (pK) AND ISOELECTRIC POINTS (pI) OF SELECTED AMINO ACIDS[1]

Amino Acid	pK'_1	pK'_2	pK'_3	pK'_4	pI
Glycine	2.34 (α-COOH)	9.60 (α-NH_2)			5.97
Alanine	2.34 (α-COOH)	9.69 (α-NH_2)			6.00
Aspartic acie	1.88 (α-COOH)	3.65 (β-COOH)	9.60 (α-NH_2)		2.77
Glutamic acid	2.19 (α-COOH)	4.25 (δ-COOH)	9.67 (α-NH_2)		3.22
α-ϵ-Diaminopimelic acid (2,6-Diaminoheptanedioic acid)	1.8 (α-COOH)	2.2 (ϵ-COOH)	8.8 (α-NH_2)	9.9 (ϵ-NH_2)	5.5

[1] Tabulated values for the dissociation constants (pK') and isoelectric points (pI) taken from Cohn and Edsall (1943, pp 84-85) except for those values reported for α-ϵ diaminopimelic acid which were taken from Rhuland (1960).

Fraenkel-Conrat and Cooper (1944) used dyes to determine the acidic and basic groups of various proteins. The behavior of proteins, amino acids, and peptides as ions and as dipolar ions were summarized in a valuable text by Cohn and Edsall (1943). In a later work, Steinhardt and Zaiser (1955) presented a detailed picture of the hydrogen ion equilibrium of both native and denatured proteins. The solubilities of proteins, as affected by acid-base combinations, was the subject of two papers by Green (1931a, 1931b). A theory was proposed to explain the shapes of the titration curves of a variety of native proteins.

The solubility of a protein may increase if the ionic strength of a salt, such as ammonium chloride, in a protein solution is also increased. This phenomenon is known as "salting-in" and is due to a dispersive effect of the added salt. This effect generally is rather minor. As the salt concentration is increased further, however, the solubility of the protein may reach a maximum and then rapidly decrease to a point below the original solubility limit. This solubility of the protein is apparently affected in an adverse manner by the formulation of insoluble complexes or by the partial denaturation of the protein. A wide range of protein-salt interactions have been studied with their mutual solubilities being a function of the ionic strength of the dissolved salt, the molecular size of the added salt, and the type of protein.

Interactions between proteins and salts are not limited to inorganic compounds. Stearn (1931) observed that the reactions between certain dyes and nucleic acids or proteins were stoichiometric. Goldstein (1949) made an extensive survey of the interactions occurring between

drugs and proteins. Several methods of studying such interactions were discussed and some quantitative data were interpreted. The specificities of these interactions were illustrated in a systematic tabulation of pertinent data obtained for 108 substances, or classes of substances, including both inorganic ions, and organic compounds found in plasma, steroids, vitamins, detergents, dyes, anesthetics, alkaloids, and antibiotics.

The interactions of proteins with many different ions were also reviewed by Klotz (1953). Seventeen methods of investigating protein interactions were described. A theory of multiple equilibria and methods of correlating data obtained for the binding of ions of proteins were also presented. Two methods of extrapolating the maximum number of ions of a particular type bound to a protein molecule were mentioned. Specific interactions of proteins with small anions, small cations, small neutral molecules, and other proteins were considered.

The interactions of proteins and synthetic detergents have been discussed by Putnam and Neurath (1944, 1945) and by Neurath and Putnam (1945). The binding of detergent ions to proteins was considered to be a stoichiometric process. Jaffe (1943) determined the isoelectric points of several soluble proteins by titrating solutions with water-soluble cationic surface-active agents until precipitation occurred. Chinard (1948) studied several chemical interactions that occur between quaternary ammonium compounds and proteins. Antonopoulos et al. (1961) described the precipitation of polyanions by long-chain aliphatic ammonium compounds.

Proteins can be reversibly adsorbed onto the surfaces of ion exchange resins. The chromatography of proteins is complicated by the sensitivity of the proteins to denaturation during adsorption. Boardman and Partridge (1955) and Partridge (1954) separated neutral proteins by displacement chromatography with ion exchange resins. Davies et al. (1950) obtained a separation of amino acids using strongly basic anion exchange resins. The separation of amino acids by ion exchange was also reviewed by Hamilton (1957). The chromatography of proteins and nucleic acids was also reviewed by Sober and Peterson (1957). The adsorption and separation of many compounds of biological interest using ion exchange resins were discussed in great detail in the text authored by Calmon and Kressman (1957).

2. Chemistry of the bacterial cell surface

The nature of the bacterial cell wall has been the subject of considerable research by many investigators. The pertinent literature has been surveyed in an attempt to advance a possible explanation of, and to propose a mechanism for, the ionic interaction between bacteral cells and particles of ion exchange resins. The nature of the cellular surface, its chemical composition, electrical charge, and the behavior of extracellular ions that are capable of adsorbing onto or diffusing through the cell wall, are of particular concern. The emphasis of the current discussion, therefore, is directed toward the chemical interactions of the cell wall with its surrounding ionic environment. The biosynthesis and functioning of the bacterial cell wall are related areas of interest which will be considered only as they apply to the study of cell-resin interactions.

The surface layers of a typical bacterial cell have been differentiated into six concentric shells by Salton (1964). These six layers are:

1. Ionic layer
2. Capsules, microcapsules
3. Adsorbed slimes and gums
4. Cementing layer in cell aggregates
5. Cell wall or outer envelope component
6. Cell membranes, plasma membranes

The net electrical charge of the outermost ionic layer is produced by the interaction of various charges components of the cellular surface. This charge is particularly important in determining the behavior of the bacterial cell as a "macro-ion" in its surrounding environment. A description of each of the remaining surface components underlying the ionic layer has been presented by Salton (1964). He also considered methods of cell wall isolation, electron microscopy of cell wall components, physico-chemical properties and composition of isolated cell wall components, and the occurrance and synthesis of various cell wall structures. Additional references in which the cell wall has been described in detail are those of Rogers (1963), Work (1961), Lammana and Mallette (1959, Chapter VII), Salton (1960a, 1960b), and Miles and Purie (1949).

One structural component that is common to all bacterial cell walls is a mixture of two amino sugars (hexosamines): N-acetylglucosamine and N-acetylmuramic acid, which are combined with three amino acids: alanine, glutamic acid, and either lysine or α-ϵ-diaminopimelic acid.

In addition, glycine or aspartic acid is usually present. Two of these compounds, muramic acid and diaminopimelic acid are compounds which appear to be specific to bacteria. Muramic acid is essentially glucosamine with an ether linkage through its 3-position with lactic acid. Diaminopimelic acid is unlike other naturally-occurring amino acids in that it has two amino and two carboxyl groups.

The precise structure and arrangement of these compounds in the bacterial cell wall are still in question. The amino sugars are probably joined in polysaccharide chains which in turn are linked together by peptides attached to the carboxyl group of the muramic acid. Structural rigidity is imparted to the cell walls of both Gram-positive and Gram-negative microorganisms by these mucopeptides. A major portion of the walls of Gram-positive organisms may be comprised of mucopeptides but only 5-10% of the walls of Gram-negative organisms may be comprised of these compounds.

Another class of compounds found in bacterial cell walls are the teichoic acids. These compounds are ribitol or glycerol phosphate polymers. They are generally considered to be a component only of Gram-positive bacteria but a glycerol polymer has also been detected in the cells of Escherichia coli, a Gram-negative bacterium (Salton, 1964). The mode of attachment of the teichoic acids with other compounds in the cell wall is still in debate.

A general comparison between Gram-positive and Gram-negative bacteria is made in Table XVIII. The characteristics compared in this table have been previously reported by Salle (1961b, p.60), by Lamanna and Mallette (1959, p.155) and by Salton (1964). The Gram-positive

TABLE XVIII

DISTINGUISHING CHARACTERISTICS OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

Characteristic	Gram-Positive	Gram-Negative
Description of the cell wall		
Thickness	150-800 Å	60-100 Å
Type	Simple	Multilayered
Principal components	Glycosaminopeptides Polysaccharides Oligosaccharides Teichoic acids Teichuronic acids Glycolipids and mycosides	Glycosaminopeptides Polysaccharides Proteins Lipids Lipo-polysaccharides Lipo-protein
Amino acid content	Small variety	Wider variety
Aromatic or sulfur-containing amino acids	Absent	Present
Amino sugar content	Generally lower	Generally higher
Reducing substances	Lower	Higher
Lipid content	Low	High
Magnesium content	Higher	Lower

TABLE XVIII (CONT'D)

DISTINGUISHING CHARACTERISTICS OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

Characteristic	Gram-Positive	Gram-Negative
Distinguishing behavior		
Apparent isoelectric point by staining	pH 2-3	pH 4-5
Amounts of acid and basic dyes retained at apparent isoelectric point	Higher	Lower
Optical changes with increased salt content	None or little	Generally increasing
Leakage of P^{32}-labelled compounds from cells exposed to 100 % ethyl alcohol (% loss of max.)	10-43% (13 species)	54-96% (13 species)
Resistance to physical effects		
Heat	No rupture	Rupture
Pressure and sonic energy	More resistant	Less resistant
Low surface tension	Greater	Less
Resistance to chemical effects		
Acriflavine dyes	Marked susceptibility	Less marked susceptibility
Triphenylmethane dyes	Very susceptible	More resistant

TABLE XVIII(CONT'D)

DISTINGUISHING CHARACTERISTICS OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

Characteristic	Gram-Positive	Gram-Negative
Resistance to chemical effects		
Anionic detergents	Very susceptible	Much less susceptible, only in acid media
Penicillin	Susceptible	Less susceptible
Iodine	More susceptible	Less susceptible
Strong acid	Resistant	Less resistant
Strong alkali	Not dissolved	Dissolved

bacteria are generally more susceptible to chemical agents which combine directly with the cells, e.g. dyes, drugs, and detergents, while the Gram-negative bacteria are more susceptible to physical effects, e.g. heat, pressure, and sonic energy, to chemical agents not involving combination, e.g. acid and alkali, and increased salt concentration.

The walls of Gram-negative bacteria are more complex than those of Gram-positive bacteria. The previously described constituents of mucopeptides, glucosamine, muramic acid, and diaminopimelic acid, are also present. The bulk of the Gram-negative cell wall is accounted for by protein, lipid, and polysaccharide constituents. The composition of the cell walls of several species of Gram-positive and Gram-negative bacteria have been described in detail by Salton (1964).

Gram-positive bacteria appear to have a more limited complement of amino acids then do Gram-negative bacteria. Cell walls of Gram-positive bacteria have been found to be lacking in aromatic and certain sulfur-containing amino acids, arginine and proline, while Gram-negative bacteria contain these compounds. Generally speaking, Gram-negative cell walls are richer in lipids than Gram-positive cell walls. Polysaccharides have been observed to occur in both types. These compounds are determined as reducing substances followind acid hydrolysis of the cell walls. Glucose, galactose, mannose, rhammose, and arabinose, have been specifically identified. These compounds occur in various combinations and individually, depending upon the particular bacterial species, and the polysaccharides that establish antigenicity can be partially characterized from analyses for these compounds.

The fractional compositions of the cell walls of several bacterial species are presented in Table XIX. All values are expressed in percentages of the total dry weight. The cell wall can be assumed to contain approximately 20% of the dry weight of the entire cell although this figure will vary with the age of the culture. The characteristic differences between Gram-positive and Gram-negative bacteria with respect to P^{32} leakage, reducing substances, amino sugars, and lipids, previously reported in Table XVIII, are reported in detail in Table XIX. The phosphorous fraction may be larger for those Gram-positive bacteria containing substantial amounts of teichoic acids. The nitrogen fraction is about the same for both Gram-positive and Gram-negative bacteria.

Identifications of free amino acids and C-terminal amino acids and C-terminal amino acids in the cell walls of several bacterial species have been reported in the literature. Results that are applicable to the six bacterial species considered in this report are presented in Table XX. The limited data available are not strictly comparable since they are expressed in terms of the amount of a given amino acid per gram of cell wall. The fraction of the total cell weight contributed by the cell wall is a function of the individual species. Nothing can be implied on the basis of these results regarding the spatial arrangement of the amino or carboxyl groups on the cell wall, or their availability for reaction with oppositely charged surfaces external to the cell. Nevertheless, differences are found among Staphlococcus aureus, S. albus, and S. citreus. Similar differences are apparent among Bacillus cereus, B. licheniformis, and B. megaterium. It is probable that a very specific technique for the distribution of the available charged surface groups of bacterial

TABLE XIX

COMPOSITION OF THE CELL WALLS OF SELECTED BACTERIAL SPECIES

Species	Gram reaction	Cell wall, % dry weight[1]	P^{32} leakage[2]	% Nitrogen[3]	% Phosphorous[3]	% Reducing substances[3]	% Amino sugar[4]	% Lipid[5]
Pseudomonas aeruginosa	-		(96)	9.3	1.65	10.1		15
Escherichia coli	-	15	84	10.1	1.52	16	3	20
Proteus vulgaris	-		90				5.1	17.6
Staphlococcus aureus	+	20	26		high[6]		17	
Bacillus cereus	+	(20-25)	11				31	0
Bacillus subtilis	+	(20-25)		5.1	5.35	34	6.8	0.7

1 Percent ge dry weight of whole cell, Salton (1964, Table 10). Values for B. cereus and B. subtilis estimated to be the same as for B. megaterium.

2 Leakage of P^{32}-labelled compounds from bacteria exposed to 100% ethyl alcohol, % relative to maximum, Salton (1963, Table 3), value for P. aeruginosa estimated to be the same as for P. sp.

3 Salton (1964, Tables 15 and 16), reducing substances expressed as glucose equivalent, estimated after hydrolysis with 2 N HCl for 2 hours.

4 Salton (1964, Table 40), determined after hydrolysis of walls with 2 N HCl for 2 hours at 100 C.

5 Salton (1964, Table 50).

6 Reported by Salton (1964, p 97).

TABLE XX

FREE AMINO GROUPS AND C-TERMINAL RESIDUES OF CELL WALLS OF SELECTED BACTERIAL SPECIES

Species	Free Amino Groups[1]									C-Terminal Residues[2]						
							ϵ-NH_2									
	Ala	Asp	Glu	Gly	Lys	DAP	Lys	DAP	Total	Ala	Glu	Gly	DAP	Lys	Unknown	Total
Pseudomonas sp.	7					2	55	140	204	17	11	6	10	+	1-2	46
Escherichia coli	2	0	tr.	tr.	0	0			2	14	13	5	10	+	1	43
Proteus vulgaris[3]																
Staphlococcus aureus	180	0	0	32	0	0	2	0	214	18	0	8	0	0	None	26
Staphlococcus albus	20	0	0	5	0	0	160	0	185	94	0	tr.	0	26	None	120
Staphlococcus citreus	7	0	tr.	35	0	0	5	0	47	58	0	7	0	tr.	None	65
Bacillus cereus	4	0	0	0	0	1	0	35	39	34	8	0	5	0	None	37
Bacillus licheniformis	50	0	0	0	0	1	0	130	181	8	tr.	0	4	0	1-2	14
Bacillus megaterium	11	0	0	0	0	1	0	230	242	68	20	0	125	0	3	216

1 Determined by reaction with fluorodinitrobenzene, μ moles/ gram wall (corrected for losses), Salton (1961, Table 6).

2 Liberated by hydrazinolysis at 100 C. for 8 hours, μ moles/gram wall (corrected for losses), Salton (1961, Table 5).

3 No comparable data found.

cells regardless of whether complete analyses were available or not. Cummins and Harris (1956, 1958), Goulden and Sharpe (1958), and Norris and Greenstreet (1958) attempted to distinguish bacterial species on the basis of cell wall differences.

3. Bacteria as macro-ions

The removal of bacterial cells from an aqueous suspension by adsorption onto ion exchange resins is analgous to the usual exchange reaction occurring when dissolved ions in a solution come into contact with particles of an ion exchange resin. Certain reservations must be made in this analogy since bacterial cells are essentially "ions" in a suspended state rather than in a dissolved state. In a true solution, the particles of solute distributed in the solvent are believed to be of molecular size, whereas in a true suspension, the particles maybe large enough to be visible to the naked eye, or at least under a microscope. Ideally, the particles are uniformly distributed in the liquid medium but they may agglomerate as a result of the interaction of electrostatic forces.

The size ranges of particles suspended in a fluid are described in a somewhat arbitrary manner. An approximate classification of the ranges of particle diameters might be: settlable ($> 100\ \mu$), supra-colloidal ($100\ \mu - 1\ \mu$), colloidal ($1\ \mu - 1\ m\mu$), and dissolved ($< 1\ m\mu$). The usual approach has been to consider suspensions containing bacterial cells as colloidal systems. The behavior of bacterial cells in suspension are governed by their surface to volume ratio to an extent similar to that of true colloids. The bacterial cell as a whole is rather

large and complex to class as a simple colloid, but certainly it is composed of many colloidal subsystems of proteins and amino acids.

Certain physical and chemical phenomena which have been observed to occur in suspensions of bacterial cells have been associated with the isoelectric points of the suspended cells. The following compilation of such phenomena has been slightly modified from similar lists reported in the literature by Yamaha and Ishii (1933) and later by Kölbel (1949).

1. Minimum net charge of the cells
2. Maximum total charge of the cells
3. Minimum adsorption of other ions onto the cells
4. Maximum flocculation or minimum dispersion of the cells
5. Minimum permeability of the cells to other ions
6. Minimum growth or germination of the cells
7. Minimum viscosity of the suspension
8. Minimum osmotic pressure of the suspension
9. Maximum refractive index of the suspension
10. Maximum surface tension of the suspension

The first four items in the above listing are of particular concern in the present discussion. The isoelectric point will be defined according to the zwitterion hypothesis to be that pH at which the ionization of the amphoteric bacterial cell is maximum. A fundamental description of the significance of the isoelectric point has been given by Hitchcock (1936).

It is assumed that the isoelectric points of each of the many compounds present in and on a bacterial cell can be represented by

a single average value. This assumption is complicated by the wide ranges of isoelectric points exhibited by the individual compounds. Microorganisms are normally negatively charged at physiological pH values because the isoelectric points of their surface components are located in the acidic pH range. As previously shown in Figure 1, a protein molecule or bacterium will behave as a cation at pH values below its isoelectric point and as an anion at pH values above its isoelectric point.

A distinction must now be made between the various methods of determining the isoelectric points of bacterial cells. The most reliable estimate of the true isoelectric point is obtained by electrophoretic techniques, i.e. determination of the pH at which the cells remain stationary in an electric field. Isoelectric points, determined by electrophoresis, are the most representative values that can be correlated with the phenomenon of adsorption of bacterial cells onto charged surfaces such as those of ion exchange resins. The quantity being measured by electrophoresis is due to the electrical interaction of charged surface components with the surrounding environment.

Apparent isoelectric points have also been determined by measuring the stoichiometric combination of dyes with whole cells. Dyes can permeate the cell, however, and combine with internal structures such as the cytoplasmic membrane. The values of the isoelectric point determined by dye adsorption, therefore, may be considerably different from values determined by electrophoresis. The flocculation or agglutination of suspended bacterial cells may also be observed by optical methods. The point of maximal or minimal optical effect can be determined

by measuring turbidity as a function of pH during titration of the suspension with acid or base.

The isoelectric points of many bacterial species have been reported in the literature (Stearn and Stearn, 1928; Yamaha and Ishii, 1933; Yamaha and Abe, 1934; Kölbel, 1949; Harden and Harris, 1953; and James, 1957). Isoelectric points of fungii (Yamaha and Ishii, 1933), algae (Yamaha and Ishii, 1933), viruses (Oster, 1951; Brinton and Lauffer, 1959, p.467), red blood cells (Coulter, 1921), plant cells (Yamaha and Ishii, 1933), and amino acids, peptides, and proteins (Cohn and Edsall, 1943; and Alberty, 1953) have also been reported. Isoelectric points of selected bacterial species determined by electrophoretic techniques are presented in Table XXI. Variations within species may be due to different or unknown conditions of ionic strength, culture, etc.

The combination of various chemical compounds with bacterial cells is dependent upon the net electrical charges of both reactants. The ion exchange behavior of bacterial cells is also a function of the pH of their suspending medium. At pH values below their isoelectric points, bacterial cells will behave as cations and are capable of reacting with various anionic materials such as acidic dyes (Yuri, 1928a, 1928b; Tolstoouhov, 1929; Harris, 1949; and Harris, 1951). The interactions of bacterial cells and anionic polyelectrolytes have also been studied (Terayama, 1954; and Busch, 1966). At pH values above their isoelectric points bacterial cells, and cells of other microorganisms as well, will behave as aniona and are capable of combining with cationic materials such as inorganic cations (McCalla, 1941b; and Harris and McCalla, 1951), basic dyes (Yuri, 1928a, 1928b; Kennedy and Woodhour,

TABLE XXI

ISOELECTRIC POINTS OF SELECTED BACTERIAL SPECIES[1]

Preferred Classification	Reference Classification[2]	Page[3]	Buffer	Γ/2[4]	pI[5]	Reference
Gram Positive Species						
Rhodospirillum rubrum		59	Acetate	0.01	3.46	Harden & Harris (1953)
Staphlococcus aureus		465	None	.4	3.4	Yamaha & Abe (1934)
" "		"	Phosphate	-	3.0	Kölbel (1949)
" "	Micrococcus citreus	"	Acetate	.1	1.90	Harden & Harris (1953)
" sp.		-	Phosphate	.067	.7	Verwey & Frobisher (1940)
Sarcina lutea		470	None	.4	2.6	Yamaha & Abe (1934)
" "		"	Acetate	.01	2.20	Harden & Harris (1953)
Diplococcus pneumoniae		507	PAG[6]	.27	2.7-3.3	Falk & Jacobson (1936)
" "		"	Phosphate	.067	3.5-4.7	Thompson (1932)
" "		"	Several	-	2.0-3.0	Mudd (1933)
Streptococcus faecalis		522	Acetate	.1	1.90	Harden & Harris (1953)
Leuconostoc mesenteroides		531	Acetate	.01	2.25	Harden & Harris (1953)

1 All isoelectric points determined by electrophoretic means.

2 Reference classification if different from preferred classification.

3 "Bergey's Manual of Determinative Bacteriology," 7th Ed., Breed, Murray, and Smith (1957).

4 Ionic strength if available.

5 Isoelectric point on pH scale.

6 Phosphate-acetate-glycine buffer (Northrup and DeKruif, 1922a).

TABLE XXI (CONT'D)

ISOELECTRIC POINTS OF SELECTED BACTERIAL SPECIES

Preferred Classification	Reference Classification	Page	Buffer	Γ/2	pI	Reference
Gram Negative Species						
Alcaligenes faecalis		297	Acetate	0.01	3.28	Harden & Harris (1953)
Escherichia coli	Bacillus coli	336	PAG, others	-	13.6-13.8	Winslow & Shaugnessy (1924)
" "	Bacterium coli	"	None	-	2.5	Winslow & Upton (1926)
" "	Bact. coli	"	Phosphate	-	2.3	Kolbel (1949)
Aerobacter aerogenes		342	Acetate	.01	2.42	Harden & Harris (1953)
Klebsiella pneumoniae		344	Acetate	.01	2.48	Harden & Harris (1953)
Erwinia amylovora		351	Acetate	.01	3.3	Frampton & Hildebrand (1944)
" carotovora		355	Acetate	.01	2.99	Harden & Harris (1953)
Serratia marcescens		361	Acetate	.01	2.17	Harden & Harris (1953)
Proteus vulgaris	Bacillus proteus	365	None	.4	2.1	Yamaha & Abe (1932)
" "		"	Acetate	.01	2.67	Harden & Harris (1953)
Salmonella typhosa	Salmonella typhii (S)	372	Acetate	.02	5.0	Joffe & Mudd (1935)
" gallinarum	Salmonella gallinorum	375	Phosphate	.013	6.9	Moyer (1936)
" "	Bact. pullorum	"	Phosphate	-	3.4	Kolbel (1949)
" "	Salmonella pullorum	"	Acetate	.01	2.12	Harden & Harris (1953)
Bordetella pertussis	Bacillus pertussis	402	None	.4	.5	Yamaha & Abe (1934)

TABLE XXI (CONT'D)

ISOELECTRIC POINTS OF SELECTED BACTERIAL SPECIES

Preferred Classification	Reference Classification	Page	Buffer	Γ/2	pI	Reference
Gram Negative Species						
Brucella abortus		404	Acetate	0.2	3.0-4.0	Mudd (1934)
" "		"	Acetate	.01	2.3-3.3	Stearns & Roepke (1941)
Haemophilus influenzae	Hemophilus influenzae	407	Several	-	3.0-4.0	Mudd (1933)
Moraxella bovis		420	Acetate	.01	3.47	Harden & Harris (1953)
Neisseria gonorrhoeae	Diplococcus gonorrhoeae	481	None	.4	2.5	Yamaha & Abe (1934)

1956; Borzani and Vairo, 1959; Finkelstein and Bartholomew, 1960; Borzani, 1961; Leman, 1964; and Giles and McKay, 1965), and cationic polyelectrolytes (Terayama, 1954; and Tenney and Stumm, 1965).

The exchangeable nature of various ions capable of being adsorbed by bacterial cells was discussed by McCalla (1940a, 1941b). Hydrogen ions adsorbed onto bacterial cells, which had previously been titrated with acid and washed with distilled water, could be displaced by various electrolytes. The displacement of magnesium ions from bacterial cells by methylene blue, and the displacement of hydrogen ions from bacterial cells by basic dyes, were additional observations made by McCalla (1941a). McCalla and Clark (1941) observed a decrease in the pH of a bacterial cell suspension upon the addition of crystal violet which they interpreted to be a displacement of hydrogen ions by the dye.

The capacities of the cells of several selected bacterial species to adsorb various cations are tabulated in Table XXII. All values are expressed as milliequivalents of the particular cation adsorbed per 100 grams of dried bacterial cells. Quantitative comparisons between the results obtained using the cells of different bacterial species is difficult because of differences in the pH, concentration of cation, and the amount of time allowed for adsorption. A value of 50 meq/100 grams dry cells can be assumed to be an average cation adsorption capacity for the cells of most bacterial species.

4. Nature of bonding forces and reactive sites

A complete description of the possible types of bonding forces which may be involved in cellular interactions is beyond the scope of this thesis. The nature of several of the more significant forces and

TABLE XXII

CATION ADSORPTION CAPACITIES OF SELECTED BACTERIAL SPECIES

Species	Cation[1]	Adsorption Capacity[2]	pH	Reference
Pseudomonas aeruginosa	Hydrogen ion	34.	4-7	Harris & McCalla (1951)
Escherichia coli	Hydrogen ion	47.4	5.0	McCalla (1940a)
" "	" "	56	2.82	McCalla (1941b)
" "	" "	25	4-7	Harris & McCalla (1951)
" "	Methylene blue	27	5.0	McCalla (1940a)
" "	Micramine	(113)	7.0	Terayama (1954)
Proteus vulgaris	Hydrogen ion	44	4-7	Harris & McCalla (1951)
Staphlococcus aureus	Hydrogen ion	7	6.37	McCalla (1941b)
" "	Crystal violet	46	7.0	Harris (1953)
" "	Micramine	(92)	7.0	Terayama (1954)
Bacillus cereus	Crystal violet	72	7.0	Harris (1953)
Bacillus subtilis	Hydrogen ion	57	4-7	Harris & McCalla (1951)
" "	Crystal Violet	69	7.0	Harris (1953)
" "	Micramine	(30)	7.0	Terayama (1954)
Bacillus megaterium	Hydrogen ion	33	4-7	Harris & McCalla (1951)

1. Micramine is N-polymethylated chitosan, a cationic polyelectrolyte.

2. Capacities are expressed as milliequivalents of cation adsorbed per 100 grams of dry bacterial cells. Parathetical quantities (113) were estimated from corresponding values basen upon wet weight by assuming 80% moisture composition.

the character of the reactive sites located on the cellular surface are briefly outlined. Particular areas of interest in this discussion are: electrophoretic analysis of cellular surface groups, uptake of inorganic ions, ion-protein interactions, flocculation, adsorption of drugs, and phage-host cell combination.

Pethica (1961) assessed the relative importance of many of the forces that may be involved in the adhesion and repulsion of cells. The possible attractive and repulsive forces are listed below. Those having short range effects and high specificity are presented first.

Forces of attraction

1. Chemical bonds between the opposed surfaces, e.g. hydrogen bonds, thiol, amide, and ester bonds.
2. Ion-pair and ion-triplet formation, e.g. $-NH_3^+ \ldots {}^-OOC-$ and $-COO^- \ldots Ca^{+2} \ldots {}^-OOC-$, involving energies and entropies of solution.
3. Forces due to fluctuations in electrical charges
4. Charge mosaic on surfaces of like or unlike overall charge, specificity resulting from electrostatic attraction of geometrically arranged charges.
5. Charge attraction of opposite signs
6. Electrostatic attractions between surfaces of like charges
7. Electrostatic attraction due to image forces, i.e. due to solvation of intermembrane ions as other membranes are brought into proximity
8. Surface tension or surface energy
9. van der Waals' forces

Forces of repulsion

10. Charge repulsion between surfaces of like charge
11. van der Waals' forces of repulsion
12. Hinderance to attraction due to steric barriers, such as inert capsules or solvated layers

Pethica described each of these forces in general terms and attempted, "to see the physico-chemical wood despite the biological trees," in assessing their relative importance to adhesive phenomena exhibited by cells. Conformal changes and reversible or successive adhesions were not considered in detail. Forces due to the diffusion potentials which probably exist around cells as a result of the diffusion of ionic nutrients and metabolites are also absent from the above list. The dispersion forces of London and those forces due to the interactions of permanent or induced dipoles are usually included in the van der Waals' forces.

Huggins (1962) discussed the physico-chemical aspects of hydrogen bonds and their application to biology. Morowitz (1960) attempted to explain cellular division in terms of the limitations imposed by the second law of thermodynamics and presented some entropy calculations. The distribution of sixteen types of covalent bonds in nutrient medium and in cells of Escherichia coli were also tabulated. Weiss (1963a) stated that the net negative electrical charges of cells suspended in an aqueous medium were due to ionogenic surface groups and to ion-induced dipoles. The repulsive barrier preventing adsorption of cells onto surfaces can be suppressed by: decreasing the radius of curvature of the cells, lowering the surface potentials of the cells, or reducing the thickness of the ionic double layer.

The exact nature of the sorptive interactions occurring between bacterial cells and materials in their environment is open to much speculation. Absorbable or adsorbable ions, such as those of salts, dyes, drugs, surface-active agents, and particulate matter, such

as other cells, soil particles, resins, etc., are undoubtedly subjected to many kinds of attractive and repulsive forces. Most phenomena involving these substances have been attributed to three types of forces: hydrogen bonding, electrostatic charge interaction, and van der Waals' attraction. All other forces usually are considered to be of a minor or unknown importance. These viewpoints are elaborated in the subsequent discussion of several specific areas of interest.

The electrophoretic behavior of microorganisms has been an especially useful characteristic for determining the nature of cellular surfaces. The electrophoretic mobility of many species is independent of the pH over certain limited ranges within which the presence of certain nonionogenic surface components can be determined. Electrophoretic studies are also found to be useful in providing insights into the nature of amphoteric and polar material present on cellular surfaces. The charges of the amphoteric substances are intimately related to the ionization of specific chemical groups on the cellular surface. An excellent discussion of the various factors which influence the electrophoretic behavior of bacterial cells has been provided by James (1957). The electrochemistry of the bacterial cell surface was reviewed in detail.

Gittens and James (1963a) determined the variation of the zeta potential of normal and ethylenimine-treated cells of Aerobacter aerogenes in different salt and buffer solutions. They concluded that the zeta potential was dependent upon: (1) adsorption of anions or cations from the solution onto the nonionogenic areas of the surface or onto ion pairs, (2) neutralization of the surface charge of the ionogenic groups

by association with ions of opposite sign as the ionic strength is increased, (3) decrease in the thickness of the electrical double layer as the ionic strength is increased, and (4) alteration of the position of the shearing plane due to changes of viscosity in the double layer associated with the increase in field strengths at high ionic strengths, i.e. the effect of surface conductance upon the zeta potential of the ionogenic surfaces.

Davies, Haydon, and Rideal (1956) determined the concentrations of various electrolytes required to exactly neutralize the surface charge of bacterial cells by experiment or by extrapolating the curve of zeta potential versus the logarithm of the electrolyte concentration to zero potential. The negatively charged bacterial cells were more susceptible to combination with cations than with anions. Cations of higher valence were more strongly adsorbed. The partition of an ion between the bulk solution and an interface was dependent upon the surface ionogenic groups and upon the nature of the uncharged portions of the interface. The electrical properties of the surfaces were not affected by osmotic changes.

According to Davies et al. (1956) the surfaces of cells of Escherichia coli are composed of an acid polysaccharide, possible an arabinate. The recorded isoelectric point of arabic acid of approximately pH 1 is higher than that of the strongly acidic phosphatidic group. Haydon (1956) found that the concentration of phenol required to sterilize a culture of bacterial cells was markedly increased by the presence of electrolytes. The cations of higher valence were most effective in increasing the resistance of the cells of phenol. The concentration of phenol necessary for sterilization was a function of zeta potentials of the bacterial cells.

Haydon (1961a) discussed several of the possible relationships that could exist between the zeta potential and the surface charge of a bacterial cell. Haydon (1961b) later interpreted the particular electrophoretic behavior of cell surfaces to be due to large areas of nonionogenic material interspersed with small numbers of ionizable groups. The effects of ions of different valence upon the surface potential, the position of the reversal of charge concentration, and the occurrence of maxima in charge density at certain electrolyte concentrations, were considered to be due to interactions of these ions with both types of surface material and not just the ionizing groups. Bridging of the electrolyte ions between two or more surface carboxyl groups that were widely, but uniformly, distributed over the cellular surface was not considered possible. Neutralization of negativel charged surface groups by multivalent cations can lead to an excess of positive charge. The cells can therefore assume a zero or positive overall charge even through the nonionogenic regions remain negatively charged. Specific agglutination effects, however, are likely to be governed by the actual number of surface ionogenic groups and their character of interaction rather than simply by the net electrical charges.

Douglas and Shaw (1957, 1958) illustrated the general applicability of mobility-pH curves for determining the nature of unknown materials of cellular surfaces. They prepared a variety of model surfaces consisting of natural or synthetic polymers with carboxyl groups as the sole or primary ionizable group adsorbed onto hydrocarbon carrier droplets. The concentrations of various multivalent cations required to produce a

reversal of charge of the model particles were determined by measuring the electrophoretic mobilities as functions of the pH of the solution. The resulting mobilities were found to be dependent upon the surface carboxyl groups; changes in the backbone materials had only secondary effects.

Douglas and Parker (1957) determined the cation charge reversal spectra of spores and cells of several species of the genus Bacillus. They concluded that the carboxyl group was the principal charge group of the cellular surfaces while the main underlying surface structure may possibly be polysaccharide-peptide. Douglas (1957) emphasized the limiting features of a method of differentiating and identifying the nature of colloidal surfaces in which he determined the concentration of a variety of oppositely charged ions required to reduce the net charge of the surfaces to zero. The nature of the ionogenic groups can be established with some degree of certainty by this method but the underlying matrix may not be so clearly defined.

A mechanism to describe the adsorption of various inorganic cations by bacteria was advanced by McCalla (1940a) and McCalla and Foltz (1941). They suggested that the presence of a cation adsorption complex on the surface of a living cell could be accounted for by the presence of carboxyl groups. The physico-chemical relationship between bacteria and soil colloids was extended by McCalla (1940b) to be a process involving ion exchange. A similar mechanism was proposed by Williams (1962) to explain the anion exchange properties of plant root surfaces.

Abram and Gibbons (1961) hypothesized that the cell walls of certain halophilic bacteria were loosely bound by hydrogen bonds, coulombic forces, or "salt" linkages. In the presence of high concentrations of sodium chloride, the electrostatic forces were screened and the organisms were held in rod shapes. As the salt concentration was decreased the electrostatic force became inoperative and the cells assumed spherical shapes due to the weakened or stretched bonding. The halophilic character of bacteria was increased by increasing the number of carboxyl groups in the cellular membrane according to Brown (1964). Conformal changes of the membranes of halophilic bacteria were initiated through predominately electrostatic effect by cations upon exposure of negatively charged carboxyl groups.

Klotz and Curme (1948) noted a decrease with decreasing pH in the extent of binding of cupric ions to bovine serum albumin. Anionic carboxyl groups of the protein molecule were assumed to be involved in the bond formation with the cation. Chinard (1948) reasoned in a similar manner that positively charged quaternary ammonium compounds were attracted to the negatively charged surfaces of protein molecules by coulombic forces. The role played of van der Waals' forces depended upon the substituent groups of the nitrogen atom. Aggregation of the protein molecules was possible through van der Waals' forces if the negative groups of the protein were effectively "neutralized" by the positively charged quaternary ammonium ions. Feitelson (1963) studied several effects specific to the interaction between ionized gels and amino acids. A gel-solution system was found to be particularly suitable for the differentiation between electrostatic interactions and other more specific

interactions. The latter can be postulated to be dispersion forces that are operative between the side chains of the amino acids and the matrix of the polyelectrolyte gel.

Giles and McKay (1965) studied the adsorption of cationic dyes by fixed yeast cells. The adsorption mechanism was considered to be predominantly ionic in character. The most probable sites for adsorption were: the carboxyl groups in polypeptide chains, the phosphoric aicd residues in nucleic or teichoic acids, and possibly even the sulfuryl residues in polysaccharides. Based upon an analysis of adsorption isotherms, their results were found to be consistent with an ion exchange mechanism. The attraction of the weakly acidic carboxyl groups was considered to be overshadowed by the powerful attraction of the strongly acidic phosphoric acid residues.

McKinney (1956) proposed a theory of bioflocculation by treating the bacterial cell as a hydrophobic colloid which can be influenced by four major factors: electrokinetic forces, van der Waals' forces, Brownian movement, and cellular motility. He concluded that bacterial cells possessing a common negative charge were normally repelled from one another by electrokinetic forces and attracted by weaker van der Waals' forces. Flocculation commenced when the balance of these forces was reversed. The failure of the cells present in some cultures to flocculate at potentials below the critical potential at which hydrophobic colloids should flocculate was explained on the basis of the active motility of the cells. The polysaccharide slime layer was considered to have little chemical reactivity and take part in negligible chemical bonding. Other factors, such as pH and agitation,

were also considered to affect flocculation. The adsorption of bacteria onto the surfaces of negatively charged colloids was rapid.

Chesbro and Hedrick (1957) considered the flocculation of cells in microbial suspensions to be analgous to the destabilization of metallic suspensoids by electrolytes. Aggregation occurred when the zeta potential, or columbic repulsion that existed between particles, was depressed by the action of the electrolytes. Hodge and Metcalfe (1958) studied the flocculation of bacterial cells by hydrophilic colloids. They supported the acceptance of a mechanism by which the colloids adsorbed onto the hydrated surfaces of the cells and acted as bridges between the suspended particles through subsequent aggregation. Hydrogen bonding between hydroxyl groups and nonionized carboxyl groups was apparently involved in the adsorption process.

Mill (1964a, 1964b) concluded that calcium ions were an essential requirement for the flocculation of yeast cells. Two roles were envisaged for calcium: steric blocking of surface groups that ordinarily maintain the cells in a non-flocculent state, or formation of a salt bridge between two cells. The salt bridge hypothesis is supported by observation of an antagonistic action of sodium ion which causes deflocculation of cells to occur. Mill suggested that an ionic bond may form between a calcium ion in solution and a carboxyl group situated on the cell surface.

Flocculation and deflocculation of cells by urea was dependent upon the temperature of the suspension. The presence of weak secondary bonds, probably hydrogen bonds, was suspected. Complementary patterns of carbohydrate hydroxyl and hydrogen groups over the cell surface were suggested from observations of a selective dispersing activity displayed

using different hexoses. The general picture of yeast flocculation, therefore, is that of a calcium ion bridge forming between carboxyl receptor sites situated on different cells. The bonds between cells initially are ionic. Once contact is made between cells, hydrogen bonds are established between complementary carbohydrate structures in the walls of the two cells. The resulting complex has the character of a chelate with a definite spatial arrangement of the calcium-complexing groups.

The negative character of the surface charge of bacterial cells was dramatically demonstrated by Terayama (1954). Almost all of the species investigated showed little or no tendency to combine with polyvinyl alcohol sulfate, an anionic polyelectrolyte. The cells of several bacterial species did show considerable affinity for N-polymethylated chitosan, a cationic polyelectrolyte, at pH values about 5.7. This affinity was sharply decreased at lower pH values.

According to Tenny and Stumm (1964, 1965) dispersed microorganisms behave as hydrophilic colloids. Such cells were flocculated by cationic polyelectrolytes and by hydrolyzed metal ions, but not by the free metal cations or by their hydrous metal oxides. These flocculations were influenced by pH, extent of agitation, temperature, flocculent dose, and ionic composition of the system. The mechanism was considered to be stoichiometric and to result in the formation of a flocculant bridge between the cells. The stability of bacterial suspensions could not be explained upon the basis of electrostatic forces alone. The interaction of the hydrophilic surfaces of the bacterial cells with the solvent was also considered to be an important factor in the establishment of a stable suspension.

Busch (1966) extended the earlier work of Tenny and Stumm (1965) to include anionic and nonionic polymers and further defined the specific interactions occurring between polymers and biocolloids. Bacteria do not necessarily aggregate at their isoelectric point. The mobilities of dispersed and aggregated cells usually are not too different. The flocculation of biocolloids with synthetic or natural polymers appears to be initiated by specific adsorption of certain functional groups of the polymers onto specific sites of the biocolloid. The resulting aggregation is then promoted by polymer bridging.

A direct electrostatic attraction between polymers and bacterial cells at physiological pH values is precluded by the anionic or nonionic character of both reactants. Polymers can sorb onto the bacterial surface by hydrogen bonding or coordinate bonding. The bridging mechanism proposed by LaMer and Healy (1963) may be operative. The reaction of a macromolecule at the surfaces of two or more colloid particles is followed by subsequent flocculation due to a bridge formation. A minimal quantity of divalent cations was found to be necessary to achieve flocculation of bacteria by a partially hydrolyzed polyacrylamide, an anionic polyelectrolyte. A change in the structural configuration of the polymer, or a coordinate bridge between the polymer and an active site on the bacterial surface, or both, may possibly be initiated by the added cations. A similar effect was not observed during flocculation of bacteria by another anionic polyelectrolyte, sodium polystyrene sulfonate, or by nonionic dextrans.

Best and Durham (1965) concluded that the antibiotic vancomycin was adsorbed onto the cell walls of Bacillus subtilis through ionic

bonding. Additional attractive forces, such as those of van der Waals' or hydrogen bonding, were considered to be of secondary importance. The reaction was found to be reversible, and to involve acidic groups on the cell wall and one or more basic groups associated with the antibiotic.

Free carboxyl groups and phosphate esters associated with teichoic acid on the cell surface were considered likely to be involved in the ionic bonding. Vancomycin did not adsorb at all to carboxyl groups of the cell wall that had previously been esterified with diazomethane in methanol-ether under mild conditions. Decreased adsorption of vancomycin was also observed for cells contacted only by the methanol-ether solvent suggesting that phospholipids may also be involved in the adsorption. Magnesium and other cations competed with vancomycin for free anions on the cell surfaces of Bacillus subtilis. This competition was also observed by Russell and Thomas (1966) using cells of Escherichia coli.

The quantitative and irreversible decrease of the surface charge of bacteria by diazomethane was also studied by Maccaro and James (1959) and by Gittens and James (1963b). The latter two authors also chemically modified bacterial cells using seven other reagents. Diazomethane and methanolic hydrochloric acid were found to be most suitable for modification of carboxyl groups on alcohol-stable surfaces; fluoro-2-4-dinitrobenzene and p-toluenesulphonyl chloride were most suitable for amino groups. The electrophorotic mobilities of both normal and chemically treated cells were functions of the pH values of the suspending media. James (1965) also discussed the modification of bacterial surfaces by both chemical and enzymatic treatment.

The binding of bacteriophage to host cells is a phenomenon which is closely parallel to the interaction between bacterial cells and ion exchange resins. Adams (1959) comprehensively reviewed many aspects of this phenomenon. The nature of the mechanism has been expounded by many investigators. They are in general agreement that the interaction between bacteriophages and host cells proceeds in two distinct steps: a reversible attachment between a positively charges virus-cation complex and a negatively charged host cell, followed by an irreversible binding of these two entities.

Tolmach and Puck (1952) concluded from the influence of pH upon the attachment of T_2 bacteriophages to cells that amino groups of the viruses and carboxyl groups of the host cells are involved in the bonding. Treatment of the cells with carboxyl-blocking reagents suppressed their ability to bind the virus. Treatment of cells with amino- or sulfhydryl-blocking reagents had no effect upon the binding. The ability to bind T_1 virus was lost, however by chemically blocking the amino groups on the surfaces of host cells. Cell attachment was promoted by esterification of the carboxyl groups because the repulsive forces between virus and cell due to excess negative charges of both bodies were reduced. Participation of weaker hydrogen bonding or van der Waals' forces in the union of cell and virus is not excluded but is deemed to be of secondary importance.

Puck and Sagik (1953) state that two conditions must be fulfilled before virus and host cell attachment can occur. The electrostatic repulsion between the two surfaces must be sufficiently reduced to allow approach within a critical distance where binding is possible.

The first condition is initiated by the action of cations in the medium. Puck (1953) described the first step as involving only electrostatic bond formation. This diffusion-controlled reaction was found to be reversible and its activation energy very low.

The two-step reaction was further considered by Puck, Garen, and Cline (1951), Garen (1954), and Garen and Puck (1951). The first step is the reversible interaction between virus and cations in the solution to form a positively charged complex. The second step is the irreversible binding of this complex with negative sites distributed over the surface of the host cell. The second reaction is almost certain to necessitate electrostatic bonding since the very high reaction velocity observed cannot be a result of van der Waals' forces or other weak bonding forces although such weak forces can be highly specific. The correspondingly low efficiency of random collisions between the reacting surfaces is dictated by the accompanying requirement of a close steric fitting.

Puck and Tolmach (1954) summarized certain evidence that the attachment of a T_2 bacteriophage onto its host cell is a result of bond formation between amino groups of the virus and carboxyl groups of the host cell. Several conclusions were made: (1) bond formation is electrostatic rather than covalent, (2) the attachment of virus to host cell is similar to attachment of ions to a cation exchanger, (3) the possibility of a multivalent cation bridge between virus and host cell was disproven by sequestering the cations with ethylenediamine tetraacetate, (4) attachment is zero at pH values where carboxyl or amino groups are not ionized, (5) the ability of cells to bind T_2 virus was

suppressed by blocking the cellular amino groups, (6) the ability of T_2 virus to attach to cells was not affected by treatment of the virus with a carboxyl-blocking reagent but was destroyed by treatment with amino-blocking reagents, and (7) the sequence of events initiated by the attachment of T_2 virus to its host cell could be duplicated by introducing a synthetic polyamino compound to a cell suspension.

The T_1 virus may also bind to its host cell through carboxyl and amino groups but both types of groups may be present on either reacting body. Excess negative charges on a virus particle can be effectively neutralized by hydrogen ions or other strongly bonding cations so that the amino groups of the virus are sufficiently exposed to allow nonspecific bonding with carboxyl groups of the host cell. The attachment and penetration of cells by viruses was also reviewed by Tolmach (1957). Sagik and Levine (1957) concluded that the attachment of Newcastle disease viruses to chicken erythorocytes was dependent upon the pH, required salt, and involved carboxyl and amino groups on both reactants.

Zago (1956) supported the scheme of a two step reaction occurring between viruses and host cells. He found that the reaction was inhibited by increasing the concentration of divalent cations in suspending medium. These cations apparently combine in a reversible manner with negatively charged sites located on the surfaces of the host cells. In order for the viruses to adsorb, therefore, the cells must first possess appropriate receptor sites on their surfaces. The suspending medium must also contain multivalent cations and be at a suitable pH value. Under these conditions cations can then reversibly

adsorb onto the negatively charged surfaces of the virus particles thereby forming virus-cation complexes. These complexes, in turn, can reversibly attach to acidic groups of the host cells by means of ionized amino groups on the complexes. The role of cations in the virus-cell interaction, therefore, is to neutralize the acidic groups of the virus surfaces enabling them to approach the negatively charged surfaces of the host cells and bind. Rountree (1951) also noted that divalent cations were required for the adsorption of bacteriophages onto host bacterial cells. He also found that this adsorption was inhibited by the addition of sodium citrate.

Beumer, Dirkx, and Beumer-Jochmans (1957) explained the role of cations in the adsorption of phages onto bacterial cells in terms of the Verwey and Overbeek (1948) theory of the stability of lyophobic colloids. The total energy of interaction between a phage particle and a bacterial cell was expressed as a function of the interlying distance between the two reactants. Adsorption of phage particles onto cells is possible in certain cases in which the energy barrier is reduced by increasing the ionic strength of the medium. The existance of a mechanical barrier, in addition to the electrical barrier, was postulated in order to explain a lack of fixation by the cells at low ionic strengths in which case no energy barrier existed. The adsorption of phage particles was also inhibited by high concentrations of divalent cations. The possible explanation was presented in terms of the fraction of the bacterial surface occupied by the cations and the binding energy of these ions.

The adsorptions of a number of different viruses onto columns containing cholesterol were studied by Younger and Noll (1958). The bond between the virus surface and the cholesterol was quite strong and was independent of the ionic environment. The observed interaction was therefore considered due to van der Waals' forces rather than to either hydrogen bonding or electrostatic forces. Virus particles were adsorbed onto cholesterol through van der Waals' interactions occurring between the large hydrocarbon nucleii of cholesterol molecules and non-polar lipophilic sites at the surface of the virus particles. Because of their strongly hydrophilic nature, proteins do not adsorb onto cholesterol. The explanation of virus-lipid interactions can be contrasted to the previously described interactions occurring between viruses and host cells.

Noll and Younger (1959) classified viruses as lipophilic and hydrophilic according to their ability to interact with lipids. The lipophilic viruses were subdivided according to their affinities for lipids having different capacities for adsorption. Very strong, irreversible adsorptions may result from the combined effects of van der Waals' forces and electrostatic interactions upon the interaction of lipophilic viruses and lipids having ionizable groups. The attachment of hydrophilic viruses to cells may be explained on the basis of a predominance of ionic interactions.

According to Zelkowitz and Noll (1959), the combination of T_1 bacteriophages with neutral and acidic lipids, such as cholesterol, and steric acid, respectively, presumably was through weak van der Waals' interactions. In the case of a basic lipid, such as hexadecylamine, the

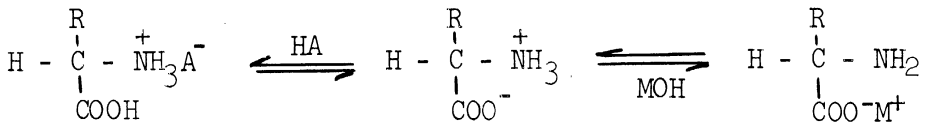
binding was irreversible. This was attributed to reinforcement of the binding by an additional electrostatic attraction between positively charged groups on the lipid surfaces and negatively charged groups at the end of the tail of the bacteriophages.

5. Proposed mechanism of cell-resin interactions

The aim of the present discussion is to postulate a reasonable mechanism to explain the adsorptive ion exchange of bacteria cells with particles of ion exchange resin. The multitude of forces participating in cellular natures of the various reactive groups located on the surface of a bacterial cell have also been considered. Based on this discussion a mechanism is now proposed to explain the anion and cation exchange of bacterial cells with synthetic resins. In the present study, the mechanism by which bacterial cells become attached to the surface of an ion exchange resin is postulated to involve a predominant electrostatic charge phenomenon. A cell-resin combination should result only when the reacting particles are of opposite electrical charge. This idealization can be complicated by hydrogen bonding and van der Waals' forces contributing to the overall binding, and the variety of different ionizable groups on the bacterial surface that are available for interaction. These complications are not considered in detail in the present discussion.

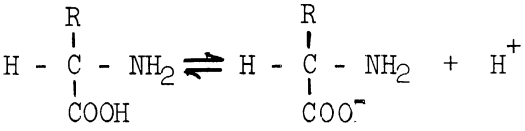
According to the "zwitterion" hypothesis, the isoelectric point is the pH at which maximum ionization of the acidic and basic groups of a dipolar compound occurs. An overall neutrality as an inner salt is assumed if these groups are completely ionized. The addition of an acid, HA, or a base, MOH, to a bacterial cell that is behaving as a

dipolar ion in suspension can be illustrated as follows:

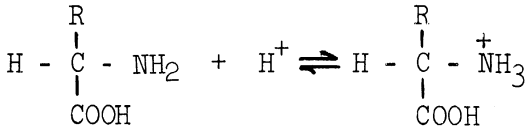


The radical, R , is used to represent the complex surface structure of the bacterial cell.

Polar groups of proteins are oriented in the aqueous phase whenever possible. Ionizable hydrogen ions can thus be produced if the pH of a suspension containing bacterial cells is above the isoelectric point (pH > pI) of the carboxyl groups in the bacterial cells or on the cellular surface. These hydrogen ions can conceivably be replaced by any other cation:



Alternatively, at pH values below the isoelectric point of the amino groups in the bacterial cells or on the cellular surface (pH < pI), the bacterial cells can assimilate additional hydrogen ions, exhibit a net positive charge, and be capable of combination with any other anion:



The above representations are greatly simplified. An idealized terminal amino acid has been depicted in both of the latter two equations. Such active sites on the bacterial surface can function as cationic or anionic adsorption complexes in a living cell and participate in typical ion exchange reactions.

A formal reaction mechanism now can be proposed to describe the exchange adsorption of bacterial cells from liquid suspension by synthetic ion exchange resins. The large complex structure of the bacterial cell can behave either as a cation or as an anion and react, respectively, with the charged groupd of either a cation (anionic) or an anion (cationic) exchange resin. The exchange resin can be represented as a large polymeric network which assumes either a positive or negative charge in association with small, dissociable counter ions of opposite charge. Typical anion and cation exchange resins are described in Section III-B-2 of this report. A typical anion exchange resin can be represented as $R' - \overset{+}{N}(CH_3)_3Cl^-$, where R' is a large polymeric structure. A typical cation exchange resin can be represented as $R'' - SO_3^-H^+$, where R'' is also a large polymeric structure.

The exchange between a negatively charged (anionic) bacterial cell and a positively charged (cationic) ion exchange resin can be depicted in the following manner:

$$H - \underset{\underset{COO^-}{|}}{\overset{\overset{R}{|}}{C}} - NH_2 \quad + \quad Cl^-(H_3C)_3N^+-R' \rightleftharpoons H - \underset{\underset{COO^-(H_3C)_3\overset{+}{N}-R'}{|}}{\overset{\overset{R}{|}}{C}} - NH_2 \quad + Cl^-$$

This reaction is represented more completely in the schematic of Figure 2a. The corresponding exchange between a positively charged (cationic) bacterial cell and a negatively charged (anionic) ion exchange resin can be depicted in a similar fashion:

$$H - \underset{\underset{COOH}{|}}{\overset{\overset{R}{|}}{C}} - N^+H_3 \quad + \quad H^+-O_3S-R'' \rightleftharpoons H - \underset{\underset{COOH}{|}}{\overset{\overset{R}{|}}{C}} - N^+H_3^-O_3S-R'' \quad + H^+$$

This reaction is represented more completely in the schematic of Figure 2c.

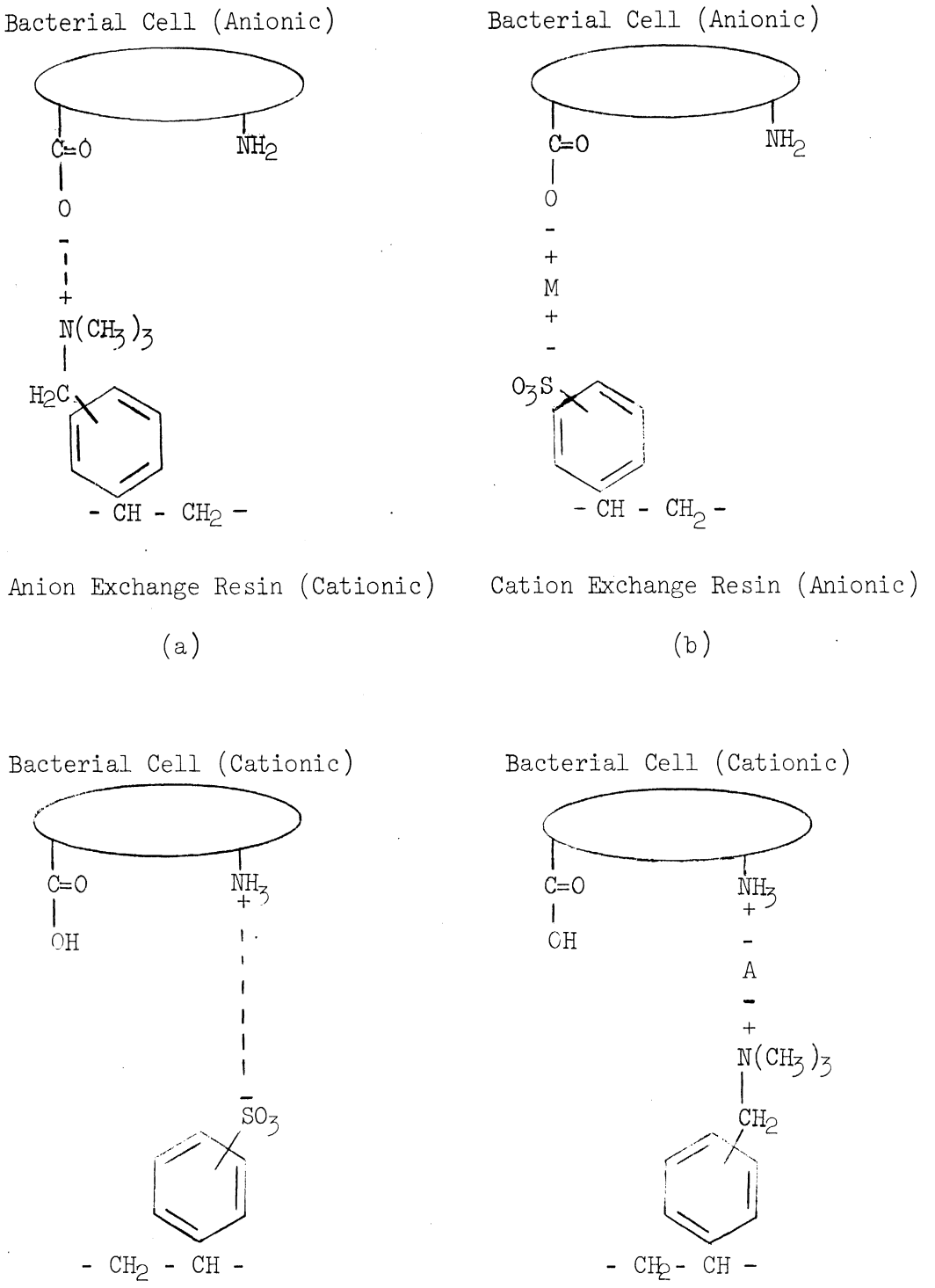
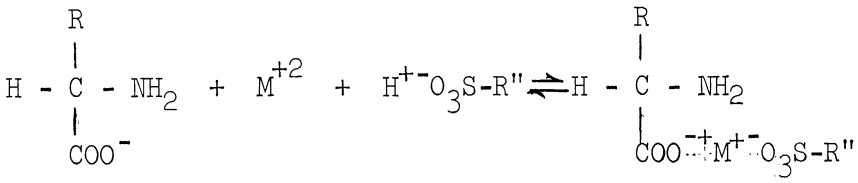
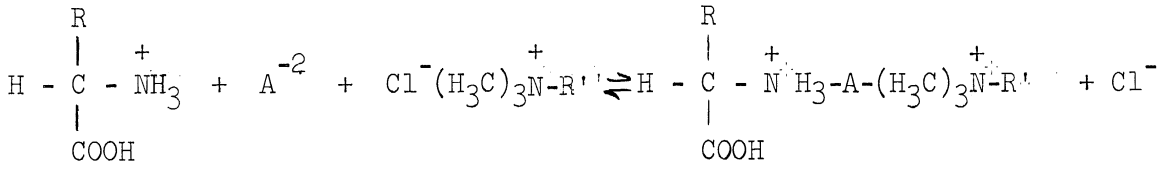


Figure 2. Schematic Representation of the Binding of a Bacterial Cell onto the Surfaces of Anion and Cation Exchange Resins. (a) Direct Attachment to an Anion Exchange (b) Attachment to a Cation Exchange Resin Through a Multivalent Cation Bridge (c) Direct Attachment to a Cation Exchange Resin (d) Attachment to an Anion Exchange Resin Through a Multivalent Anion Bridge.

Two additional reactions can be postulated if mediation by either a multivalent cation or a multivalent anion is assumed. The exchange between a negatively charged (anionic) bacterial cell and a negatively charged (anionic) ion exchange resin is therefore possible if a multivalent cation, M^{+2}, is present in the system to act as a bridge between the two similarly charged reactants. This reaction can be depicted as follows:



This reaction is represented more completely in the schematic of Figure 2d. The corresponding exchange between a positively charged (cationic) bacterial cell and a positively charged (cationic) ion exchange resin is also possible if a multivalent anion, A^{-2}, is present. This reaction can also be depicted by a bridge mechanism:



This reaction is represented more completely in the schematic of Figure 2b. The multivalent ions depicted in the latter two equations can be either simple inorganic ions or organic polyelectrolytes.

Admittedly, the four reactions proposed above are greatly simplified versions of actual exchanges. The individual reactions may be multiplied by a factor of 10^8, the approximate number of active exchange sites per bacterial cell based upon calculations involving ions (McCalla, 1940a). Carboxyl and amino groups are by no means the only charged groups on the bacterial cell that can participate in the indicated

indicated reactions. In addition, the contributions of van der Waals' forces and hydrogen bonding should not be neglected in the total picture of the adsorption.

The proposed mechanisms of exchange presented here has been kept as general as possible. No attempt has been made to explain the selective exchange of cells of different species on the basis of subtile differences in the compositions of the surfaces of the individual cells. This possibility has been tacitly suggested in an earlier section of this report. The combined effects of many distinct charged components of the cellular surface are almost certainly involved in the actual exchange behavior of the cells of a particular bacterial species. The adsorption of bacterial cells onto the surfaces of ion exchange resins can be represented by an electrostatic mechanism only in certain limiting cases.

6. Predicted resolutions of mixed suspensions

Predictions can be made of the relative difficulties to be encountered in resolving several binary combinations of the six bacterial species considered in the current report. The adsorption of the cells of these species from individual suspensions onto separate qualities of an anion exchange resin are shown in Figure 12 of Section V. Predictions can be formulated on the bases of two characteristics of the cells of the component species present in a given binary mixture: (1) the difference in the apparent isoelectric points of the cells of the two species, and (2) the relative affinities of the cells of the two species for a particular ion exchange resin.

The apparent isoelectric points of the six bacterial species listed above are presented in Table XXIX. Values of the apparent isoelectric points were determined to be those pH values at which: a maximum or a minimum was observed in the absorbance of a cellular suspension, or a maximum was observed in the adsorption affinity of the cells for the anion exchange resin used in the current investigation. These apparent isoelectric points are compared with other representative values that were determined by electrophoretic methods and reported in the literature.

The same six bacterial species can be divided into four classes according to their relative affinities of adsorption onto an anion exchange resin. These four classes are: (1) limited adsorption which is self-reserving (Ec), (2) strong adsorption with desorption occurring upon reduction of pH (Sa, Po, Bc), (3) strong adsorption with desorption occurring upon the addition of salt (Pv), and (4) very strong adsorption with desorption occurring only upon the reduction of pH and the addition of salt (Bs).

Qualitative predictions of the ease of resolving various binary mixtures can be made on the basis of knowledge of the apparent isoelectric points and of the relative affinities of the individual species for adsorption. These predictions are summarized in Table XXIII for the case of anion exchange, and in Table XXIV for the case of cation exchange. The types of resolution which are proposed (I or II) correspond to the definitions given in Section IV-D of this report. Those mixtures labelled as resolvable (R) have been experimentally verified. Those mixtures labelled feasible (F) contain the cells of two species

TABLE XXIII

RESOLUTION OF BINARY MIXTURES BY ANION EXCHANGE

Species 1	Species 2				
	Bs	Sa	Bc	Pv	Po
Ec	R(I)	R(I)	F(I)	F(I)	R(I)
Po	F(II)	D(II)	D(II)	-	
Pv	D(II)	-	-		
Bc	F(II)	D(II)			
Sa	R(II)				

R Resolved

F Feasible as predicted

D Difficult as predicted

\- Insufficient data for prediction

I,II Type of resolution

TABLE XXIV

RESOLUTION OF BINARY MIXTURES BY CATION EXCHANGE

Species 1	Species 2				
	Bs	Sa	Bc	Pv	Po
Ec	F(I)	-	-	F(I)	-
Po	-	-	-	-	
Pv	R(I)	-	-		
Bc	-	-			
Sa	-				

R Resolved

F Feasible as predicted

D Difficult as predicted

\- Insufficient data for prediction

I,II Type of resolution

that have large differences in apparent isoelectric points or in relative adsorption affinities. Those mixtures labelled difficult (D) contain the cells of two species of very similar character. Insufficient data is available to permit any predictions of the resolution of several of the possible combinations (-).

The techniques used to resolve binary mixtures may be extended to more complex mixtures as well. One such example is the resolution of the quaternary mixture containing the cells of Ec, Po, Sa, and Bs. The scheme proposed for the resolution of this mixture is presented in Figure 3. This scheme is a combination of the basic Type I and Type II resolution schemes previously defined for binary mixtures. Initially, the cells of one species will remain unadsorbed while those of the other three are adsorbed onto an anion exchange resin following a Type I resolution. The cells of the three adsorbed species will then be desorbed in sequence following a series of Type II resolutions.

The cells of Po, Sa, and Bs, are first selectively adsorbed by the anion exchange resin at pH 5. The cells of Ec remain unadsorbed in the suspension. The resin is washed and then contacted with an aqueous solution adjusted to pH 3. The cells of Po are desorbed while those of Sa and Bs remain adsorbed on the resin. The resin is washed a second time and then contacted with a second aqueous solution adjusted to pH 1.5. The cells of Sa are desorbed leaving only the cells of Bs adsorbed on the resin. The resin is washed a third time and then contacted with a molar solution of sodium chloride adjusted to pH 1.5. The cells of Bs are desorbed by this final treatment. The cells of the four component species of the original quaternary mixture are thus resolved into four separate fractions.

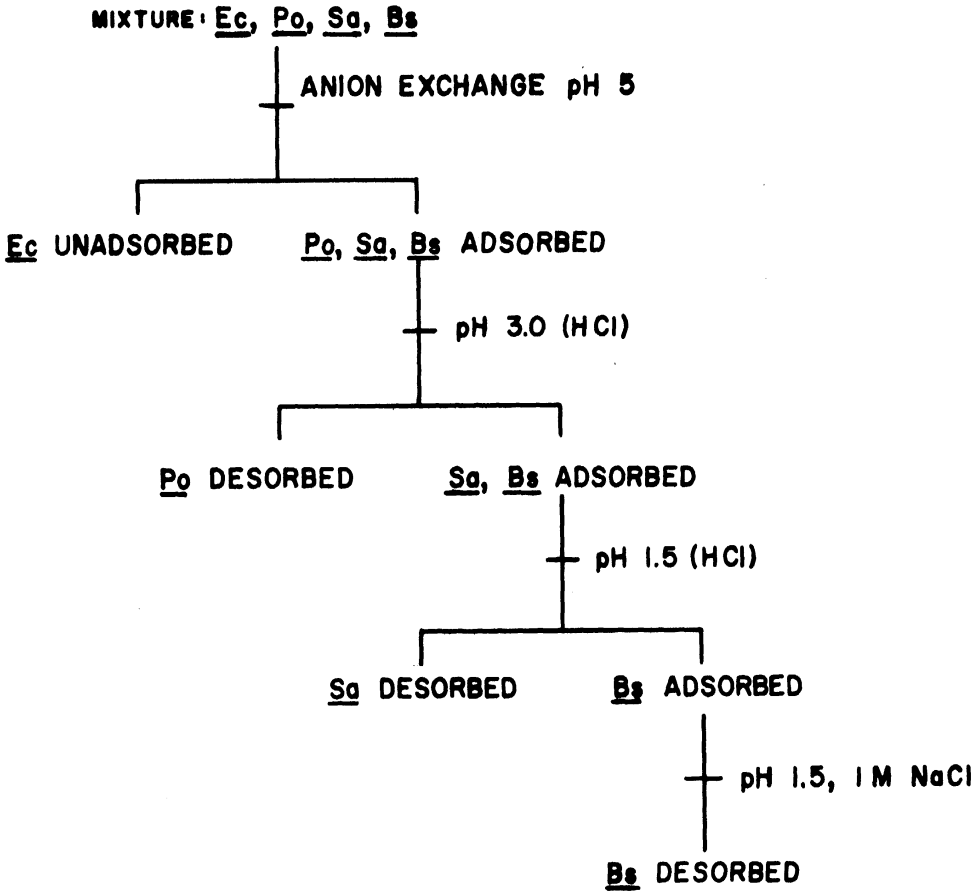


Figure 3. Proposed Resolution of a Quaternary Mixture of Bacterial Cells (Escherichia coli, Pseudomonas ovalis, Staphlococcus aureus, and Bacillus subtilis).

Several assumptions are associated with this proposed scheme: (1) the resin must have sufficient capacity to adsorb all cells of Po, Sa, and Bs, (2) the cells of each species must adsorb and desorb independently of those of the other species, and (3) no premature desorption must occur during the agitation or washing processes. The quantitative limitations of this scheme are probable due more to the purely mechanical treatment than to any lack of selectivity of the resin. The degree of resolution may be improved by the use of column chromatography if the attendant problem of filtration of the cells can be overcome.

7. Variables associated with the exchange bacterium

The adsorptions and desorptions of the cells of a particular bacterial species are greatly affected by the chemical and physical state of the cells. The exchange behavior of the cells is influenced by the conditions of their culture and the preparation of their suspending medium. Several of the variables associated with the exchange organism are briefly outlined below. Those variables marked by an asterisk (*) have been investigated in detail in the experimental portion of this thesis. The results are reported in Section V.

*(1) Species of bacterium

Variations in the sorptive behavior of the cells of different bacterial species with ion exchange media can be expected on the basis of observed differences in the chemical nature of the surfaces of the cells. Resolutions of mixed suspensions containing the cells of several different species of bacteria can be based upon these observed differences.

(2) Culture media

The type and magnitude of the surface charge exhibited by a bacterial cell is a function of the type and the composition of its culture medium. The distribution of electrical charge over the surface of a bacterial cell may possibly be controlled by adjusting the various nutrients and buffer salts of the medium prior to or during growth.

(3) Culture age

The surface charges developed by bacterial cells during growth are often a function of the age of the culture. The assumption that an average cell is representative of the total population with regard to age, surface charge, and sorptive behavior usually must be made. The stability of a suspension of cells must also be considered if the experiment is of long duration.

(4) Suspension media

The type of medium in which the bacterial cells are suspended is important in determining their sorptive behavior. The suspending medium may also be the culture medium. Alternatively, the cells can be harvested, partially purified by washing and filtering, and then resuspended in a fresh medium. The pH and the concentration of salt ions in the suspending medium can be controlled in part by adjusting the concentration of buffer salts. The magnitude and distribution of the surface charges, and hence the sorptive behavior of the bacterial cells, are strongly influenced by the ionic environment of the growth medium.

*(5) Concentration of cells

The rate of the adsorption of bacterial cells onto a surface

is a function of the concentration of cells initially present in the suspension. The equilibrium distribution of cells between the freely suspended state and the adsorbed state is also dependent upon the initial concentration of cells.

B. Ion Exchange Processes

1. Ion exchange reactions

Ion exchange can be broadly defined as a methetical (transposition) chemical reaction between an electrolyte in solution and an insoluble electrolyte with which the solution is contacted. Essentially, it is a reversible solid-liquid reaction with a resulting transfer or exchange of ions between the two phases. The kinetics of this reaction, and the techniques involved in conducting it, closely resemble adsorption For this reason ion exchange is generally thought of as a special case of adsorption although the attractive forces are ionic in character rather than molecular, as is the case with materials such as activated carbon or silica gel. Ion exchange, in contrast to sorption, is a stoichiometric process with every ion removed from solution being replaced by an equivalent amount of another ionic species of the same sign. A solute is removed without replacement during the usual type of sorptive process.

The exchange reaction is classified as being either of two distinct types depending upon the sign of the electrical charges involved in the exchange. Cation exchange is the transfer of positively charged ions or cations; anion exchange is the transfer of negatively charged ions or aniona. Certain amphoteric exchangers are capable of

exchanging both anions and cations. There are also separations based on the principle of exclusion of certain neutral or non-polar molecules. The exchange of ions can also be conducted in nonaqueous solvents with quite different ionic behavior resulting than would occur in aqueous solution.

The first materials used for ion exchange were natural clays and earths (generally oxides and silicates), and plant gums and resins. Charcoal, cellulose, and other carbonaceous materials were also used. Practical applications, however, were limited to water softening by greensands and zeolites. Beginning about 1935, the naturally occurring exchangers have been largely superseded by synthetic resinous ion exchangers which have greater capacity for exchange, improved selectivity, and better hydraulic properties. The synthetic resins are generally polymeric compounds with various associated functional groups having dissociable ions.

Synthetic ion exchange resins can be described as elastic, three-dimensional hydrocarbon networks that contain large numbers of ionizable groups. The chemical behavior of an ion exchange resin is governed by the nature of these ionizable groups. If the functional groups are sulfonic, carboxylic, or phenolic in structure, the resin is negatively charged and therefore anionic in its behavior. A typical resin consists of a very large, non-diffusable anion, R'', and a small, replicable or exchangeble cation that diffuses easily, e.g. H^+, Na^+, Ca^{++}, etc. Such a resin is called a cation or an acid exchanger. The cation exchange reaction proceeds typically as follows

$$Na + \overline{HR}'' \rightleftharpoons \overline{NaR}'' + H^+$$

where the solid phases are indicated by the bared quantities.

Cationic behavior can be produced in a resin by functional groupings of a quaternary ammonium or polyamine nature. These positively charged resins consist of a very large cation, R', and a replaceable anion, e.g. Cl^-, OH^-, $CO_3^=$, $PO_4^{\equiv}$, etc. This type of resin is called an anion or a basic exchanger. The anion exchange reaction proceeds typically as follows

$$OH^- + \overline{R'Cl} \rightleftharpoons \overline{R'OH} + Cl^-$$

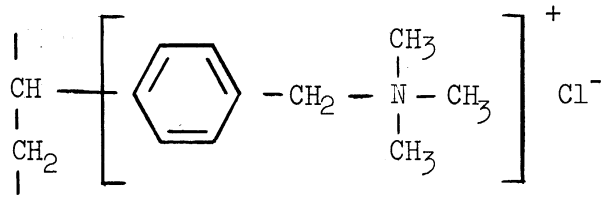
with the solid and liquid phases as previously defined.

The above reactions illustrate the definition of a metathetical chemical reaction. In such a reaction two chemical compounds react together in such a way as to form two other compounds by a transposition of the reactive groups.

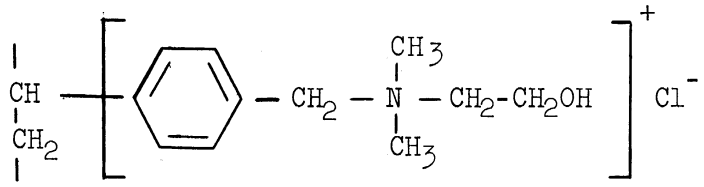
2. Ion exchange materials

Strong-base anion exchange resins are available as two main types that are arbitrarily designated as Types I and II. The basic structures of both types are of the quaternary ammonium configuration but differ slightly in the substituent groups. The chemical activities of these two types differ only in relative affinities for chloride and hydroxide ions and in stability toward higher temperatures and acid decomposition. Resins of Type II can be converted more easily to the hydroxide form; those of Type I are more chemically stable.

The four substituents of the nitrogen atom of a typical Type I anion exchange resin ("Dowex" 1, The Dow Chemical Company, Midland, Michigan) are a polymeric benzyl group and three methyl groups. This type I resin can be represented in the following manner.



The substituents of the Type II ("Dowex" 2) are the same except that one of the methyl groups is replaced by an ethanol group. The Type II resin can be represented in the following manner.



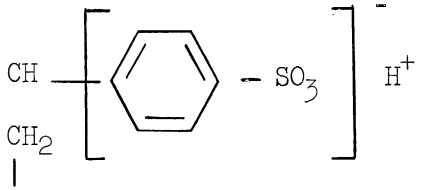
These quaternary ammonium anionic resins are highly ionizable and can be used over the entire pH range. The decreasing selectivity of Dowex 1 for anions can be qualitatively expressed (Dowex, 1964) by the series:

$$CNS^- > I^- > NO_3^- > Br^- > CN^- > HSO_4^- \simeq HSO_3^- > NO_2^- > Cl^- > HCO_3^- > CH_3COO^- > OH^- > F^-$$

A similar series can be described for Dowex 2 except that the OH^- ion is intermediate between the Cl^- and HCO_3^- ions. More complete listings of selectivities have been provided by Peterson (1953), Gregor et al.

(1955), Helfferich (1962), and Samuelson (1963). Anion exchange resins that have been reported in the literature as being capable of adsorbing bacterial cells are listed in Table XII in Section II.

The principal types of cation exchange resin of commerical importance are the strong-acid resins with sulfonic acid groups and the weak-acid resins with carboxylic acid groups. A typical sulfonic acid type ("Dowex" 50W) can be represented in the following manner.



This resin is highly ionized in both acid and salt forms, and is thus capable of exchange over the complete pH range. The decreasing order of selectivity for cations by Dowex 50W can be qualitatively represented (Dowex, 1964) by the series for univalent cations.

$$Ag^{+} > Cs^{+} > Rb^{+} > K^{+} > NH_4^{+} > Na^{+} > H^{+} > Li^{+}$$

and for divalent cations by

$$Ba^{++} > Sr^{++} > Ca^{++} > Mg^{++} > Be^{++}$$

Generally speaking, the cations of higher valence are more tightly bound, i.e. trivalent > divalent > monovalent. More complete listings of cation selectivities are provided by Bonner and Smith (1957), Helfferich (1962), and Samuelson (1963). Cation exchange resins that have been reported in the literature as being capable of adsorbing bacterial cells are listed in Table XIII of Section II.

3. Variables associated with the exchange resin

Several variables associated with the ion exchange resin can significantly affect the adsorption of bacterial cells. These variables will be briefly discussed with reference to ion exchange processes of a general nature. The list of variables begun in Section III-A is continued. Those variables marked by an asterisk (*) have been experimentally investigated. Results are presented in Section V of this report.

*(6) Type of resin

The behavior of a resin toward the exchangable ions in a solution is determined by the arrangement of certain functional groups that are fixed on the resin surface. Exchange of anions is promoted by a positively charged resin; exchange of cations is promoted by a negatively charged resin. The selectivity of a particular resin for different ions of the same charge is determined to some degree by the chemistry of the functional groups.

*(7) Ionic form

The selectivity of a resin for various ions in solution is determined by the type of counter (exchangable) ion initially associated with the resin. Selectivity, and the various factors promoting it, have been summarized by Helfferich (1962). The counter ion possessing the following properties is preferred by the resin: higher valence, smaller (solvated) equivalent volume, greater polarizability, strongest interaction with the fixed ionic groups or with the matrix of the resin, and least participation in complex formation with the co-ion.

*(8) Particle size

The particle size of the ion exchange resin in contact with a solution is important in determining the rate of exchange and the final degree of exchange of dissolved ions from the solution. Both of these factors are improved by decreasing the particle size of the resin. Smaller resin particles are also more physically stable toward temperature fluctuations. They are also less susceptible to expansion and contraction due to variation in the ionic strength of the solvent or to the type of solvent. The pressure drop experienced in columns can substantially increase, however, if the particle size of the resin is decreased.

*(9) Crosslinkage

The expansion-contraction behavior of a polymeric resin is a function of the degree of crosslinking with another polymer. More water is absorbed by resins of low crosslinkage causing them to swell considerably. The wet volume capacity increases and the dry weight capacity decreases with an increased amount of crosslinkage. Selectivity as a rule can be enhanced by increasing the crosslinkage as the diffusion of the larger ions into the interior of the resin particles is then restricted.

*(10) Concentration

Both the exchange rate and the ionic equilibrium are significantly affected by the concentration of resin particles in a given solution. The rate at which exchanged ions are removed from the contacting solution is increased by a greater concentration of resin particles. A direct measure of the resin capacity can be made if the

amount of exchangable material in solution is greater than that which the resin can accomodate.

C. Mathematics of Diffusional Processes

1. Introduction

The nature of most chemical and physicochemical processes, in which heterogeneous transformations in liquids and gases are of primary importance, are largely determined by hydrodynamic factors. Transformations occurring at phase boundaries and at surfaces having catalytic properties include: catalytic reactions, adsorption and desorption at solid and liquid surfaces; dissolution, and precipitation of crystals from solutions and melts; electrochemical reactions at electrode surfaces; evaporation, sublimation, and condensation; and gas adsorption by liquid and solid surfaces.

Three steps are involved in all heterogeneous reactions: a transfer of reacting particles to the surface at which reaction occurs, the heterogeneous reaction itself (chemical transformation, adsorption of desorption, the discharge and formation of ions, etc.), and the transfer of reaction products from the reaction site. The reaction is diffusion controlled if the introduction or removal of reactants is rate determining, e.g. absorption of gases in liquids, dissolution of solids in liquids, and electrochemical and fast catalytic reactions.

The kinetics of diffusion occurring in the liquid phase and, more specifically, heterogeneous reactions at the liquid-solid interface are areas of current interest. Particular aspects of these areas that have been reported in the literature include: the coagulation of colloidal particles, and quenching of fluorescence in solution, the

process of condensation polymerization, the kinetics of enzyme-substrate reactions, the adsorption of viruses onto host cells, the coagulation of aerosols, and the nucleation and growth of crystals. The theoretical treatments of such diffusion-controlled reactions can be divided into two catagories: considerations of the concentration gradients present in such systems, or statistical analyses of the movements of pairs of diffusing particles.

Smoluchowski (1916, 1917a, 1917b) developed an original kinetic theory to describe the coagulation of colloidal particles that were uncharged, spherical, and monodispersed. These particles were assumed to move randomly by Brownian motion in a stagnant or uniformly moving continuum and coagulate, or stick together, upon collision. Two types of coagulation were recognized: perikinetic coagulation due to particle diffusion, and orthokinetic coagulation due to fluid motion. The solutions of the boundary value problem describing the coagulation of colloids that were derived by Smoluchowski have become classic. They have been only slighly modified in recent derivations to account for more general boundary conditions.

Jablczynski (1924a, 1924b) considered the coagulation of colloids to follow first and second order kinetics. Müller (1926) and Wiegner (1926) extended Smoluchowski's theory of coagulation to include polydispersed colloidal systems. In a more detailed report Müller (1928) considered the effects of polydispersity, particle geometry, and fluid motion upon coagulation. Harper (1934) took exception to Smoluchowski's solution but proposed an alternative one that differed only by a simple numerical factor. Smoluchowski's development of the subject has served

as the classical treatment and although certain features have been criticized it has been widely accepted in the literature.

Chandrasekhar (1943) authored an excellent tretice (73 references, 676 equations) dealing with stochastic problems of physics and astronomy. Very erudite discussions of the theories of Brownian movement and of colloid coagulation were included. Overbeek (1952) considered the kinetics of flocculation and carefully reviewed the theories for both rapid (Smoluchowski) and slow coagulation of colloidal particles. Rajagopal (1959) also surveyed the literature (85 references) dealing with the time-variation of particle size distributions during coalescence, dispersion, and emulsification. Convective diffusion and certain problems encountered in the theory of coagulation of dispersions involving liquids and gases were treated by Levich (1962) in his authoritative book dealing with physicochemical hydrodynamics. Fuchs (1964, Chapter VII) gave an extensive treatment of the diffusion and coagulation of aerosols.

Higuchi et al. (1963) compared some experimental results obtained from kinetic studies of rapid aggregation of suspensions to values predicted by the Smoluchowski theory. Hiestand (1964) reviewed (107 references) the theory of coarse suspension formation including fundamentals, forces between particles, flocculation rates, and methods of promoting or preventing flocculation and sedimentation.

Debye (1942) considered reaction rates in ionic solutions to be diffusion controlled process. Waite (1957, 1958, 1960) and Noyes (1961) discussed the kinetics of diffusional processes to great length. Sveshnikoff (1935) extended the Smoluchowski theory to ordinary biomolecular

reactions in connection with the quenching of fluorescence in solution. The kinetics of diffusion-controlled reactions in solutions were specifically applied to the quenching of fluorescence by Umberger and Lamer (1945), Montroll (1946), Yguerabide et al. (1964), and Ware and Novros (1966).

Oster (1947) considered the scattering of light from polymerizing and coagulating systems. Condensation polymerization was considered to be analgous to the rapid coagulation of colloids. The rate constants of the former process could be determined by a technique involving diffusion. Zimmerman (1949) reviewed diffusion and activation control in heterogeneous reactions. Harricott (1962a, 1962b) and Howarth (1963) considered the mass transfer of solute to suspended particles in a agitated vessel to be a diffusional process.

Collins and Kimball (1949a, 1949b) and Collins (1950) critically evaluated the Smoluchowski diffusion equation on the basis of random walk theory and proposed alternative solutions with more general boundary conditions. Goodrich (1954) also used random walk techniques to modify the Smoluchowski model to include the case of imperfect adsorption.

Müller (1928) considered the coagulation of monodisperse plate and rod colloids in the absence of fluid flow. Plate-like colloids were found to coagulate in approximately the same manner as spherical particles. The rate of coagulation of cylindrical particles can be much faster than that of spherical particles. Booth (1954) also used the Smoluchowski model and developed a quantitative theory for the mutual coagulation of systems containing two distinct types of particles, either or both

spheroidal. Fairly exact expressions of the rate of coagulation could be derived if the two types of particles differed appreciably in size.

Reiss and LaMer (1950) and Reiss (1951) considered the mechanics of the growth of uniform colloidal particles. Collins and Leineweber (1956) studied the kinetics of the homogeneous precipitation of barium sulfate and developed a defining equation. Frisch and Collins (1952, 1953) and Collins and Frisch (1953) considered the diffusional process involved in the growth of aerosol particles.

Fair and Gemmell (1964) developed numerical solutions to the equations derived by Smoluchowski which describe orthokinetic coagulation. This type of coagulation process can be represented either as a set of nonlinear ordinary differential equations or as a nonlinear integro-differential equation. Complete analytical solutions to those equations have not been developed. Swift and Friedlander (1964) used a similarity transform technique to describe the particle size distribution and reduced the nonlinear integro-differential equation to an ordinary differential equation. Hidy and Lilly (1965) compared the classical solution of Smoluchowski with alternate forms developed by assuming that the particle size distribution to be of a similar or self-preserving form.

Quon (1964) conducted an experimental study of perikinetic coagulation. The equivalent radius of a coagulating particle was used as a parameter of size in the characterization of a polydispersed aerosol. Quon and Mockros (1965) investigated the equilibrium size distribution of an aerosol continually reinforced with new particles. Equilibrium size distributions were numerically computed for inputs of particles of uniform size and for particles whose concentration size curve is

represented by a skewed distribution. Mockros, Quon, and Hjelmfelt (1967) made further calculations of particle size distributions of continually reinforced aerosols undergoing coagulation.

Reid (1953) considered the enzyme-substrate reactions occurring in metabolizing systems to be diffusion-controlled. Trurnit (1953) used a recording ellipsometer to measure diffusion coefficients of the adsorption and reaction of an enzyme system occurring at a solid-liquid interface. Trurnit (1954) studied the kinetics of the adsorption of chymotrypsin from solution onto a solid surface and compared experimental results with those theoretically predicted by Fick's first law of diffusion. Mackor and van der Waals (1952) considered the adsorption of rod-shaped molecules to be associated with colloidal stability. LaMer and Healy (1963) investigated the adsorption and flocculation of macromolecules at solid-liquid interfaces.

Rashevsky (1960) presented several applications of the equations of diffusion as they related to vegetative cells and cellular aggregates. The adsorption of viruses onto host cells and the adsorption of other particles of virus size onto non-biological surfaces were discussed in a series of papers by Valentine and Allison (1959) and Allison and Valentine (1960a, 1960b). A rate equation was developed assuming that adsorption was diffusion-controlled. Hiatt (1964) found that the interaction of viruses with host cells could be described by first order kinetics. Christenson (1965) presented several solutions to the differential equations representing the kinetics of reversible and irreversible attachment of bacteriophages onto host cells. Two different hypotheses were considered: a sequential reaction scheme and a competative reaction

scheme. Cookson (1966) described the adsorption of viruses onto activated carbon in terms of reversible second-order kinetics.

Schlesinger (1932a, 1932b) and Garen (1954) considered the reversibility of the interaction between bacteriophages and homologous bacteria. Polson and Shepard (1949) determined values for the diffusion rates of bacteriophages. Delbrück (1940), Stent and Wollman (1952), and Valentine and Allison (1959) interpreted the adsorption of viruses onto their host cells in terms of the Smoluchowski coagulation theory. Hershey (1957), however, pointed out the limitation of this treatment with regard to the efficiency of collision. Koch (1960) also took exception to this limitation as did Ogston (1963). The latter author attempted to destroy the mythical acceptance of a unit efficiency of collision between a virus and a cell. All of these critics strongly supported the more general development of Collins and Kimball (1949a) and Collins (1950).

The kinetics of diffusion have been applied to a diversity of natural phenomena. Predictions of reaction rates have been made on the basis of analytical solutions to the partial differential equation by which Fick's second law of diffusion is mathematically described. The beneficial extensions of these mathematical solutions to the diffusion equation from the molecular level to encompass much larger particles are evidenced in the successes obtained with systems of colloids, aerosols, crystals, and viruses. The prediction of the kinetics of adsorption of bacterial cells in suspension onto particles of an ion exchange resin is a natural continuation of this development.

2. Comparison of diffusion equations

Diffusion can be defined as the process by which matter is

transported in a single-phase system from a region of high concentration to another of low concentration as a result of random molecular motions eventually leading to an equilization of concentrations. This process has an obvious analog in the transfer of heat by conduction which also is considered to occur by a random process. The overall mechanism of mass transfer by diffusion, however, may be obscured by the contributions of additional factors. If the medium in question is not at rest then concentration changes may be promoted by convection. Diffusion may also be influenced by external forces, such as those produced by gravitational, centrifugal, or electrical forces.

The mathematical representation of such a system, therefore, can be made as complex as desired to account for all possible contributions to mass transfer. Mathematical representations of diffusional processes have appeared in many variations and their solutions tabulated (Barrer, 1941; Crank, 1956; Jost, 1960; and Rashevsky, 1960). The entirely parallel solutions of heat conduction problems have also been extensively treated (Carslaw and Jaeger, 1959). The general form of the partial differential equation describing diffusion will now be presented and its importance discussed.

The general equation of continuity for each chemical species present in a nonisothermal, multicomponent fluid relative to a mass-average velocity $\vec{v}$ is given by Equations (III-1) which is expressed in terms of the mass fraction, w_i, where ρ is the density of the fluid.

$$\frac{\partial(\rho w_i)}{\partial t} = -(\nabla \cdot \rho w_i \vec{v}) - (\nabla \cdot \vec{J}_i) + r_i \, , \quad i = 1, 2, \ldots, n \qquad \text{(III-1)}$$

The mass flux is represented in general by $\vec{J}_i$ and chemical reaction by $\mathcal{R}_i$. The mass density of species i contained in a given fluid element can change for three reasons: fluid expansion, $-(\nabla \cdot \rho w_i \vec{v})$, diffusional processes, $-(\nabla \cdot \vec{J}_i)$, and homogeneous chemical reaction, $\mathcal{R}_i$. Many special cases of Equation (III-1) have been considered by Bird, Stewart, and Lightfoot (1960, 1965).

The mass flux, $\vec{J}_i$, can be expressed explicitly in terms of the gradients and the transfer coefficients, or in general by Equation (III-2), where ordinary (concentration) diffusion, $\vec{J}_i^{(x)}$, pressure diffusion, $\vec{J}_i^{(P)}$, forced diffusion, $\vec{J}_i^{(q)}$, and thermal diffusion, $\vec{J}_i^{(T')}$, are described respectively by the individual terms.

$$\vec{J}_i = \vec{J}_i^{(x)} + \vec{J}_i^{(P)} + \vec{J}_i^{(q)} + \vec{J}_i^{(T')} \qquad \text{(III-2)}$$

Most treatments are restricted to the single flux term, $\vec{J}_i^{(x)}$, which is defined by Equation (III-3), commonly known as Fick's first law.

$$\vec{J}_i^{(x)} = -D \, \text{grad} \, c \qquad \text{(III-3)}$$

This equation is valid for the case of an isothermal system which is independent of pressure and not subject to any external forces.

In cases involving mass transfer by ionic exchange a form more general than that of Equation (III-3), known as the Nerst-Planck equation (Helfferich, 1962, p.267ff), should be applied. An external force term equal to the product of the ionic charge and the local electric field strength is included in this more general form. Although such a force is recognized, this refinement is not warranted for the present state of the art of bacterial adsorption by ion exchange resins.

Considering a stationary fluid in which no chemical reaction is occurring, and assuming a constant coefficient of diffusion, , then the mass transfer due to diffusion alone is described by Equation (III-4), commonly known as Fick's second law of diffusion.

$$\frac{\partial c}{\partial t} = -\,div\,\vec{j_i} = D\nabla^2 c \qquad \text{(III-4)}$$

This equation has been extensively solved for a great variety of boundary conditions. Solutions have also been obtained in which the convection or reaction terms of Equation (III-1) have also been included.

If diffusion is symmetric to a sphere of radius, R, suspended in an infinite medium, then Equation (III-4) can be expressed in one-dimensional form by Equation (III-5).

$$\frac{\partial c}{\partial t} = D\nabla_r^2 c = D\left[\frac{\partial^2 c}{\partial r^2} + \frac{2}{r}\frac{\partial c}{\partial r}\right] \qquad \text{(III-5)}$$

The initial and boundary conditions must be specified before Equation (III-5) can be solved. A typical initial condition for a fluid of uniform concentration is given by Equation (III-6).

$$c(r, 0) = c_0\,, \quad r \geqslant R \qquad \text{(III-6)}$$

The heterogeneous reaction occurring at the surface of the suspended sphere is accounted for by the boundary condition of Equation (III-7a).

$$c(R, t) = 0\,, \quad t > 0 \qquad \text{(III-7a)}$$

This boundary condition was originally proposed by Smoluchowski (1917a). Complete reaction at the surface of the adsorbing sphere, and in essence complete removal of the diffusing system from the system, is thus assumed.

An alternative boundary condition is given by Equation (III-7b).

$$c(R,t) = \xi \left(\frac{\partial c}{\partial t}\right)_{r=R}, \quad t > 0 \qquad \text{(III-7b)}$$

The concentration of the reacting species present at the reaction surface is proportional to the gradient in concentration existing at the surface, where $\xi = D/K$ is the transmission coefficient, γ , of Collins (1950), and K is of the nature of a specific reaction rate constant. Equation (III-7b) is therefore more general than Equation (III-7a) since the case of less than perfect adsorption is covered.

The boundary value problem defined by Equations (III-5, III-6, and III-7) can now be solved by the standard techniques (viz. Carslaw and Jaeger, 1959; Crank, 1956). The solution is of the general form of Equation (III-8).

$$c = f(r,t) \qquad \text{(III-8)}$$

This solution can be expressed in either series form or as a combination of exponential and error functions for the cases to be encountered in the subsequent derivations.

The flux normal to the spherical boundary, $r = R$, can be defined by Equation (III-9), where M_t is the total amount of diffusing substance which has accumulated in the sphere by time, t .

$$J = -\frac{d M_t}{dt} = 4\pi R^2 D \left(\frac{\partial c}{\partial r}\right)_{r=R} \qquad \text{(III-9)}$$

The functional relationship between the amount of substance which has diffused to, and reacted at, the surface of the particle, and the time for this diffusion to occur is given by Equation (III-10).

$$M_t = \int_0^t J\,dt = f(t), \quad r = R \tag{III-10}$$

Many particular solutions of this boundary value problem have been derived. Several of these will now be outlined and their significance discussed. The general format will follow the series of Equations (III-5) through (III-10). Other special forms will be introduced as needed.

Case A (Smoluchowski, 1917a): Diffusion of adsorbable particles from a fluid of infinite extent, initially containing a uniform concentration of adsorbable particles, to a spherical adsorbing surface at which a "zero" concentration of adsorbable particles is maintained.

Partial differential equation

$$\frac{\partial c}{\partial t} = D\nabla_r^2 c \quad , \quad r > R \tag{III-5-A}$$

Initial condition

$$c(r, 0) = c_0 \quad , \quad r > R \tag{III-6-A}$$

Boundary condition

$$c(R, t) = 0 \quad , \quad t > 0 \tag{III-7-A}$$

Solution

$$c(r,t) = c_0\left[1 - \frac{R}{r}\,\mathrm{erfc}\left(\frac{r-R}{\sqrt{\pi D t}}\right)\right] \tag{III-8-A}$$

Flux

$$J = 4\pi R D c_0\left[1 + \frac{R}{\sqrt{\pi D t}}\right] \tag{III-9-A}$$

Cumulative transfer

$$M_t = 4\pi R D c_o \left[t + \frac{2R\sqrt{t}}{\sqrt{\pi D}} \right] \qquad \text{(III-10-A)}$$

This derivation is admirable in its conception and has been widely quoted as the classical treatment in the subsequent development of coagulation theory. Equation (III-7-A), however, is a rather restrictive boundary condition. The assumption of a "perfect" adsorption has been criticized. The generality of the flux expression given in Equation (III-9-A) will be apparent, however, in the derivations subsequently presented in this section. Many of the more complex forms, derived by other investigators with various boundary conditions, can be reduced to the simple form of Equation (III-9-A) as a limiting case.

Another limiting form given in Equation (III-11) is formed by neglecting the transient term in Equation (III-9-A), i.e. $R/\sqrt{\pi D t} \ll 1$.

$$J = 4\pi R D c_o \qquad \text{(III-11)}$$

The significance of this transient term will be considered in subsequent discussion. The probability that a certain particle, existing anywhere in the fluid of finite volume, V, has adsorbed onto the spherical surface is given by Equation (III-11a).

$$X = \frac{4\pi D R}{V} \left[t + \frac{2R}{\sqrt{\pi D}} \sqrt{t} \right] \qquad \text{(III-11a)}$$

The probability that this same particle has not adsorbed is $U = 1 - X$. The probability that no accumulation of particles on the spherical surface has occurred is given by Equation (III-11b).

$$\ln U = -4\pi DRN\left[t + \frac{2R}{\sqrt{\pi D}}\sqrt{t}\right] \qquad \text{(III-11b)}$$

The two limiting forms of Equations (III-11a) and (III-11b) are also due to Smoluchowski (1917a).

Case B (Valentine and Allison, 1959): Diffusion of adsorbable particles from a fluid of finite volume, initially containing a uniform concentration of adsorbable particles which slowly fall, to a spherical adsorbing surface at which a "zero" concentration of adsorbable particles is maintained the volume of the adsorbing particle being considerably smaller than that of the fluid.

Partial differential equation

$$\frac{\partial c}{\partial t} = D\nabla_r^2 c \,, \quad r > R \qquad \text{(III-5-B)}$$

Initial condition

$$c(r, 0) = c_0 \,, \quad r > R \qquad \text{(III-6-B)}$$

Boundary condition

$$c(R, t) = 0 \,, \quad t > 0 \qquad \text{(III-7-B)}$$

Solution

$$c(r, t) = c_0\left[1 - \frac{R}{r}\,\mathrm{erfc}\left(\frac{r-R}{\sqrt{\pi Dt}}\right)\right] \qquad \text{(III-8-B)}$$

Flux

$$J = 4\pi RDc_0\left[1 + \frac{R}{\sqrt{\pi Dt}}\right] \qquad \text{(III-9-B)}$$

Cumulative transfer

$$\ln\left(\frac{M_t}{M_0}\right) = -\frac{4\pi RD}{V}\left[t + \frac{2R\sqrt{t}}{\sqrt{\pi D}}\right] \qquad \text{(III-10-B)}$$

The boundary value problem formulated in Case B is parallel to the familiar Smoluchowski treatment of Case A up to the point where the flux equation is integrated. Valentine and Allison initially assumed an infinite volume of fluid surrounding the adsorbing particle. They later departed from this assumption and considering the fluid volume to be finite but still much larger than that of the adsorbing particles. The bulk fluid is considered to have a uniform but slowly falling concentration. Equation (III-9-B) can be integrated to form Equation (III-10-B) if the assumption, $C \simeq C_0$, is made. A factor of two is missing in the term involving $\sqrt{t}$ in the integrated form presented by Valentine and Allison.

The rate at which virus particles collide and react with a non-biological surface was predicted from this solution of the diffusion equation. This result is somewhat surprising since mass transfer in an agitated system should be affected by fluid motion as well as by Brownian motion. Valentine and Allison considered this phenomenon to be diffusion-limiting by assuming that the adsorbing surface was covered by a stagnant liquid film. Their development is a significant extension of the Smoluchowski treatment to the problem of adsorption of virus particles onto solid surfaces in spite of criticism by Ogston (1963) for perpetuating the myth of a unit efficiency of adsorption. A combined dependency of the adsorption upon both t and $\sqrt{t}$ was fully recognized in contrast to earlier work by Schlesinger (1932a) and Delbrück (1940), who also studied the adsorption of viruses. Investigators studying

the kinetics of diffusion-controlled reactions in other area of endeavor have also assumed that the transient term involving $\sqrt{t}$ in the flux equation is negligible and have accepted the simplified form of Equation (III-11). In many cases involving the diffusion of small particles or molecules this assumption is justified. Ware and Novros (1966) studied the quenching of fluorescence and recognized the importance of the transient term in this particular case.

Case C (Collins and Kimball, 1949a; Collins, 1950): Diffusion of adsorbable particles from an infinite fluid, initially containing a uniform concentration of adsorbable particles to a spherical adsorbing surface at which the concentration of adsorbable particles is proportional to the gradient in concentration.

Partial differential equation

$$\frac{\partial c}{\partial t} = D\nabla_r^2 c \quad , \quad r > R \qquad \text{(III-5-C)}$$

Initial condition

$$c(r,0) = c_0 \quad , \quad r > R \qquad \text{(III-6-C)}$$

Boundary condition

$$c(R,t) = \zeta\left(\frac{\partial c}{\partial r}\right)_{r=R}, \quad t > 0 \qquad \text{(III-7-C)}$$

Solution

$$c(r,t) = c_0\left\{1 + \frac{R-\beta}{r}\left[\operatorname{erf}\left(\frac{r-R}{\sqrt{4Dt}}\right) + \exp\left(\frac{Dt}{\beta^2} + \frac{r-R}{\beta}\right)\operatorname{erfc}\left(\frac{\sqrt{Dt}}{\beta} + \frac{r-R}{\sqrt{4Dt}}\right)\right]\right\} \qquad \text{(III-8-C)}$$

where

$$\beta = R\zeta/(R+\zeta) \qquad \text{(III-12)}$$

Flux

$$J = 4\pi D c_0 \left\{ \frac{\alpha R^2}{\alpha R + \rho'} + \frac{\alpha^2 R^3}{\rho'(\alpha R + \rho')} \right.$$
$$\left. \cdot \exp\left[\frac{Dt(\alpha R + \rho')^2}{(R\rho')^2} \right] \operatorname{erfc}\left[\frac{\sqrt{Dt}(\alpha R + \rho')}{R\rho'} \right] \right. \qquad \text{(III-9-C)}$$

where $$\zeta = D/K = \rho'/\alpha \qquad \text{(III-13)}$$

The quantities, α and ρ', in the nomenclature of Collins (1950) are, respectively, the probability of being adsorbed, and the ratio of the mean-squared and the mean jump lengths.

The case of imperfect reaction has been considered in this derivation by replacing the Smoluchowski boundary condition of Equation (III-7-A) with the more general form of Equation (III-7-C). The quantity K is of the nature of a specific reaction rate constant. The transmission coefficient, ζ, defined by Equation (III-13) is also used by Goodrich (1954) in a slightly different argument that is beyond the scope of the present discussion. He was in agreement with other investigators, however, that the boundary condition of Equation (III-7-C) is of the correct form for the case of imperfect adsorption.

Two limiting forms of the flux defined by Equation (III-9-C) are of particular interest. The second term of Equation (III-9-C) may be neglected for small values of α unless R is very large. Equation (III-14) is the reduced form which is independent of t.

$$J = 4\pi D c_0 \left(\frac{\alpha R^2}{\alpha R + \rho'} \right) \qquad \text{(III-14)}$$

It is similar in form to Equation (III-11) if the modifications of Equation (III-15) are made.

$$R' = \alpha R^2/(\alpha R + \rho') \qquad \text{(III-15)}$$

For moderate values of α and large R the transcient term is increasingly important. For large values of t the complementary error function can be replaced by the asyptotic series given by Equation (III-16).

$$erfc(y) = \frac{1}{\sqrt{\pi}} \exp(-y^2)\cdot\left[\frac{1}{y} - \frac{1}{2y^3} + \frac{3}{4y^5} - \cdots\right] \qquad \text{(III-16)}$$

If higher order terms are neglected, then Equation (III-9-C) can be reduced to the form of Equation (III-17) which is identical with the Smoluchowski flux defined by Equation (III-9-A).

$$J = 4\pi R' D c_o\left[1 + \frac{R'}{\sqrt{\pi D t}}\right] \qquad \text{(III-17)}$$

Case D (Reid, 1953): Diffusion of adsorbable particles from an infinite fluid initially containing a uniform concentration of adsorbable particles in which the adsorbable particles are autocatalytically produced, to a spherical adsorbing surface at which the concentration of adsorbable particles is proportional to the gradient in concentration at the surface.

Partial differential equation

$$\frac{\partial c}{\partial t} = D\nabla_r^2 c + \nu c, \quad r > R \qquad \text{(III-5-D)}$$

Initial condition

$$c(r, 0) = c_o, \quad r > R \qquad \text{(III-6-D)}$$

Boundary condition

$$c(R,t) = \xi\left(\frac{\partial c}{\partial r}\right)_{r=R}, \quad t > 0 \qquad \text{(III-7-D)}$$

Solution

$$c(r,t) = c_0 e^{\nu t}\left\{1 + \frac{R-\beta}{r}\left[\operatorname{erf}\left(\frac{r-R}{\sqrt{4Dt}}\right)\right.\right.$$
$$\left.\left. + \exp\left(\frac{Dt}{\beta^2} + \frac{r-R}{\beta}\right)\operatorname{erfc}\left(\frac{\sqrt{Dt}}{\beta} + \frac{r-R}{\sqrt{4Dt}}\right)\right]\right\} \qquad \text{(III-8-D)}$$

where again $\beta = R\xi/(R+\xi)$.

Flux

$$J = 4\pi D c_0 e^{\nu t}\left\{\left(\frac{\alpha R^2}{\alpha R+\rho'}\right) + \frac{\alpha^2 R^3}{\rho'(\alpha R+\rho')}\right.$$
$$\left. \cdot \exp\left[\frac{Dt(\alpha R+\rho')^2}{(R\rho')^2}\right]\operatorname{erfc}\left[\frac{\sqrt{Dt}\,(\alpha R+\rho')}{R\rho}\right]\right\} \qquad \text{(III-9-D)}$$

This derivation is similar to Case C with the exception that the adsorbable particles are assumed to be produced at a rate proportional to their concentration, i.e. autocatalytically. The various equations are identical with case C if $\nu = 0$. The significance of the assumption of an infinite radius was discussed by Reid. It is evident from this solution that in such a system the concentration of adsorbable particles must increase without bound. The application of the resulting solution to metabolizing systems is not prevented by this unrealistic assumption since the main concern is the behavior of the concentration and the rate of reaction for small values of t . This solution of primary interest in a system in which the adsorbable particles must initially be "activated" before reaction can occur. Such applications are found for enzymesubstrate reactions occurring in metabolizing systems if such reactions are assumed to be diffusion-controlled.

The applicability of the previous derivations in representing rates of adsorption occurring in agitated suspensions of finite extent which contain large adsorbing particles of ion exchange resin can be questioned from several standpoints. The obvious effects of external forces, such as agitation and gravitation, have been discussed by many investigators. Müller (1928) showed that only the coagulation of the largest particles in a suspension is influenced by sedimentation or stirring. Manley and Mason (1952) studied the motions of uniform spherical particles in sheared suspensions. Davies (1952) incorporated the effects of turbulent diffusion into a hyperbolic differential equation. Trurnit (1954) derived equations governing the initial phase and the steady state of adsorption and reaction of enzymes occurring at solid liquid interfaces with and without stirring.

Reich and Vold (1959) described two regions of particle size: (1) colloidal dispersions, where Brownian motion is predominant in governing coagulation, and (2) suspensions, where agitation is predominant in governing coagulation. A particle diameter of approximately 1 μ is the transition between these two regions. This dimension is the size of many bacteria. Koch (1960) discussed the effects of various types of mixing upon the adsorption of viruses by host bacteria. The rate of maximum adsorption was not markedly changed upon consideration of Brownian motion or gravitational settling. Cellular motility and mechanical mixing were of much greater consequence.

The amount of solute adsorbed at a reactive surface may also be a significant fraction of the total amount of solute initially present in the suspension. The concentration of unadsorbed solute will remain

uniform but gradually decrease with time until equilibrium is attained. The volume of the adsorbing particles, in addition, may represent an appreciable fraction of the total fluid volume. The assumption of a unit efficiency of collision may not be valid in systems containing smooth, hard spheres. Several of these factors have been included in the complexity of the following derivation.

Several investigators (Wilson, 1948; Crank, 1948; Soldano, 1953; and Carman and Haul, 1954) have considered the problem of diffusion of mass from a limited volume of a "well-stirred" solution, i.e. a solution having a uniform composition at all times. An excellent summary has been presented by Crank (1956, sections 4.35, 5.33, and 6.33). The analgous situation of heat transfer to a solid from a perfect conductor has been treated by Carslaw and Jaeger (1959, sections 1.9F, 3.13, 9.6, and 12.4 III).

Case E (Paterson, 1947; Crank, 1948; Carman and Haul, 1954; Crank, 1956, 6.33; and Carslaw and Jaeger, 1956, 9.6): Diffusion of adsorbable particles from a well-stirred fluid of finite extent initially containing a uniform concentration of adsorbable particles to a spherical adsorbing surface at which the concentration of adsorbable particles is maintained equal to that of the fluid.

Partial differential equation

$$\frac{\partial c}{\partial t} = D\nabla_r^2 c \quad , \quad r < R \quad , \quad t > 0 \qquad \text{(III-5-E)}$$

Initial conditions

$$c'(r, 0) = 0 \, , \quad r < R \tag{III-6a-E}$$

$$c\,(r, 0) = c_o \, , \quad r \geqslant R \tag{III-6b-E}$$

Boundary conditions

$$c'(R, t) = K' c\,(R, t) \, , \quad t > 0 \tag{III-7a-E}$$

$$-4\pi R^2 D \frac{\partial c'}{\partial r} = V \frac{\partial c}{\partial t} \tag{III-7b-E}$$

It should be noted that in Cases A through D the concentration of adsorbable particles has been described as a function of the radius, r, for the region, $r > R$. The concentration of adsorbable particles in the region, $r \leq R$, was considered to be constant albeit zero. In contrast, for Case E a concentration profile was assumed to exist in the region, $r < R$, and a uniform concentration was assumed in the region, $r \geqslant R$. The concentration of adsorbable particles present in the solution, c, is distinguished from the concentration of adsorbable particles present in the absorbing sphere, c'. The distribution of adsorbable particles between the solid and fluid phases at equilibrium is described in terms of the sorption or partition coefficient, K'.

The two boundary conditions given by Equations (III-7a-E) and (III-7b-E) can be combined as Equation (III-7c-E).

$$-D \frac{\partial c}{\partial r} = \frac{\alpha^* R}{3} \frac{\partial c}{\partial t} \, , \; r = R, \; t > 0 \tag{III-7c-E}$$

The effective volume ratio, α^*, is defined by Equation (III-18).

$$\alpha^* = 3V / 4\pi K' R^3 N' m \tag{III-18}$$

The number of absorbing spheres per unit weight of adsorbent, N', is included in the denominator of this expression. The time of sorption, t, is given in dimensionless form by Equation (III-19).

$$\sqrt{\tau} = (\sqrt{D}/R)\sqrt{t} \qquad \text{(III-19)}$$

The solution of this particular boundary value problem in terms of the concentration of adsorbable particles present in the fluid, c, is given by Equation (III-8a-E) for large values of τ.

$$c/c_0 = \frac{\alpha^*}{\alpha^*+1} - \sum_{n=1}^{\infty} \frac{6\alpha^* \exp(-s_n^2 \tau)}{9(\alpha^*+1) + \alpha^{*2} s_n^2}, \quad \tau \geq 0.1 \qquad \text{(III-8a-E)}$$

The quantities, s_n, are the roots of the subsidary Equation (III-20).

$$\tan s_n = 3s_n / (3 + \alpha^* s_n^2) \qquad \text{(III-20)}$$

For small values of τ the alternative solution given by Equation (III-8b-E) is applicable.

$$c/c_0 = \frac{\psi_1}{\psi_1+\psi_2} \exp\left(\frac{3\psi_1\sqrt{\tau}}{\alpha^*}\right)^2 \operatorname{erfc}\left(\frac{3\psi_1\sqrt{\tau}}{\alpha^*}\right) + \frac{\psi_2}{\psi_1+\psi_2} \exp\left(-\frac{3\psi_2\sqrt{\tau}}{\alpha^*}\right)^2 \operatorname{erfc}\left(-\frac{3\psi_2\sqrt{\tau}}{\alpha^*}\right), \quad \tau \leq 0.1 \qquad \text{(III-8b-E)}$$

The functions, ψ_1 and ψ_2, are defined by Equations (III-21a) and (III-21b).

$$\psi_1 = \frac{1}{2}\left[\sqrt{1 + \frac{4}{3}\alpha^*} + 1\right] \qquad \text{(III-21a)}$$

$$\psi_2 = \psi_1 - 1 \qquad \text{(III-21b)}$$

The series solution of Equation (III-8a-E) is rapidly convergent for times, $\tau \geq 0.1$, and only two terms are usually required. The speed of convergence falls off rapidly for smaller values of τ. The exponential-error function solution of Equation (III-8b-E) is therefore preferable for times, $\tau \leq 0.1$.

The amounts of adsorbable particles adsorbed from the solution after times, t and t_∞, are denoted, respectively, as M_t and M_∞. The expressions of Equations (III-10a-E) and (III-10b-E) are applicable, respectively, for large and small times.

$$(1-M_t/M_\infty) = \sum_{n=1}^{\infty} \frac{6\alpha^*(\alpha^*+1)\exp(-s_n^2\tau)}{9(\alpha^*+1)+\alpha^{*2}s_n^2}, \quad \tau \geq 0.1 \qquad \text{(III-10a-E)}$$

$$(1-M_t/M_\infty) = (\alpha^*+1)\left[\frac{\psi_1}{\psi_1+\psi_2}\exp\left(\frac{3\psi_1\sqrt{\tau}}{\alpha^*}\right)^2 \operatorname{erfc}\left(\frac{3\psi_1\sqrt{\tau}}{\alpha^*}\right) + \frac{\psi_2}{\psi_1+\psi_2}\exp\left(-\frac{3\psi_2\sqrt{\tau}}{\alpha^*}\right)^2 \operatorname{erfc}\left(-\frac{3\psi_2\sqrt{\tau}}{\alpha^*}\right)\right], \tau \leq 1. \qquad \text{(III-10b-E)}$$

The large time solution can also be expressed by Equation (III-22a).

$$\ln(1-M_t/M_\infty) = \ln \sum_{n=1}^{\infty} \frac{6\alpha^*(\alpha^*+1)}{9(\alpha^*+1)+\alpha^{*2}s_n^2} - \sum_{n=1}^{\infty} s_n^2\tau \qquad \text{(III-22a)}$$

The small time solution can be expressed in series form by Equation (III-22b).

$$(1-M_t/M_\infty) = 1-(\alpha^*+1)\left[\frac{6}{\sqrt{\pi}}\frac{\sqrt{\tau}}{\alpha^*} - \left(1+\frac{\alpha^*}{3}\right)\frac{9\tau}{\alpha^{*2}} + \left(1+\frac{2\alpha^*}{3}\right)\frac{36\tau^{3/2}}{\sqrt{\pi}\alpha^{*3}}\right] \qquad \text{(III-22b)}$$

If $\alpha^* >> 1$, then the further reduction to the form of Equation (III-22c) is possible.

$$M_t/M_\infty = -3\tau + \frac{6}{\sqrt{\pi}}\sqrt{\tau} \qquad \text{(III-22c)}$$

This form of the solution can be modified to the form of Equation (III-22d).

$$M_t/M_o = -\frac{4\pi DR}{V}\left[t + \frac{2R\sqrt{t}}{\sqrt{\pi D}}\right] \qquad \text{(III-22d)}$$

This last equation is similar in form to the solutions previously derived for Cases A through D which show a combined dependence upon t and $\sqrt{t}$.

Diffusion coefficients of adsorbable particles suspended in a well-stirred fluid can be easily determined. The progress of the sorption is followed by observing the rate of change of the concentration of adsorbable particles remaining unsorbed in the agitated fluid. Measurement of the concentration of adsorbable particles in the fluid is usually much easier than measurement of the amount accumulated on the adsorbent.

Several applications of this technique have been reported in the literature: diffusion of solute from a liquid solution into a gel (March and Weaver, 1928), diffusion of dye into a fiber or film (Wilson, 1948; and Crank, 1948), diffusion by isotopic exchange between a solid and a gas (Wagner, 1945), and sorption of gas or vapor by permable materials (Haul and Schöning, 1953). Carman and Haul (1954) have described the procedure for determining the diffusion coefficient, D, for cases involving a solid slab, sphere, or cylinder suspended in a well-stirred fluid.

3. The rate equation of bacterial adsorption

The adsorption of freely-suspended bacterial cells onto the surfaces of particles of an ion exchange resin suspended in the same fluid medium is similar in many respects to the processes for which kinetic models were developed in the previous section. Several

additional factors must be considered, however, in defining the kinetics of adsorption of bacteria. Cells suspended in a well-stirred fluid of finite extent can diffuse to the surface of a suspended solid where a reversible chemical reaction or adsorption can occur. The cells undergoing this adsorption, however, are in suspension rather than solution. The adsorbable particles (bacterial cells) can be considered to be much smaller than the adsorbing particles (ion exchange resin) although the former may greatly outnumber the latter, i.e. $\ell \gg d/2$ but $N \ll n$. Although the bacterial cell-resin particle system is polydisperse and multiple particles are formed, the rate is a function of the disappearance of the smaller particles, i.e. the bacterial cells.

The bacterial cells are effectively removed from suspension when they come into contact with the surfaces of the adsorbing particles. The surface concentration of freely dispersed cells may therefore be considered to be zero. No diffusion into the interior of the adsorbing particles is assumed to occur. A short distance away from the surface, however, a uniform concentration of cells is maintained throughout the well-stirred fluid. The diffusion through a thin stagnent film surrounding each resin particle is considered to be the rate-limiting step. The diffusion of the bacterial cells due to Brownian motion is much greater than that of the resin particles; the latter are more susceptible to sedimentation than are the former. The average diffusion coefficient of both species, therefore, is almost entirely that of the bacterial cells alone, i.e. $D_{avg.} \simeq D_{bacteria}, D_{bacteria} \gg D_{resin}$.

A flux expression of the general form of Equation (III-23)

$$J = -\frac{df(M_t)}{dt} = k + \frac{k'}{2} \cdot \frac{1}{\sqrt{t}} \qquad \text{(III-23)}$$

and a cumulative transfer expression of the simple form of Equation (III-24)

$$f(M_t) = kt + k'\sqrt{t} \qquad \text{(III-24)}$$

have been developed from solutions of Cases A through E presented in the previous section. The quantity, $f(M_t)$, is s function of the amount of solute sorbed after time, t. Two definitions of this function which have been developed are: $f(M_t) = M_t/M_0$ for Cases A, C, and D, and for Case E ($\tau \leq 0.1$), and $f(M_t) = \ln(M_t/M_0)$ for Case B and for Case E ($\tau \geq 0.1$). The latter definition is more easily adapted to the mathematical forms to be developed in the following paragraphs.

The amount of solute sorbed after infinite time, M_∞, is related to the initial amount of solute in solution, M_0, through Equation (III-25).

$$M_\infty/M_0 = 1/(\alpha^* + 1) \qquad \text{(III-25)}$$

If the partition coefficient, K', is very large, i.e. the solid phase is favored during sorption, then $\alpha^* << 1$ and $M_\infty = M_0$. If the volume of the fluid is constant then the ratio, $1 - M_t/M_\infty$, can be replaced by Equation (III-26), where the concentration of "solute" in the fluid, c, can be expressed in terms of the number of unadsorbed bacterial cells per unit volume of suspension, n.

$$1 - M_t/M_\infty = 1 - M_t/M_0 = c/c_0 = n/n_0 \qquad \text{(III-26)}$$

The correlation between total cell number, n, and light absorbance, A, of a cell suspension is given by Equation (A-15) which

is derived in Appendix A.

$$\log (n/n_o) = (1/\beta_o) \cdot \log (A/A_o) \qquad \text{(A-15)}$$

The general form of the rate equation for the adsorption of bacterial cells onto particles of ion exchange resin is then given by Equation (III-27).

$$f(A/A_o) = kt + k'\sqrt{t} \qquad \text{(III-27)}$$

The significance of the rate constants, k and k', is dependent upon the particular mathematical model. Logarithms to the base ten, rather than natural logarithms, are used in calculating the experimental constants.

The sorption rate curve described by Equation (III-27) will assume different forms depending upon the parameters of time chosen and the relative magnitudes of the rate constants as shown in Figure 4. If $k >> k'$ then a plot of $\log(A/A_o)$ versus t will be linear of slope, k ; a plot of $\log(A/A_o)$ versus $\sqrt{t}$ will have a downward curvature. Alternatively, if $k << k'$ then a plot of $\log(A/A_o)$ versus $\sqrt{t}$ will be linear of slope, k' ; a plot of $\log(A/A_o)$ versus t will have a upward curvature. The actual experimental rate data will reflect contributions by both kt and $k'\sqrt{t}$, and a curve intermediate between the two extremes of Figure 4 will be described.

Equation (III-27) can be expressed advantageously in difference form by either Equation (III-30) or (III-31)

$$\frac{\Delta \log f(A/A_o)}{2\Delta\sqrt{t}} = k\sqrt{t} + \frac{k'}{2} \qquad \text{(III-30)}$$

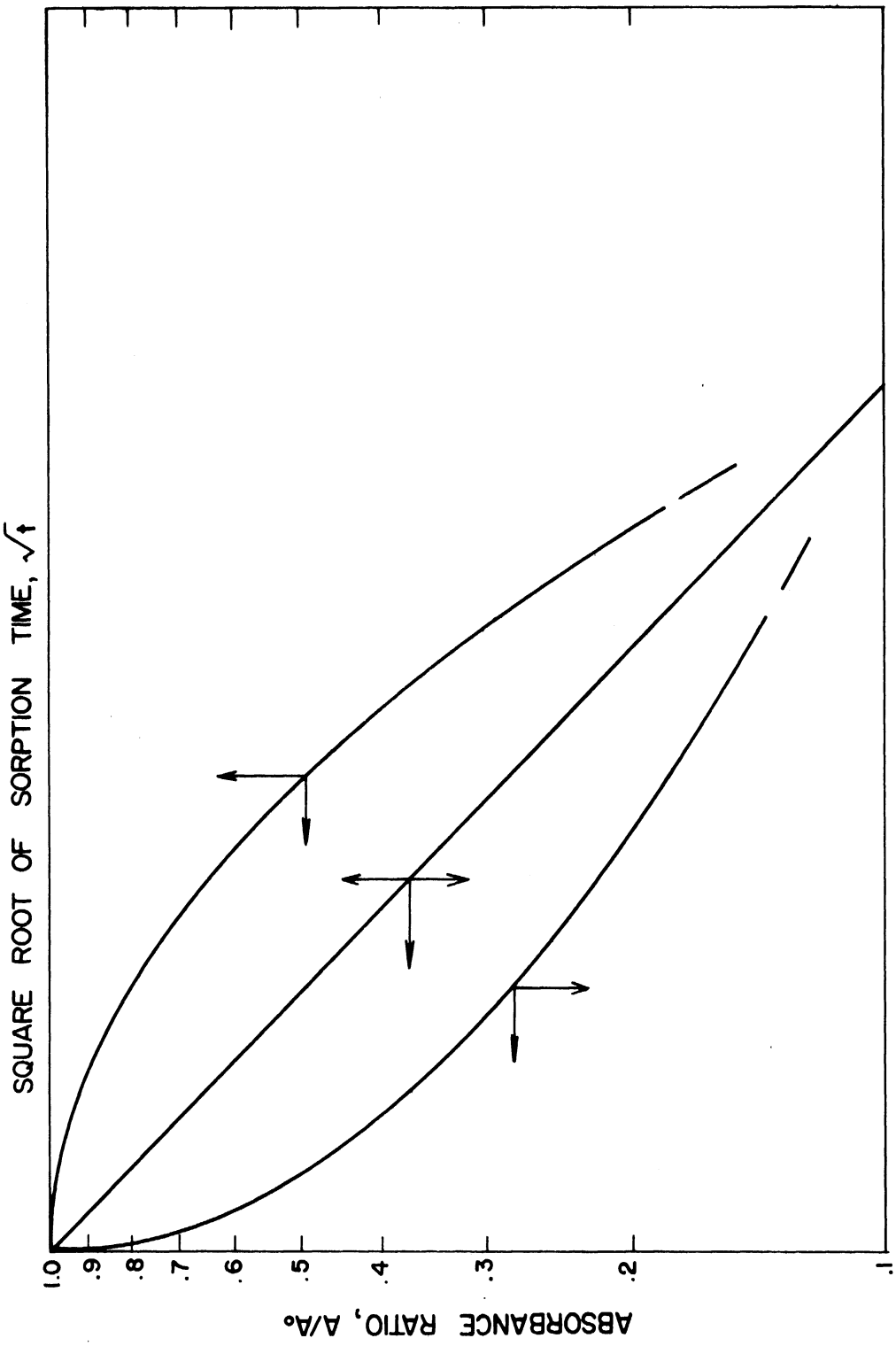


Figure 4. Idealized Sorption Curves of the General Form, $\log A/A_o = kt + k'\sqrt{t}$.

$$\frac{\Delta \log f(A/A_0)}{\Delta t} = k + \frac{k'}{2} \cdot \frac{1}{\sqrt{t}} \qquad \text{(III-31)}$$

Although both of these forms are equally correct, the former is somewhat more convenient for calculating purposes. Absorbance data collected for equal intervals of $\sqrt{t}$, e.g. t = 0.25, 1, 2.25, 4, 6.25, 9, ... minutes, corresponding to $\sqrt{t}$ = 0.5, 1, 1.5, 2, 2.5, 3, ... minutes$^{\frac{1}{2}}$, can be easily differenced according to Equation (III-30). A plot of the differenced quantity, $\Delta \log f(A/A_0)/2\Delta\sqrt{t}$, versus $\sqrt{t}$ should be linear of slope, k, and intercept, $k'/2$, if the sorption is correctly described by Equation (III-27). A typical difference plot of this kind is illustrated in Figure 5. A series of rate constants calculated by this method are presented in Table XXXI and their significance is discussed in Section V of this report. Example calculations are included in Appendix E.

The diffusion coefficient, D, can be experimentally determined by either of two methods. Theoretical values may be calculated from the Einstein-Stokes expression given by Equation (III-32).

$$D = kT'/3\pi\mu d \qquad \text{(III-32)}$$

This equation is applicable to particles of colloidal size of diameter, d, moving by Brownian motion of a fluid of temperature, T', and viscosity, μ. The quantity k is Boltzmann's constant. The diffusion coefficient for a bacterial cell 1.0μ in diameter suspended in water at 25 C. is D = 0.0487 x 10^{-7} cm^2/sec. Experimental values of D are compared in Table XXX.

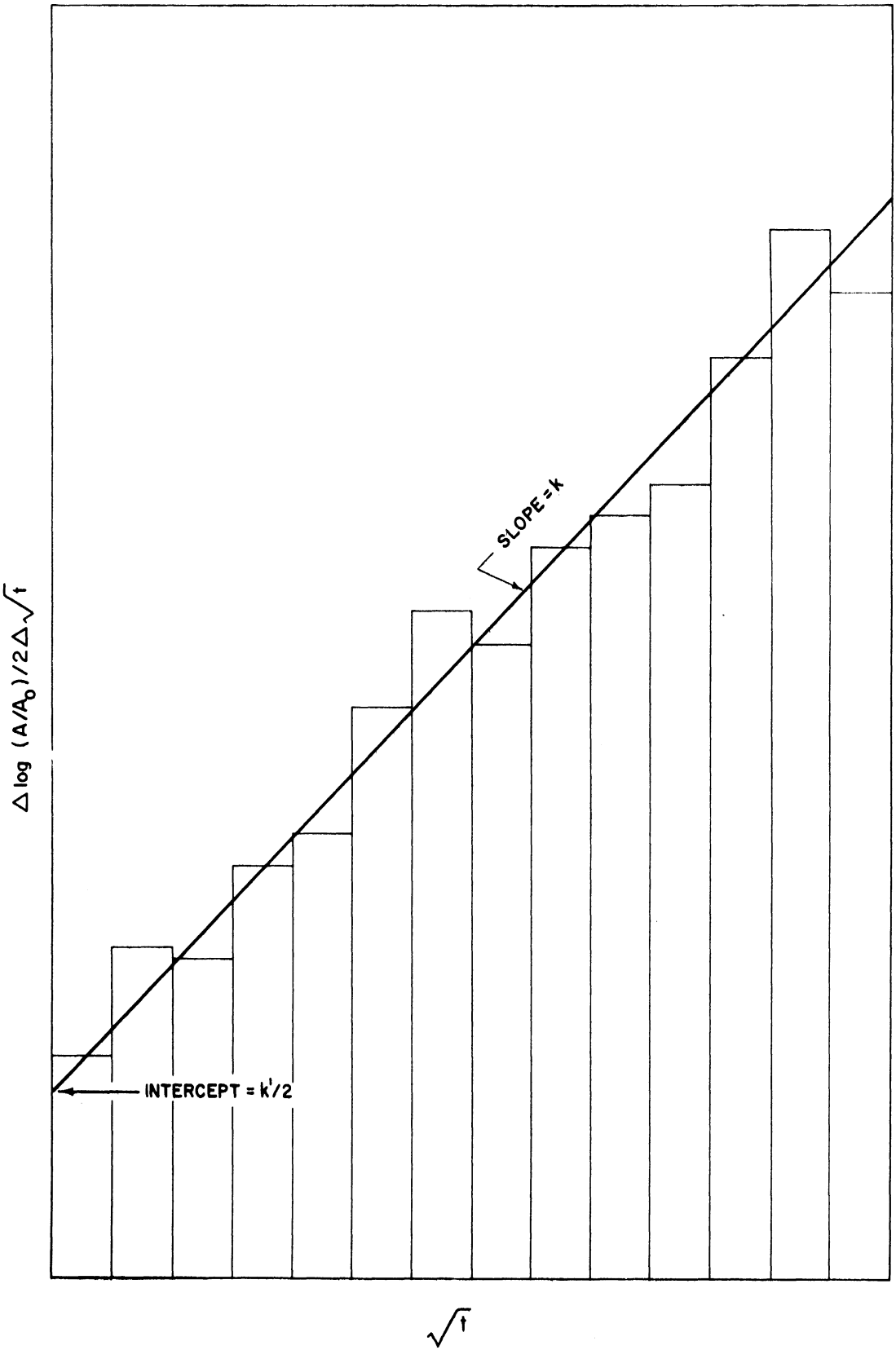


Figure 5. Typical Plot for the Determination of the Rate Constants Defined by the Difference Equation $\Delta\log(A/A_o)/2\Delta\sqrt{t} = k/t + k'/2$.

The rate constants for Case B are defined by Equations (III-33a) and (III-33b), where N is the number of adsorbing particles (ion exchange resin) per unit volume of suspension, and β_o is the slope of the logarithmic plot of absorbance, A, versus total cell number, n, presented as Figure 3-A in Appendix A.

$$k = -4\pi\beta_o R N D \qquad \text{(III-33a)}$$

$$k' = 2k R/\sqrt{\pi D} \qquad \text{(III-33b)}$$

Values of the ratio of the two rate constants can be determined without soption data by use of Equation (III-34a) which is formed by combining Equations (III-33a) and (III-33b).

$$|k/k'^2| = 1/16\beta_o R^3 N \qquad \text{(III-34a)}$$

The ratio can alternatively be expressed by Equation (III-34b) which does not include the radius, R, of the adsorbing particle.

$$|k/k'^2| = 1/12\beta_o(1-\epsilon)m \qquad \text{(III-34b)}$$

The ratio can thus be calculated knowing only the volume of the suspension, V, the mass, m, and void fraction, ϵ, of the adsorbing particles, and the slope constant, β_o. Values calculated from Equation (III-34b) can be compared with those experimentally determined from the slopes and intercepts of difference plots of the sorption data.

The diffusion constant, D, can be determined from rearrangement of Equations (III-33a) and (III-33b). Two values of D can be calculated from one set of rate data by application of Equations (III-35a) and (III-35b).

$$D_k = -k/4\pi\beta_0 RN \qquad \text{(III-35a)}$$

$$D_{k'} = (1/\pi)[-k'/8\beta_0 R^2 N]^2 \qquad \text{(III-35b)}$$

The effects of agitation, temperature, pH, salt concentration, and the concentration of the adsorbable particles (bacterial cells) are not fully accounted for in Case B. The functional dependency of the concentration of the adsorbing particles (ion exchange resin), however, is included.

The rate constants for Case E are defined over two regions of the dimensionless time variable, τ. The rate constants, k and k', are defined by Equations (III-36a) and (III-36b) for the region of small time, $\tau \leq 0.1$.

$$k = -4\pi RND \qquad \text{(III-36a)}$$

$$k' = 2Rk/\sqrt{\pi D} \qquad \text{(III-36b)}$$

In the region of large time, $\tau \geq 0.1$, $k' = 0$, and $k = \sum_{n=0}^{\infty} s_n^2$. An additional constant defined by the first term of Equation (III-22a) is also required. Although the rate constants for Case E must also be determined by a differencing technique, the diffusion coefficient, D, can be determined by a more straightforward procedure and compared with values determined by the previously described techniques.

A master plot of the quantity, $1 - M_t/M_\infty = A/A_0$, versus $\sqrt{\tau}$ can be constructed from Equations (III-10a-E) and (III-10b-E) for a particular values of α^*. The values of $\sqrt{\tau}$ corresponding to experimental values of A/A_0 can then be extrapolated from this calculated curve and plotted against the actual experimental values of t. This plot should be linear of slope, $\sqrt{D}/R$, as seen from Equation (III-19) if the model is correctly

followed. Further details of this method have been outlined and some experimental data have been processed by Carman and Haul (1954). Calculations based on this method are presented in Appendix E and the experimental data graphically presented in Section V of the current report.

The capacity of an ion exchange resin for bacterial cells, q_∞, in units of cells per gram can be determined knowing the value of the saturation ratio of infinite time, γ_∞ . The saturation ratio is defined by Equation (III-37), and is actually M_0/M_∞ .

$$\gamma = \frac{1}{1-A/A_0} \overset{t\to\infty}{=\!=} \frac{n_0 V}{q_\infty m} \qquad \text{(III-37)}$$

If sufficient resin capacity is available for complete adsorption of all bacterial cells in a given suspension then the saturation ratio is unity. If more cells are present than the available resin can accomodate then the saturation ratio is less than unity. The saturation ratio, γ_∞, can be determined by extrapolating a plot of $\log(\gamma)$ versus $1/t$ to infinite time. The capacity of the resin, q_∞ , can then be calculated from Equation (III-37) if the initial concentration of bacterial cells, n_0 , the volume of the suspension, V , and the total mass of resin in the suspension, m, are known.

4. Variables Associated with the Exchange Environment

The interaction occurring between bacterial cells and particles of exchange resin is strongly influenced by several variables of the exchange environment. Five of these variables will be briefly discussed. The numbered sequence is a continuation of the list given in Sections III-A and III-B. The experimental results for the variables investigated in this report (*) are presented in Section V.

*(11) Hydrogen ion concentration

The pH of the system is probably the most important single variable with the exceptions of the species of bacteria and the type of resin. Since bacterial cells in suspension may be assumed to act as dipolar ions, their behavior as anions or as cations is determined by the pH of the suspension.

*(12) Salt concentration

The optical character and the buffer capacity of a bacterial suspension can be affected by the concentration of salt ions. The rate of adsorption and desorption of cells to an ion exchange resin and the equilibrium established between the adsorbed and unadsorbed phases are also strongly dependent upon the type of dissolved ions and their relative concentrations.

*(13) Agitation

The rate of reaction occurring at the surfaces of particles suspended in a fluid is affected by the degree of agitation. The interaction between bacterial cells and particles of ion exchange resin is considered to be diffusion controlled. The thickness of a stagnant diffusion layer assumed to be surrounding each resin particle is reduced by any increase in agitation.

*(14) Time of contact

The time of contact between a bacterial suspension and a large number of ion exchange particles is particularly important if the system is agitated and sorption rates are to be determined. Abrasive damage

to sensitive particles such as bacterial cells may result from extended contact times. A gradual breakdown of cellular systems may also be expected for old cultures devoid of nutrients.

*(15) Temperature

An increased rate of reaction usually results from a increase in temperature. This increase may be offset by degradation of heat-labile materials of bacterial cells and a deterioration of adsorption ability.

IV. MATERIALS AND METHODS

A. Preparation of Bacteria

Six bacterial species, Pseudomonas ovalis, Escherichia coli, Proteus vulgaris, Staphlococcus aureus, Bacillus cereus, and Bacillus subtilis, were studied in the current work. Five of the eight genera most often reported in the literature dealing with adsorption (Tables II-X) were represented in the test group. These particular species were selected because of recognizable differences, such as Gram-staining behavior, cell size, and cell shape. In addition, the cells of the selected species could be easily cultured and harvested from media of the same composition. Because of their relatively low pathogenicity, the cells of these species could be handled with comparative ease. The morphological and physiological characteristics of the test species are listed in Table XXV. The procedures used for their identification are described in detail in Appendix D.

Cultures of the six bacterial species were grown under identical conditions in trypticase soy broth of the composition given in Table XXVI. Broth cultures of each species were grown for twenty-four hours at 30°C. in a controlled environmental room (Lab-line Module-Lab, Series 914). These cultures were then frozen and stored at -10°C. for reference purposes. This procedure was repeated at various intervals throughout the research. Cultures of each species were also carried on slants of trypticase soy agar. These cultures were stored at 4°C. and transferred about every three months. When an experimental run was scheduled, 10 ml of fresh broth were directly inoculated from an agar slant or from a

TABLE XXV

CHARACTERISTICS OF BACTERIAL SPECIES TESTED FOR ADSORPTIVE BEHAVIOR

Bacterium	Symbol	Reference[1]	Size, μ	Growth in nutrient broth	Action on milk
Pseudomonas ovalis	Po	108	0.5 x 2.5 rods[2]	Turbid, with pellicle and white sediment, faintly pink	Alkaline, litmus not reduced
Escherichia coli	Ec	336	0.5 x 1.0-3.0 rods	Turbid, heavy greyish sediment, no pellicle	Rapid acid production, litmus reduced, curd formation
Proteus vulgaris	Pv	365	0.5-1.0 x 1.0-3.0 rods	Markedly turbid, possibly faint pellicle	Slightly acid, becoming markedly alkaline
Staphlococcus aureus	Sa	465	0.8-1.0 spheres	Turbid, becoming clear, yellowish ring and sediment	Acid, coagulated, curd formation, litmus reduced
Bacillus cereus	Bc	617	1.0-1.2 x 3.0-5.0 rods	Heavy uniform turbidity with soft, easily dispersed sediment, no pellicle	Alkaline, litmus not reduced, some sediment
Bacillus subtilis	Bs	620	0.7-0.8 x 2.0-3.0 rods	Clear, with heavy wrinkled, waxy, tough pellicle	Alkaline, litmus not reduced, some sediment

[1] See designated pagination in "Bergey's Manual of Determinative Bacteriology," 7th Edition (Breed, Murray, and Smith, 1957) for comparison of descriptions and other results reported in this table.

[2] Bennett (1963, p.45) wet-mounted, unstained cells.

TABLE XXV (Cont'd)[3]

Bacterium	Gram reaction	Motility[4]	Lactose	Mannitol	Glucose	Sucrose	Citrate	Urea	Gelatin	Nitrate	Starch	7% NaCl	Lecithinase	Coagulase
Pseudomonas ovalis	-	+	-	-	A	-	-	-	-	-	-	±	-	
Escherichia coli	-	±	AG	AG	AG	NC	-	-	-	+	-	+	-	
Proteus vulgaris	-	+	-	-	AG	AG	-	+	+	+	-	±	-	
Staphlococcus aureus	+	-	A	A	A	A	-	-	+	+	-	+	-	+
Bacillus cereus	+	+	-	-	A	A	±	±	+	+	+	±	+	
Bacillus subtilis	+	+	-	-	A	A	+	-	+	+	+	+	-	

[3] A, acid production; G, gas production; NC, not certain; - negative; + positive; ± variable.

[4] Motility not experimentally determined; description taken from reference 1 of this table.

TABLE XXV (Cont'd)

Bacterium	Odor of broth[5]	Centrifugation[6]	Filtration[7]	Odor of purified suspension[8]
Pseudomonas ovalis	Sour-sweet	Incomplete, pink-white cake	Slow	Slightly-sweet
Escherichia coli	Earthy-sweet	Incomplete, dirty white cake, layered	Slow	Sweet
Proteus vulgaris	Slightly fishy	Complete, white cake	Slow	Earthy
Staphlococcus aureus	ND	Complete, pink to golden cake	Rapid, some curdy residue	ND
Bacillus cereus	ND	Complete, dirty white cake	Rapid	Earthy
Bacillus subtilis	ND	Complete, dirty white cake	Rapid	Earthy

[5] ND, nondescript or masked by normal odor of nutrient broth.

[6] 1500 rpm, 20 minutes.

[7] Filtration of 50 ml of suspension through 40 mm, #4 Whatman paper; slow: > 10 minutes, rapid < 10 minutes.

[8] Suspension of harvested cells following centrifugation, washing, and filtration.

TABLE XXVI

COMPOSITION OF CULTURE MEDIUM

Trypticase Soy Broth[1]	
Trypticase[2]	17.0 grams
Phytone[3]	3.0 "
Sodium chloride	5.0 "
Dipotassium phosphate	2.5 "
Dextrose	2.5 "
Distilled water	1.0 liter
pH	7.3 ±

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[1] B-B-L 01-162, an all purpose medium
Baltimore Biological Laboratories,
Baltimore, Maryland

[2] Pancreatic digest of caesin

[3] Soy peptone

thawed frozen broth culture. An actively growing seed culture developed after 24 to 48 hours of incubation at 30°C. The seed culture was aseptically transferred to 190 ml of sterile broth contained in a 500 ml erlenmeyer flask fitted with a gauze plug. The total culture volume of 200 ml was then incubated for 24 hours at 30°C. under continuous agitation provided by a motor-driven shaker fitted with an excentric drive (New Brunswick Model VS).

The bacterial cells were harvested by centrifugation in 250 ml glass centrifuge bottles (Corning #1260) for 20 minutes at 1500 rpm (International Centrifuge, Size 2, Model K). The supernatant liquid, containing any soluble nutrients, metabolic products, or buffer salts, was discarded. The cell cakes and inside surfaces of the centrifuge bottles were lightly washed with distilled water using a plastic squeeze bottle. These washings were also discarded. The cells were then resuspended in 200 ml of distilled water and centrifuged a second time for 20 minutes at 1500 rpm. The supernatant liquid was again discarded. The washed cells were next resuspended in a minimum volume of distilled water, usually less than 50 ml total, and filtered through paper. Particulate contaminants and cell clumps were removed by this process. The filter medium was an open-textured paper (Whatman #4) placed in a buchner funnel. The filtrate from the coarse paper was then filtered twice through another filter paper of finer texture (Whatman #42). The purified suspension containing uniformly distributed bacterial cells was finally diluted with distilled water or selected salt solutions to the desired initial concentration.

B. Preparation of Ion Exchange Resins

Ion exchange resins were chosen as the test adsorbents because of their commercial availability, sphericity, well-defined electrical charge, high exchange capacity, and selectivity of reaction. The particular ion exchange resins evaluated in the current study were all copolymers of styrene and divinylbenzene. They included a strong-acid cation exchange resin of the sulfonic acid type, Dowex 50W, and two strong-base anion exchange resins of the quaternary ammonium type, Dowex 1 and Dowex 2. Characteristics of these resins are listed in Table XXVII. The bulk of the investigations were conducted using ion exchange resins having a particle size of 100/200 mesh (149/74 μ). This size fraction is small enough to settle from suspension in a reasonable time period yet have adequate exchange capacity per unit weight. The cation exchange resin was tested almost exclusively in its hydrogen form. The anion exchange resin were principally tested in their chloride form; hydroxide, acetate, and nitrate forms were investigated to a lesser extent. Only the eight-percent cross-linked materials were studied in the current work. The most commonly used resin was the anion exchange resin, Dowex 1 x 8, 100/200 mesh, chloride form.

The ion exchange resins to be evaluated in the batch adsorptions were first weighed in their commercially available forms. The anion exchange resins were available in the chloride form; the cation exchange resin was available in the hydrogen form. The weighed resin was placed on a filter paper (Whatman #4) supported in a buchner funnel. It was then washed with five, 50 ml portions of distilled water to remove any residual color or soluble salts. The water droplets clinging to the resin particles

TABLE XXVII

CHARACTERISTICS OF ION EXCHANGE RESINS TESTED FOR ADSORPTIVE ACTION

	Resin	Ionic form	Mesh size[4]	Estimated avg. mesh	Estimated avg., μ	Measured avg., μ	Standard deviation, μ
Anion:	Dowex 1 x 8[1]	Chloride	20/50	35	500	537	44.
			50/100	70	210		
			100/200	140	105	97.2	29.0
			200/400	270	53	62.8	23.9
			< 400				
		Hydroxide	20/50				
			100/200				
		Nitrate	20/50				
			100/200				
		Acetate	20/50				
			100/200				
	Dowex 2 x 8[2]	Chloride	20/50				
			50/100				
			100/200				
			200/400				
			< 400				
Cation:	Dowex 50Wx8[3]	Hydrogen	50/100				
			100/200				
			200/400				
		Sodium	50/100				
		Calcium	50/100				

[1] Strong base quaternary ammonium type I, $-N(alkyl)_3$
[2] Strong base quaternary ammonium type II, $-N(alklol)(alkyl)_2$
[3] Strong acid sulfonic, $-SO_3$
[4] U.S. Standard Seive Size

after the washing were removed by aspiration. The damp resin was then transferred to a waxed paper in preparation for rapid addition to a bacterial cell suspension. Most experiments were conducted using 50 ml of cell suspension to which 4 grams of ion exchange resin were added.

The resin to be evaluated in the columnar adsorptions was weighed and transferred to a glass column. The column consisted of a 10 x 300 mm Pyrex chromatographic tube (Corning #38450) with a 250 ml erlenmeyer flask welded to the top to serve as a reservoir. Standard 50 ml burettes were also incorporated as columns during some of the initial studies. The resin was contained in the columns by coarse-fritted glass plates or glass wool plugs. The resin was then washed with distilled water to remove any residual color or soluble salts. Conversion to other ionic forms was accomplished by passing solutions of appropriate regenerating agents through the column until the effluent was free of the displaced ion. Displacement of chloride ion was visually monitored by precipitation with silver nitrate; hydroxide and hydrogen forms were monitored with pH test papers. The converted resins were then thoroughly washed with distilled water. Resins of ionic forms other than chloride or hydrogen form used in the batch adsorption experiments were converted to the other forms in columns. The converted resin was transferred to a buchner funnel, aspirated to a damp condition, and then transferred to a waxed paper ready to be added to a suspension of bacterial cells.

C. Experimental Measurements

Paramount among the variables associated with the exchange reaction between bacterial cells and ion exchange resins are: the concentration of bacterial cells per unit volume of suspension, and the hydrogen

ion concentration of the suspension. The concentration of various salt ions, and the effects of temperature and agitation upon the experimental rates of adsorption and desorption must also be determined. Descriptions of the experimental techniques used to a measure these variables are contained in the following paragraphs.

1. Cell concentration

The concentration of bacterial cells was determined during the experimental work by measuring the absorbance of the suspension at 420 mμ with a photoelectric colorimeter (Klett-Summerson Model 900-3). Absorbances of the distilled water blank and the cell suspension were measured over the 40 mm path length of a rectangular 20 x 40 mm glass cuvette. Absorbance measurements were usually made using 30 ml samples. An optically-matched set of 15 x 125 rimless test tubes (Corning #9820) was also used during the preliminary investigations.

The concentration of bacterial cells was correlated with the absorbance of a given suspension. Beer's Law was modified to account for the deviation from its linear form at high concentrations of bacterial cells. This correlation can be expressed by Equation (A-14), where the quantities α_o and β_o are empirical constants, A is the

$$A = \alpha_o n^{\beta_o} \qquad \text{(A-14)}$$

absorbance of the suspension measured at 420 mμ, and n is the total number of bacterial cells/cm^3 as determined by direct counts using a hemocytometer. The length of the light path and the "molar" absorptivity of the cellular material in suspension are incorporated into the proportionality constant α_o.

This correlation is an empirical extension of the customary linear form of Beer's law for which the empirical constant β_o has a value of unity. Values of β_o were experimentally determined as the slopes of logarithmic plots of absorbance versus total cell number (viz. Figure A-3). A value of $\beta_o = 0.83$ was found to be applicable for the cells of Bacillus subtilis suspended in either distilled water or 0.25 M sodium chloride in the range $0.001 \leq A \leq 1.0$. A more detailed discussion of this correlation and its generality is included in Appendix A.

2. Hydrogen ion concentration

The hydrogen ion concentration (pH) of a suspension of bacterial cells, or of a mixed suspension containing both bacterial cells and particles of ion exchange resin, was measured with a direct recording pH meter (Beckman Expandomatic #76004). Provisions were available for full-scale deflection of the meter over any range of two pH units. The glass electrode (Beckman #39045) and the calomel electrode (Beckman #41239) were both $2\frac{1}{2}$ inches in length. A thermocompensator (Beckman #39096) was also incorporated into the system.

The rates and equilibria of both adsorption and desorption were considered to be very dependent upon the pH of the suspension. Initial adjustment of the pH to a predetermined value was done in certain cases. This was accomplished by dipping a Teflon spatula into a concentrated solution of hydrochloric acid or sodium hydroxide and then briefly plunging the wetted tip into the agitated suspension of bacterial cells. Addition of acid or base was continued until the desired pH value was obtained.

3. Salt concentration

Changes in the hydrogen ion concentration often exceeding two pH units can occur during the adsorption of bacterial cells onto ion exchange resins. Buffer salts were added to the cell suspensions to alleviate this condition in several experiments. Sodium acetate was added in solid form and allowed to equilibrate with the agitated suspension for one-half hour prior to the addition of any ion exchange resin. Any changes in the absorbance of the cell suspension due to the salt occurred within a few minutes after addition. Any acid or base released during the exchange of bacterial cells was sufficiently buffered by 0.1 M sodium acetate.

Desorption of some bacterial species was promoted in certain cases by the addition of salt to the equilibrated adsorption complex. The salt was weighed and added directly to the suspension in solid form. Solution was rapid. Sodium chloride solutions of molar concentration were usually of sufficient strength to desorb most cells from the resins. The ions displaced from the resin upon adsorption of the cells were measured during some experiments. The release of these ions into the bulk solution were determined by pH changes, or in the case of chloride ion, by precipitation with silver nitrate.

4. Temperature

The rate and equilibrium of the reaction between bacterial cells and ion exchange resins are both affected by the temperature of the mixture. The majority of the rate experiments were therefore conducted in a constant temperature bath maintained at 25°C. The bath was a small aluminum tank, 6 x 6 x 12 inches, filled with 3 inches of water, and fitted with a

heating coil, thermoregulator, and bladed agitator. The reaction vessel was a 150 ml beaker (Corning #1000) suspended in the center of the bath. Thermometers were placed in the reaction vessel and in the bath adjacent to the reaction vessel. The difference in temperature between these points was never greater than 0.5°C. at 25°C. even though 60 per cent of the reaction mixture was periodically withdrawn for absorbance measurements.

5. Agitation

The reaction mixtures in the rate experiments were agitated with a cylindrical Teflon-coated stirring bar driven by a magnetic unit (Magnestir Cat. No. 1250, Labline, Inc., Chicago, Ill.). The most common size of stirring bar used was 3/8 x 1-3/4 inches. The aluminum constant temperature bath was placed over the stirrer drive unit and separated by a layer of asbestos of 1/8 inch thickness. The distance between the top of the housing of the drive unit and the bottom of the reaction vessel containing the stirring bar was about 2 cm. The rotational velocity of the stirring bar was correlated with the setting of the drive unit for conditions of 50 ml of distilled water at 25°C. contained in a 150 ml beaker and agitated by a 3/8 x 1-3/4 inch stirring bar. The agitation speed (rpm) as a function of the index numbers of the drive unit is presented in Appendix B.

D. Experimental Procedures

A technique of sampling the bacterial cell-resin particle complex was required before absorbance measurements could be made. A specialized nomenclature was also developed in order to designate the absorbed and

unadsorbed fractions of a suspension containing one or more bacterial species. The reaction of bacterial cells and ion exchange resins can be classed as batch or continuous depending upon the mode of contact. A description of the procedure used in the batch technique is described in the present section. A discussion of the continuous, or columnar, technique has been relegated to Appendix F. The resolution of mixed suspensions containing the cells of two or more species was accomplished by procedures that are direct extensions of the simple batch procedure.

1. Sampling technique

A measure of the relative proportions of adsorbed and unadsorbed bacterial cells existing in a mixed suspension of cells and ion exchange resins at any given time is necessary for evaluation of the process of adsorption. A sample containing only the freely-dispersed, unadsorbed cells must somehow be separated from the larger particles of ion exchange resin with the adsorbed cells attached to their surfaces. The relative merits of the techniques used by other researchers are discussed in Appendix C. These techniques suffer from certain disadvantages of requiring excessive periods of time for sampling, or of introducing extraneous effects, such as filtration. The sampling technique developed in the current work was found to be acceptable for the investigation of liquids containing suspended particles having two widely differing average diameters.

A syringe filter (Becton-Dickinson #423-FA) was modified for application to the cell-resin mixtures as shown in Figure 6. The filter adapter was altered so that a 270 mesh screen, in addition to the usual

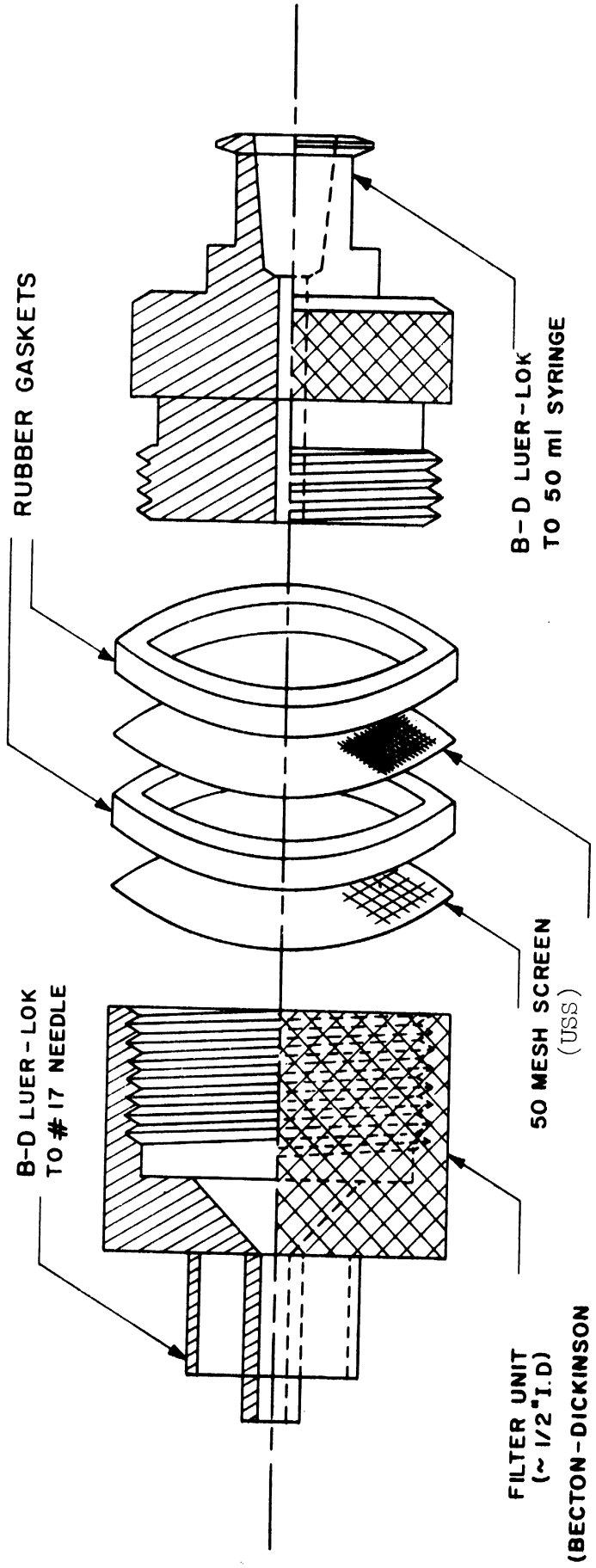


Figure 6. Detail of the Assembly of the Syringe Filter used to Separate the Unadsorbed Cell Fraction from the Adsorbed Cell-Resin Particle Complex.

50 mesh screen (U.S. Standard Seive size), was enclosed by rubber gaskets in the stainless steel housing. A #17 needle and a 50 ml glass syringe were then attached at opposite ends of the filter assembly.

The cell-resin complex formed in a reacting mixture is first allowed to settle after agitation is halted. A sample is then collected by inserting the syringe needle into the supernatant liquid. A liquid volume of 30 ml is then drawn up through the filter assembly. Any resin particles with cells adsorbed onto their surfaces are retained by the screens and the filtered suspension, containing only the freely-dispersed, unadsorbed cells, is collecting in the body of the syringe. The syringe is then disconnected from the filter unit and the filtrate transferred to a glass cuvette. The absorbance of the filtered sample can now be determined without interference from the resin particles.

After sampling is completed,the syringe is reassembled and a small portion of the remaining reaction mixture is drawn into the collection chamber and then flushed back through the filter unit. Any resin particles lodged on the filter screens are thus returned to the reaction vessel. The filtered sample is returned directly from the cuvette to the reaction vessel and agitation immediately resumed. The flow rate through the syringe filter may be reduced during repeated sampling if the screens clog with accumulated resin. This is corrected by back-flushing occasionally during sampling.

The cumulative adsorption, or desorption, time is a summation of all periods of agitated contact between bacterial cells and resin particles. The periods of sampling, when agitation is halted and the cell-resin complex is quiescent except for the supernatant liquid, are

not included in the cumulative time. Two assumptions associated with this method of sampling have been verified by separate experiments. These are: (1) negligible adsorption, or desorption, occurs in the residual suspension left in the reaction vessel during the time agitation is halted and the absorbance of the filtered supernatant liquid is being measured, and (2) no measureable quantities of bacterial cells are removed from the suspension by filtration through the syringe or by adsorption onto the surfaces of the equipment.

2. Nomenclature

The initial absorbance of a suspension of bacterial cells prior to the addition of any ion exchange resin is denoted as A_o ; those absorbances determined subsequent to the addition of the ion exchange resin are denoted as A . The fraction of unadsorbed cells existing at any point in time during adsorption or desorption is therefore expressed by the dimensionless ratio of absorbances, A/A_o . The fraction of adsorbed cells is simply $1-A/A_o$. Absorbance, alternatively designated as optical density or extinction, itself is a dimensionless quantity.

The absorbance of a suspension containing a single pure component does not necessarily follow Beer's law. The usual linear dependency of absorbance upon concentration can be modified, however, to yield the exponential form described in a previous section of this report and in Appendix A. The absorbances of the component species in a mixture are also not strictly additive. The ratio of the absorbance of the mixutre to that of either pure component diluted to the same volume, however, can be a useful parameter. The extent of adsorption achieved in a binary mixture

can be determined by observing the asymptotic approaches of the ratios, A_{1-2}/A_1 or A_{1-2}/A_2, to zero of unity, respectively for complete adsorption of both species or complete adsorption of either species as a function of time t. In the present nomenclature the absorbance of the mixture is designated as A_{1-2} and the absorbances of suspensions containing cells of species (1) and species (2) as A_1 and A_2, respectively.

Ratios formed in a similar manner can be used to estimate the extent of desorption. Absorbance values of suspensions of the pure component species determined under conditions of dilution, pH and salt content comparable to those existing during the desorption are required as reference quantities. If a series of desorptions is to be conducted, the first is followed by observing the approach of the ratio, A'_{1-2}/A'_1, from zero to unity, and the second is observed by the approach of the ratio, A''_{1-2}/A''_2, from zero to unity in a like manner. The adjusted absorbance values of the first and second desorptions are designated, respectively, by the prime and double prime markings and corresponding to desorption times, t' and t''.

The progressive movement of bacterial cells onto the resin surfaces during adsorption, or from the resin surfaces during desorption, can thus be followed simply by observing the changes in the absorbance of a suspension containing one or more species.

The resolution of a mixture containing two species of bacteria, with each possessing distinct but homogeneous adsorption properties, can be classed as either of two types. These types, denoted Type I and Type II, are shown, respectively, in Figures 7 and 8. The type of exchange can be specified further with regard to the ability of the bacterial cells to be adsorbed as anions or as cations. Each type of resolution will now be defined in detail.

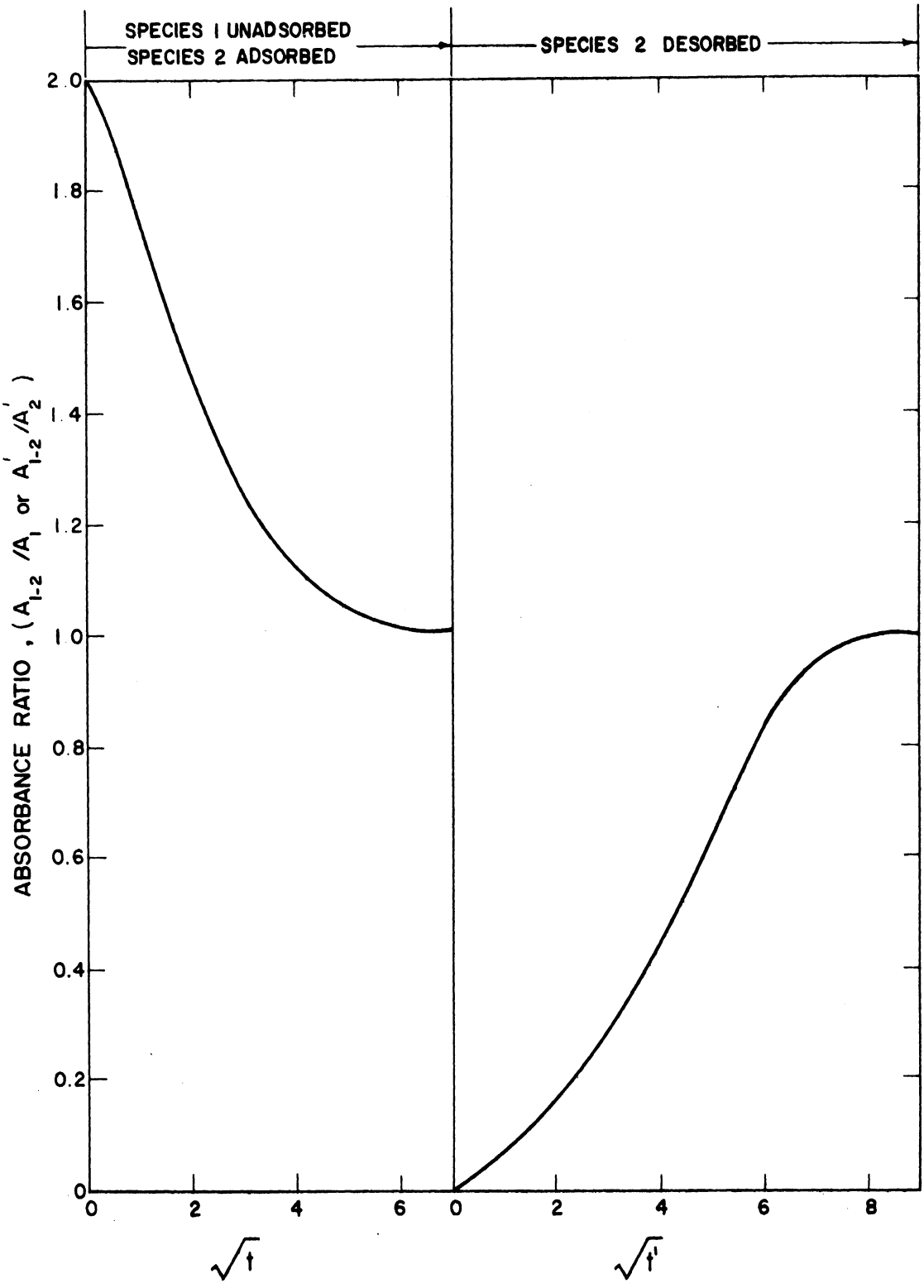


Figure 7. Resolution of a Binary Mixture: Type I. Cells of one Species Selectively Adsorbed and Desorbed.

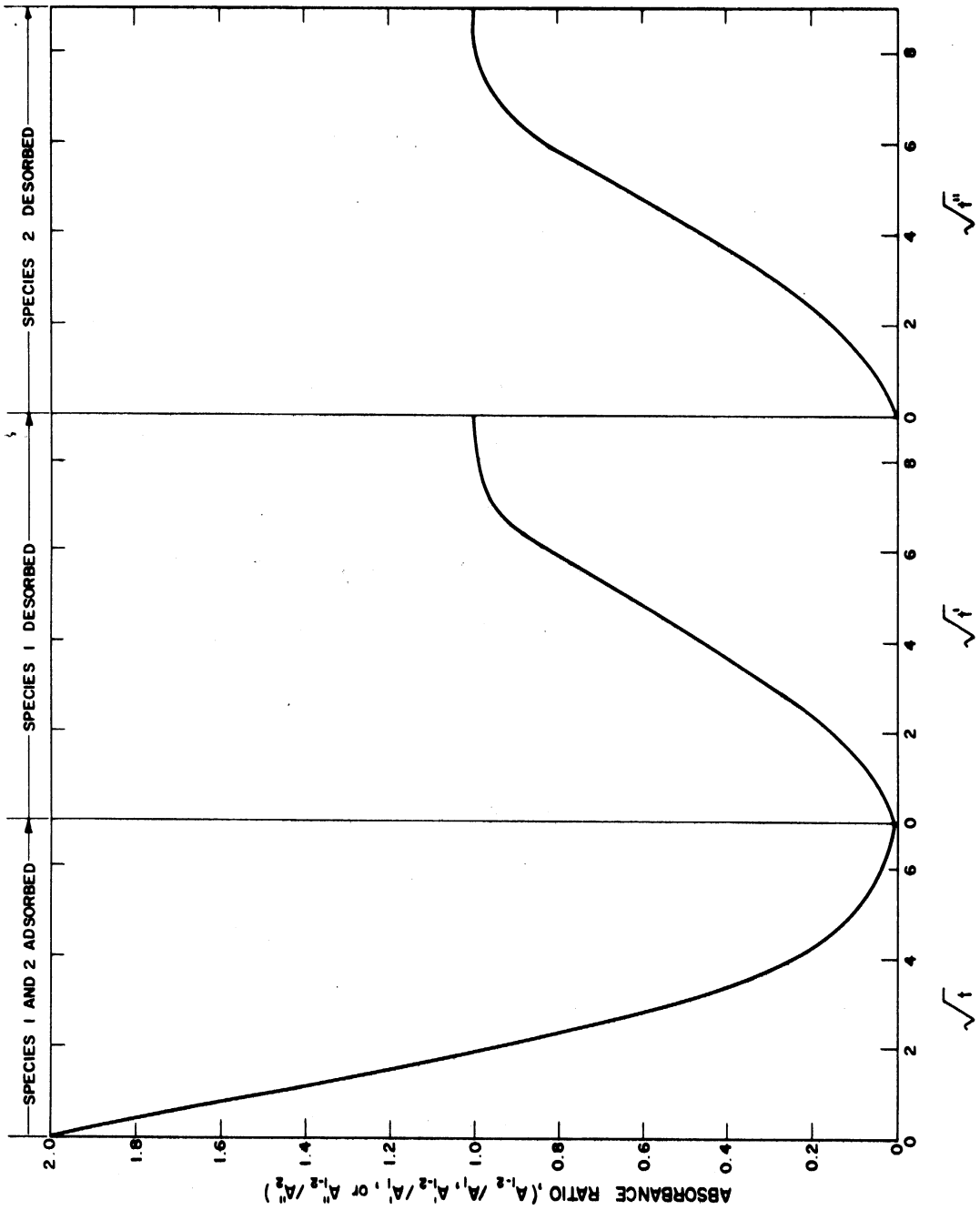


Figure 8. Resolution of a Binary Mixture: Type II. Cells of Both Species Adsorbed and Sequentially Desorbed.

In a resolution of Type I, selective adsorption of the cells of one species occurs while the cells of the other species remain unadsorbed. The cells of the adsorbed species are subsequently desorbed from the resin into a fresh solution. The selective adsorption of the cells of species (2) from a binary mixture containing the cells of both species (1) and (2) is illustrated in Figure 7. The ratio, A_{1-2}/A_1, initially at a value of two if Beer's law is followed, is observed to decrease toward a value of unity at equilibrium as the adsorption of species (2) is completed. The suspension containing only the cells of species (1) is then removed and the resin with only the cells of species (2) adsorbed onto its surface is washed to remove any clinging, unadsorbed cells. The resin is then placed in a solution containing a suitable agent to initiate desorption of the cells of species (2). The ratio, A'_{1-2}/A'_2, is then observed to increase from zero toward unity as the cells of species (2) becomes completely desorbed.

The resolution of Type II involves the nonselective adsorption of the cells of both species followed by the selective desorption of the cells of each species in sequence as shown in Figure 8. Complete adsorption of the cells of species (1) and (2) is indicated by both ratios A_{1-2}/A_1 and A_{1-2}/A_2 approaching zero values. The resin, containing the cells of both species adsorbed onto its surfaces, is then transferred to a fresh solution containing an agent capable of selectively desorbing the cells of species (1). This first desorption is indicated by the increase in the ratio, A'_{1-2}/A'_1, from zero to unity. The cells of species (2) remains adsorbed onto the resin. The resin is then transferred to a second solution containing an agent capable of desorbing the cells of

species (2). This second desorption is indicated by an increase in the ratio, A''_{1-2}/A''_2, from zero to unity.

3. Adsorption-desorption of a single species

The adsorption and desorption of the cells of a single bacterial species using an ion exchange resin placed in a cell suspension was accomplished by a straightforward process. The bacterial suspension and the ion exchange resin were separately prepared according to the procedures previously outlined. The cell suspension was initially diluted to an absorbance of 0.6. A total volume of 50 ml was then transferred by volumetric pipette to a 150 ml (tall form) beaker suspended in the constant temperature bath. The pH electrodes, thermocompensator, thermometer, and magnetic stirring bar were all inserted into the suspension and agitation commenced. The initial pH was measured and then agitation was halted while a 30 ml sample of the suspension was withdrawn for measurement of the absorbance. This sample was subsequently returned to the reaction vessel and agitation resumed. Both the pH and the absorbance were checked at intervals of five minutes. Equilibrium values for both quantities were usually established within one-half hour. Equilibration was particularly important if the pH at which the adsorption was to be conducted was considerably different from the pH of the initially neutral suspension. Adjustment of the pH was made with concentrated acid or base following the procedure previously outlined. The absorbance of the suspension at pH 7 was correlated with the total numerical concentration of bacterial cells. The extent of adsorption, however, was calculated on the basis of the absorbance measured at the equilibrated pH value denoted as A_o.

After equilibrium of both pH and absorbance in the resin-free cell suspension was established, the timer (Time-it, Precision Scientific Company, Chicago, Illinois) was set at zero, agitation was resumed, and the pH electrodes were activated. The ion exchange resin, previously weighed, washed, and aspirated to a damp condition, was then rapidly added to the cell suspension and the timer started. At predetermined intervals, the pH was measured and the agitation and timer halted. A sample of the suspension was then collected for measurement of the absorbance according to the technique previously described. After determination of the absorbance, the sample was returned to the reaction mixture, agitation was resumed, and the timer restarted.

This procedure was repeated several times throughout the duration of an experiment. The course of the ensuing reaction was thus followed by monitoring the absorbance of the suspension, A, as a function of the cumulative time, $\sqrt{t}$, of agitated contact between the ion exchange resin particles and the bacterial cells in suspension. The square root of the cumulative time was used as a convenient plotting variable and was expressed in units of $\text{minutes}^{\frac{1}{2}}$. The reaction was considered complete when the measured absorbance reached an equilibrium value. Adsorption was usually complete by $\sqrt{t} = 7\ \text{min}^{\frac{1}{2}}$; desorption was sometimes much slower and in some cases was still not complete even after $\sqrt{t'}$ or $\sqrt{t''} = 20\ \text{min}^{\frac{1}{2}}$.

Desorption was accomplished by either of two techniques: adjustment of the pH or the addition of salt to the suspension containing the cell-resin complex. The pH of the reaction mixture could be sharply increased or decreased, respectively, upon the addition of concentrated sodium hydroxide or hydrochloric acid. The amount of acid or base required to

titrate a given cell-resin complex to a desired pH value was predetermined in a separate experiment. The acid or base was then added dropwise from a burette as a concentrated solution. The volume of the reaction mixture was diluted to a negligible extent by this titration. Minor adjustment of the pH to an exact value was done by adding small amounts of acid or base with a Teflon spatula as previously described.

Desorption could also be induced by adding a weighed amount of solid salt, usually sodium chloride, directly to the reaction mixture while agitation was in progress. Solution of the salt was rapid. A final salt concentration of one molar or less was used. The settling rate of the resin particles was often retarded due to the increased density of the salt solution. Repeated flushings of the syringe filter were often required during the sampling of these salt solutions in order to collect the full 30 ml liquid volume needed for an absorbance measurement.

4. Resolution of a binary mixture

Several additional steps were required to resolve a mixed suspension containing the cells of more than one bacterial species into its component parts. Binary mixtures were the most complex systems investigated in the current work. The same techniques can be extended to mixtures of higher orders. The five binary mixtures studied in the present work were: Escherichia coli - Bacillus subtilis (Ec-Bs), Escherichia coli - Staphlococcus aureus (Ec-Sa), Escherichia coli - Pseudomonas ovalis (Ec-Po), Staphlococcus aureus - Bacillus subtilis (Sa-Bs), and Bacillus subtilis - Proteus vulgaris (Bs-Pv).

Measured volumes of pure suspensions of cells of two bacterial species, previously adjusted to absorbance values of 0.6, were combined to form a binary mixture. Most of the binary mixtures studied in the current work were prepared by mixing equal volumes of the two component suspensions to yield a total volume of 50 ml. The absorbance ratios, A_{1-2}/A_1 and A_{1-2}/A_2 varied from 1.6 to 1.8. Values of A_1 and A_2 were obtained by diluting portions of the pure component suspensions to twice their original volume. The ion exchange resins used as a selective adsorbent to resolve the binary mixtures were prepared by the same procedures previously described.

The initial phase of adsorption occurring in the resolution of a binary mixture, by either a Type I or a Type II procedure, was similar to the procedure described in the preceding section for the adsorption of a single species. Only the cells of one of the two species present in a binary mixture were adsorbed and then sequentially desorbed in a resolution of Type II. Samples were periodically collected for absorbance measurements during the adsorption phase of either type of resolution. Gram stains were also made at frequent intervals to aid in determining the relative proportions of the cells of the two species present in the suspension phase.

Once equilibrium in the adsorption had been reached, the procedure used in resolving a binary mixture was different from that previously described for the adsorption of the cells of a single species. At this point the cells of one or both of the two species originally present in the binary mixture were considered to be completely adsorbed onto the ion exchange resin. A separation of the resin phase and the suspension phase

was required in either type of resolution. This was achieved by first allowing the resin particles to settle after agitation was halted and then decanting the supernatant liquid. In difficult cases where the resin particles tended to float on the liquid surface, the syringe filter was used to withdraw the liquid. The resin phase, containing the adsorbed bacterial cells and other unadsorbed cells trapped in the interstices of the cell-resin floc, was then transferred to a filter paper placed in a buchner funnel. A series of five 10 ml volumes of distilled water were sequentially passed through the resin. These washings were sufficient to remove any vestige of unadsorbed cells from the resin. These washings were assumed not to contain prematurely desorbed cells and were discarded. The resin was next transferred in a damp condition to 50 ml of a fresh solution containing a suitable agent to promote desorption of the cells of one of the bacterial species.

Desorption was promoted by changes in pH, the addition of salt, or both methods were simultaneously applied. The course of the desorption was followed by measuring the absorbance and preparing Gram stains at predetermined intervals as was done in the adsorption phase of the resolution. In a resolution of Type II, the second desorption was accomplished in a manner identical to that used in the first desorption. The resin phase was separated from the suspension containing the cells of the first species to be desorbed and then washed free of any desorbed cells still clinging to the particles. The damp resin was finally transferred to 50 ml of a second solution containing a suitable agent to promote desorption of the cells of the remaining bacterial species.

The extent of resolution was determined according to several criteria. The equilibrium absorbances of the separated fractions were compared to similar absorbances of the pure component suspensions measured at comparable dilutions, pH and salt conditions. The criterion of absorbance is of limited value, however, if the fractions obtained during the resolution are not of high purity, i.e. containing a predominance of the cells of one species.

Microscopic analyses of the size, the shape, and other morphological characteristics of the cells of each species present can be of value in certain cases. Gram stains of the cells of two species of differing reaction can also be an excellent criterion for evaluating the extent of resolution of a mixture. The most conclusive tests used to distinguish cells of different species are based upon physiological differences. Sterile conditions must prevail during such tests, however, to prevent contamination by extraneous organisms. Physiological tests based upon growth of cells in a given medium or reaction of certain metabolic products are not always advantageous since incubation of the samples is required. Physiological tests were not used in the current work other than for identification of the individual species.

V. EXPERIMENTAL RESULTS

A. Introduction

The four divisions of the statement of intent of the current research were previously outlined in Section I. They are:

1. Establishment of Adsorption
2. Determination of Variables
3. Elucidation of Mechanism and Model
4. Development of Separation Techniques

Each of these divisions will be considered in detail in the present section. The conclusions extended are supported by experimental data and calculations. The nomenclature is consistent with the other sections of this report and is alphabetically listed in Appendix G.

B. Establishment of Adsorption

1. Exchange of bacteria as a surface phenomenon

Aqueous suspensions containing either bacterial cells or particles of ion exchange resin are usually well dispersed when kept under constant agitation. Such dispersions of cells and of resin particles are illustrated, respectively, in Figures 9a and 9b. These dispersed forms are to contrasted with the situation shown in Figure 9c. An immediate flocculation is evident upon the addition of particles of an anion exchange resin to a suspension already containing bacterial cells. The resulting complex of resin particles and adsorbed cells can be easily separated from the unadsorbed cells still remaining in the bulk suspension.

The surfaces of the resin particles are covered in a "pincushion" fashion by the adsorbed bacterial cells. In the higher magnifications of

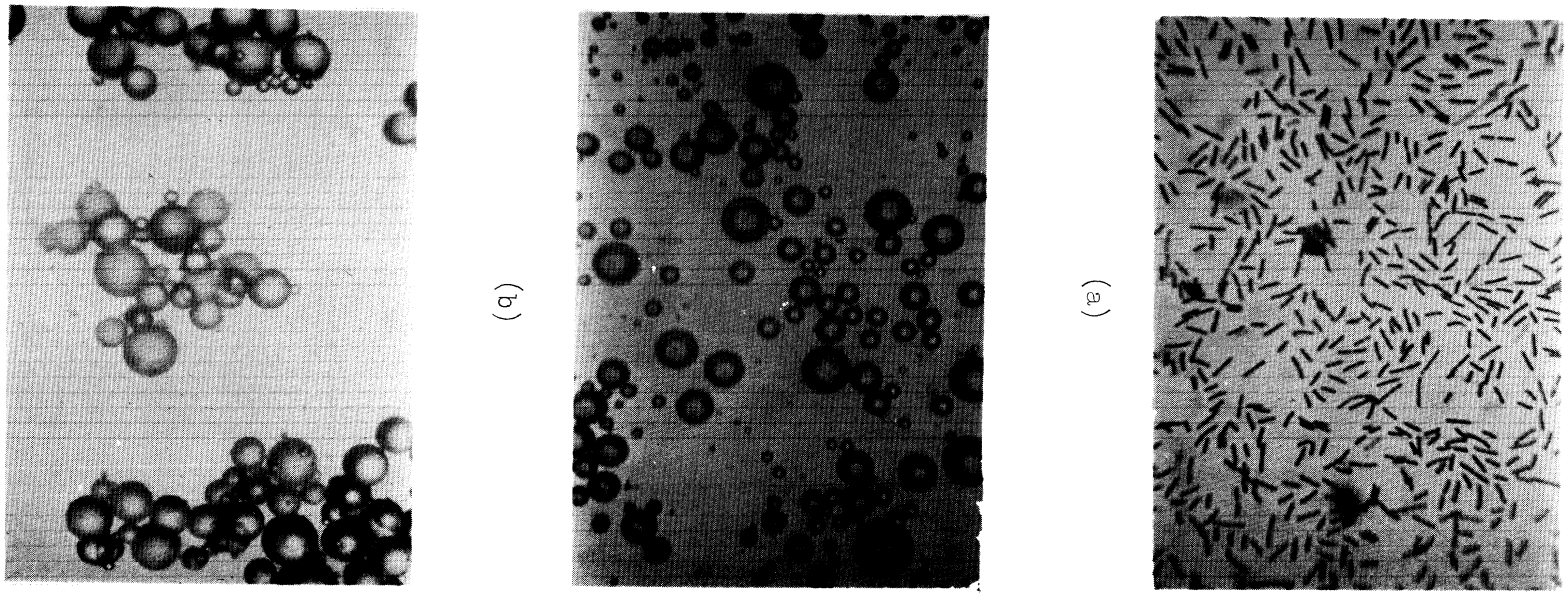
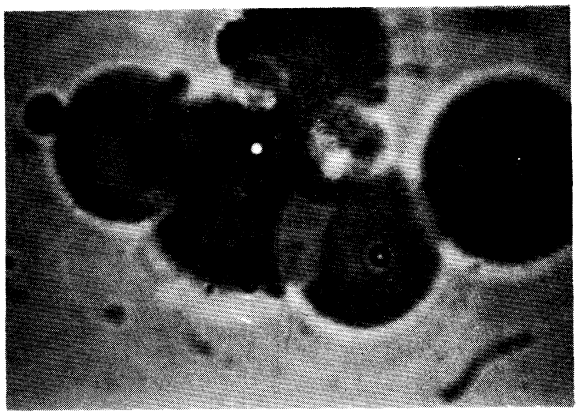
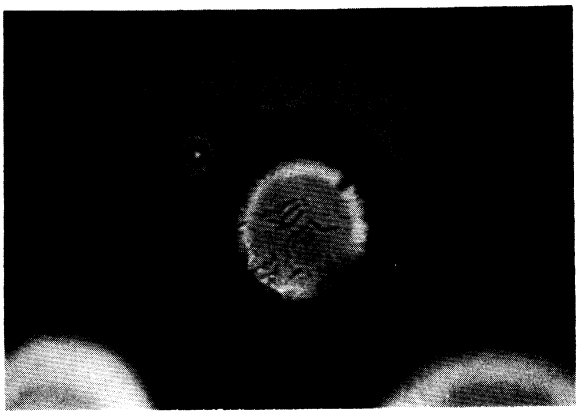


Figure 9. Pictorial representation of the adsorption of bacterial cells onto an anion exchange resin: a. dispersion of bacterial cells in water (X 970), b. dispersion of particles of an anion exchange resin in water (X 100), c. flocculation of particles of an anion exchange resin upon addition to a suspension of bacterial cells (X 100), d. bacterial cells adsorbed onto the surfaces of resin particles (X 430), e. bacterial cells adsorbed onto the surfaces of resin particles (X 970), f. bacterial cells adsorbed onto the surfaces of resin particles (X 970). (Bacterial species: Bacillus subtilis; anion exchange resin: Dowex 1 x 8, 200/400 mesh, chloride form).



(d)



(e)

(f)

Figure 9. Continued.

Figures 9c, 9d, and 9e, the individual bacterial cells can be observed as short, rod-shaped particles that are randomly distributed over the surfaces of the much larger resin particles. The bacterial cells become attached to the resin surfaces in stable longitudinal orientations. The surfaces of the resin particles are impermeable to particles of bacterial size.

Particular note is made of the absence of freely dispersed bacterial cells in the bulk of the liquid surrounding the particles of ion exchange resin. No bacterial cells were allowed to settle since the suspensions were maintained under constant agitation until just before the photomicrographs were taken. A few cells may have physically enmeshed themselves within the existing cell-resin floc, however, if they were not otherwise capable of adsorption. It is concluded upon the bases of these observations that bacterial cells can be successfully withdrawn from suspension by a process of true adsorption involving ion exchange.

The rate of adsorption of bacterial cells onto particles of ion exchange resin is a function of the average particle diameter of the resin. The motion of a given particle of resin and its frequency of collision with bacterial cells are governed by the size of the much larger resin particle. The rate of adsorption should increase, therefore, if the particle diameter is decreased since a greater expanse of surface area is made available per unit mass of resin. The capacity of a given resin for bacterial cells, determined after the adsorption has reached a state of equilibrium, is also a function of the available surface area of the resin. This available area is inversely proportional to the average particle diameter.

A series of adsorptions of bacterial cells onto equal masses of several resin fractions of differing average particle diameter is shown in Figure 10. The surface area available for adsorption of bacterial cells is increased by a factor of four-fold for each of the fractions proceeding in the order of increasing mesh number (decreasing particle diameter). The previous prediction of increased rates of adsorption for the smaller particle sizes is substantiated by the results obtained for this series. The prediction of greater capacity of the resin for bacterial cells upon decreasing the particle size is not readily apparent. The concentrations of bacterial cells in suspension used in this series were such that only ten percent of the available surface area of the resin fractions having the largest average particle diameter would be saturated if all of the bacterial cells initially in suspension became absorbed. The degrees of saturation of the surfaces of the resin fractions of smaller average particle diameter are correspondingly less. The concentrations of bacterial cells still remaining in suspension after equilibria have been attained in the cases of the four smaller particle size fractions were not determined because of instrumental limitations. This should also have been the case for the adsorption involving the largest particle size fraction.

The considerable difference between the equilibrium concentrations of cells determined for adsorption times approaching one hour for both the largest fraction and the second largest fraction may be due to two factors. The rate of adsorption for the former may have been relatively slow due to the excessive turbulence or abrasive action created by the larger particles. The average diameter of the particles in the

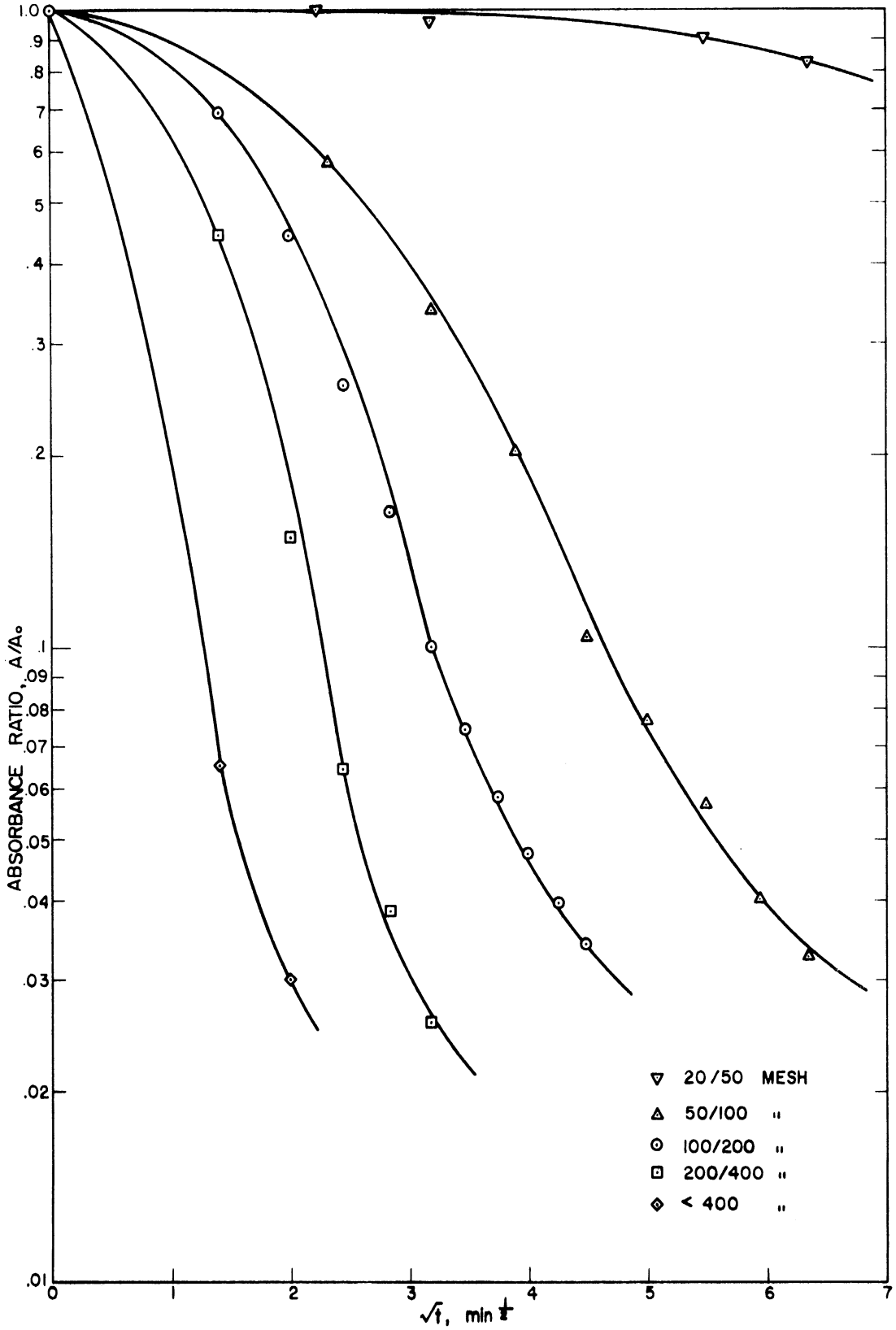


Figure 10. Adsorption of Bacteria onto an Anion Exchange Resin as a Function of Resin Particle Size (Bacterial species: Bacillus subtilis; anion exchange resin: Dowex 1 x 8, chloride form, 10 grams/50 ml, pH 3.5).

former may also have been larger than a critical size beyond which a stable resin-floc could not develop. These conjectures can only be proven through additional experimentation.

The surface-limiting character of the adsorption of bacterial cells by ion exchange resins is further depicted in Figures 15 and 18. In Figure 15 both the mass and the particle size of the ion exchange resin are held constant while the concentration of bacterial cells is increased to a point beyond saturation of the available surface area. In Figure 18 the particle size of the resin and the concentration of bacterial cells are held constant while the mass of resin is increased to a point beyond which saturation is impossible.

The capacity, q_∞, of an ion exchange resin for adsorbing cells of a particular bacterial species can be determined for those experiments in which the total number of bacterial cells initially present in suspension was greater than the number that could be accomodated on the available surface area of the resin. The saturation ratio, γ, defined by Equation (III-37) must first be extrapolated to infinite time. Two

$$\gamma = \frac{1}{1 - A/A_o} \overset{t \to \infty}{=} \frac{n_o V}{q_\infty m} \qquad \text{(III-37)}$$

examples of this procedure are illustrated in Figure 11. The data obtained for the adsorptions shown in Figure 18 are replotted as $\log(\gamma)$ versus $1/\sqrt{t}$. The extrapolation to $1/\sqrt{t} = 0$ corresponds to infinite time. The calculations of the capacity of this resin for bacterial cells is described in Appendix E of this report.

An average value of $q_\infty = 0.610 \times 10^{10}$ cells/gram was calculated for the cells of Bacillus subtilis adsorbing onto the anion exchange

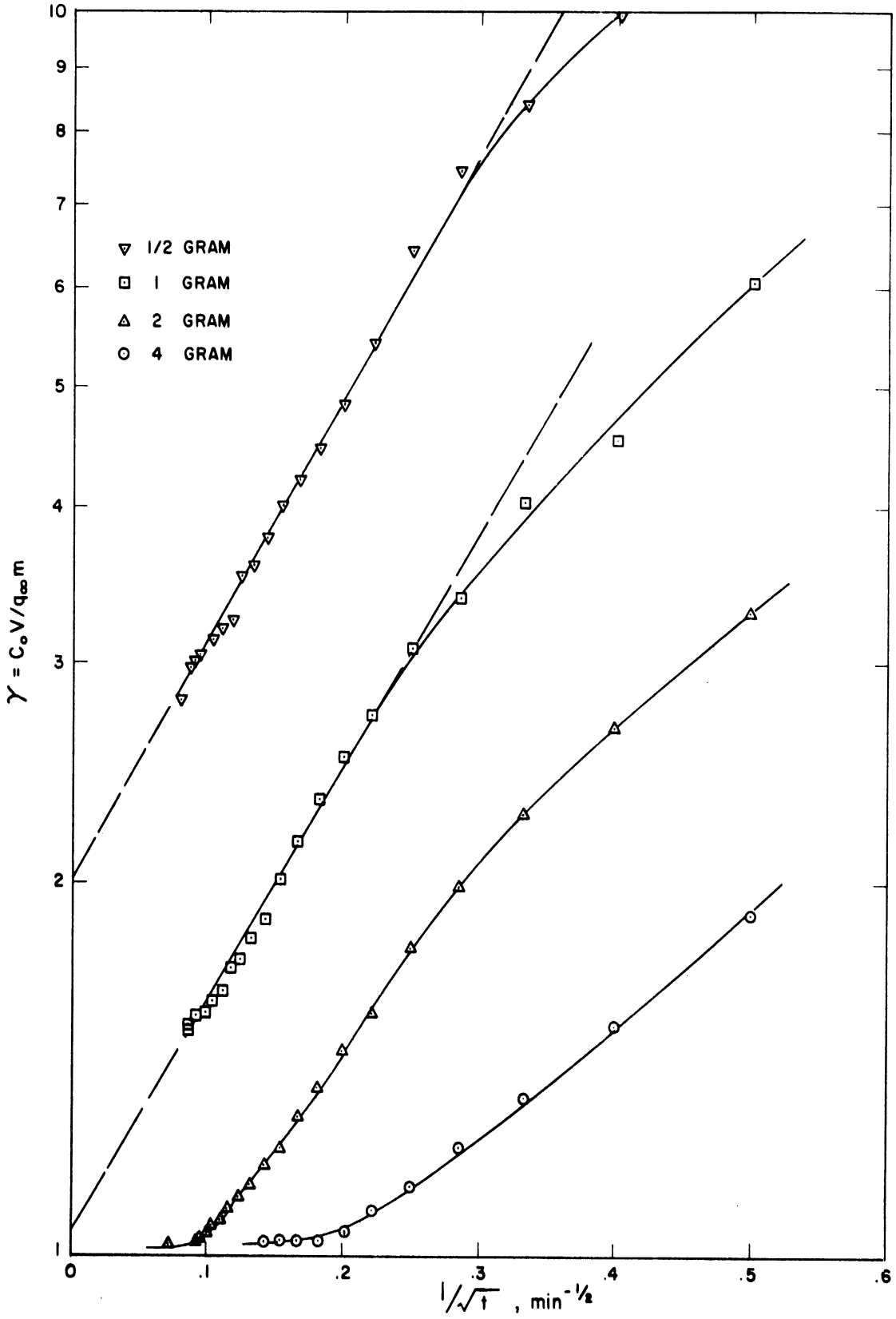


Figure 11. Determination of the Capacity of an Anion Exchange Resin for Bacterial Cells (Bacterial species: Bacillus subtilis, A_o = 0.600, pH 3.5; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form. The corresponding adsorptions are depicted in Figure 18).

resin, Dowex 1 x 8, 100/200 mesh, chloride form at pH 3.5. The predicted capacity for this same system assuming an average cell size of 0.75 x 2.5 μ is $q_\infty = 1.98 \times 10^{10}$ cells/gram. Only 30.8% of the predicted capacity of the resin was saturated in the experimental adsorption. Each bacterial cell which adsorbed onto the resin surface apparently occupied slightly more than three times its projected cross-sectional area if the longitudinal orientation was assumed for the most stable adsorption. The capacity of the resin for adsorbing bacterial cells is probably reduced by the random distribution of the adsorbed cells. The available surface area is therefore less efficiently covered. Small areas that remain uncovered are too small to permit additional bacterial cells to gain a "foothold" for adsorption.

The removal of suspended cells in columns containing ion exchange resin is similar to the adsorption of dissolved ions except that interpolation of the process may be obscured by attendant filtration. Sorptions in batch systems, where filtration is an insignificant factor, were of primary concern in the present research. A discussion of column sorption has been relegated to Appendix F of this report.

2. Adsorption as a reversible exchange process

The adsorption of bacterial cells onto particles of an ion exchange resin can be considered to be a reversible process. Curves describing the adsorptions of cells from suspensions of six different bacterial species onto separate fractions of the same anion exchange resin are presented in Figure 12. The pH values of the suspensions during the adsorptions of these bacteria were assumed to be at or above the

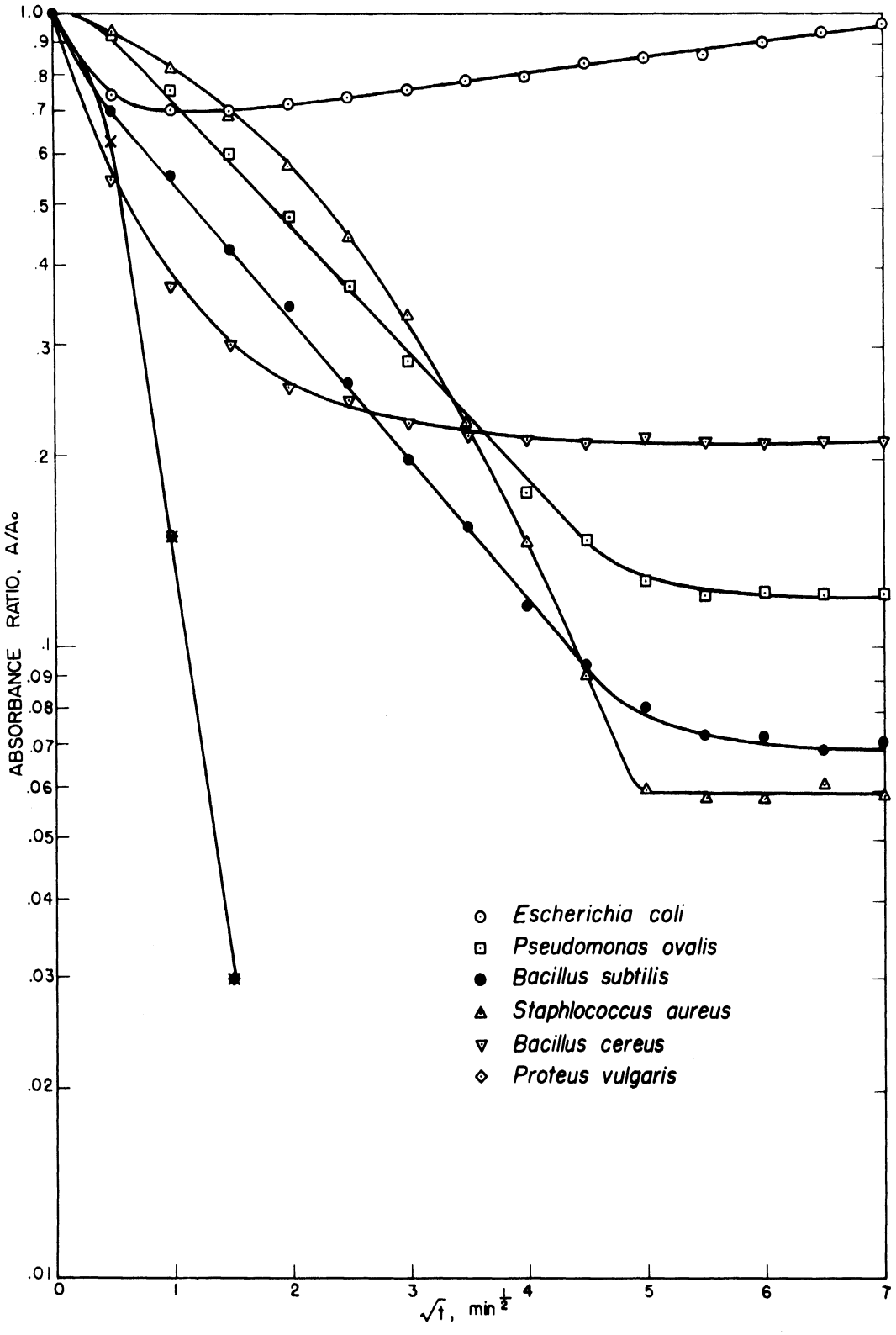


Figure 12. Adsorption of the Cells of Six Bacterial Species onto an Anion Exchange Resin (Bacterial species: Escherichia coli, A_o = 0.596, pH 3.5, Pseudomonas ovalis, A_o = 0.371, pH 4.0, Bacillus subtilis, A_o = 0.616, pH 1.5, Staphlococcus aureus, A_o = 0.313, pH 3.5), Bacillus cereus, A_o = 0.598, pH 3.5, and Proteus vulgaris, A_o = 0.596, pH 4.5; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml. The corresponding desorptions are depicted in Figure 13).

effective isoelectric points of the cells of the respective species with the possible exception of Escherichia coli. The cells of the other five bacterial species, Bacillus subtilis, Staphlococcus aureus, Pseudomonas ovalis, Bacillus cereus, and Proteus vulgaris, were all strongly adsorbed under similar conditions.

The cells of Escherichia coli were initially adsorbed onto the resin to a slight degree. As time progressed the process was reversed and the cells became totally desorbed as indicated by the return of the absorbance ratio, A/A_o , to its original value of unity. This behavior may be due to a reversal of charge, an increase in the salt concentration in the bulk suspension, or a mechanical desorption as a result of extended agitation. The latter explanation is the most plausible since cellular debris was observed in the suspension upon microscopic analysis. The cells of the other five species can be also dislodged from the resin during extended periods of agitation. The time required for the mechanical desorption of bacteria to become significant is relatively long for the cells of Bacillus subtilis and Staphlococcus aureus compared to the cells of Escherichia coli as shown in Figure 25.

The adsorptions depicted in Figure 12 can be reversed by purely chemical means. These desorptions are illustrated in Figure 13. The previous adsorption-desorption curve described by the cells of Escherichia coli is repeated only for comparison. The cells of the other bacterial species were desorbed by altering the pH of the individual suspensions, by adding solid salt to the suspensions, or by conducting both procedures simultaneously. Mechanical desorption was not considered to be a significant factor in the desorptions of the cells of these species.

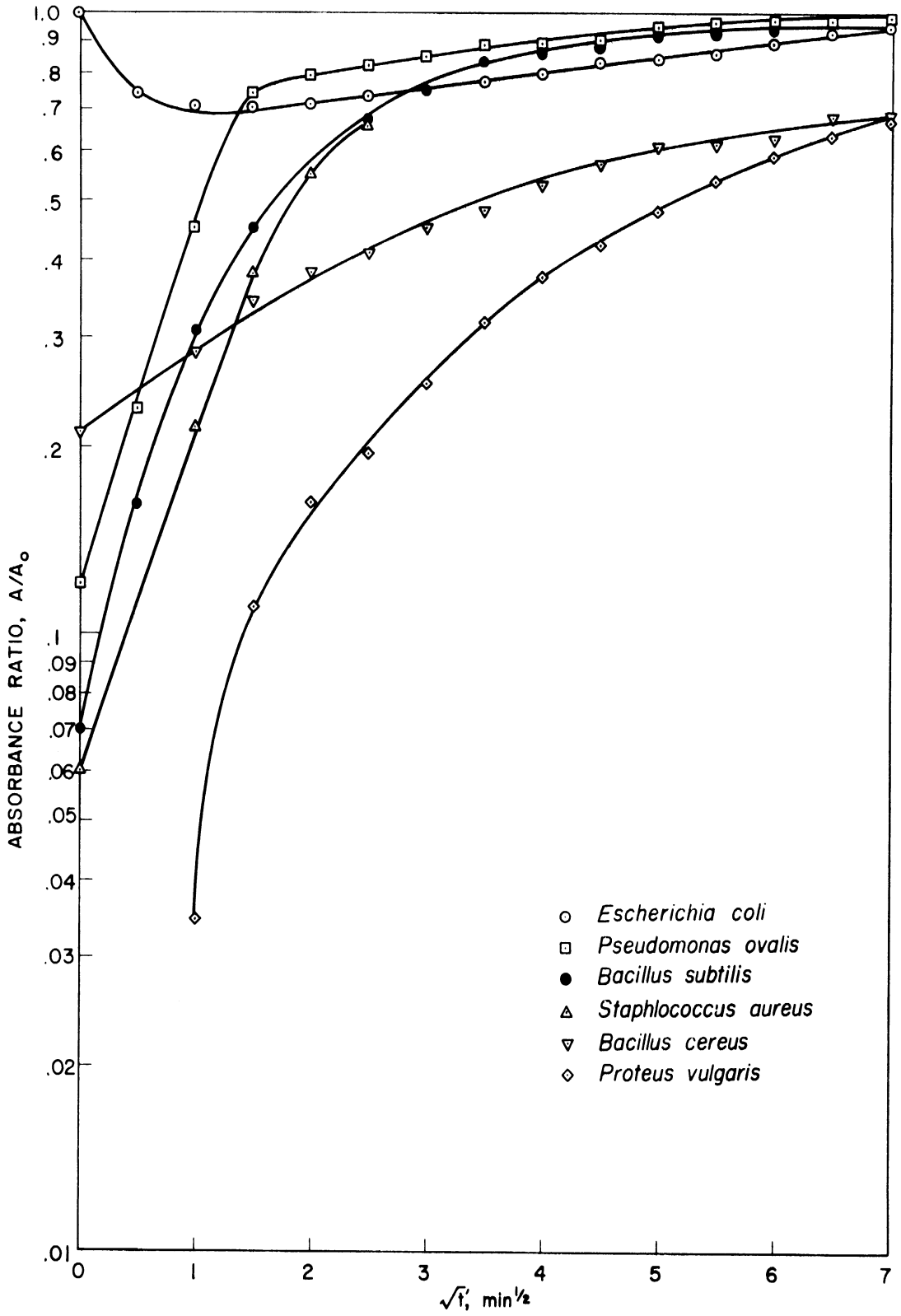


Figure 13. Desorption of the Cells of Six Bacterial Species from an Anion Exchange Resin (Bacterial species: Escherichia coli, A_0 = 0.596, pH 3.5, Pseudomonas ovalis, A_0 = 0.371, pH 1.0, Bacillus subtilis, A_0 = 0.616, pH 1.5, 1M KCl, Staphlococcus aureus, A_0 = 0.313, pH 1.5, Bacillus cereus, A_0 = 0.598, pH 1.5, and Proteus vulgaris, A_0 = 0.596, pH 5.5, 1M KCl. The corresponding adsorptions are depicted in Figure 12.)

Desorption of the cells of Bacillus cereus, Pseudomonas ovalis, Staphlococcus aureus, were affected by lowering the hydrogen ion concentrations of each suspension to approximately pH 1.5 by the drop-wise addition of concentrated hydrochloric acid. A reversal of the surface charges of cells of these three species was thus promoted as the pH values of the suspensions were lowered below the apparent isoelectric points of the cells. The bacterial cells then behaved as cations rather than anions and showed no interest ionically in the anion exchange resin. The cells of Proteus vulgaris, because of their susceptibility to coagulation by acid alone, were desorbed at pH 5.5 with 1 M potassium chloride. The cells of Bacillus subtilis could not be readily desorbed by simply lowering the pH or by adding solid salt to the suspension. The combined conditions of low pH and the addition of potassium chloride were required to effect a reasonable rate of desorption of the cells of this species.

On the bases of these observations a relatively high apparent isoelectric point is indicated for the cells of Escherichia coli and a very low one for the cells of Bacillus subtilis. The cells of the remaining four bacterial species are considered to have apparent isoelectric points in a range intermediate between those of Escherichia coli and Bacillus subtilis. The apparent isoelectric points of the cells of these six bacterial species are tabulated in Table XXIX.

The rate and the degree of both the adsorption and the desorption of bacterial cells contacted with anion exchange resins are found, therefore, to be strongly dependent upon the pH and the salt content of the surrounding medium. The sorption of bacterial cells by a cation exchange resin is a similar process. Adsorption onto a cation exchange

resin, however, must occur below, rather than above, the apparent isoelectric points of the contacted cells. Desorption of these cells from a cation exchange resin can then be effected by increasing the pH of the suspension by the drop-wise addition of a concentrated base such as sodium hydroxide, the addition of solid salt, or by a combination of the two procedures.

Four distinct types of sorption exhibited by bacterial cells in contact with an anion exchange resin are recognized in the pH range represented in Figures 12 and 13. These are: limited adsorption which becomes self-reversing (Escherichia coli), strong adsorption with desorption occurring by charge reversal upon pH reduction (Staphlococcus aureus, Pseudomonas ovalis, and Bacillus cereus), strong adsorption with desorption promoted by the addition of salt (Proteus vulgaris), and very strong adsorption with desorption promoted only by the combined actions of low pH and the addition of salt (Bacillus subtilis).

A discharge of soluble ions from the resin simultaneously occurs upon the adsorption of bacterial cells onto the resin. This phenomenon of ion exchange is illustrated in Figure 14 by the reduction in pH upon the adsorption of bacterial cells from a series of suspensions containing equal quantities of resin but different concentrations of cells. The simultaneous decrease in the numbers of unadsorbed cells present in the suspensions of the same series as determined by absorbance measurements are shown in Figure 15. As the proportion of adsorbed cells is increased, the respective pH value of the suspension is decreased. The reduction in pH is partially compensated in the more concentrated cell suspension by the increased buffering capacity of the suspended cells. A loss of

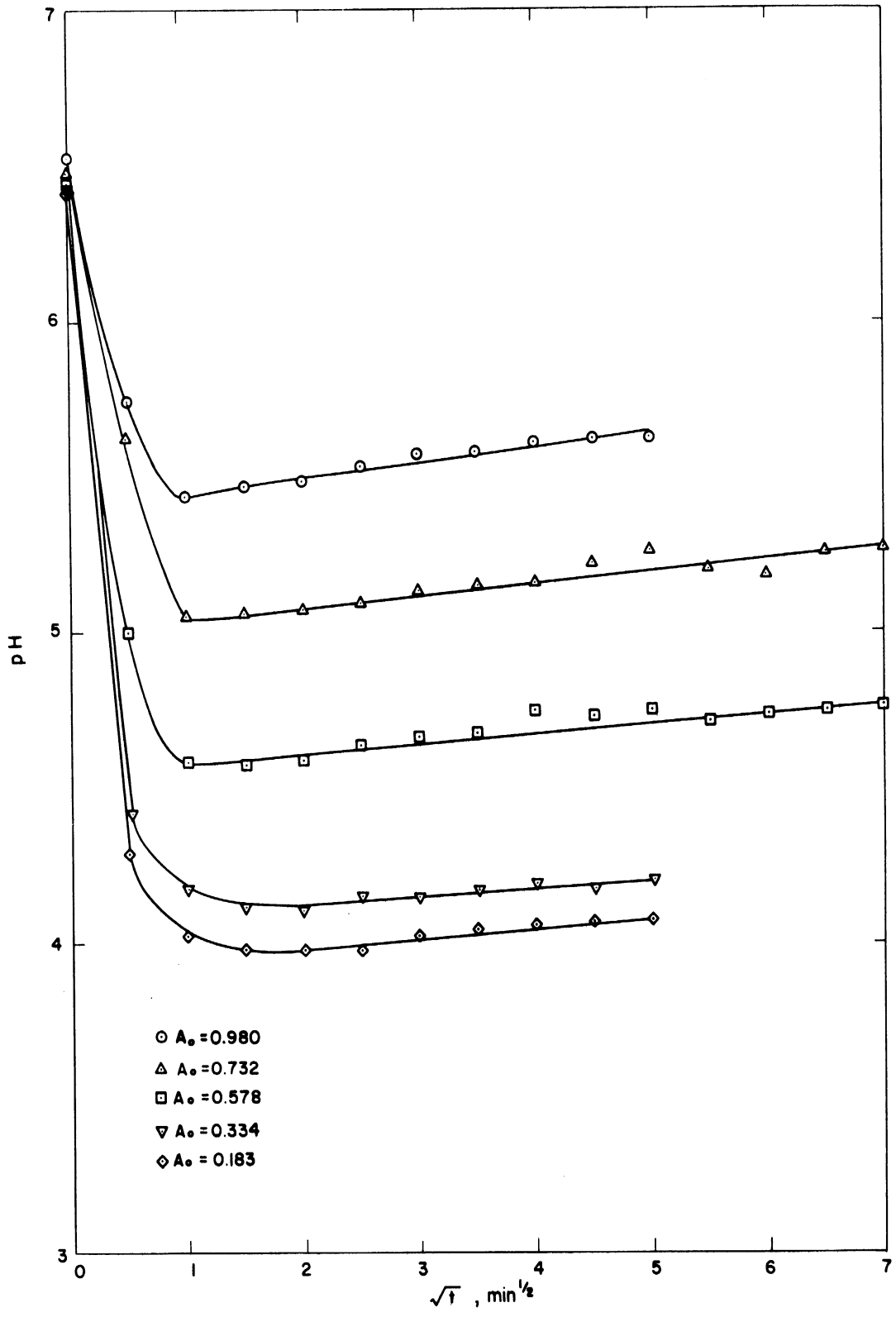


Figure 14. Changes in pH Accompanying the Adsorption of Bacterial Cells onto an Anion Exchange Resin as a Function of the Initial Concentration of Bacterial Cells (Bacterial species: Bacillus subtilis; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml. The corresponding adsorptions are depicted in Figure 15).

hydrogen ions from the bulk solution may also occur through permeation into the interiors of the cells or of the resin particles.

C. Determination of Variables

The variables that affect the interactions occurring between bacterial cells and particles of an ion exchange resin can be associated with: (1) the organism undergoing the exchange adsorption, (2) the absorbent promoting the exchange, and (3) the environment in which the process is occurring. A list of selected variables to be considered in this section is given in Table XXVIII.

1. Species of bacteria

The species of the bacterial cells adsorbing onto a given ion exchange resin is the most important single variable to be considered. The adsorptions and desorptions of the cells of six representative species of bacteria were previously presented in Figures 12 and 13. The development of the separation techniques discussed in Section V-E of this report are based upon differences in the characteristic sorptions of the cells of these species.

2. Culture medium

All six species of bacteria studied were grown in culture media of the composition described in Section IV of this report. Various dissolved or suspended nutrients, buffers, etc., can be very influential in establishing the magnitude of the surface charges of the developing bacterial cells. The effects of varying the type and composition of the growth medium upon the subsequent sorption of the cultured cells

TABLE XXVIII

VARIABLES AFFECTING THE SORPTION OF BACTERIA BY ION EXCHANGE RESINS

Variables associated with the bacterium

* 1. Species of Bacterium

2. Culture medium

3. Culture age

4. Suspension medium

* 5. Concentration of cells

Variables associated with the ion exchange resin

* 6. Type of resin

7. Ionic form

* 8. Particle size

*10. Concentration of resin

Variables associated with the environment

*11. Hydrogen ion concentration

*12. Salt concentration

*13. Agitation

*14. Time of contact

*15. Temperature

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* Considered in some detail in this report

were not investigated. The related literature, however, has been reviewed in Section III-A of this report.

3. Culture Age

All bacterial cells used in these experiments were grown for 24 hours in an agitated medium. There have been some statements made in the literature reviewed in Section III-A that the electrical charges of bacterial cell surfaces vary considerably throughout the early stages of cell growth. The magnitude and distribution of these charges usually stablize within 24 hours or less in rapidly growing cultures. The cell suspensions in the present study were stable for at least one eight-hour day of experimentation following culture, centrifugation, washing, and filtration. The "shelf-lives" of these purified suspensions can be extended by refrigeration. The effects of the age of the suspended cells upon their subsequent sorption were not evaluated in detail in the current research.

4. Suspension medium

Bacterial cells suspended in distilled water were used in the majority of the experiments reported in this thesis. In a few cases dilute buffers were used to counteract any pH changes that were produced by the ions released from the ion exchange resins during the sorption of the bacterial cells. Buffer salts were used sparingly, however, since changes in pH were excellent indices of the degree of adsorption or desorption of cells. Desorption of cells from a resin was accomplished in some instances by the addition of solid salt, such as sodium chloride. The absorbance of a cell suspension is often altered by the addition of

salt as a buffer or as an agent for desorption. Such changes in absorbance must therefore by recognized if absorbance is to be a valid index of adsorption or desorption.

The sorption of bacterial cells suspended in other media, such as culture broth, waste-water, etc., were not evaluated in detail. Other ions present in these more complex suspensions can interfere to some degree with the sorption of bacterial cells. Such difficulties are not insurmountably in actual practice since bacteria in their native state have been observed to interact with soil particles, bacteriophages, etc.

5. Concentration of cells

The effects of varying the concentration of cells of Bacillus subtilis in suspension upon the subsequent adsorption of the cells are shown in Figure 15. The instrumental limitations are apparent for absorbances less than 0.02. The background absorbance, due to dissolved substances, cellular debris, etc, becomes significant below this level. The corresponding reductions in pH values for this same series of adsorptions were previously presented in Figure 14.

The rate of adsorption was found to increase upon decreasing the initial concentration of bacterial cells in the suspension. This effect may be due to the steeper gradients in pH values developed upon adding resin to the more dilute suspensions. If the degree of agitation is held constant, and the concentration of cells is increased, the rate of adsorption theoretically should increase since the probability of the cells colliding with a resin particle reaching stable adsorption sites is

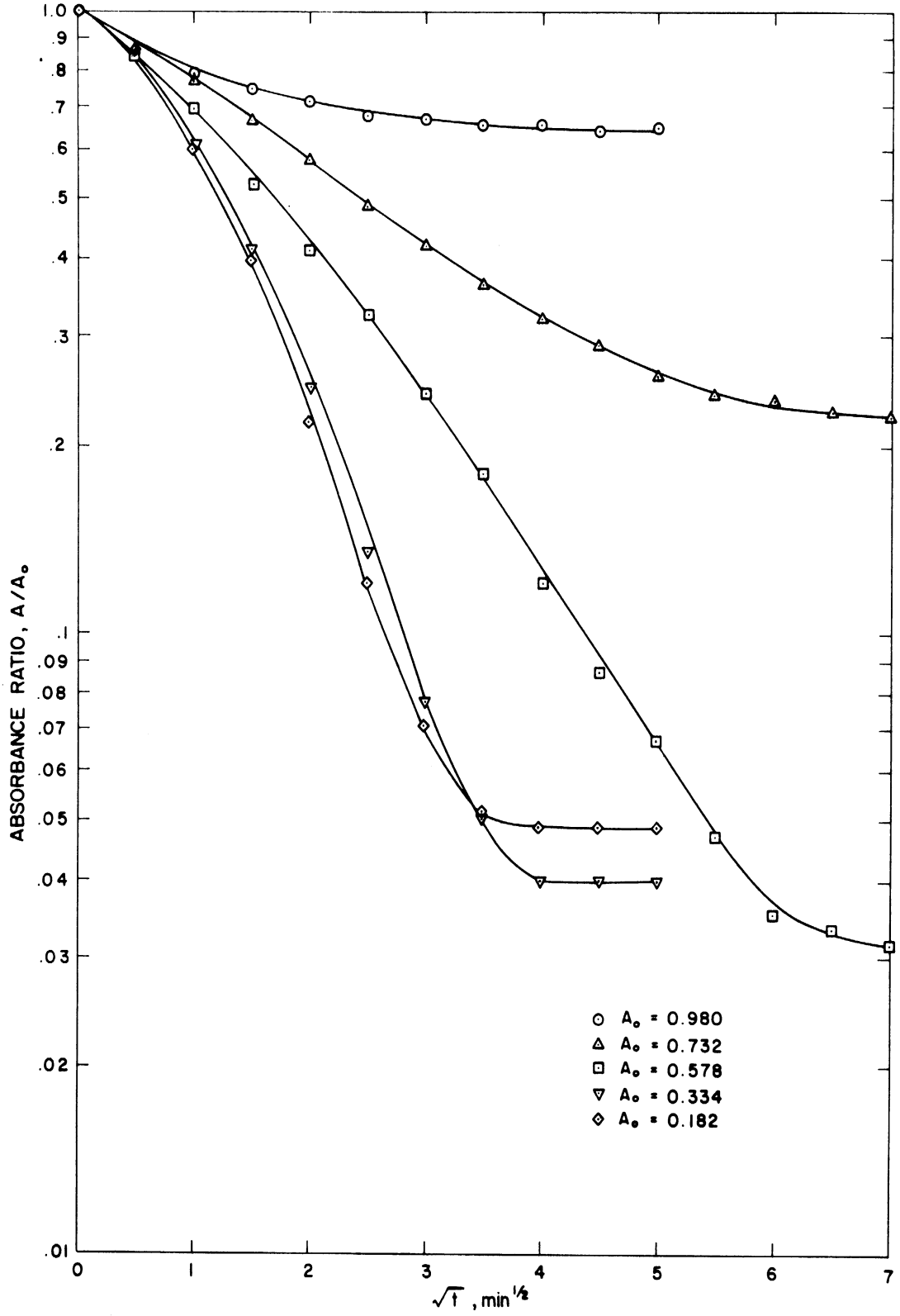


Figure 15. Adsorption of Bacterial Cells onto an Anion Exchange Resin as a Function of the Initial Concentration of Bacterial Cells (Bacterial species: Bacillus subtilis; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml. The corresponding pH changes for these adsorptions are depicted in Figure 14.)

also increased. The final equilibrium value of the absorbance ratio is a function of the degree of saturation of the available resin capacity. Rate constants obtained for varying cell concentrations are presented in Table XXXI.

6. Type of resin

Cells of a particular bacterial species are theoretically capable of adsorbing onto either an anion or a cation exchange resin. The adsorptions of cells of Bacillus subtilis onto the anion exchange resin, Dowex 1 x 8, and onto the cation exchange resin, Dowex 50W x 8, at pH values of 1.47 and 0.98, respectively, are shown in Figures 16 and 17. The particle sizes and concentrations of both resins were identical in these experiments.

If the pH of a suspension is above the isoelectric points of the suspended cells then a decrease in the pH will generally lead to a corresponding decrease in the degree of anion exchange and to an increase in the degree of cation exchange. The cells of Bacillus subtilis behave more as anions than as cations at pH 1.47 and, therefore, preferentially adsorb onto the anion exchange resin at this pH as shown in Figure 16. This situation is reversed at pH 0.98 as shown in Figure 17. At this pH very little affinity for adsorption onto the anion exchange resin is shown by the cells of Bacillus subtilis which behave almost exclusively as cations.

7. Ionic form

Conclusions of a limited nature can be made regarding the effect of the ionic form of the resin upon the adsorption of bacteria.

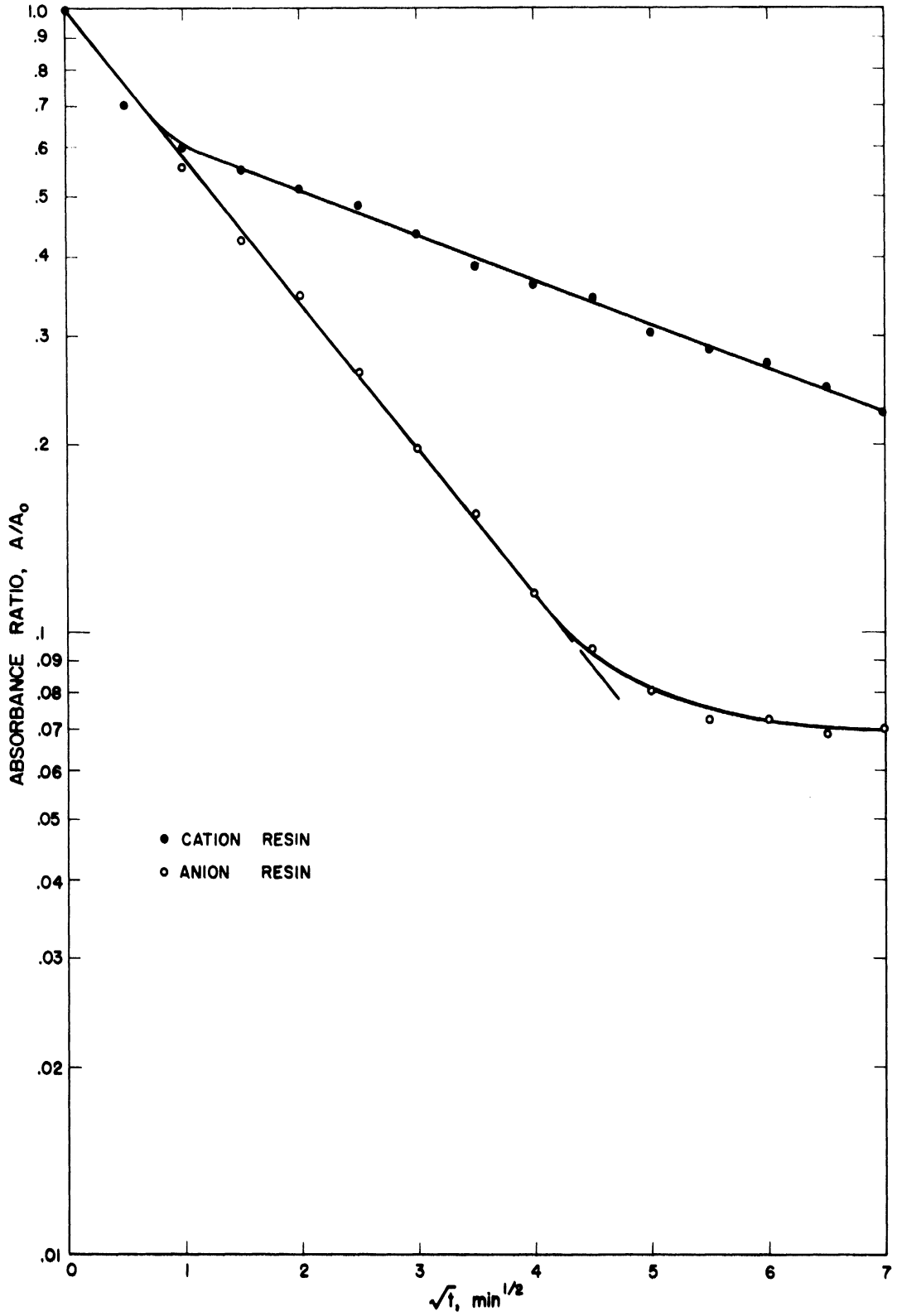


Figure 16. Preferential Adsorption of Bacterial Cells onto an Action Exchange Resin vis-a-vis a Cation Exchange Resin (Bacterial species: Bacillus subtilis, A_o = 0.616, pH 1.47; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml; cation exchange resin: Dowex 50W x 8, 100/200 mesh, hydrogen form, 4 grams/50 ml.

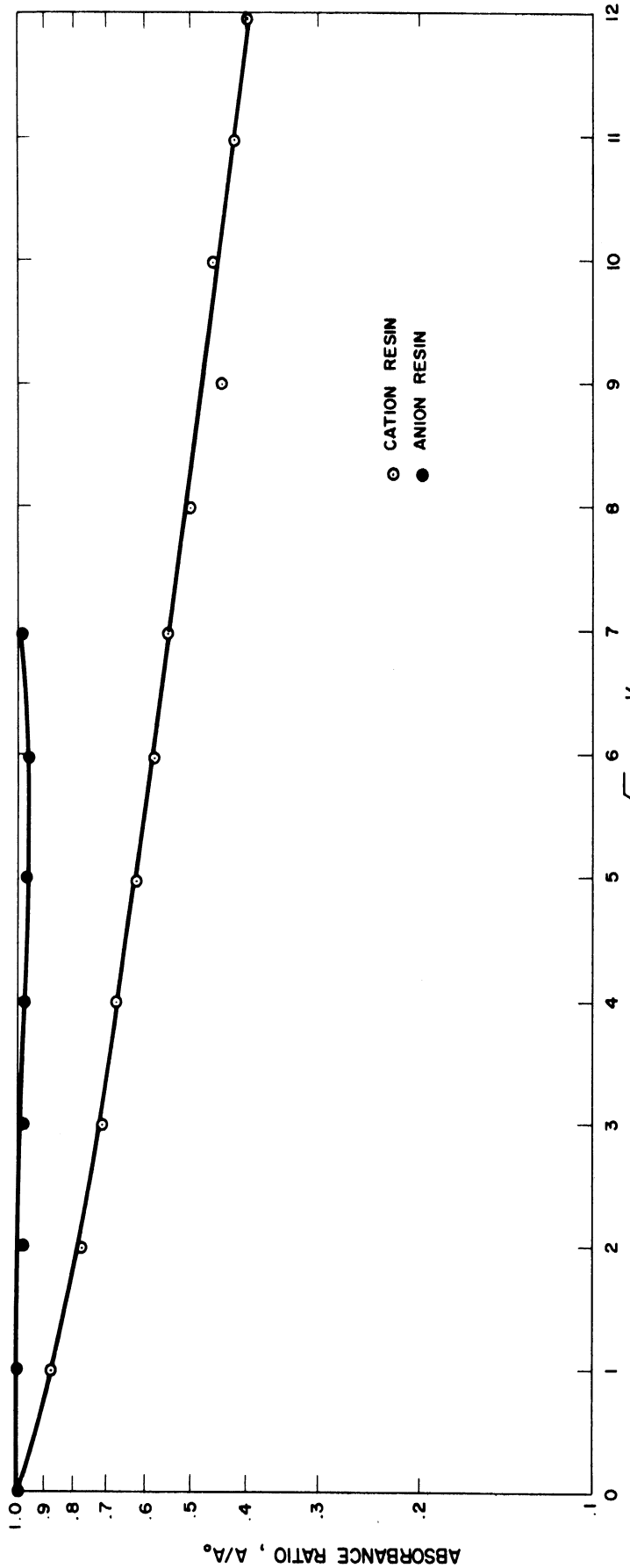


Figure 17. Preferential Adsorption of Bacterial Cells onto a Cation Exchange Resin vis-a-vis an Anion Exchange Resin (Bacterial species: Bacillus subtilis, A_o = 0.600, pH 0.98; cation exchange resin: Dowex 50W x 8, 100/200 mesh, hydrogen form, 4 grams/50 mL; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 mL).

The chloride form of the anion exchange resin, Dowex 1 x 8, was used in most of these experiments. The cell-resin mixtures were sampled by the syringe filter technique. The chloride form was found to be compatible with suspensions of bacterial cells. Changes in the pH of suspensions contacted with this resin were excellent indices of the degree of adsorption.

The hydroxide, nitrate, and acetate forms of Dowex 1 x 8 were also investigated during the initial stages of this research. The cell-resin mixtures tested using these forms were sampled by removing the resin containing the adsorbed cells by filtration through paper and measuring the absorbance of the filtrate containing the unadsorbed cells. This method is not considered to be acceptable since unadsorbed cells were also removed by the filtration. The following series of decreasing affinities of these anionic forms for cells of Bacillus subtilis is therefore qualitative:

$$Cl^- \geqslant NO_3^- > OH^- > CH_3COO^-$$

The exact order of this series may also vary with the species of bacteria undergoing adsorption. Several anion exchange resins reported in the literature that appear to be capable of adsorbing bacteria are listed in Table XII.

The hydrogen form of the cation exchange resin, Dowex 50W x 8, was tested in the majority of the cation exchange experiments. Sampling was accomplished by the syringe filter technique. Contact of the hydrogen form with suspensions of bacterial cells resulted in considerable reductions in pH if adsorption occurred.

Little capacity for adsorption of bacterial cells was observed for either the sodium or calcium forms of Dowex 50W x 8. The cell-resin mixtures in the experiments involving the latter two ionic forms were sampled by decanting off the supernatant liquid containing the unadsorbed cells. This method was also found to be unacceptable since fine particles of the resin were also entrained in the supernatant liquid. The following series of decreasing affinities of these cationic forms for cells of Bacillus subtilis is therefore qualitative

$$H^+ \gg Na^+, Ca^{++}$$

Again, the exact order of such a series of selectivity is dependent upon the species of bacteria undergoing adsorption. Several cation exchange resins reported in the literature that appear to be capable of adsorbing bacteria are listed in Table XIII. Qualitative series of adsorption affinities of several bacterial species for cation exchange resins were also reported by Zvyagintsev (1962).

8. Particle size

The adsorption of bacteria by an ion exchange resin is a function of the particle size of the resin. The adsorptions of cells of Bacillus subtilis by equal weights of five resin fractions of differing particle size were previously illustrated in Figure 10. The adsorption of bacterial cells onto particles of an ion exchange resin was therefore concluded to be a true surface phenomenon.

9. Crosslinkage

The effects of the degree of crosslinkage of the ion exchange resin upon the adsorption of bacterial cells were not investigated in the

current study. Resins of low crosslinkage have greater affinities for bacterial cells according to the literature reviewed in Section II. Pertinent references are those of Kuwajima et al. (1957) and Kurozumi et al. (1965).

10. Concentration of resin

The adsorption of bacterial cells by an ion exchange resin can be influenced by the concentration of resin particles. This is illustrated in Figure 18 for the case of the adsorption of cells of Bacillus subtilis from a series of suspensions each containing 1.25×10^8 cells/ml onto $\frac{1}{2}$, 1, 2, and 4 grams, respectively, of the anion exchange resin, Dowex 1 x 8. Complete adsorption of almost all cells was achieved in the suspensions containing 2 and 4 grams of resin; incomplete adsorption occurred in the suspensions containing 1 and $\frac{1}{2}$ grams of resin. The capacity of this resin for bacterial cells was computed with the aid of Figure 11 using values of the saturation ratios determined for the adsorptions by $\frac{1}{2}$ and 1 gram of resin. The rate of adsorption increased upon increasing the resin concentration. Rate constants for all four tests are presented in Table XXXI.

11. Hydrogen ion concentration

The pH of the suspension containing the cell-resin mixture is very important in determining the character of the resulting exchange. Cells of a particular bacterial species can be either positively or negatively charged depending upon the pH of the suspension. The cells are positively charged and behave as cations at pH values acidic to their apparent isoelectric points; at pH values basic to their isoelectric points they are negatively charged and behave as anions.

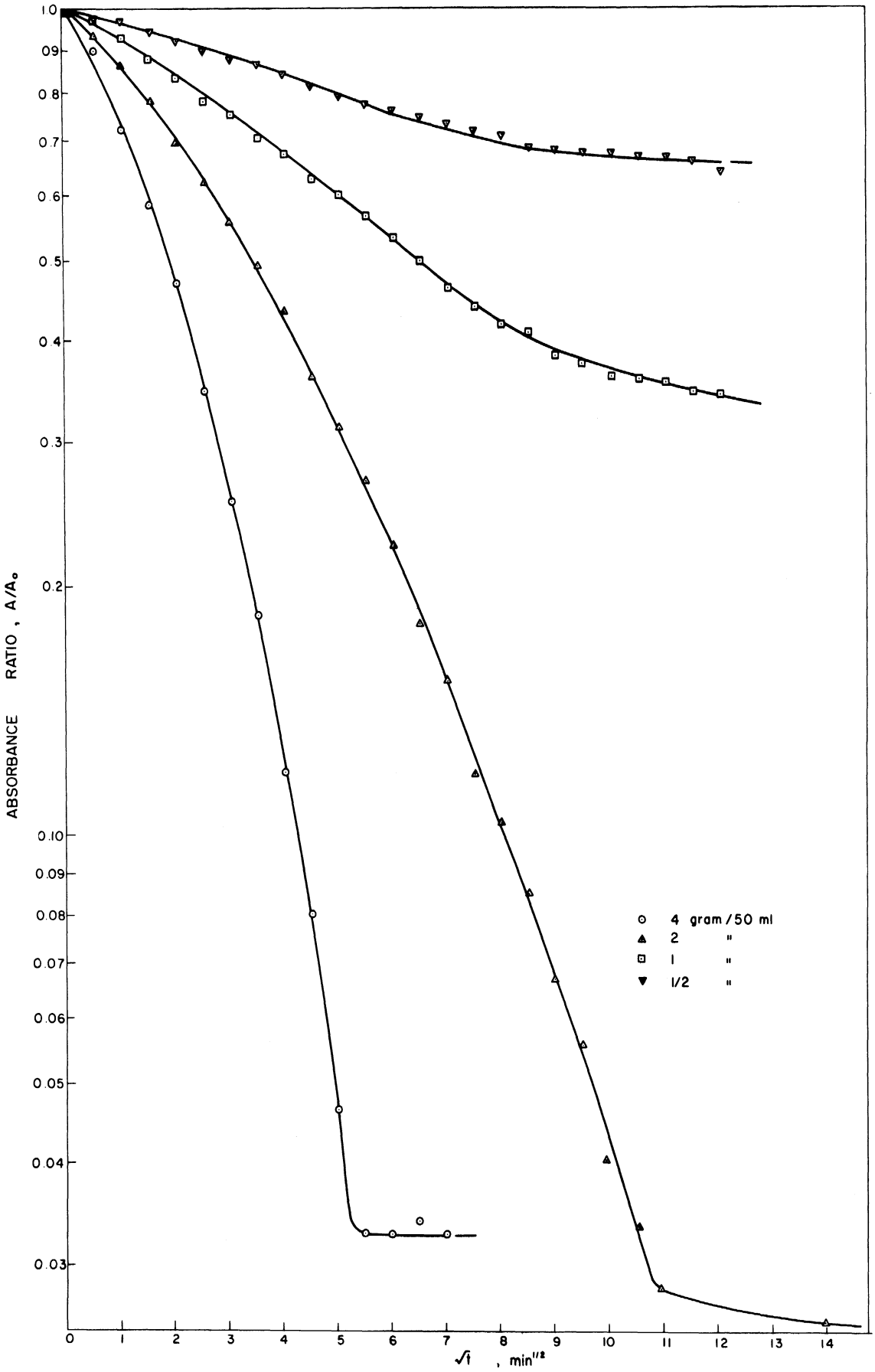


Figure 18. Adsorption of Bacterial Cells onto an Anion Exchange Resin as a Function of the Concentration of Ion Exchange Resin (Bacterial species: Bacillus subtilis, A_o = 0.596, pH 3.5; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form).

The dependence of the rates of adsorption and desorption upon the pH of the suspension are illustrated, respectively, in Figures 19 and 20. The experimental systems contained cells of Bacillus subtilis and particles of the anion exchange resin, Dowex 1 x 8. All variables other than the initial pH values of the suspensions were held constant. Changes in the pH of the bulk solutions produced by the ions exchanged from the resin were overshadowed by the initial adjustment of the pH with concentrated acid. The rates of adsorption were comparable in the range pH 1.5 to 5.5.

The effect of the pH upon the desorption of cells of Bacillus subtilis was somewhat more pronounced than for adsorption as shown in Figure 20. The cells adsorbed in the previous series of experiments were desorbed by adding sodium chloride in solid form to make each suspension 1 M in salt concentration. The average pH values for the corresponding adsorptions are in parentheses. The increases in pH that occurred upon desorption of the cells may be due to "readsorption" of the ions producing the reduction in pH during the initial adsorption of the cells.

The degree of adsorption is a function of the pH of the reacting mixture of bacterial cells and resin particles. This is shown in Figure 21 for the system Bacillus subtilis - Dowex 1 x 8. The absorbances of a cell suspension without resin present are shown in the upper curve. The absorbances of a series of cell suspensions in which the pH values were adjusted prior to the addition of the ion exchange resin are represented in the lower curve. The cells of Bacillus subtilis strongly adsorb onto the anion exchange resin over a wide range of pH values.

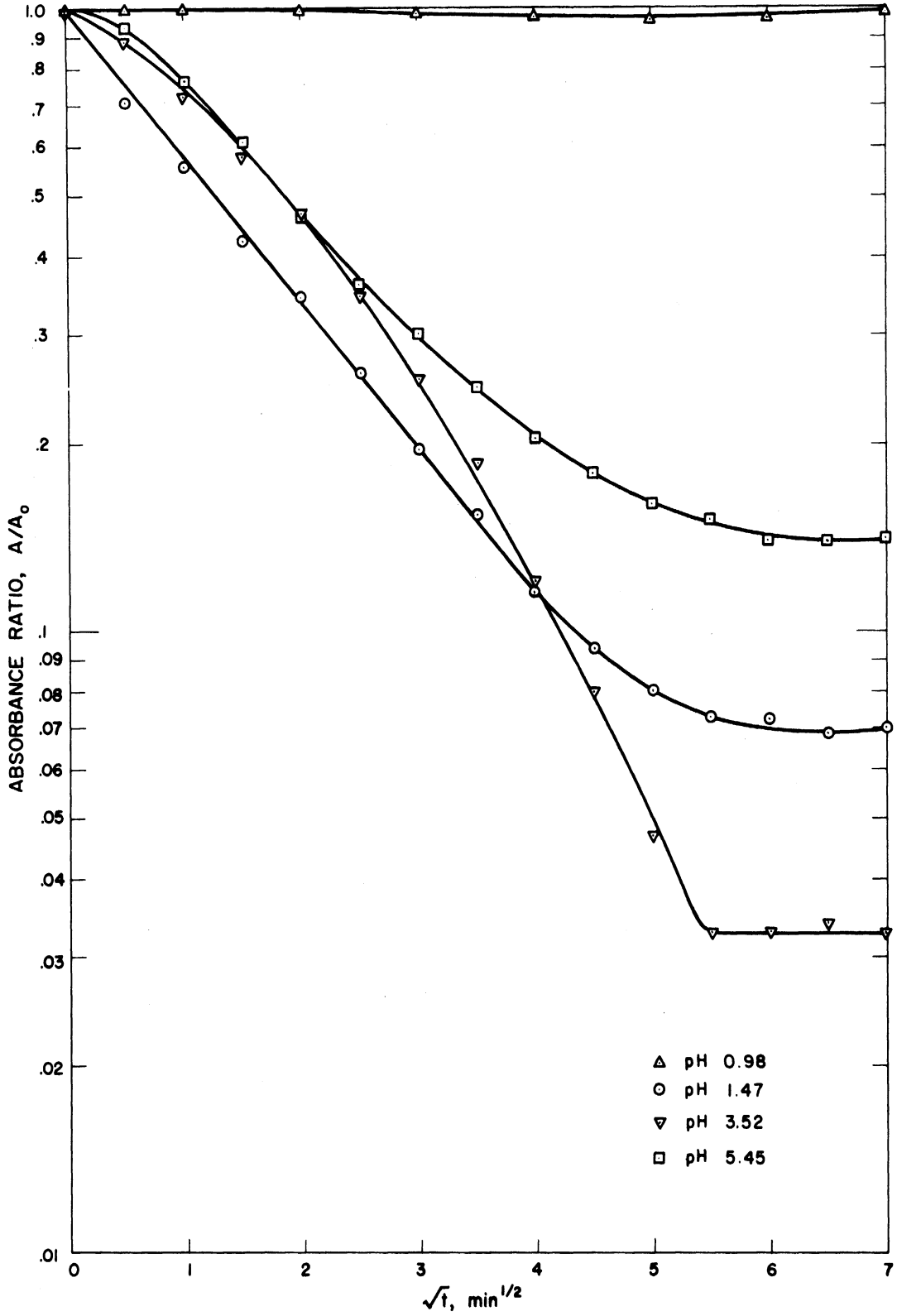


Figure 19. Adsorption of Bacterial Cells onto an Anion Exchange Resin as a Function of pH (Bacterial species: Bacillus subtilis, $A_o \cong 0.6$; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml. The corresponding desorptions are depicted in Figure 20.)

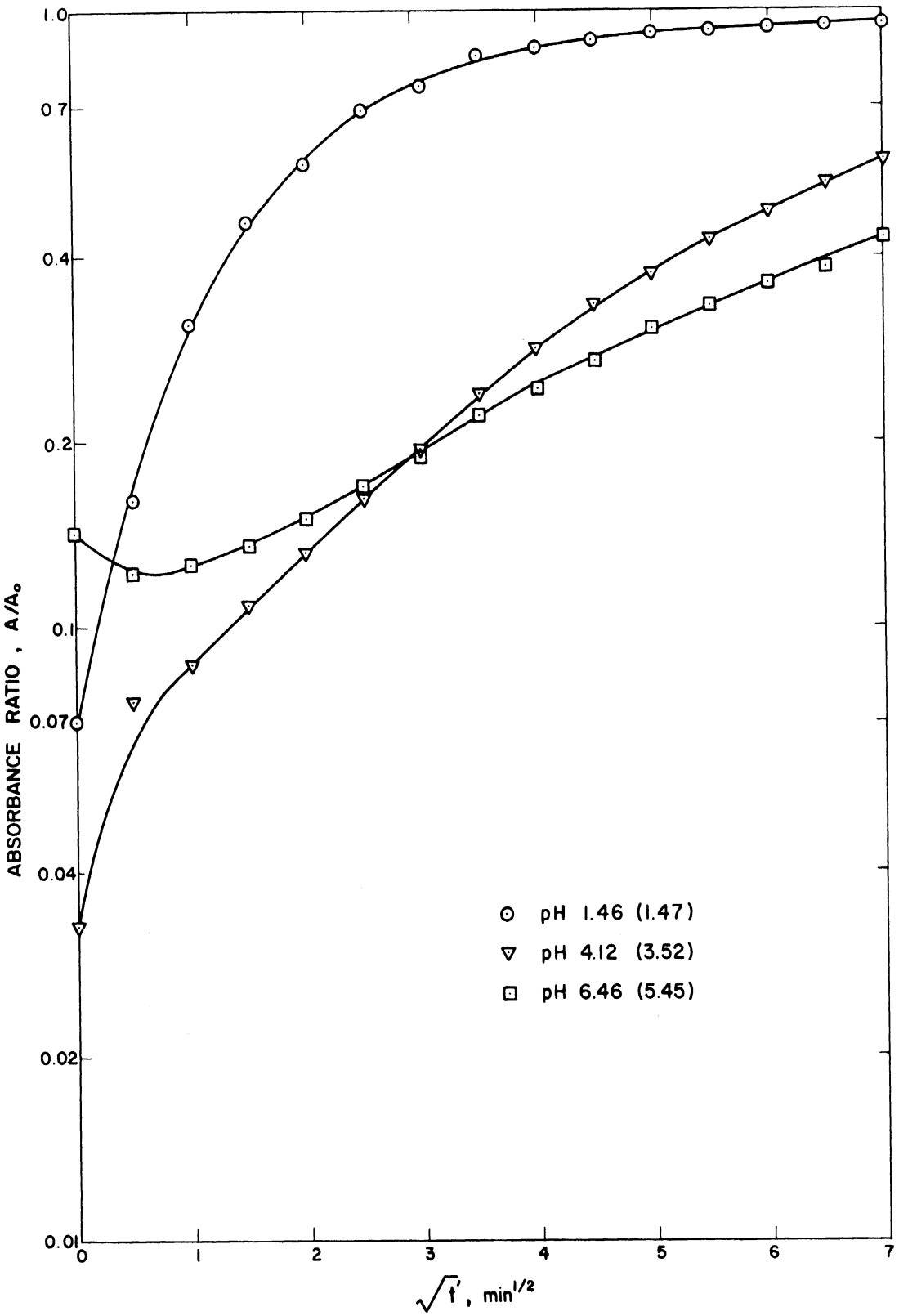


Figure 20. Desorption of Bacterial Cells from an Anion Exchange Resin as a Function of pH (Bacterial species: Bacillus subtilis, $A_o \cong 0.6$; anion exchange of resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml. The corresponding adsorptions are depicted in Figure 19.)

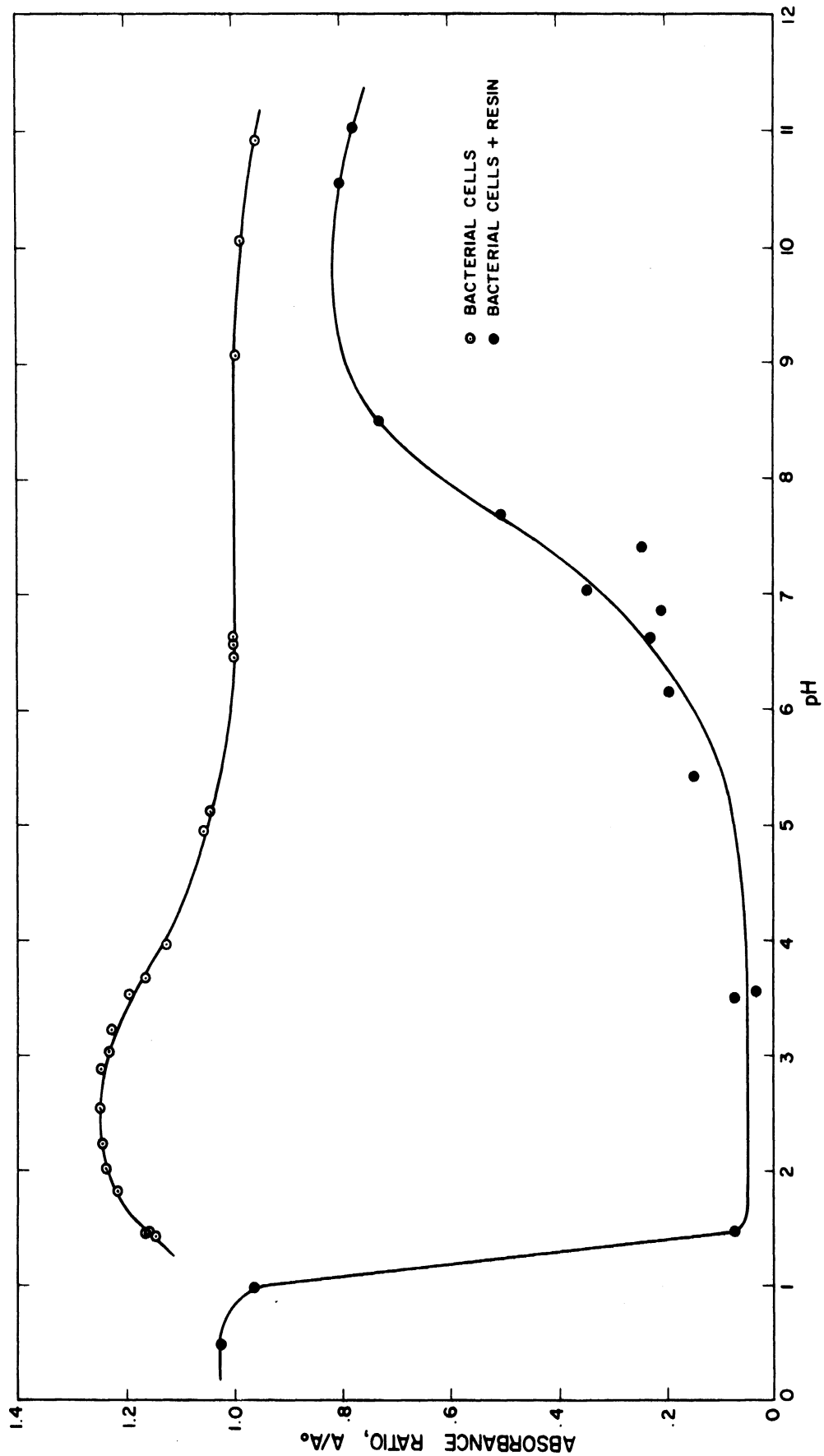


Figure 21. Equilibrium Adsorption of Bacterial Cells onto an Anion Exchange Resin as a Function of pH (1) (Bacterial species: Bacillus subtilis, A_0 = 0.6; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 mL).

Greater than 90% adsorption occurred over the range of pH 1.5 to 5.5 in the case of the system shown in Figure 21. Cells of Bacillus subtilis appear to behave as anions even in the pH range above 5.5. The failure of the cells of this species to adsorb more strongly above this pH value is probably due to the presence of buffer salts required to maintain the high pH values.

It is interesting to note in Figure 21 that almost no adsorption of the cells of Bacillus subtilis occurred below pH 1. The existance of an apparent isoelectric point near this pH value is suspected. This feature was previously suggested in reference to Figures 16 and 17. The pH values existing at the surfaces of cells of Bacillus subtilis may be lower than the surrounding solution according to Weiss (1963b). In the current work the cells of Bacillus subtilis are behaving partially as cations in a suspension at pH 1.5 and show decreasing interest in the anion exchange resin. Desorption is not forthcoming, however, if cells of Bacillus subtilis are first adsorbed onto an anion exchange resin at a pH basic to their apparent isoelectric points and the pH of the mixture is then rapidly lowered to pH 1 or below.

This result is in direct contrast to observations made of the adsorptions of four cells of the five other bacterial species studied in the present research. With the exception of the cells of Proteus vulgaris, which coagulate by themselves at low pH values, the cells of each of the other species can be desorbed from an anion exchange resin by simply lowering the pH of each system. Rapid desorption of the cells of Bacillus subtilis is possible, however, if salt is also added when the pH is lowered.

A similar plot of the degree of adsorption of the cells of Pseudomonas ovalis as a function of pH is described in Figure 22. The same suspension was used in developing the entire curve instead of using a fresh suspension at each pH value. The pH of the suspension was adjusted at intervals with concentrated acid or base and the absorbance was allowed to reach equilibrium. The variation of the absorbance of the cell suspension without any resin present is described by the upper curve of Figure 22. The cells of Pseudomonas ovalis are affected more severely by changes in pH than are the cells of Bacillus subtilis. The importance of variations in the absorbance of a suspension of cells due to pH changes or to salt additions are again emphasized since such optical measurements are assumed to be valid indices of the adsorption of bacterial cells. Details of these considerations are reported in Appendix A of this report.

The variation in absorbance of a mixture of cells of Pseudomonas ovalis and particles of anion exchange resin is described by the lower curve in Figure 22. Maximum adsorption is indicated by the sharp minimum in the absorbance ratio occurring near pH 3.9. This pH was assumed to be the apparent isoelectric point of the cells of Pseudomonas ovalis. Complete adsorption of the cells of this species was predicted for pH values greater than pH 3.9 where the cells behave as anions. Somewhat contrary to this predicted behavior, a slight desorption was noted in the pH range 4 to 5. This desorption may be partially attributable to the increased concentration of salt produced upon neutralization of the system during titration with concentrated base.

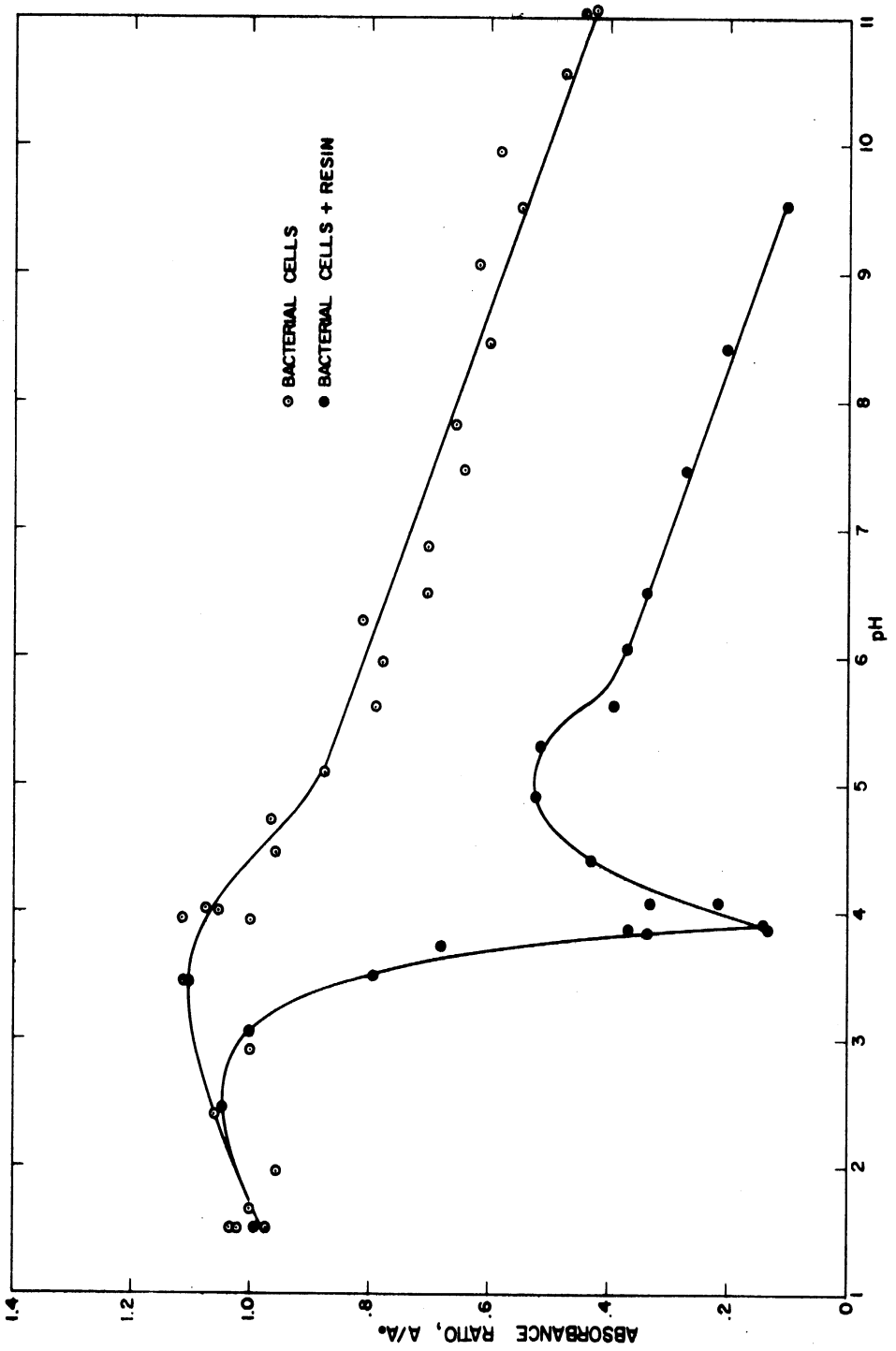


Figure 22. Equilibrium Adsorption of Bacterial Cells onto an Anion Exchange Resin as a Function of pH (2) Bacterial species: Pseudomonas ovalis, $A_0 = 0.600$; anion exchange resin: Dowex 2 x 8, 200/400 mesh, chloride form, 5 grams/50 ml).

A reversal of charge on the cell surfaces should occur if the pH is lowered below their apparent isoelectric points. The bacterial cells will then become positively charged and behave as cations. This behavior was evidenced for the cells of Pseudomonas ovalis at pH values below 3.9. In this range the cells were not attracted to the particles of anion exchange resin. The situation should be exactly reversed for the case of cation exchange.

The apparent isoelectric points of the cells of several bacterial species are presented in Table XXIX. A comparison is made of values of the isoelectric points determined by three methods: light absorbance, ion exchange, and electrophoresis. The pH values at which maxima or minima were found in the amount of light transmitted through a suspension of cells upon titration with acid or base were experimentally determined. The pH values at which maximum adsorption of cells onto an anion exchange resin occurred were also experimentally determined. Values for the apparent isoelectric points determined by electrophoretic techniques were taken from the literature.

12. Salt Concentration

The influence of the concentration of salt present in suspensions containing cells or Bacillus subtilis at constant pH values upon the adsorption of the cells by an anion exchange resin is shown in Figure 23. Both the rate of adsorption and the equilibrium adsorption are both significantly affected by the presence of sodium chloride. The rate and extent of desorption of the cells of this species are similarly affected. The cells of Bacillus subtilis apparently lose

TABLE XXIX

APPARENT ISOELECTRIC POINTS (pI) OF BACTERIAL SPECIES EXHIBITING ADSORPTIVE BEHAVIOR

Bacterium	pI by absorbance [1]	pI by anion exchange [2]	pI by electrophoresis	Degree of flocculation of cell-resin complex [3]
Pseudomonas ovalis	3.5-4.0 max.	3.9	2.17,2.95,3.25[4]	Medium
Escherichia coli	3.6 min.	~3.5	2.5[5]	Light
Proteus vulgaris	1.5-3.5 min.	>3.5	2.67[6]	Heavy
Staphlococcus aureus	3.6 min.	>1.5	1.90[6]	Medium
Bacillus cereus	2.0-3.9 min.	>1.0	3.55[6]	Medium
Bacillus subtilis	2.5 max.	1.0-1.5	2.19[6]	Heavy

1 pH of maximum or minimum light absorbance, $A = -\log T$, at 420 mμ.

2 pH of greatest affinity for adsorption onto the anion exchange resin, Dowex 1 x 8.

3 Physical appearance of flocculated cell-resin complex (relative scale).

4 Harden and Harris (1955) for P. aeruginosa, P. convexa, and P. cyanogenes, respectively (viz. Table XXIII).

5 Windslow and Upton (1926), (viz. Table XXI).

6 Harden and Harris (1955), (viz. Table XXI).

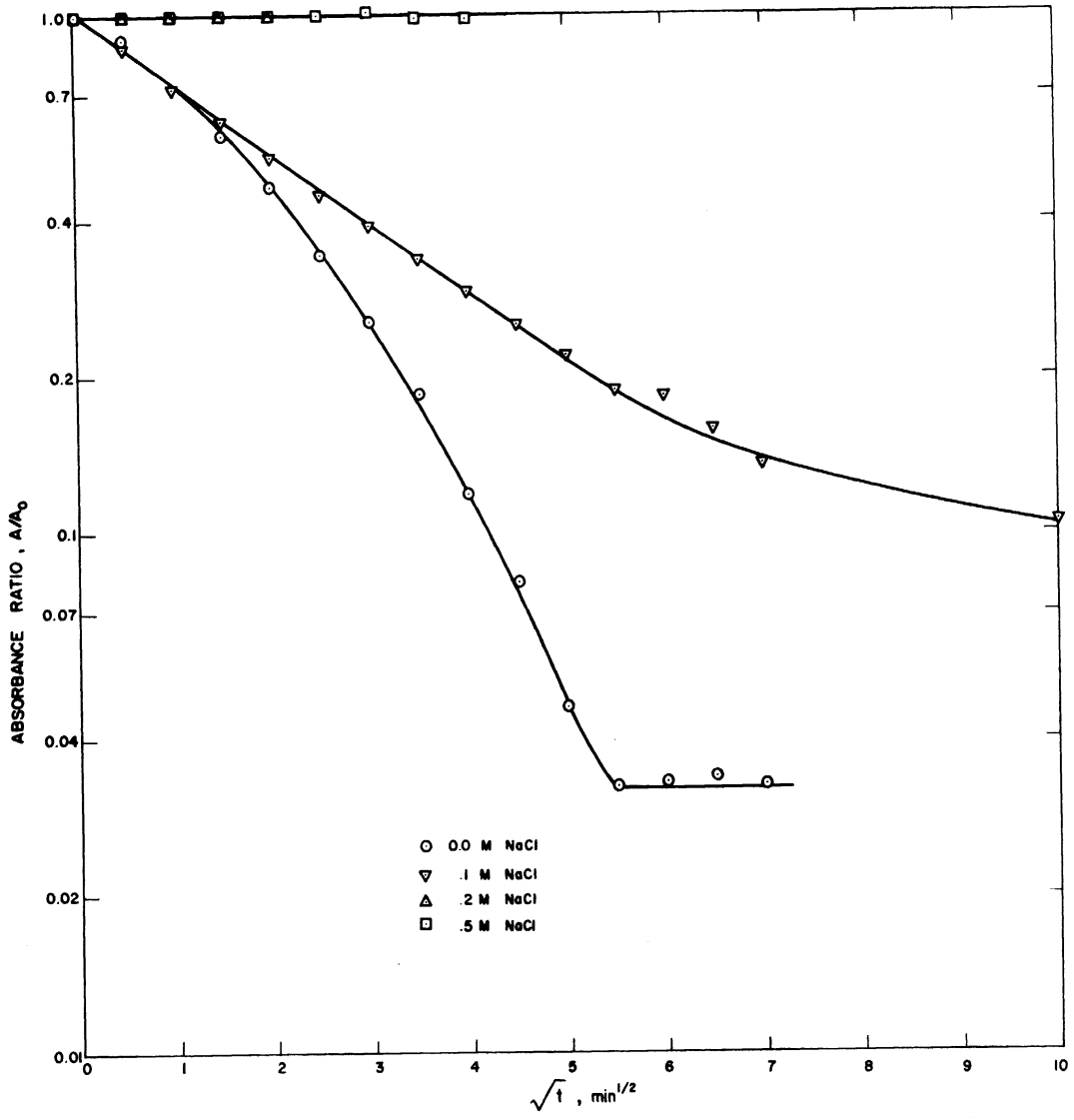


Figure 23. Adsorption of Bacterial Cells onto an Anion Exchange Resin as a Function of the Concentration of Salt (Bacterial species: Bacillus subtilis, $A_o \cong 0.6$, pH 3.5; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml).

their ability to adsorb onto an anion exchange resin at pH 3.5 if the concentration of sodium chloride is between 0.1 and 0.2 M. The cells of this species remain completely unadsorbed at salt concentrations of 0.2 M and greater.

13. Agitation

The effects of agitation upon the adsorption of cells of Bacillus subtilis by an anion exchange resin are shown in Figure 24. The rate of adsorption obtained in an experiment in which the only agitation was provided by the periodic sampling is described by the upper curve. The single point at the upper right of Figure 24 was determined for a system that was sampled only after standing undisturbed for 49 minutes. In this case, the only agitation provided was during the initial addition of the resin. The assumption that the rate of adsorption is negligible in an unagitated system is therefore supported for times of approximately one hour or less. Agitation for the cases described by the two lower curves of Figure 24 was provided by a magnetic stirrer placed in an unbaffled 150 ml beaker. The rate of adsorption was found to be significantly increased by increasing the degree of agitation. Rate constants for different agitation rates are presented in Table XXXI.

14. Time of contact

The distribution of bacterial cells between the adsorbed and unadsorbed phases is a function of the period of contact allowed between the cells and the resin particles present in an agitated suspension. The time variable has been used in a supporting role in most of the previous discussion. The absorbance ratio, A/A_o , has been plotted as a function of the cumulative time of agitated contact, $\sqrt{t}$.

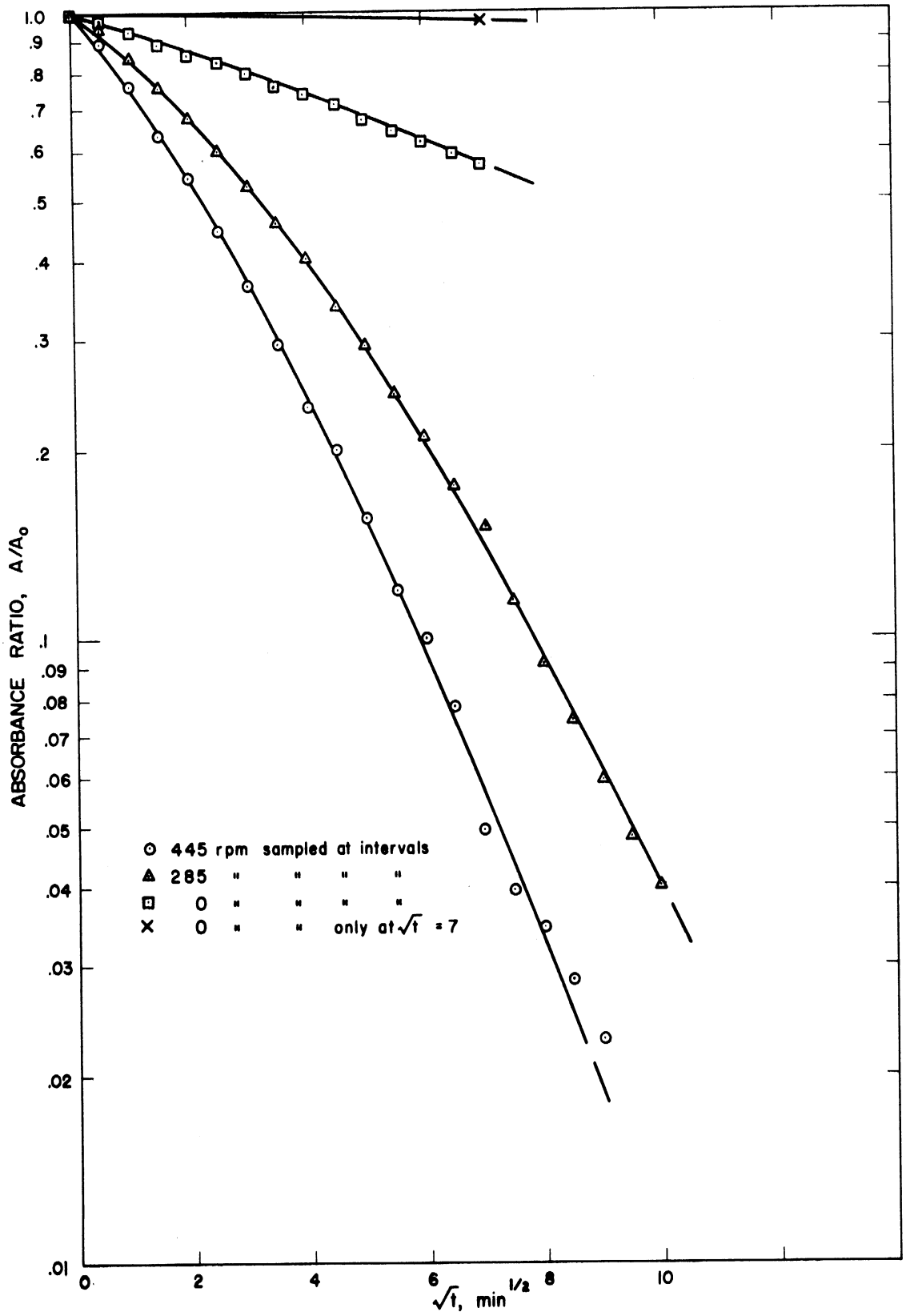


Figure 24. Adsorption of Bacterial Cells onto an Anion Exchange Resin as a Function of the Degree of Agitation (Bacterial species: Bacillus subtilis, $A_o \cong 0.6$, pH 3.5; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/ 50 ml).

A secondary effect is observed in cases involving extended periods of agitation. A gradual reversal of the cells from a state of adsorption to one of desorption is observed even though no apparent changes in the pH or the salt content of the suspension have occurred to otherwise promote desorption. Three curves of the adsorption-desorption behaviors of the cells of Escherichia coli, Staphlococcus aureus, and Bacillus subtilis, are presented in Figure 25. The process of adsorption is reversed and desorption of cells is observed to occur after different periods of agitation for each of these species.

This type of desorption is probable due to a purely mechanical action brought about by the extended agitation. The attachment sites located on the cell surfaces that are associated with their ion exchange behavior may be physically uprooted during the extended buffeting experienced with neighboring particles of ion exchange resin or the walls of the reaction vessel. The destruction of bacterial cells by agitation in the presence of small inert particles such as glass beads has been reported by Curran and Evans (1942), King and Alexander (1948), Furness (1952), and Salton (1964, pp.46-51).

15. Temperature

The rate of adsorption of Bacillus subtilis cells onto an anion exchange resin was found to be greater at a higher temperature. This effect is illustrated in Figure 26. The final adsorption equilibrium values were nearly identical. Rate constants for the adsorptions conducted at different temperatures are presented in Table XXXI.

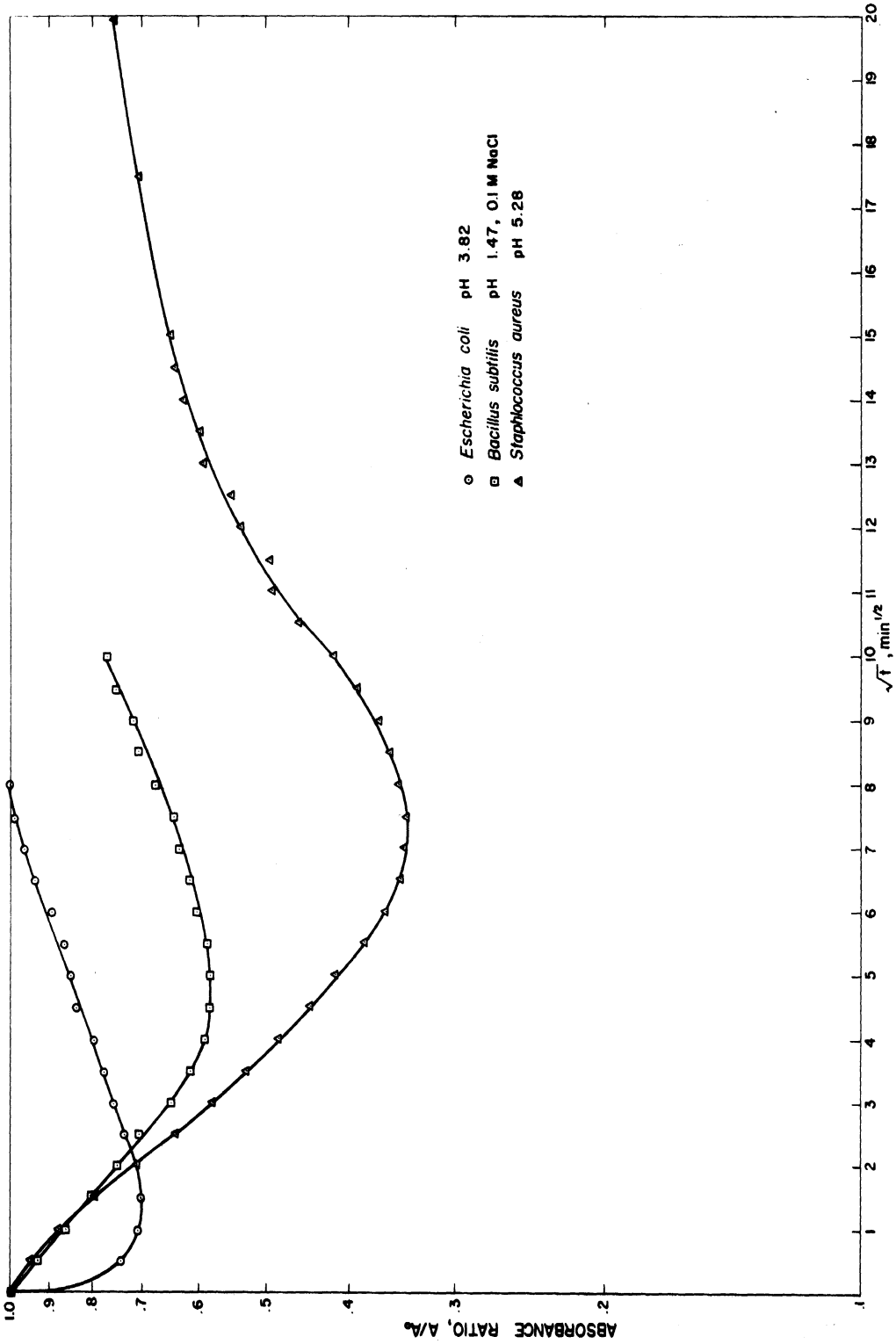


Figure 25. Adsorption of Bacterial Cells of Three Bacterial Species onto an Anion Exchange Resin as a Function of the Time of Contact (Bacterial species: Escherichia coli, A_o = 0.556, pH 3.82, Bacillus subtilis, A_o = 0.600, pH 1.47, 0.1 M KCl, Staphlococcus aureus, A_o = 0.594, pH 5.28; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml).

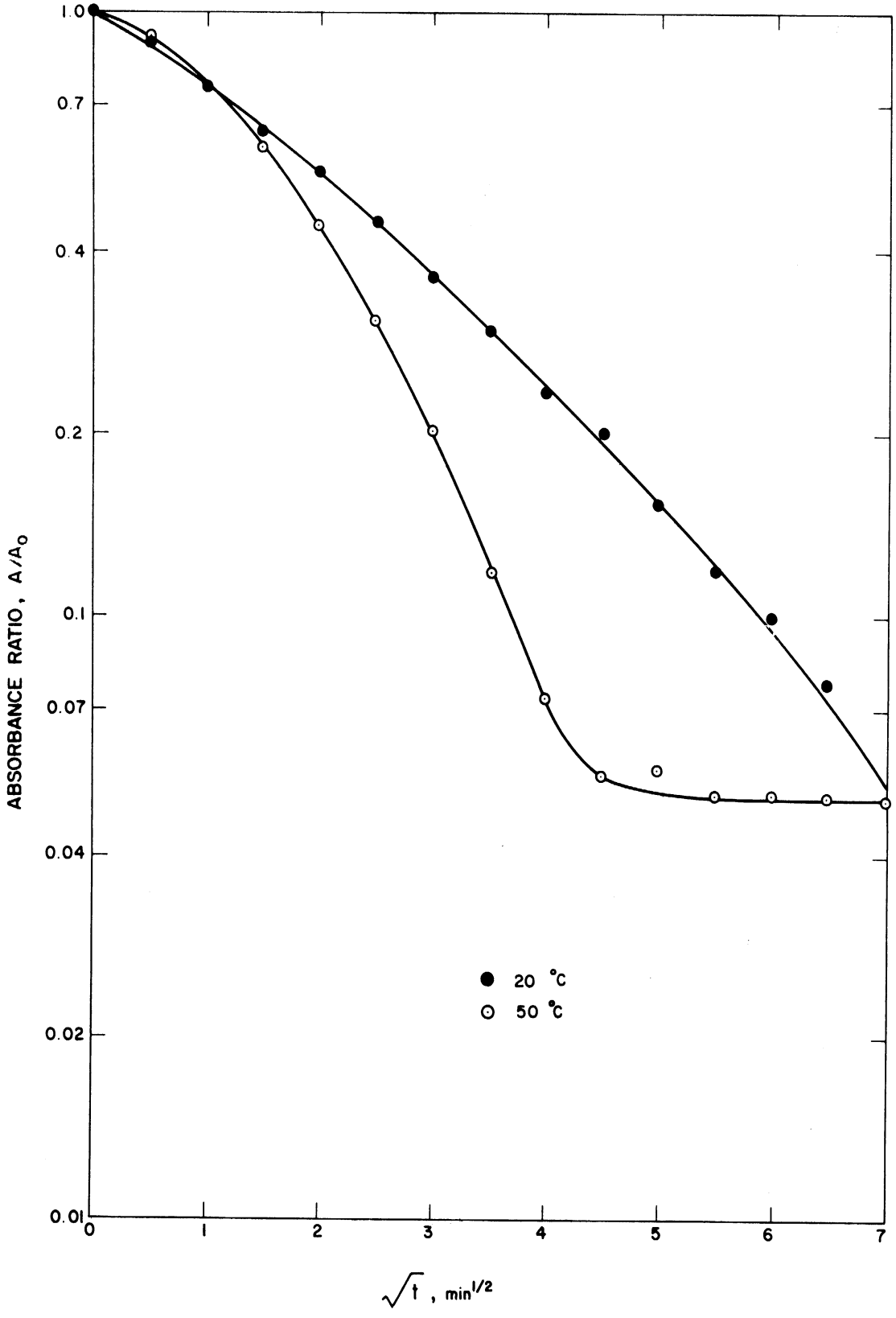


Figure 26. Adsorption of Bacterial Cells onto an Anion Exchange Resin as a Function of Temperature (Bacillus subtilis, A_o = 0.599, pH 3.5; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml).

D. Elucidation of Mechanism and Model

The current discussion is devoted to the presentation of experimental data in support of the proposed mechanism of exchange and the mathematical model that describe the interaction of bacterial cells and ion exchange resins. A typical set of data that were collected during the adsorption and desorption of bacterial cells with an anion exchange resin are analyzed. The appropriate rate constants and diffusion coefficients are determined and the experimental data are then fitted with calculated curves. A collection of rate constants derived under various conditions of cell concentration, resin concentration, pH, agitation, temperature, and salt content are presented. The significance of the effects of each of these variables upon the rate constants are discussed. Rate constants are tabulated for both adsorption and desorption.

1. Determination of rate constants

A typical set of data obtained for the adsorption and desorption of the cells of a given bacterial species using an ion exchange resin as the adsorbent can be differenced according to the procedure outlined in Section III-D. In summary, a plot of the quantity, $\Delta \log f(A/A_o)/2\Delta\sqrt{t}$, versus $\sqrt{t}$ should be linear of slope k and intercept $k'/2$. The rate constants, k and k', can then be substituted into a rate equation of the form given by Equation (III-27).

A plot of the differenced data presented in Table E-II of Appendix E for the adsorption of cells of Bacillus subtilis from 50 ml of suspension containing 1.25×10^8 cells/ml onto 4 grams of the anion exchange resin, Dowex 1 x 8, 100/200 mesh, chloride form, is presented

in Figure 27. The conditions of this experiment were: pH 3.5, 30 C., and 445 rpm. The difference plot can be improved in certain cases by fitting a smooth curve through the raw data and then calculating the difference quantities from smoothed values taken from this fitted curve. The rate constants for the case described above were determined from the slope and intercept, respectively, to be $k_a = -0.035\ min^{-1}$ and $k_a' = -0.090\ min^{-\frac{1}{2}}$.

The experimental data for this adsorption can now be compared to a calculated curve determined from the rate expression given by Equation (V-1).

$$\log (A/A_0) = -0.035\, t - 0.090\, \sqrt{t} \qquad \text{(V-1)}$$

The results are shown in Figure 28. The agreement is considered to be excellent, particularly over the initial portion of the curve. Deviation from the calculated curve at times greater than 25 minutes can be attributed either to an approach to an equilibrium condition, or, to the limited sensitivity of the instrumental techniques.

A similar plot of the differenced data collected for the same system, Bacillus subtilis - Dowex 1 x 8 , during the desorption of the adsorbed bacterial cells promoted by the addition of solid sodium chloride to produce a final salt concentration of 1 M is given in Figure 29. The corresponding rate constants for this desorption were determined to be $k_d = -0.00564\ min^{-1}$ and $k_d' = -0.0080\ min^{-\frac{1}{2}}$. The experimental data were then compared to a curve computed from Equation (V-2).

$$\log (1-A/A_0) = -0.00564\, t' - 0.0080\, \sqrt{t'} \qquad \text{(V-2)}$$

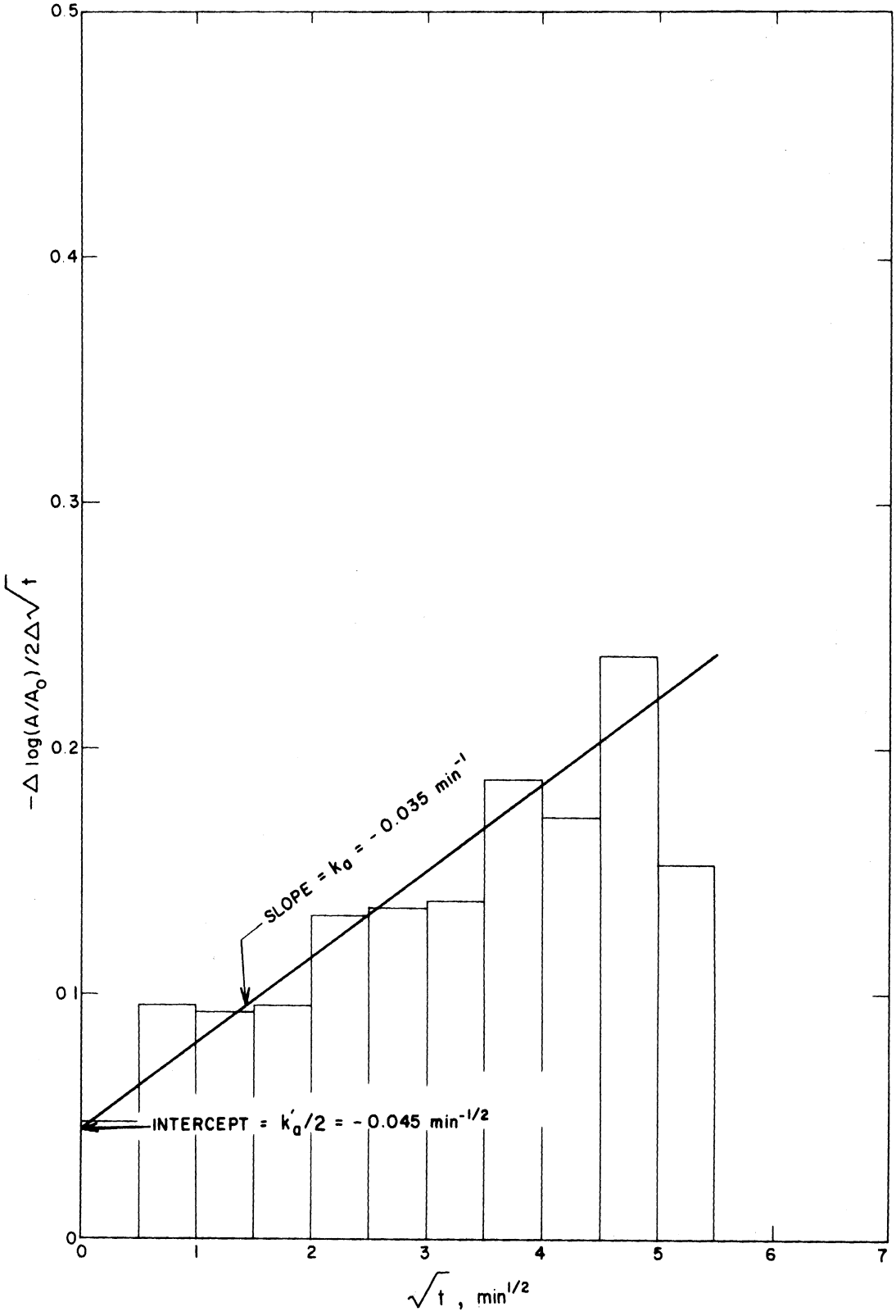


Figure 27. Determination of the Rate Constants for the Adsorption of Bacterial Cells onto an Anion Exchange Resin Using a Differencing Technique (Bacterial species: Bacillus subtilis, A_0 = 0.598, pH 3.53; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml. A similar plot of the differenced data obtained for the related desorption is depicted in Figure 29).

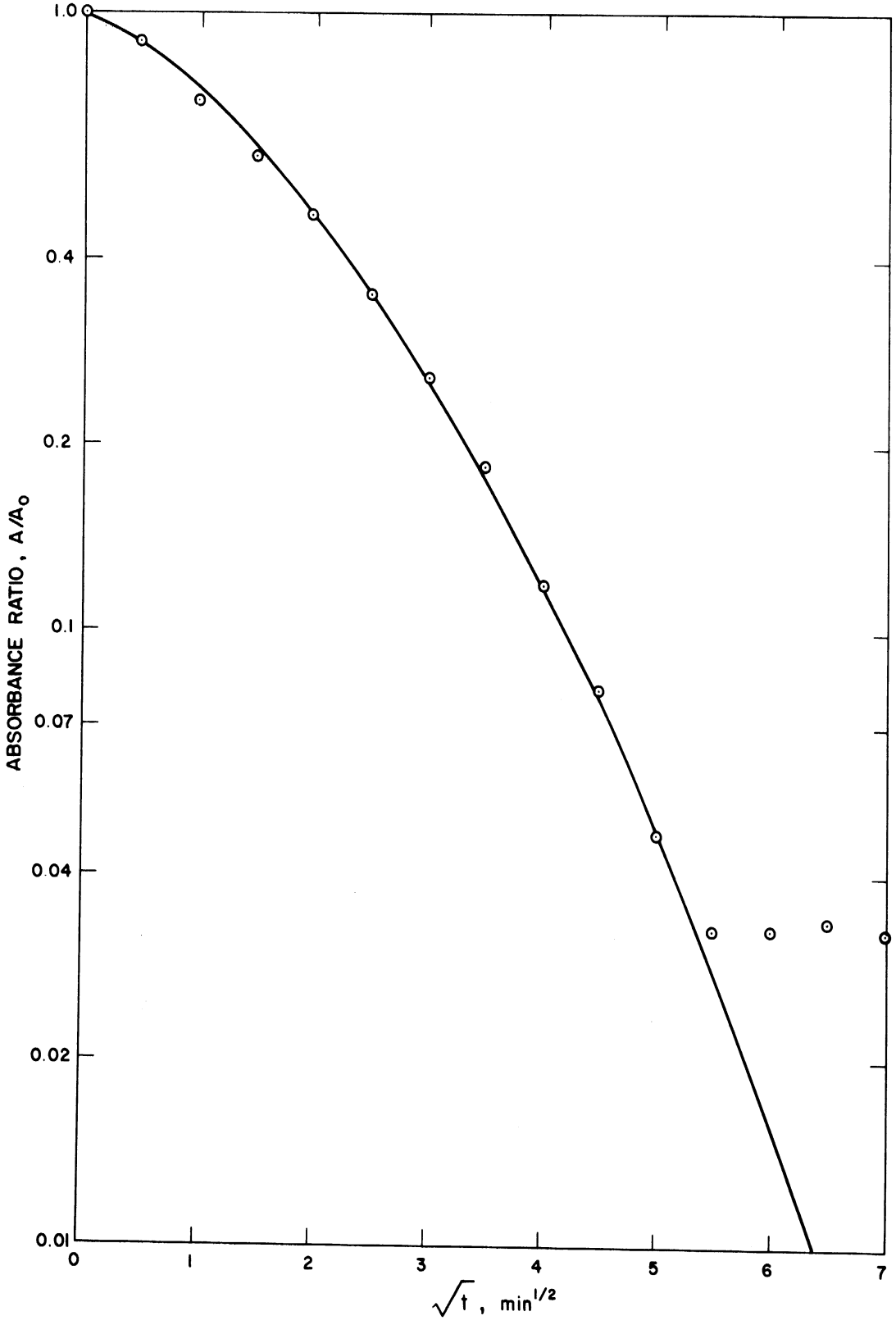


Figure 28. Adsorption of Bacterial Cells onto an Anion Exchange Resin Fitted by Equation (III-10-B) (Bacterial species: Bacillus subtilis, A_o - 0.598, pH 3.53; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml. The corresponding difference plot is depicted in Figure 27. The rate equation is: $\log A/A_o = -0.035\ t - 0.090\ /t$).

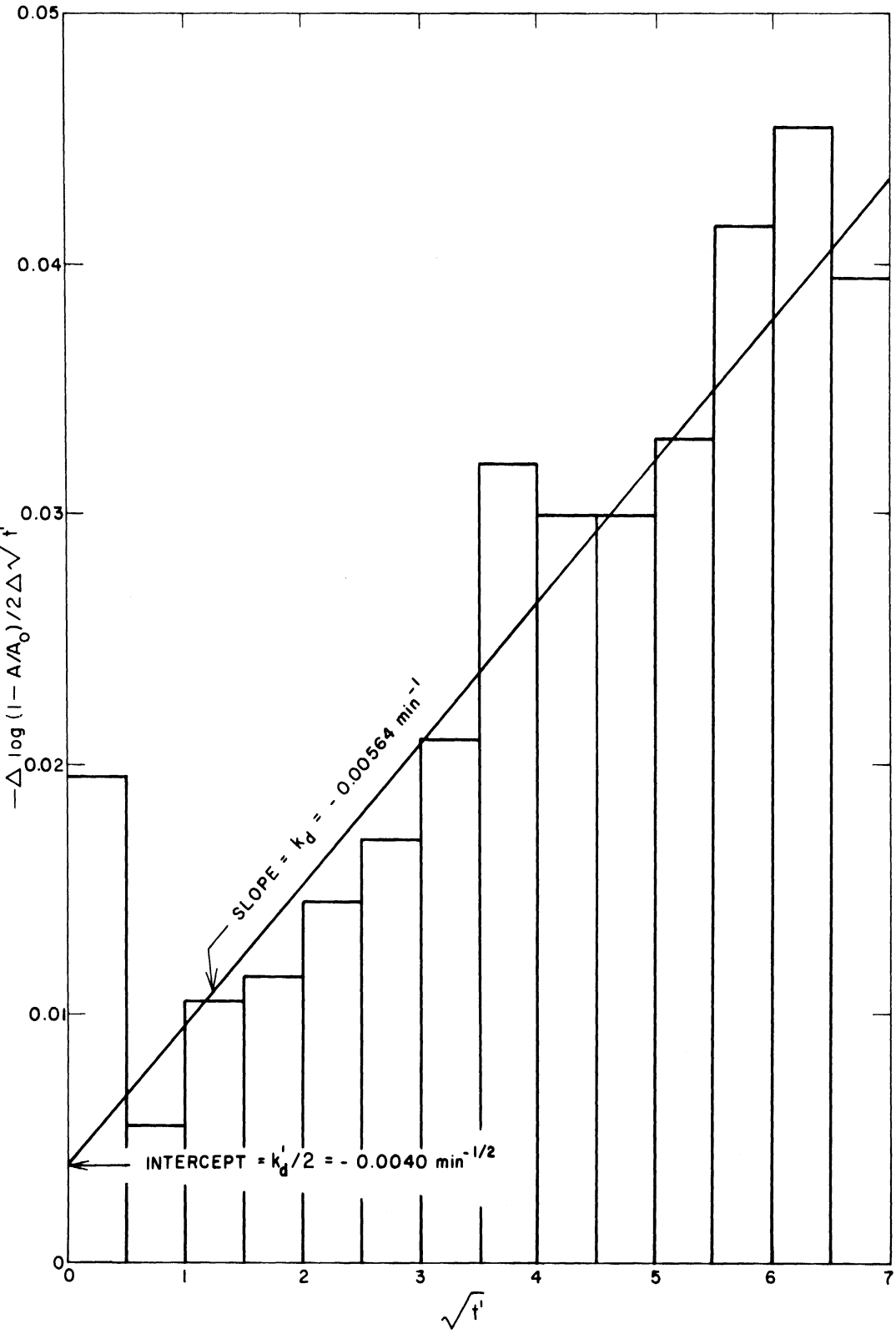


Figure 29. Determination of the Rate Constants for the Desorption of Bacterial Cells from an Anion Exchange Resin Using a Differencing Technique (Bacterial species: Bacillus subtilis, A_o = 0.598, pH 4.12, I M KCl; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml. A similar plot of the differenced data obtained for the related adsorption is depicted in Figure 27).

This comparison is made in Figure 30. The experimental data of A/A_o given in Table E-V of Appendix E were corrected for an initial absorbance of $A/A_o = 0.0328$ at $\sqrt{t'} = 0$ before plotting in Figure 30.

The agreement between the desorption data and the calculated desorption curve is considered to be good for those time values greater than nine minutes after addition of the regenerating salt. Deviation of the calculated curve from the actual data in the initial time period up to $\sqrt{t'} = 3$ may be due in part to the limitations of the instrumental measurements. Small changes in the absorbance of the suspension due to salt effects may also be reflected.

Once the rate constants, k and k', have been determined, the diffusion coefficient, D, defined by Case B of Section III-C-2 can be calculated from Equation (III-35a) or Equation (III-35b). In addition to k and k', the following experimentally derived

$$D_k = -k / 4\pi\beta_o R N \qquad \text{(III-35a)}$$

$$D_{k'} = (1/\pi)[k'/8\beta_o R^2 N]^2 \qquad \text{(III-35b)}$$

constants are required for this calculation: β_o, the slope of the A versus n plot, R, the radius of an average resin particle, and N, the number of resin particles contained per unit volume of suspension. Calculations of D according to Case B are summarized in Appendix E of this report.

An alternative method of determining the diffusion coefficient, D, is to plot the quantity, $\log (A/A_o) = \log(1-M_t/M_\infty)$ versus $\sqrt{t}$ as defined by Equations (III-10a-E) and (III-10b-E). A particular value of the ratio of the fluid to adsorbent particle volume, α^*

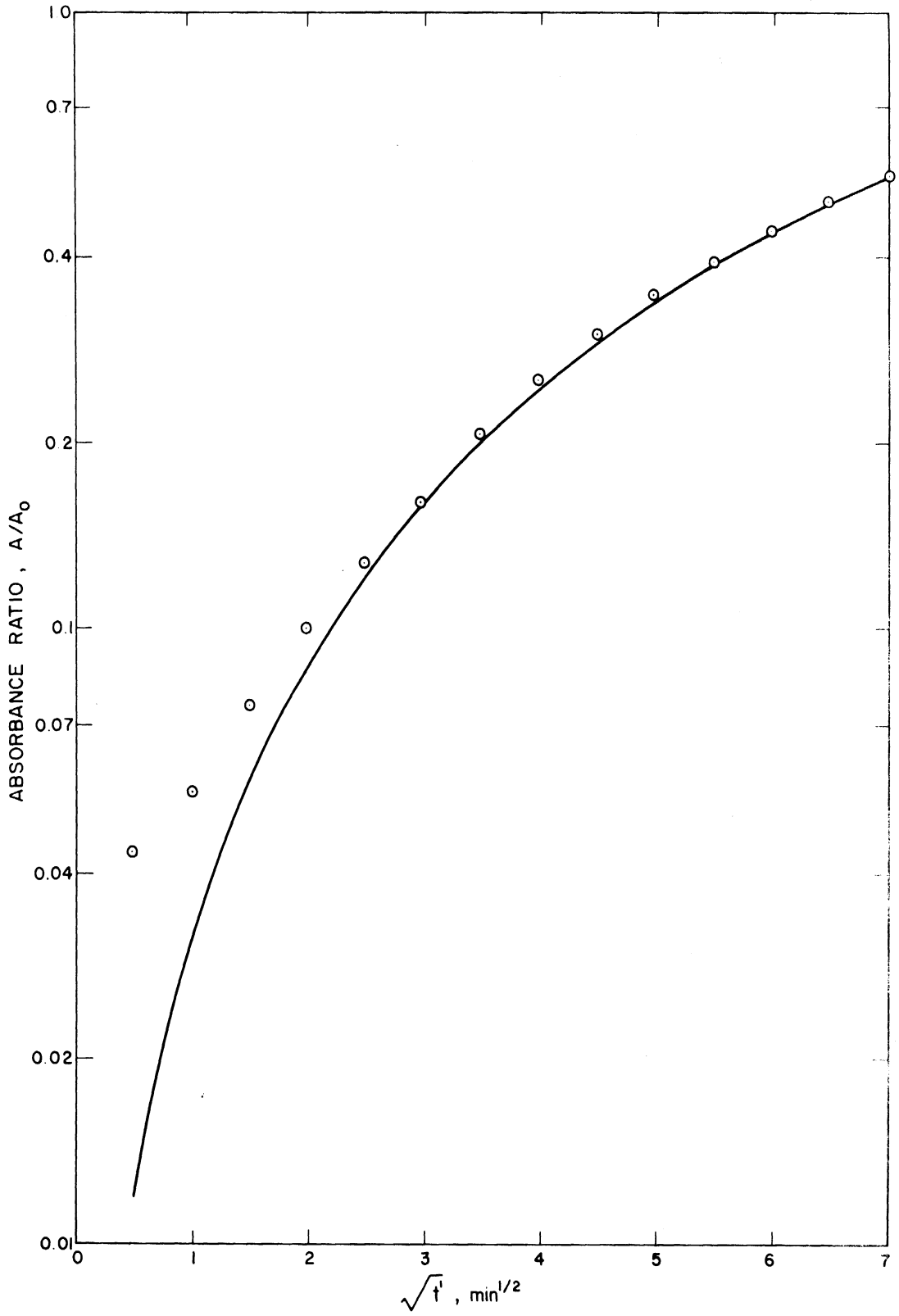


Figure 30. Desorption of Bacterial Cells from an Anion Exchange Resin Fitted by Equation (III-10-B) (Bacterial species: Bacillus subtilis, A_o = 0.598, pH 4.12, 1 M KCl; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml. The corresponding difference plot is depicted in Figure 29. The rate equation is: log $(1-A/A_o)$ = -0.00564 t -0.0080 /t).

$$(1-M_t/M_\infty)=\sum_{n=1}^{\infty}\frac{6\alpha^*(\alpha^*+1)\exp(-s_n^2\tau)}{9(\alpha^*+1)+\alpha^{*2}s_n^2}\quad,\ \tau\geq 0.1 \qquad \text{(III-10a-E)}$$

$$(1-M_t/M_\infty)=(\alpha^*+1)\left[\frac{\psi_1}{\psi_1+\psi_2}\exp\left(\frac{3\psi_1\sqrt{\tau}}{\alpha^*}\right)^2 erfc\left(\frac{3\psi_1\sqrt{\tau}}{\alpha^*}\right)\right. \qquad \text{(III-10b-E)}$$

$$\left.+\ \frac{\psi_2}{\psi_1+\psi_2}\exp\left(-\frac{3\psi_2\sqrt{\tau}}{\alpha^*}\right)^2 erfc\left(-\frac{3\psi_2\sqrt{\tau}}{\alpha^*}\right)\right]-\alpha^*,\ \tau\leq 0.1$$

must be also chosen; this quantity is defined by Equation (III-18).

$$\alpha^* = \frac{3V}{4\pi K' R^3 N' m} \qquad \text{(III-18)}$$

Calculations based upon the model of Case E of Section III-C-2 described by the above equations are summarized in Appendix E of this report. A value of $\alpha^* = 20.797$ has been used for these calculations.

Values of $\sqrt{\tau}$ corresponding to the experimental A/A_0 data presented in Figure 28 are determined from the calculated curve of Figure E-1 and plotted against the actual $\sqrt{t}$ values in Figure 31. The diffusion coefficient D_a, is then calculated from the slope, $\sqrt{D_a}/R$, of this plot. A similar plot of $\sqrt{\tau'}$ versus $\sqrt{t'}$ of the desorption data previously presented in Figure 30 is made in Figure 33. The values of D_a and D_d were calculated from the slopes of straight lines that did not pass through the origins of the $\sqrt{\tau}$ versus $\sqrt{t}$ plots shown in Figures 31 and 33. The positive intercepts for the cases of adsorption and desorption, respectively, are $\sqrt{t} = 0.23$ min$^{\frac{1}{2}}$ and $\sqrt{t'} = 1.78$ min$^{\frac{1}{2}}$. The calculated curves fitted to the data presented in Figures 32 and 34, respectively, for adsorption and desorption, have been translated by these amounts. Possible factors that make such shifts necessary are: deviations of the resin particles from spherical shapes, variation of D and/or K' during the initial stages of sorption, and thermal effects due to the heat of sorption. The latter two effects

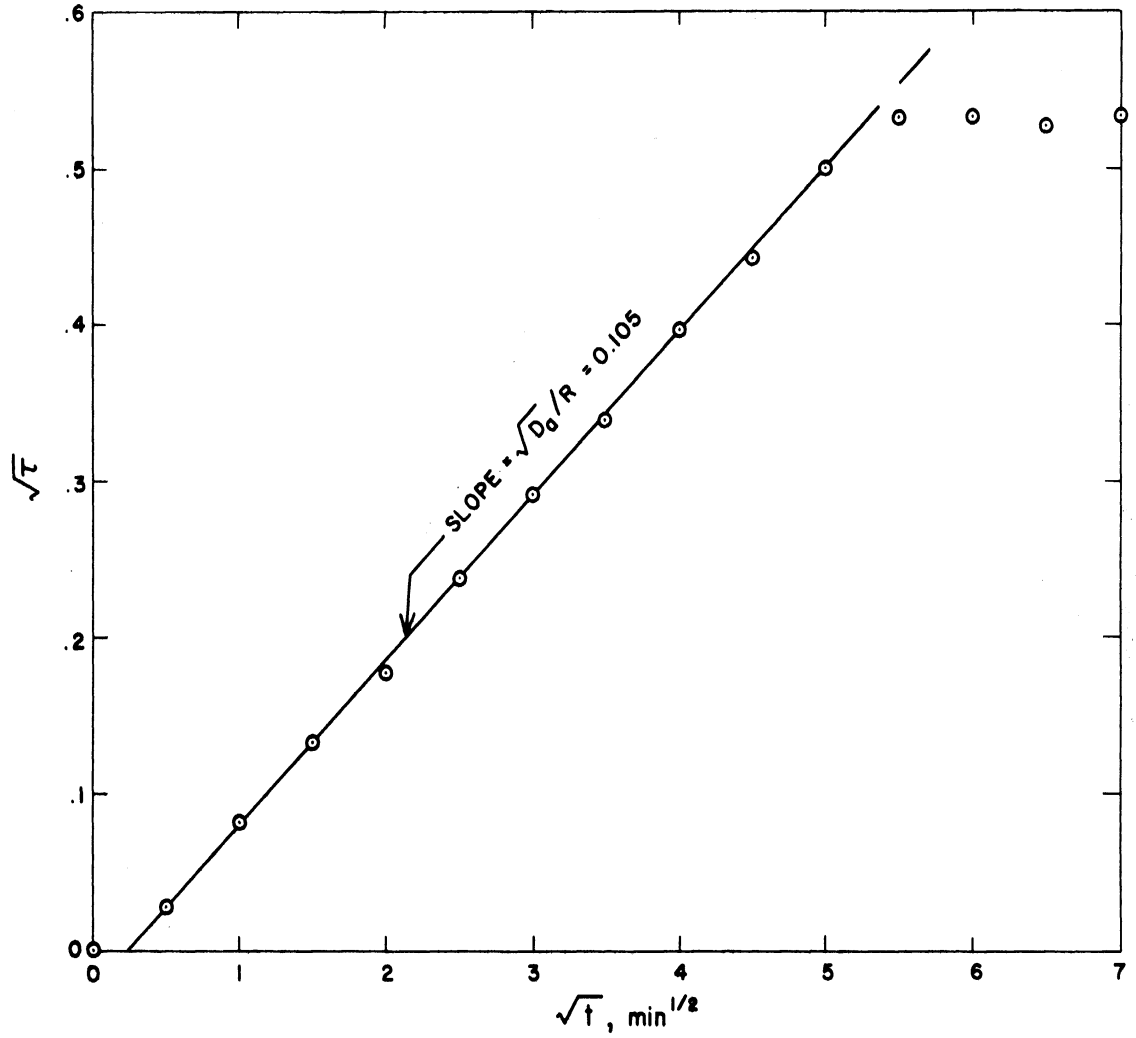


Figure 31. Determination of the Diffusion Coefficient for the Adsorption of Bacterial Cells onto an Anion Exchange Resin (Bacterial species: Bacillus subtilis, A_o = 0.598, pH 3.53; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml. A similar plot of the data obtained for the related desorption is depicted in Figure 33).

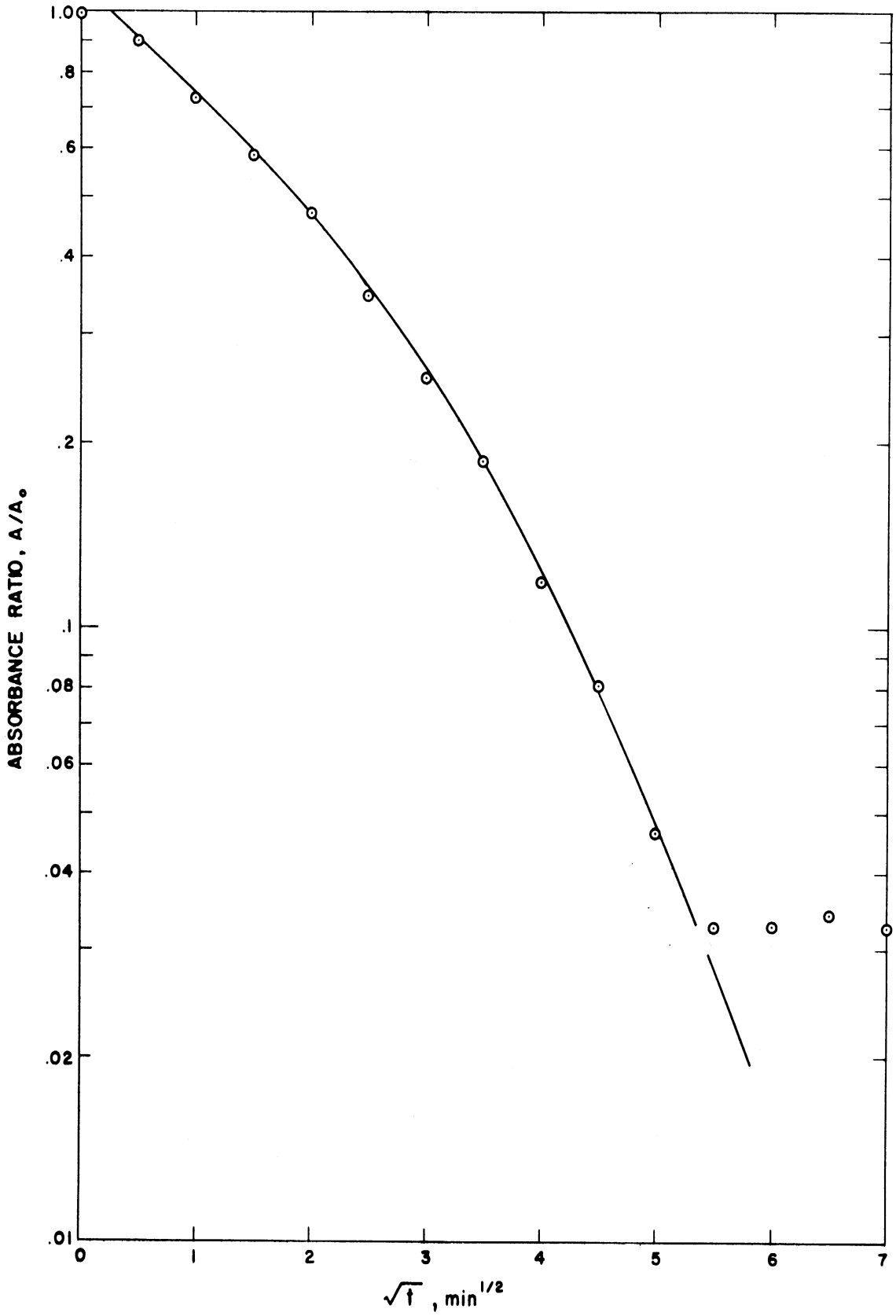


Figure 32. Adsorption of Bacterial Cells onto an Anion Exchange Resin Fitted by Equations (III-10a-E) and (III-10b-E) (Bacterial species: Bacillus subtilis, A_o = 0.598, pH 3.53; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml. Details are given in Appendix E.)

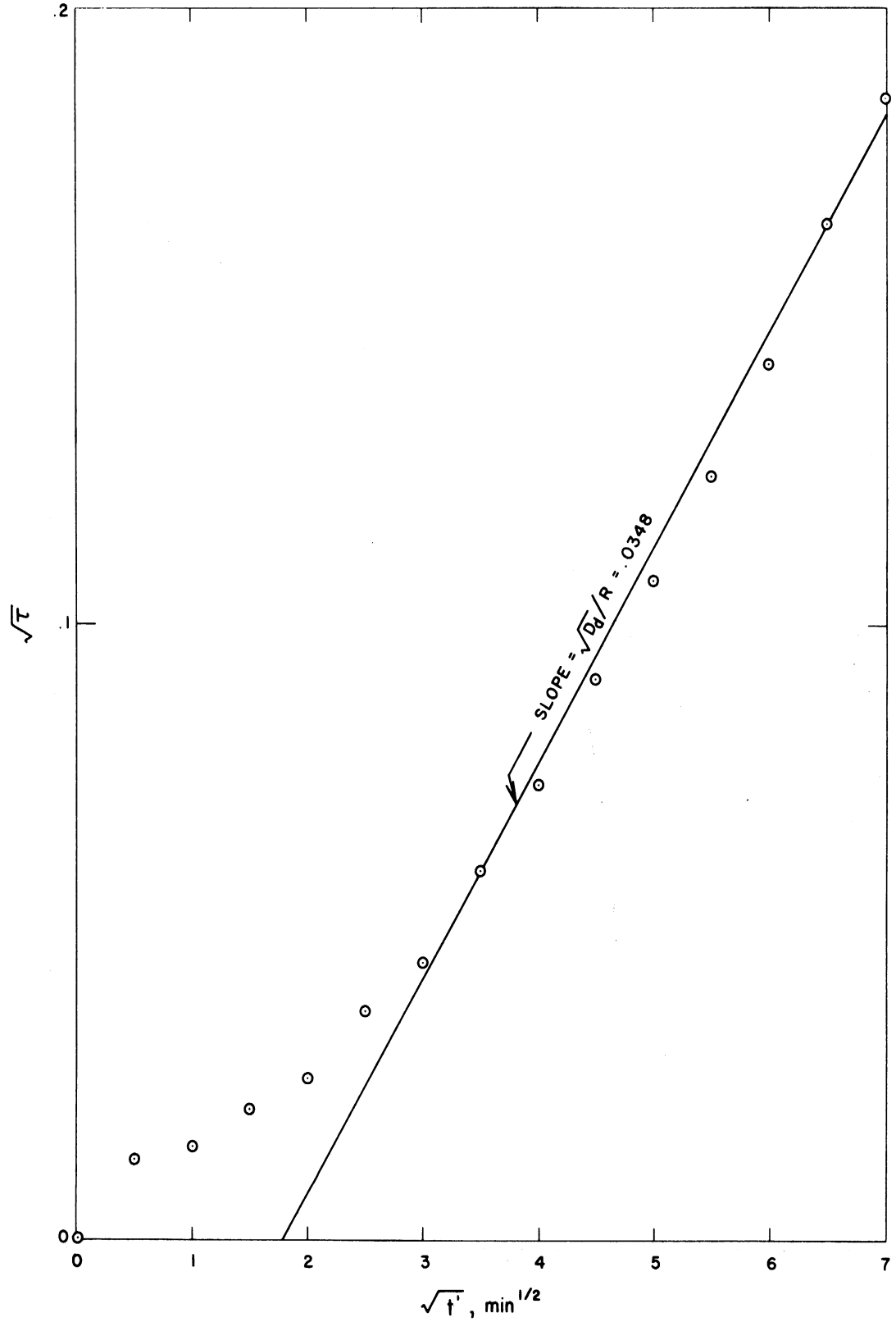


Figure 33. Determination of the Diffusion Coefficient for the Desorption of Bacterial Cells from an Anion Exchange Resin (Bacterial species: Bacillus subtilis, A_o = 0.598, pH 4.12, 1 M KCl; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml. A similar plot of the data obtained for the related adsorption is depicted in Figure 31).

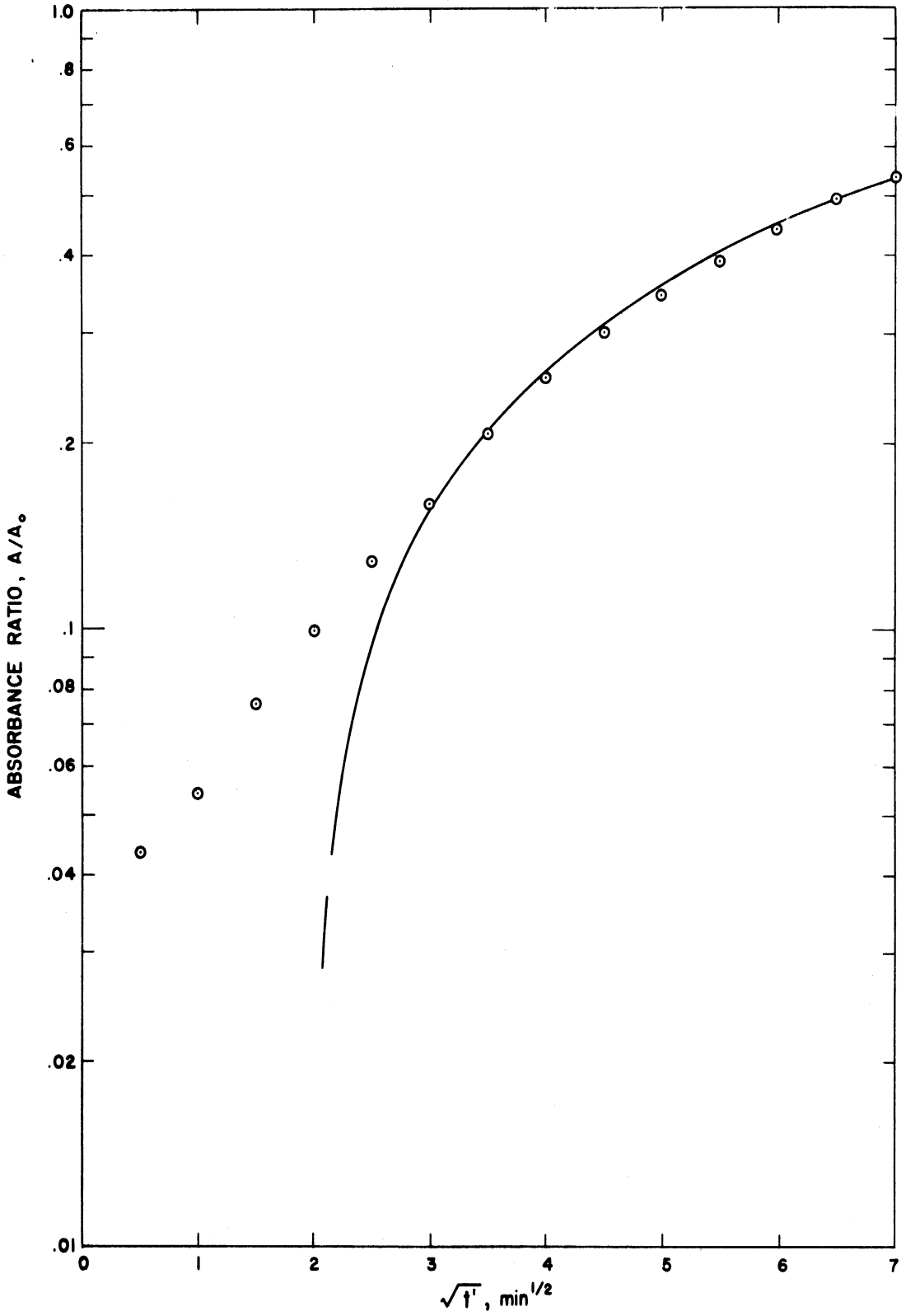


Figure 34. Desorption of Bacterial Cells from an Anion Exchange Resin Fitted by Equations (III-10a-E) and (III-10b-E) (Bacterial species: Bacillus subtilis, pH 4.12, 1 M KCl; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml. Details are given in Appendix E.)

are less marked in the later part of an experiment where concentration gradients and sorption rates are relatively small. The net result is an apparent error in the time zero. This phenomenon was recognized by Carman and Haul (1954).

Diffusion coefficients that have been determined for the typical adsorption and desorption data presented, respectively, in Figures 28 and 30 and again in Figures 32 and 34 are summarized in Table XXX. Values of D_a and D_d that were experimentally determined by the two methods previously described are compared with a theoretical value calculated for the case of Brownian motion by use of the Einstein-Stokes equation. The value of D_a calculated according to Case E is in surprisingly close agreement with that predicted from the Einstein-Stokes equation. Valentine and Allison (1959) made a similar comparison for the adsorption of latex particles and vaccinia viruses onto various surfaces. The rate of adsorption in a well-agitated system could be almost exactly predicted from Brownian theory. They postulated the existance of a stagnant liquid film surrounding each adsorbing surface. The diffusion of virus or latex particles through this stagnant film was the rate-controlling step.

The value of D_a calculated according to Case B is much larger than that determined either according to Case E or from the Einstein-Stokes equation. The differences between the experimentally determined values of D_a can not be fully explained at the present time. The physical interpretation of the diffusion coefficient for adsorption is

apparently not the same in Cases B and E. The ratio of D_a to D_d, however, is approximately ten for both Cases B and E indicating some degree of correspondence between the two models. The values of D_a and D_d are assumed identical for the case of Brownian motion. Desorption, however, is assumed to occur by a different mechanism from adsorption in the current experimental work.

TABLE XXX

DIFFUSION COEFFICIENTS FOR THE SORPTION OF Bacillus subtilis WITH DOWEX 1 x 8 at pH 3.5[1]

Method of Determination	Adsorption D_a, $cm^2/sec \times 10^7$	Desorption D_d, $cm^2/sec \times 10^7$
Case B, Section III; Equations (III-35a) and (III-35b)[2]	5.97	0.580
Case E, Section III; Equations (III-10a-E) and (III-10b-E)[3]	.0433	.00477
Einstein-Stokes Equation (III-32)[4]	.0487	.0487

1 Data are presented in Tables E-II and E-III of Appendix E.

2 Average values calculated from rate constants, k and k', presented in Table XXXI.

3 Calculated for a bacterium 1.0 μ in diameter suspended in water at 25 C.

2. Significance of the rate constants

Several sets of rate constants have been determined for various conditions of cell concentration, resin concentration, pH, agitation, temperature, and salt content. Diffusion coefficients for each condition were calculated from Equations (III-35a) and (III-35b) assuming the model of Case B to be valid. These values are tabulated in Table XXXI. The significance of each variable listed above and its effect upon the magnitudes of the rate constants will now be discussed.

The theoretical ratio of rate constants, $|k/k'^2|$ is defined by Equation (III-34a). A numerical value of this ratio can be calculated

$$\left|k/k'^2\right| = 1/16\beta_0 R^3 N \qquad \text{(III-34a)}$$

for the conditions most frequently encountered in the current work, i.e. 4 grams of 100/200 mesh resin in 50 ml of a suspension containing cells of Bacillus subtilis. The following constants are applicable: N = 10^5 particles/cm^3 of suspension, $2R$ = 97.2μ and β_0 = 0.83. The calculated ratio for these conditions is $|k/k'^2|$ = 6.56. The values of $|k/k'^2|$ experimentally determined for other conditions are listed in Table XXXI. These values are in agreement within an order of magnitude. It should be noted that the rate constants, k and k', have been determined using logarithms to the base ten rather than natural logarithms as are specified in some of the derived forms of Section III.

TABLE XXXI

RATE CONSTANTS AND DIFFUSION COEFFICIENTS FOR SEVERAL EXPERIMENTAL EXCHANGES[1]

Variable	A_o	m, grams	pH	Ω, rpm	T, °C.	$-k$, $min^{-1/2}$	$-k'$, $min^{-1/2}$	D_k	$D_{k'}$[3]	$\lvert k/k^2 \rvert$[4]
					Adsorption of bacterial cells onto the resin					
A_o	0.732	4	5.57	445	28.5	0.0052	0.110	3.94	13.8	0.187
	.578	"	4.70	"	"	.0229	.135	1.73	20.9	.546
	.334	"	4.18	"	"	.0745	.120	5.64	16.5	2.24
	.182	"	4.05	"	"	.105	.120	7.94	16.5	3.16
m	.598	4	3.53	445	30.0	.035	.090	2.65	9.28	1.88
	.596	2	3.46	"	25.0	.00725	.064	1.10	18.8	.769
	.596	1	3.55	"	"	.00162	.035	.490	22.5	.574
	.596	1/2	3.55	"	"	.000872	.0155	.527	17.6	1.58
pH	.598	4	3.53	445	30.0	.035	.090	2.65	9.28	1.88
	.594	"	5.45	"	"	.040	.064	3.03	6.61	4.24
Ω	.598	4	3.53	445	30.0	.035	.090	2.65	9.28	1.88
	.600	"	3.50	285	19.4	.00805	.068	.610	5.30	.756
	.599	"	3.50	0	20.0	.00064	.030	.483	10.3	.309
T	.600	4	3.53	445	20.0	.0147	.100	1.12	11.4	.638
	.598	"	3.53	"	30.0	.035	.090	2.65	9.28	1.88
	.599	"	3.53	"	52.6	.0524	.080	3.96	7.33	3.55
					Desorption of bacterial cells from the resin					
pH	.616	4	1.46	445	30.0	0.060	0.0526	3.98	4.12	9.41
	.598	"	4.12	"	"	.00564	.0080	.427	.733	38.3

[1] Rate constants and diffusion coefficients determined according to Case B.

[2] Rate constants calculated using base ten logarithmic difference values.

[3] Diffusion coefficients D_k and $D_{k'}$ expressed as $cm^2/sec \times 10^{-7}$, corrected, respectively, by factors of 2.303 and $(2.303)^2$.

[4] Corrected by factor of 1/2.303 .

The ratio of the rate constants, $|k/k'^2|$, has therefore been corrected by the factor of 1/2.303. The diffusion coefficients, D_k and $D_{k'}$, that are derived, respectively, from the rate constants, k and k', have been corrected by the factors, 2.303 and $(2.303)^2$.

Discrepancies between the ratio of the rate constants, $|k/k'^2|$, calculated from Equation (III-34a) and other values determined from the actual experimental adsorption or desorption data can be due to: (1) inaccuracies in the measurements of the various constants, or (2) limitations of the model to account for the effects of certain variables. The significance of these two factors will become more apparent in the subsequent discussion.

Of all the constants required for substitution into Equation (III-34a), the average radius of the resin particles, R, is subject to the greatest error in measurement. This quantity can be eliminated, however, and the rate constant ratio, $|k/k'^2|$, can be represented in the alternative form of Equation (III-34b). All of the quantities, V, β_o, ϵ, and m, are known initially or are experimental values that

$$\left|k/k'^2\right| = \pi V/12\,\beta_o(1-\epsilon)m \qquad \text{(III-34b)}$$

can be determined with a fair degree of accuracy. The only possible source of large error, therefore, is the assumption that R is truely the radius of an average resin particle. The alternative approach to this problem is to postulate the existance of a "sphere of influence" around each resin particle within which a bacterial cell must approach

in order to become firmly adsorbed onto the resin surface. The radius R_I of this "sphere of influence" would be a combination of the radius of the naked resin particle, R, and the thickness, δ, of a stagnant liquid film surrounding the resin particle. The significance of such a stagnant film has been discussed by Valentine and Allison (1959), Koch (1960), and Ware and Novros (1966).

Variations in the "sphere of influence" can be tentatively explained on the basis of changes in the flow behavior of an agitated suspension containing a mixture of resin particles and bacterial cells. The thickness of the stagnant liquid film surrounding each resin particle will be an inverse function of the rate of agitation to some power (Levich, 1962). If $R_I = 2R$ is assumed for the purposes of comparison, then the ratio of the rate constants for the same conditions previously described is $|k/k'^2| = 0.82$. A two-fold change in the radius is thus magnified to an eight-fold change in the rate constant ratio.

The radius of this "sphere of influence" may also vary with the ionic conditions of the surrounding solution. The resin particles are capable of swelling or contracting depending upon the ionic strength of the contacting solution. The thickness of the electrical double layer surrounding a resin particle is also a function of the ionic strength upon the radius of the resin particles and the thicknesses of desorption of bacterial cells from a resin in which case solid salt is added to the medium.

Values of the diffusion coefficient, D, calculated from the two rate constants, k and k', are in agreement within a factor of ten or better for most of the experimental adsorptions tabulated in Table XXXI. Some variations can be noted among the values of the rate constants themselves calculated for a series of adsorptions in which only a single variable has been changed. The rates of adsorption, and those of desorption to a somewhat lesser degree, are still very definite functions of t and $\sqrt{t}$. These dependencies can be observed in the linearity of the difference plots computed for a great variety of experimental conditions. The effects of variations in the cell concentration, resin concentration, pH, salt content, degree of agitation, and temperature upon the magnitudes of the rate constants can still be estimated even though they can not as yet be adequately incorporated into the present form of the mathematical model.

The relationships between the concentration of bacterial cells initially present in a given suspension, as expressed by the absorbance, A_o, and the rate constants, k and k', are shown in Figure 35. The rate constant, k', is essentially independent of A_o; the rate constant, k, and hence the ratio, $|k/k'^2|$, are found to decrease with increasing values of A_o. These findings are not entirely consistent with the proposed model for which both rate constants were predicted to be independent of the concentration of bacterial cells. Apparently the diffusion coefficient is not as constant as was originally assumed and is concentration-dependent.

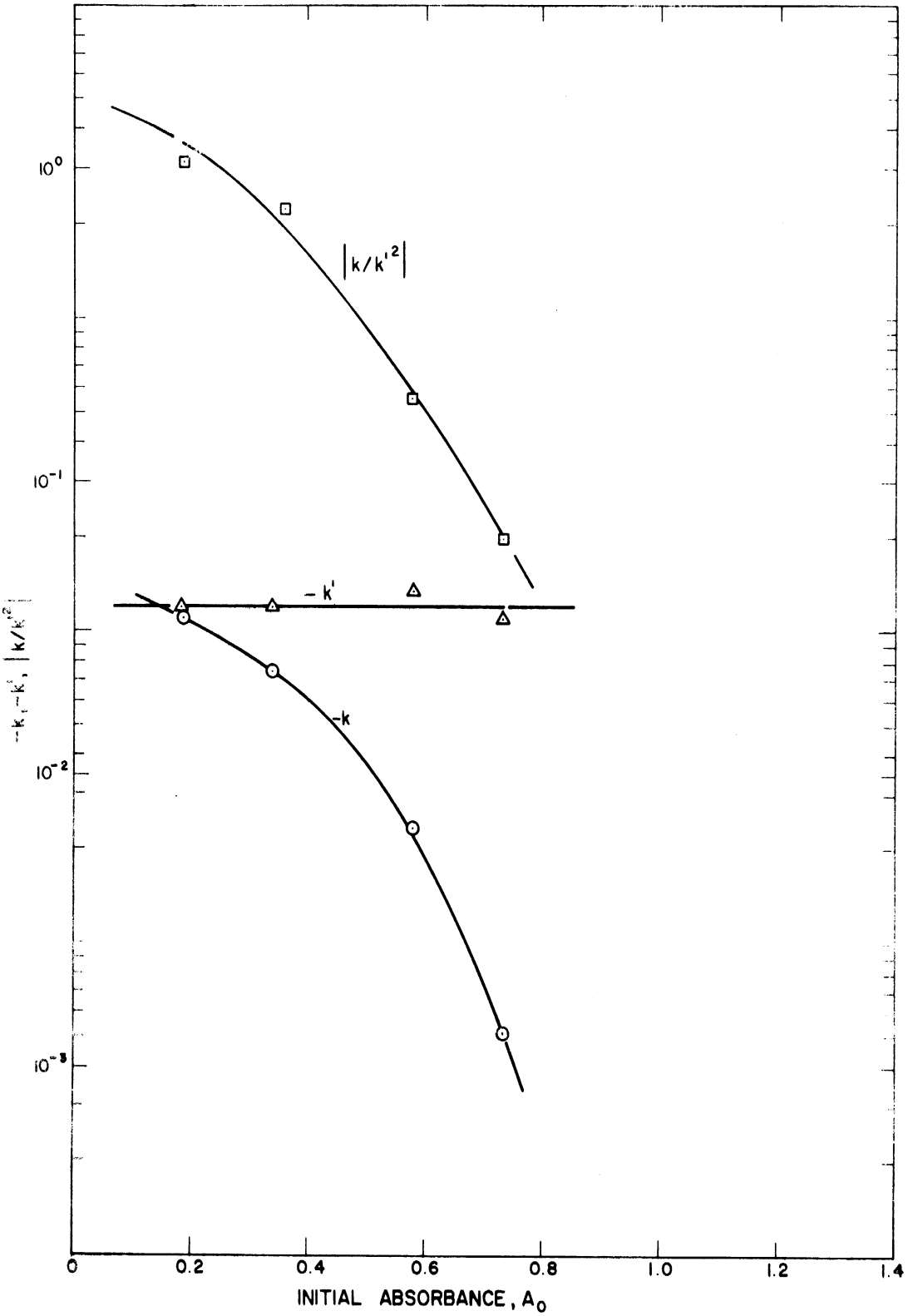


Figure 35. Rate Constants for the Adsorption of Bacterial Cells onto an Anion Exchange Resin as a Function of the Initial Concentration of Bacterial Cells (Bacterial species: Bacillus subtilis, pH 3.5; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml).

The variations of the rate constants, k and k', with respect to the concentration of resin particles, m/V, are predicted to be linear functions of the quantity of resin, m, for constant values of the suspension volume, V. The variations of k and k' as functions of m are shown in Figure 36. Theoretical curves which are based upon such linear functions are fitted to these data assuming for illustration purposes that m = 1/2 gram is the most valid point. A trend toward linearity can be seen but the data are too limited to form definite conclusions as to the validity of the proposed model with respect to this variable.

The relationships between the rate constants, k and k', and the degree of agitation Ω are shown in Figure 37. The constants determined for the condition of "zero" agitation are derived from the experimental data presented earlier in Figure 24 for the case in which no agitation was provided by the magnetic stirrer but some agitation resulted from the periodic sampling. Under static conditions, one percent or less of the total adsorption attainable in an agitated system can be attributed to Brownian motion. The rate of adsorption is markedly increased upon supplementing the Brownian motion with mechanical agitation. Both rate constants appear to be exponential functions of the degree of agitation.

The effect of temperature upon the rate constants is shown in Figure 38. The rate of adsorption is increased upon increasing the

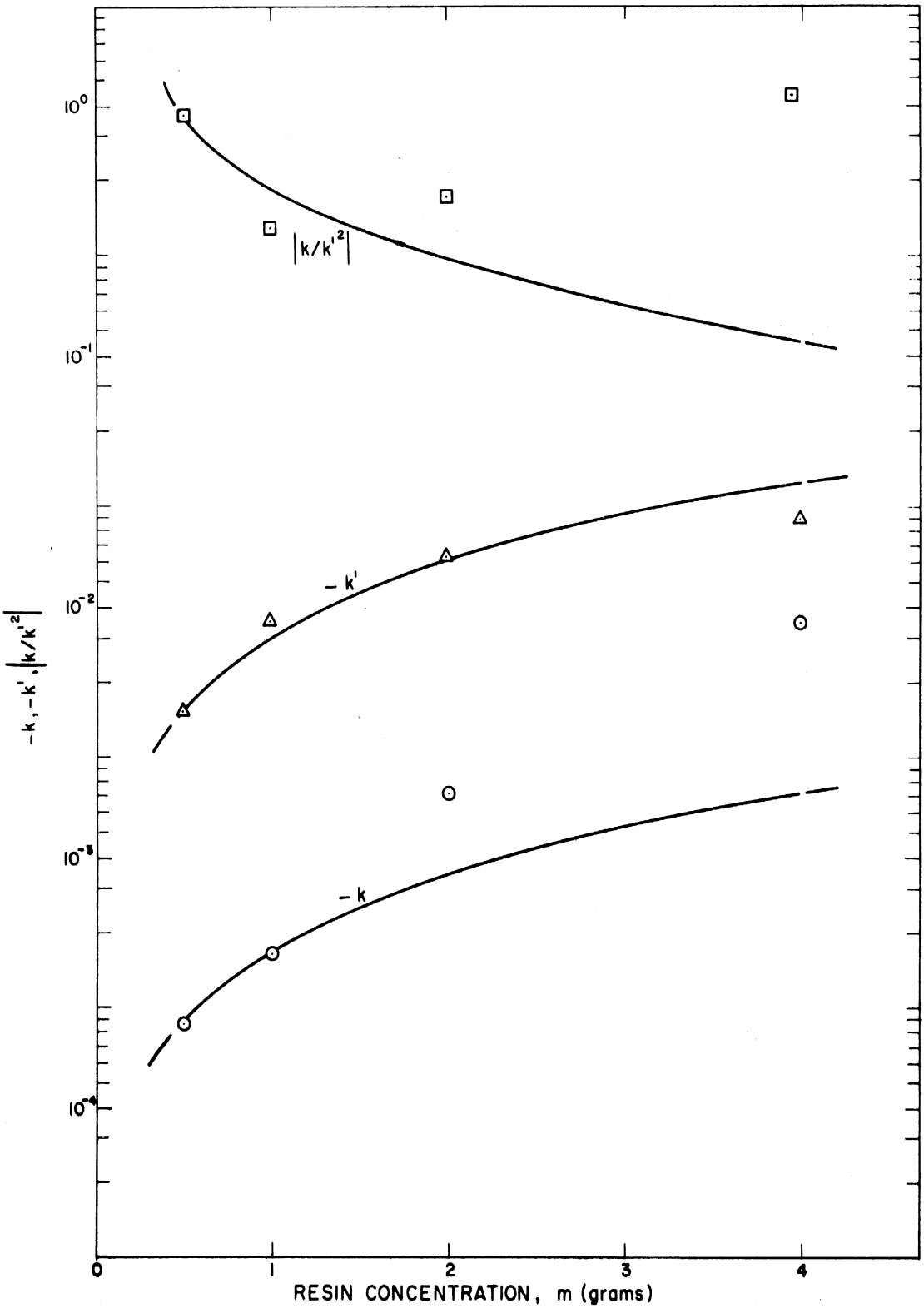


Figure 36. Rate Constants for the Adsorption of Bacterial Cells onto an Anion Exchange Resin as a Function of the Concentration of Ion Exchange Resin (Bacterial species: Bacillus subtilis, A_o = 0.6, pH 3.5; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form).

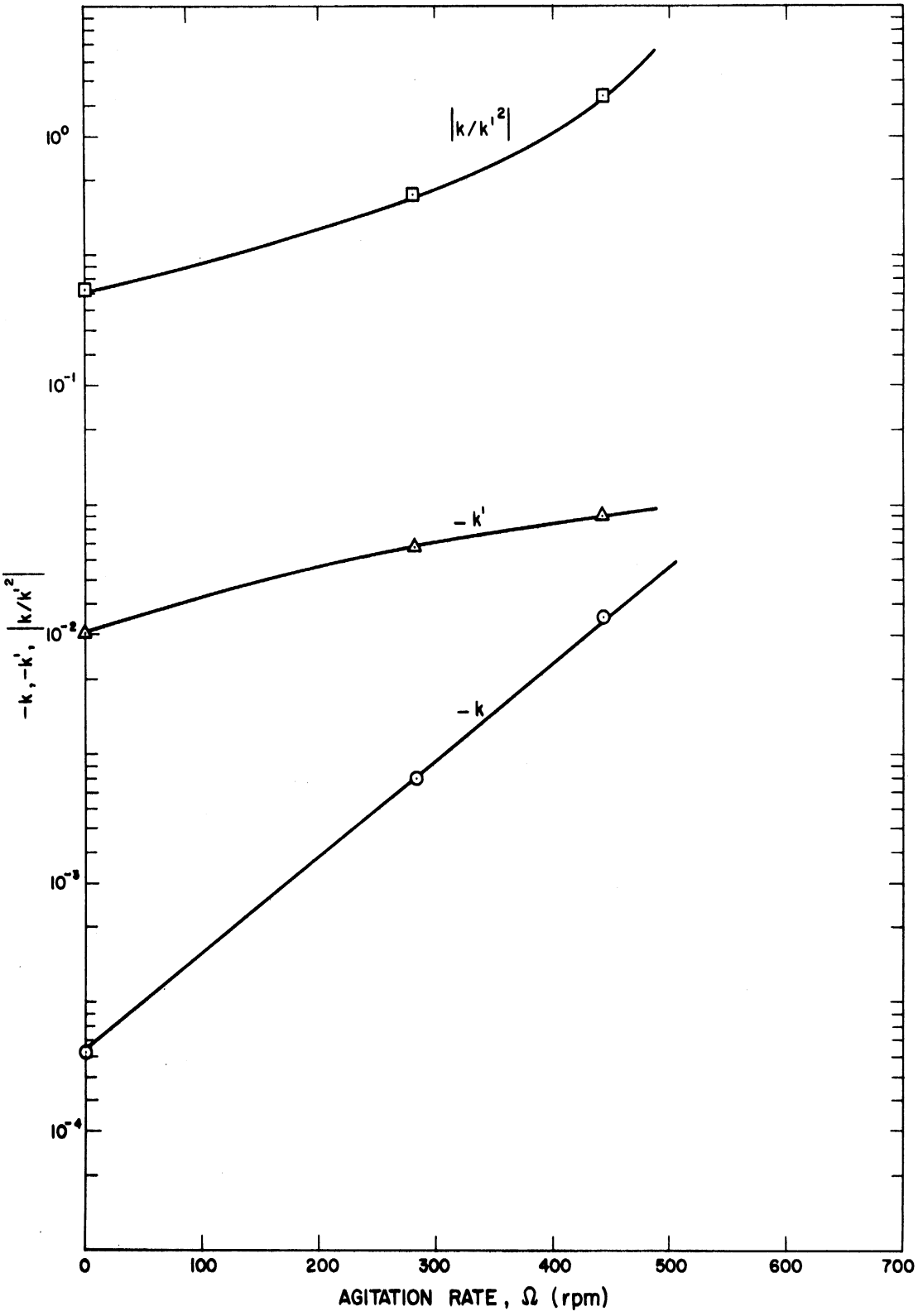


Figure 37. Rate Constants for the Adsorption of Bacterial Cells onto an Anion Exchange Resin as a Function of the Degree of Agitation (Bacterial species: Bacillus subtilis, A_o = 0.6, pH 3.5; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml).

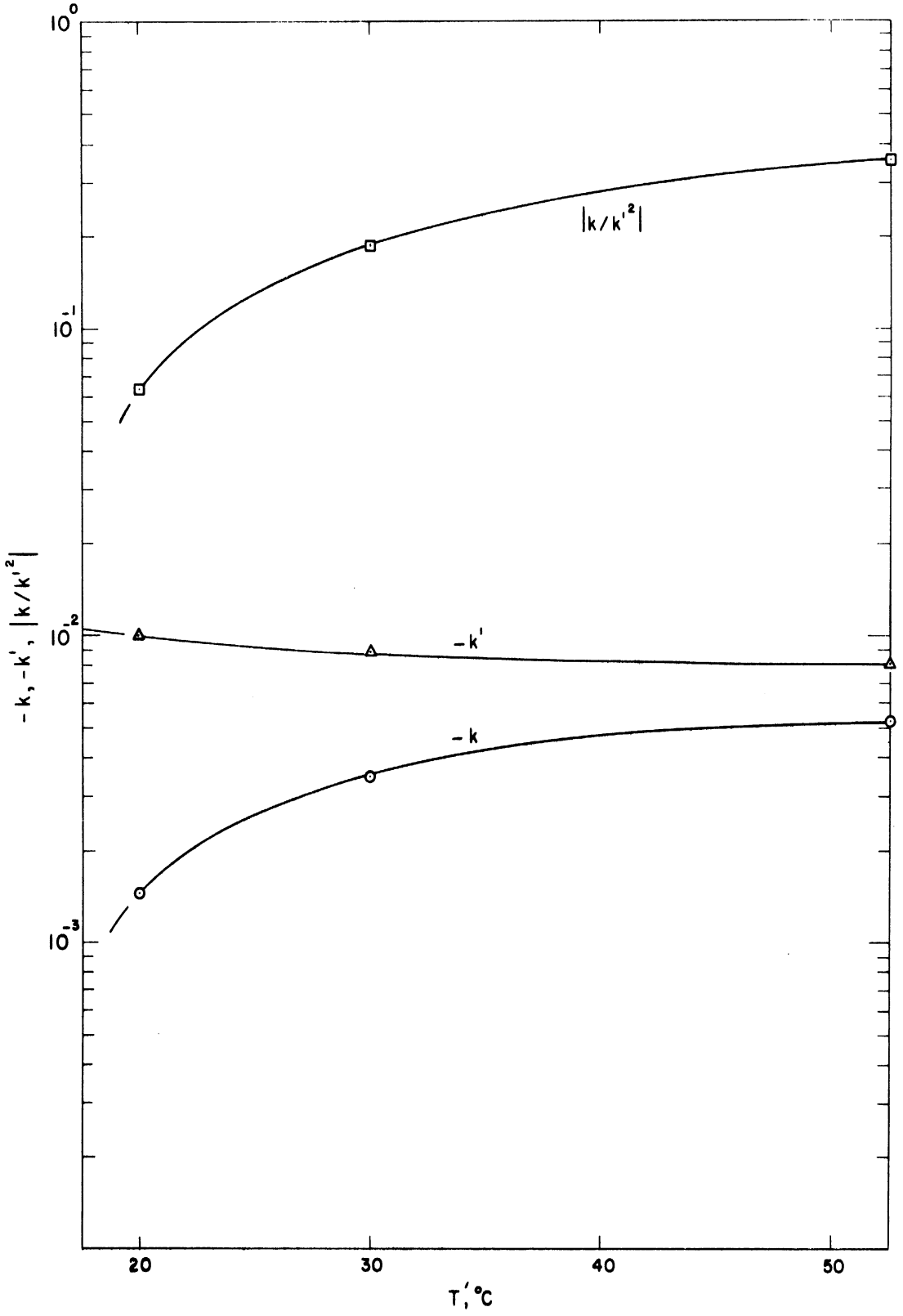


Figure 38. Rate Constants for the Adsorption of Bacterial Cells onto an Anion Exchange Resin as a Function of Temperature (Bacterial species: Bacillus subtilis, $A_o = 0.6$, pH 3.5; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml).

temperature of the system. The rate constant, k', appears to be less dependent upon temperature than does the rate constant, k, although both constants contain the diffusion coefficient, D, which is considered to be a linear function of the temperature, T', as shown in Equation (III-32) which is the Einstein-Stokes equation.

The effects of the hydrogen ion concentration and the presence of various salt ions upon the magnitude of the rate constants are also impossible to rationalize completely due to the paucity of data amenable to rate analyses. The existance of ranges of pH values in which either the adsorption or desorption rate may be maximum is acknowledged but the exact location of these ranges can not be defined at present. The pH values at which the rate and the degree of adsorption or of desorption are maximal are not necessarily identical. The rate of adsorption is decreased and the rate of desorption is increased upon increasing the concentration of salt ions in suspension.

E. Development of Separation Techniques

The preparations of cells of individual species of bacteria used in the experimental work of this thesis were suspended in distilled water or buffer salt solutions. The mixtures to be encountered in actual practice, however, can contain the cells of several bacterial species, $B_1^{\pm}$, $B_2^{\pm}$, ..., having either positive or negative surfaces charges depending upon the pH and the salt concentrations of the suspending media. In addition, other anions, A_i^-, and cations, C_j^+, as well as uncharged species, N_k^o, will be present. These additional components will be

considered to be contaminants for the purposes of this discussion. They may be organic or inorganic, or dissolved or suspended materials.

Mixed suspensions containing both bacterial cells and contaminants can be separated in three ways. They are: (1) purification of the cells by the selective adsorption of the contaminants with no attendant concentration of the cells, (2) nonspecific concentration of the cells and the contaminants with little or no purification of the cells, and (3) resolution of the cells themselves into separate fractions containing the cells of a single species. These three possibilities can be schematically represented.

Mixture		Suspension Phase	Adsorbed Phase
$B_1^{\pm}, B_2^{\pm}, \ldots$	(1)	$B_1^{\pm}, B_2^{\pm}, \ldots$	A_i^-, C_j^+, N_k^0
A_i^-, C_j^+	(2)	A_i^-, C_j^+, N_k^0	$B_1^{\pm}, B_2^{\pm}, \ldots A_i^-, C_j^+, N_k^0$
N_k^0	(3)	A_i^-, C_j^+, N_k^0	$B_1^{\pm}, B_2^{\pm}, \ldots$

The bulk of the discussion of this present section will be directed toward the resolution of mixed suspensions containing cells of several bacterial species into their component species.

1. Purification without concentration

The contaminants present in a suspension also containing bacterial cells can be removed by adsorption onto or absorption into a selective solid material that is introduced into the mixture. The bacterial cells remain undisturbed in the bulk suspension and are not concentrated to any extent. Toxic materials can thus be removed from growing

cultures of bacterial cells. No detailed experimental work was conducted in the current research with respect to the purification of cell suspensions through the selective adsorption of contaminants. The following observations, however, can be made.

Bacterial cells behave as cations at pH values below their isoelectric points and do not adsorb onto anion exchange resins. This feature is illustrated in Figure 17. Other materials that act as anions in the same pH range can therefore be removed by anion exchange without affecting the cells. Conversely, at pH values above their isoelectric points bacterial cells behave as anions and do not significantly adsorb onto cation exchange resins. This feature is shown in Figure 16. Contaminating cations can therefore be removed by cation exchange in this pH range without affecting the cells.

The anionic-cationic behavior of bacterial cells of Bacillus subtilis and Pseudomonas ovalis are shown as functions of pH, respectively, in Figures 21 and 22. Separation of the bacterial cells from the contaminating material can thus be accomplished by selective adsorption of the latter onto ion exchange resins at designated pH values where the cells are predominantly of one charge and the contaminating material is of the opposite charge. Bacterial cells can also be barred from adsorption onto the resins by maintaining a sufficiently high concentration of salt in the suspension. A concentration of 0.2 M sodium chloride is sufficient to prevent the adsorption of cells of Bacillus subtilis or to desorb cells that have already been adsorbed. This

behavior is shown in Figure 23. Contaminating material conceivably could be removed by adsorption onto the resins at this salt concentration without affecting the bacterial cells.

2. Concentration without purification

The bacterial cells and an undetermined amount of the contaminating material can also be nonspecifically removed from suspension by adsorption onto a contacting solic. Bacterial cells can thus be concentrated from dilute suspensions. Water supplies or other fluids could possibly be sterilized by the removal of bacterial cells by adsorption. Cells may also be recovered from fermentation broths and further processed to recover a natural product.

The cells of one or more bacterial species can be simultaneously adsorbed onto particles of an ion exchange resin provided that sufficient surface area is available to accomodate them. This feature is shown in Figure 42 for the case of the simultaneous adsorption of cells of Bacillus subtilis and Staphlococcus aureus. The proposed resolution of the quaternary mixture, Escherichia coli, Pseudomonas ovalis, Staphlococcus aureus, and Bacillus subtilis, is based upon the premise that the cells of the latter three species can simultaneously adsorb onto the same ion exchange resin in a reasonable period of time.

3. Resolution of mixed suspensions

The resolutions of five binary suspensions prepared from pure cultures of cells of individual bacterial species were achieved. The

five mixed suspensions were prepared by combining equal volumes of two separate cell suspensions that had been previously adjusted to equal absorbances. The mixtures prepared were: (1) Escherichia coli - Bacillus subtilis (Ec-Bs), (2) Escherichia coli-Staphlococcus aureus (Ec-Sa), (3) Escherichia coli - Pseudomonas ovalis (Ec-Po), (4) Staphlococcus aureus - Bacillus subtilis (Sa-Bs), and (5) Bacillus subtilis - Proteus vulgaris (Bs-Pv). Mixtures containing the cells of either Gram-negative (Gram(-)) species, or Gram-positive (Gram(+)) species, or both Gram (-) and Gram (+) species, are included in the above series. Examples of resolutions obtained by Type I and Type II using either anion or cation exchange resins are included.

(1) Escherichia coli - Bacillus subtilis (Ec-Bs)

The binary mixture Ec-Bs contains, respectively, Gram(-) and Gram(+) bacilli. Resolution of this mixture was attempted by a Type I anion exchange. The cells of one species (Bs) were to be selectively adsorbed onto the resin while those of the other species (Ec) were to remain unadsorbed. The adsorbed cells of the first species (Bs) were to be subsequently desorbed into a fresh solution containing a suitable regenerating agent. The experimental results obtained for the resolution of the Ec-Bs mixture are presented in Figure 39. The scheme of separation and the quantitative recoveries of the cells of the two species into separate fractions are also included.

Some Ec cells weakly adsorbed onto the ion exchange resin during the initial stages of the process but later became completely

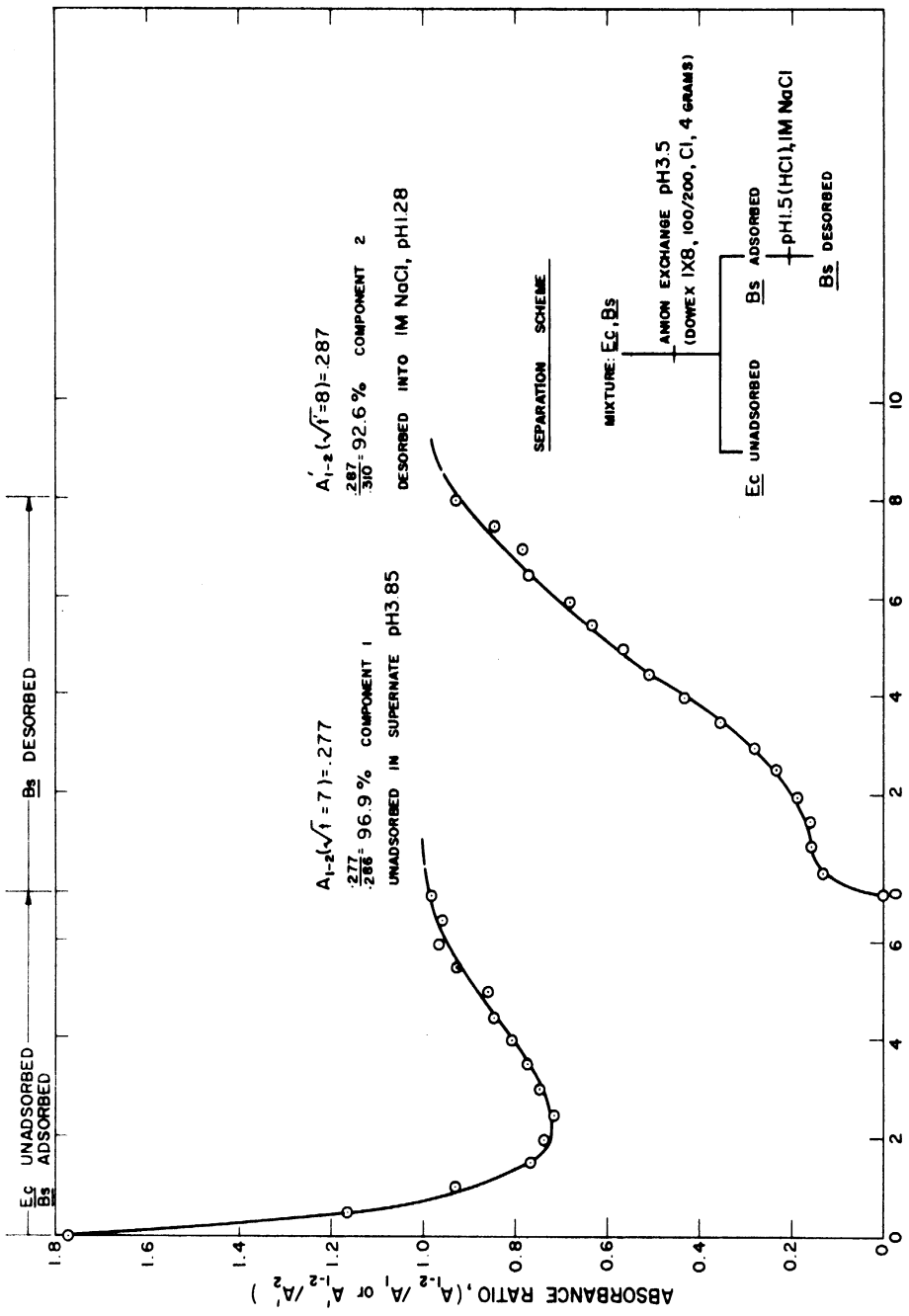


Figure 39. Resolution of the Binary Mixture: Escherichia coli - Bacillus subtilis (Ec-Bs), by a Type I Anion Exchange (Bacterial species: Escherichia coli, A_1 = 0.286, Bacillus subtilis, A'_2 = 0.310; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml).

desorbed due to the extended agitation. This behavior was evidenced by a dip in the absorbance ratio below unity followed by a gradual return to unity. Similar behavior has been shown in Figure 12 for the case of the adsorption of cells of Ec from pure suspensions. In the present example, the cells of the Gram(-) Ec were much more abundant than those of the Gram(+) Bs in a Gram stain made of the unadsorbed cells still in suspension after time, $\sqrt{t} = 7$ min$^{1/2}$. Approximately 96.9% of the cells of the pure component Ec ($A_1 = 0.286$) were represented in the suspension phase ($A_{1-2} = 0.277$). The majority of the Bs cells were strongly adsorbed onto the resin. The resin phase was then gently rinsed with distilled water to free any unadsorbed cells that had become physically entrapped in the interstices of the cell-resin floc.

Desorption of the Bs cells occurred upon transfer of the washed resin phase to a fresh M sodium chloride solution previously adjusted to pH 1.5 with hydrochloric acid. Approximately 92.6% of the cells of the pure component Bs ($A_2' = 0.310$) were represented in the desorbed fraction ($A'_{1-2} = 0.287$). The desorbed cells were predominantly Gram(+) in a stain prepared from the suspension at time, $\sqrt{t'} = 8$ min$^{1/2}$. A small amount of Gram(-) debris was also present which may have resulted from rupture of the cells during agitation or to the combined action of the salt and acid. The high degree of resolution obtained for the Ec-Bs mixture by ion exchange was considered to be good but not optimum.

(2) Escherichia coli - Staphlococcus aureus

Gram(-) bacilli and Gram(+) cocci, respectively, are contained in the binary mixture Ec-Sa. Resolution of this mixture was attempted by a Type I anion exchange similar to the procedure applied to the Ec-Bs mixture. In the present case, the Sa cells were to be selectively adsorbed while the Ec cells remained unadsorbed. The experimental results obtained for the Ec-Sa mixture are presented in Figure 40.

The adsorption-desorption behavior of Ec previously reported for the Ec-Bs mixture was repeated in the present case. Strong adsorption of the Sa cells was concluded on the basis of a complete absence of Gram(+) cocci in a Gram stain prepared of the cells still in suspension at time, $\sqrt{t}$ = 7 $min^{1/2}$. Essentially all of the cells of the pure component Ec (A_1 = 0.358) were found in the suspension phase (A_{1-2} = 0.358). The resin was washed free of the suspension containing the unadsorbed cells and then transferred to a fresh solution previously adjusted to pH 1.5. The desorption of the Sa cells was apparent from a Gram stain made at time, $\sqrt{t'}$ = 10 $min^{1/2}$, that contained only Gram(+) cocci. A recovery of 100% of the cells of the pure component Sa (A_2' = 0.312) was obtained in the desorbed fraction (A'_{1-2} = 0.312). The resolution of the Ec-Sa mixture was considered to be complete.

(3) Escherichia coli - Pseudomonas ovalis (Ec-Po)

The binary mixture Ec-Po is particularly interesting since the cells of both component species are Gram(-) becilli. Resolution was

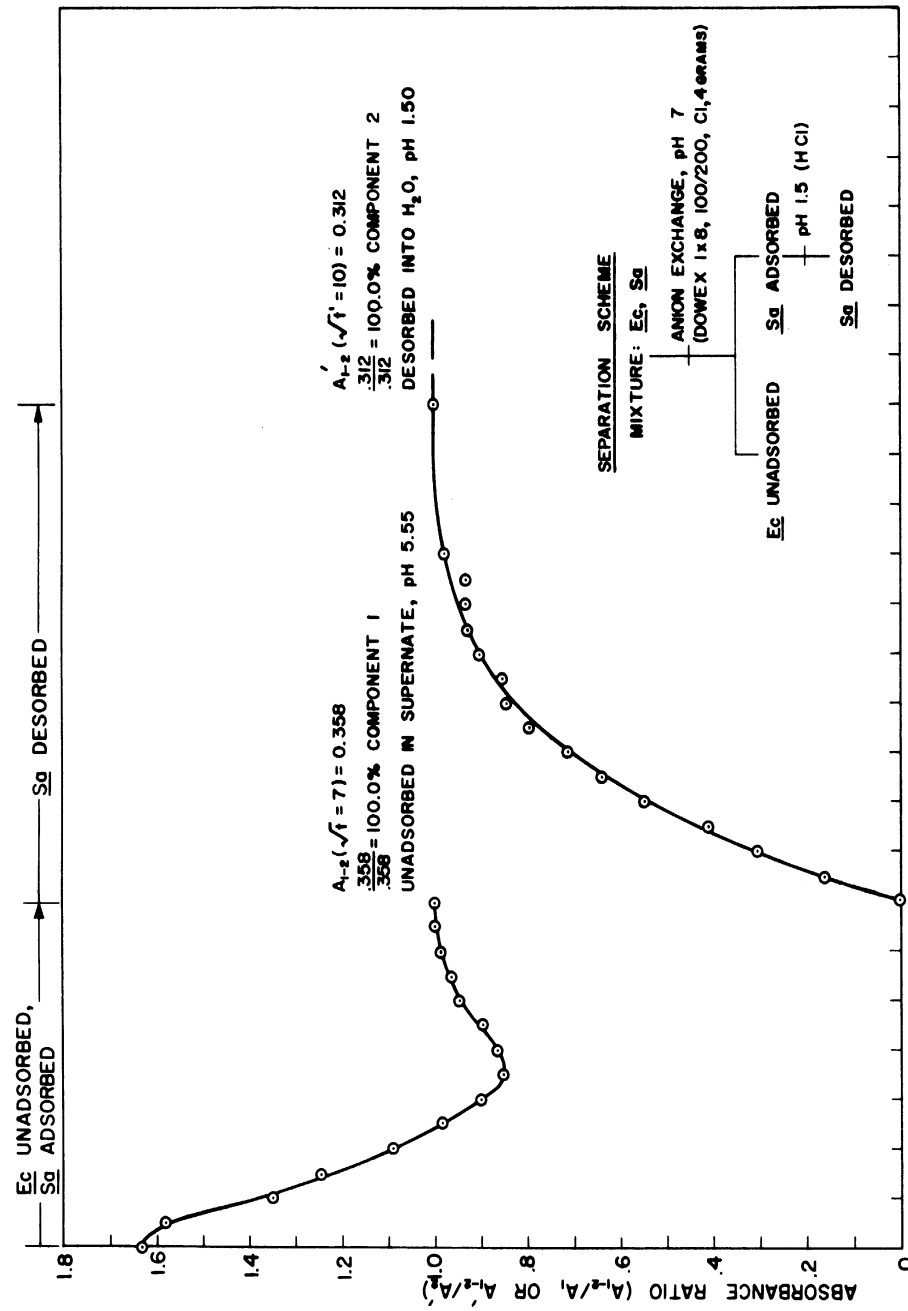


Figure 40. Resolution of the Binary Mixture: Escherichia coli - Staphlococcus aureus (Ec-Sa), by a Type I Anion Exchange, (Bacterial species: Escherichia coli, A_1 = 0.358, Staphlococcus aureus, A_2 = 0.312; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml).

again attempted by a Type I anion exchange. In the present case, however, resolution could not be verified by the Gram stain. Strong supporting evidence for the applicability of a Type I anion exchange is provided instead by previous observations of the adsorption behaviors of the cells of each component species in the present mixture in separate suspensions. Strong adsorption was noted for the cells of Po while those of Ec did not permanently adsorb as illustrated in Figure 12. It is tacitly assumed that no interaction will occur between the cells of these two component species in the present mixture. Resolution of the Ec-Po mixture is presented in Figure 41.

A Gram stain prepared from the suspension at time, $\sqrt{t}$ = 10 $min^{1/2}$, contained all Gram(-) cells. Essentially 100% of the cells of the pure component Ec (A_1 = 0.354) were considered to be present in the unadsorbed fraction (A_{1-2} = 0.354). This conclusion was based upon the observed dip in the absorbance ratio which has been shown to be characteristic of Ec. Desorption of the Po cells was promoted by contacting the washed resin phase with an aqueous solution adjusted to pH 1.5. Gram(-) bacilli and some debris were observed in a Gram stain prepared from the desorbed fraction at time, $\sqrt{t'}$ = 10 $min^{1/2}$. A recovery of 95.8% of the cells of the pure component Po (A_2' = 0.328) was realized in the desorbed fraction (A'_{1-2} = 0.314). The resolution of the Ec-Po mixture was considered to be good on the basis of the limited criteria that were applied. More conclusive differentiation of the cells of the component species in the separated fractions is warrented in future experiments.

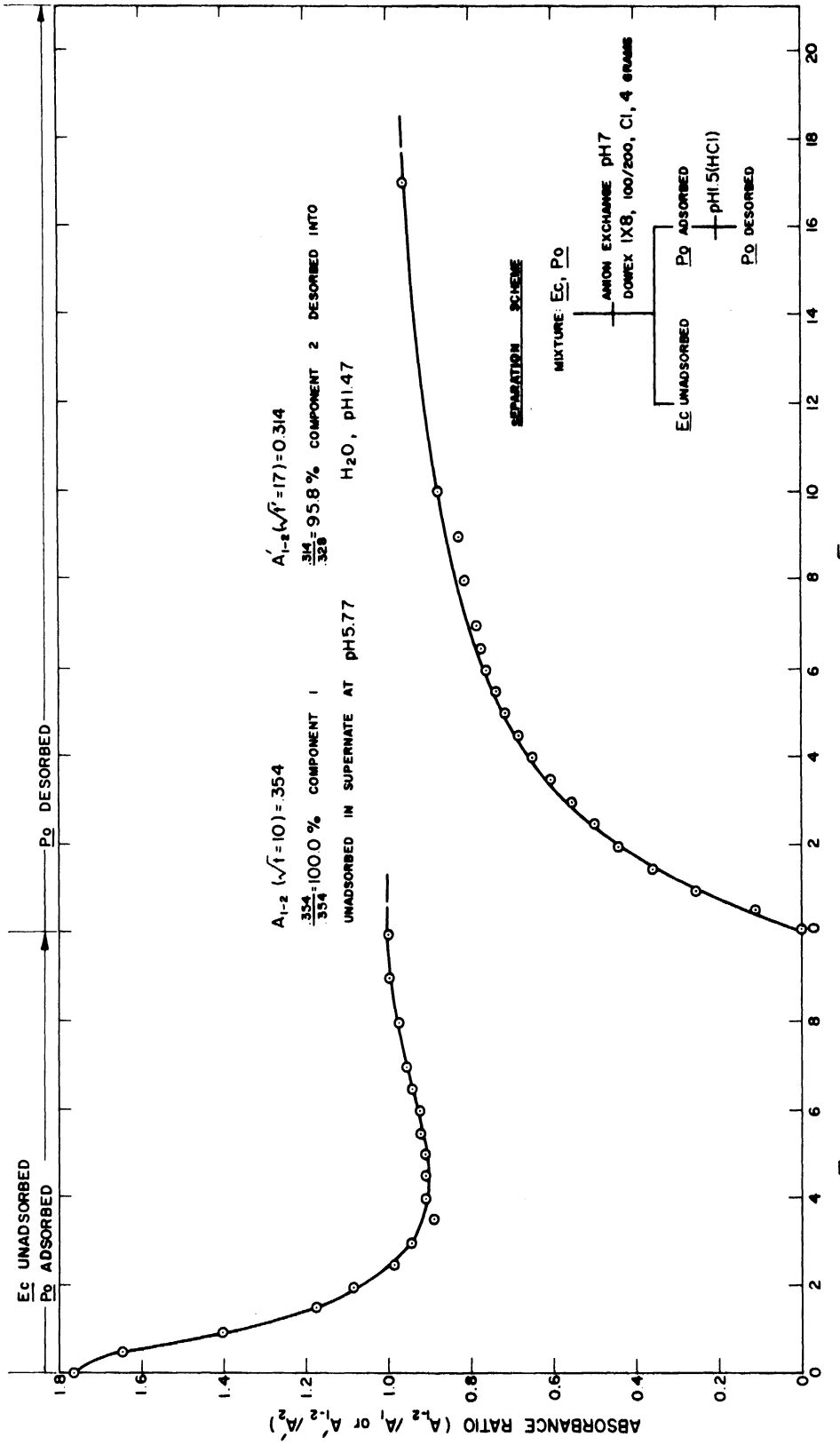


Figure 41. Resolution of the Binary Mixture: Escherichia coli - Pseudomonas ovalis (Ec-Po), by a Type I Anion Exchange (Bacterial species: Escherichia coli, A_1 = 0.354, Pseudomonas ovalis, A'_2 = 0.328; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml).

(4) Staphlococcus aureus - Bacillus subtilis (Sa-Bs)

Another interesting case is the binary mixture Sa-Bs, which contains, respectively, Gram(+) cocci and Gram(+) bacilli. Resolution of this mixture was attempted by a Type II anion exchange. Nonselective adsorption of the cells of both species (Sa and Bs) was to be followed by sequential desorption of the cells of the first species (Sa) and those of the second species (Bs). The experimental results obtained for the resolution of the Sa-Bs mixture are shown in Figure 42.

Insufficient resin capacity was available for complete adsorption of all cells of both species in a reasonable period of time. Only Gram(+) cocci were found to be present in a stain prepared from the suspension at time, $\sqrt{t}$ = 6 $min^{1/2}$. All cells of Bs were apparently adsorbed. In addition, 62.6% of the cells of the pure component Sa (A_1 = 0.342) were also adsorbed from the mixture (A_{1-2} = 0.214).

Contact of the washed resin phase with an aqueous solution adjusted to pH 1.5 resulted in the desorption of some Gram(+) cocci, which were observed in a stain made of this first desorbed fraction at time, $\sqrt{t'}$ = 4 $min^{1/2}$. About 32.4% of the cells of the pure component Sa (A_1' = 0.342) were recovered in this first desorbed fraction (A_{1-2}' = 0.101). The total percentage of Sa cells recovered in the initial suspension and in the first fraction to be desorbed was therefore 62.6% + 32.4% = 95.0%.

The resin phase was washed again, this time with a fresh aqueous solution at pH 1.5, and then resuspended in one molar sodium

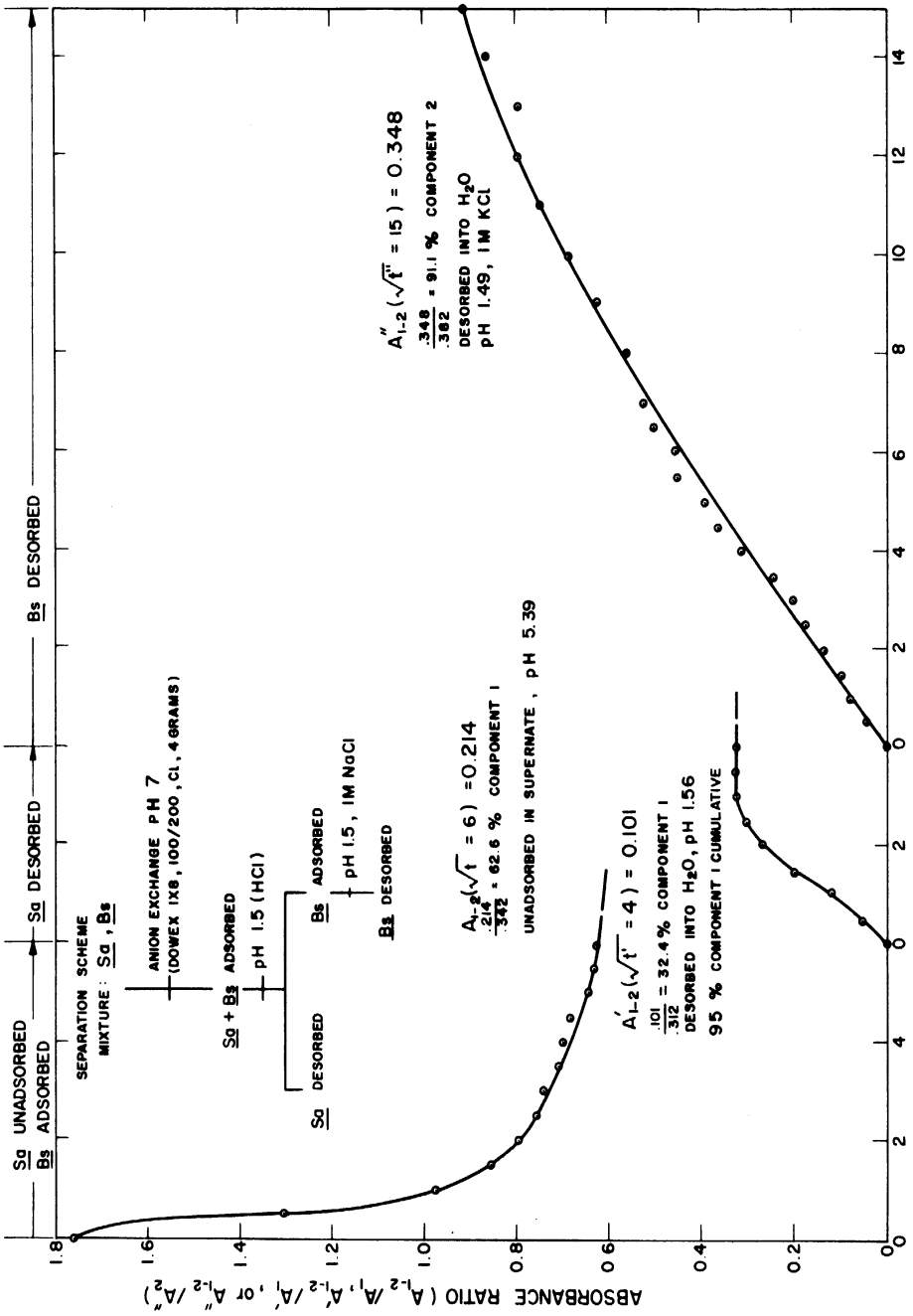


Figure 42. Resolution of the Binary Mixture: Staphlococcus aureus - Bacillus subtilis (Sa-Bs), by a Type II Anion Exchange (Bacterial species: Staphlococcus aureus, $A_1 = 0.342$, $A_1 = 0.312$, Bacillus subtilis, $A_2'' = 0.382$; anion exchange resin: Dowex 1 x 8, 100/200 mesh, chloride form, 4 grams/50 ml).

chloride solution also at pH 1.5. Analysis of the second fraction to be desorbed (A''_{1-2} = 0.348) showed that 91.1% of the cells of the pure component Bs (A''_2 = 0.382) were recovered. A Gram stain prepared at time, $\sqrt{t''}$ = 15 $min^{1/2}$, contained a majority of Gram(+) bacilli and a very limited number of Gram(+) cocci. The resolution of a mixture containing the cells of two Gram(+) species, therefore, is a distinct possibility. Additional anion exchange resin is necessary to improve the degree of resolution of the Sa-Bs mixture that has been described above.

(5) Bacillus subtilis - Proteus vulgaris (Bs-Pv)

Gram(+) and Gram(-) bacilli, respectively, are contained in the binary mixture Bs-Pv. The resolution of this mixture was attempted according to the alternative scheme of a Type I cation exchange for two reasons. Cells of Pv coagulate at the low pH values which are necessary to desorb them from an anion exchange resin. Secondly, the cells of Bs do not significantly adsorb onto a cation exchange resin above pH 3. The experimental results obtained for the Bs-Pv mixture are presented in Figure 43.

Essentially 100% of the cells of Pv and 18.0% of those of the pure component Bs (A_1 = 0.410) were adsorbed from the mixture (A_{1-2} = 0.336). Only a few scattered Gram(-) Pv cells were observed in the midst of large numbers of Gram(+) Bs cells in a stain prepared from the suspension at time, $\sqrt{t}$ = 7 $min^{1/2}$. Subsequent desorption of the Pv cells from the washed resin phase was accomplished by contacting the resin with

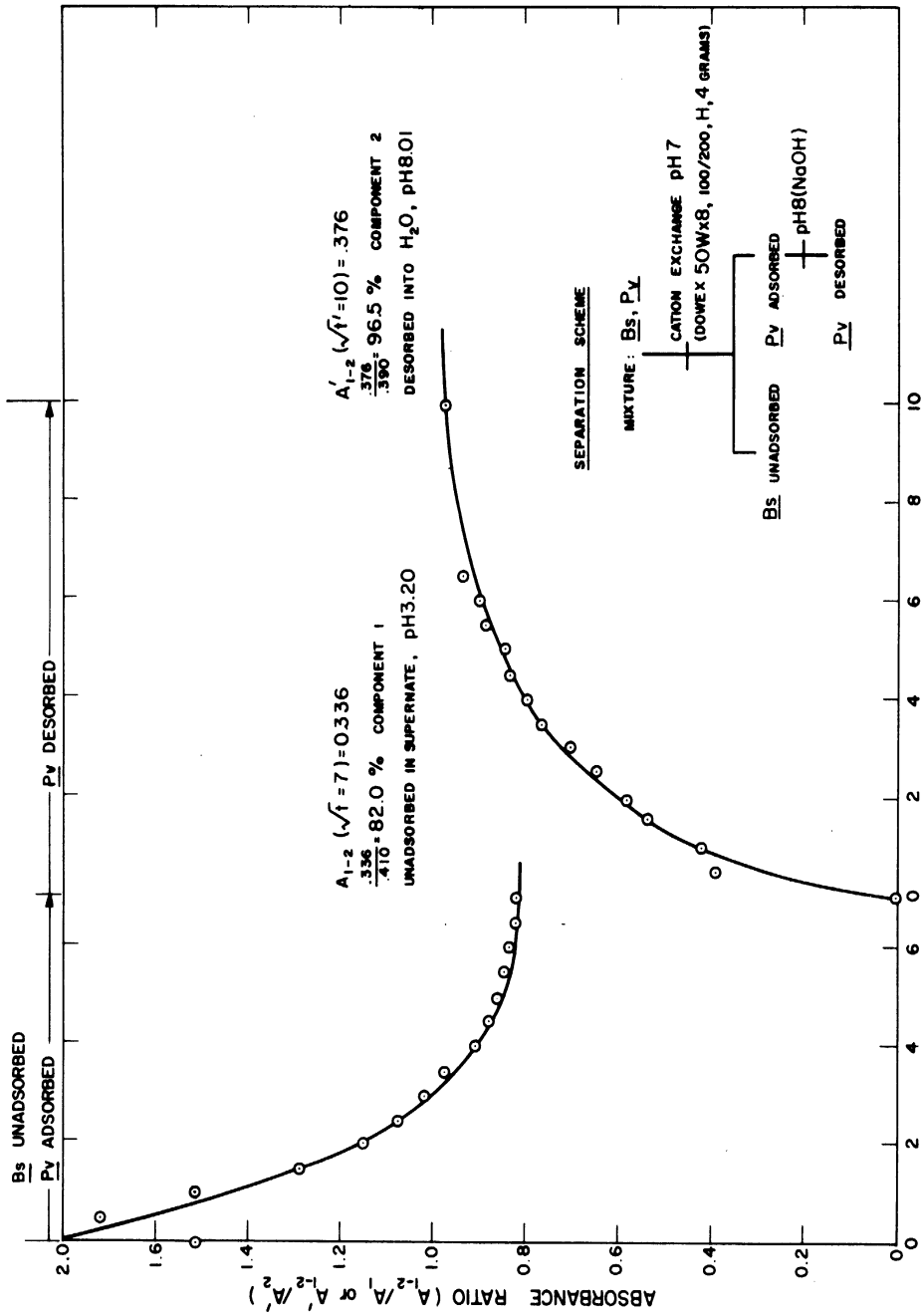


Figure 43. Resolution of the Binary Mixture: Bacillus subtilis - Proteus vulgaris (Bs-Py), by a Type I Cation Exchange (Bacterial species: Bacillus subtilis, A_1 = 0.410, Proteus vulgaris, A'_2 = 0.390; cation exchange resin: Dowex 50W x 8, 100/200 mesh, hydrogen form, 4 grams/50 ml).

a fresh aqueous solution adjusted to pH 8 with sodium hydroxide. The desorbed fraction (A'_{1-2} = 0.376) contained 96.5% of the cells of the pure component Pv (A'_2 = 0.390). A few scattered Gram(+) Bs cells and many Gram(-) Pv cells were observed in a stain made of the desorbed fraction at time, $\sqrt{t'}$ = 10 $min^{1/2}$. The small percentage of Bs cells initially adsorbed, or otherwise physically trapped in the resin phase, were apparently retained on the resin during desorption or had been lost upon washing. The resolution of the Bs-Pv mixture was not ideal, but the possibilities of the alternative technique of cation exchange are evident.

The resolutions of the five binary mixtures described in this report are summarized in Table XXXII. Particular note is made of the high degrees of recovery obtained using batch exchange techniques.

TABLE XXXII

RESOLUTIONS OF MIXED SUSPENSIONS OF BACTERIA

Species 1	Species 2	Type of Resolution	Recovery of Component Species, %[1]	
			Species 1	Species 2
Escherichia coli (Ec)	Bacillus subtilis (Bs)	Type I anion exchange: species 1 unadsorbed; species 2 adsorbed, desorbed by low pH and addition of salt	96.9	92.6
Escherichia coli (Ec)	Staphlococcus aureus (Sa)	Type I anion exchange: species 1 unadsorbed; species 2 adsorbed, desorbed by low pH	100.0	100.0
Escherichia coli (Ec)	Pseudomonas ovalis (Po)	Type I anion exchange: species 1 unadsorbed; species 2 adsorbed, desorbed by low pH	100.0	95.8
Staphlococcus aureus (Sa)	Bacillus subtilis (Bs)	Type II anion exchange: species 1 and 2 adsorbed; species 1 desorbed by low pH; species 2 desorbed by low pH and addition of salt	95.0[2]	91.1
Bacillus subtilis (Bs)	Proteus vulgaris (Pv)	Type I cation exchange: species 1 unadsorbed; species 2 adsorbed, desorbed by high pH	82.0	96.5

[1] All percentages of recovery are based upon absorbances of suspensions of the pure components measured at comparable conditions of pH, salt content, and dilution to those of the resolved fractions.

[2] Comprised of 62.6% unadsorbed cells in initial suspension and 32.4% desorbed cells.

VI. SUMMARY

Phenomena that are attributable to the surface charge on microorganisms have been observed in a number of areas of microbiology. These have included the adsorption of drugs and dyes onto microorganism, interactions between phage particles and host cells, reactions of antibodies and antigens, activities of surface-active germicides, transfer of metabolites, flocculation of cells by hydrophilic colloids and other chemical agents, and movement of cells in applied electrical fields, and the adsorption of cells onto soid particles, gas bubbles and other surfaces. The primary goals of the research reported in this manuscript are to provide explanations for the phenomena observed in the adsorption of bacterial cells onto ion exchange resins and to develop suitable mathematical correlations that can be applied to the development of practical separations.

This study involved the contact of particles of either anion or cation exchange resins with agitated suspensions of bacterial cells. The rate of adsorption and desorption in these mixed suspensions were followed by measuring the absorbances of samples containing the unadsorbed cells. The bacteria studied included Pseudomonas ovalis, Escherichia coli, Proteus vulgaris, Staphlococcus aureus, Bacillus cereus and Bacillus subtilis. Both anion and cation exchange resins were used in these experiments.

A. Establishment of Adsorption

The attachment of bacterial cells onto particles of an ion exchange resin is a surface adsorption phenomenon. Evidence in support of this conclusion can be summarized:

i. The cells and resin particles flocculate together during adsorption and form an intimate complex; no separate sedimentation of the cells due to a chemical precipitation can be observed; the number of freely-dispersed cells decreases.

ii. In photomicrographs, the cells appear attached in a "pincushion" fashion to the surfaces of the resin particles the adsorbed cells do not penetrate into the interiors of the particles.

iii. The rate of adsorption of cells is increased if the surface area of the resin is increased while the weight of resin is kept constant.

iv. The rate of adsorption of cells is dependent upon the degree of agitation of the suspending medium and becomes negligible if agitation is halted.

v. The total number of cells actually adsorbed is approximately equal to the number calculated assuming a monolayer of adsorbed cells. The value of the former is 0.610×10^{10} cells/gram, for the adsorption of the cells of Bacillus subtilis onto Dowex 1 x 8, 100/200 mesh, chloride form, at pH 3.5.

B. Determination of Variables

At least fifteen variables are associated with: (1) the organism to be exchanged, (2) the resin producing the exchange, and (3) the chemical environment of the exchange. Several variables that are significant in establishing the character of the sorption process are:

i. Species of bacterium - six species were investigated.

ii. Type of resin - both anion and cation exchange resins were used.

iii. Hydrogen ion concentration - important in determining the magnitudes and signs of the surface charges of cells relative

to their isoelectric points; index of both adsorption and desorption; promotor of desorption by charge reversal

iv. Salt concentration - direct adsorption is inhibited and desorption is promoted by increased salt concentrations; adsorption by a bridging mechanism may possibly be enhanced by multivalent salt ions.

v. Agitation - influential upon the rates of adsorption and desorption; promotor of desorption over extended periods of time.

Several variables are of lesser importance:

i. Particle size - the capacity of a resin for bacterial cells is a function of the available surface area.

ii. Concentration of cells - concentrated suspensions of cells can exert a considerable buffer capacity; the rate of adsorption is also a function of the total number of cells and the ratio of unadsorbed to adsorbed cells.

iii. Concentration of resin - the rate and degree of removal of cells are dependent upon the amount of resin per unit volume of the mixed suspension.

iv. Time of contact - used as an independent variable in association with absorbance measurements to monitor adsorption and desorption.

v. Temperature - the rate of adsorption is increased as the temperature is increased.

The effects of five other variables were not extensively evaluated:

i. Culture medium - the surface structures and charges of cells are established during growth.

ii. Culture age - the electrophoretic mobilities of cells are known to change with age.

iii. Suspension medium - distilled water and dilute buffers were used in the current work; the electrophoretic mobilities of cells are known to be a function of the suspending medium.

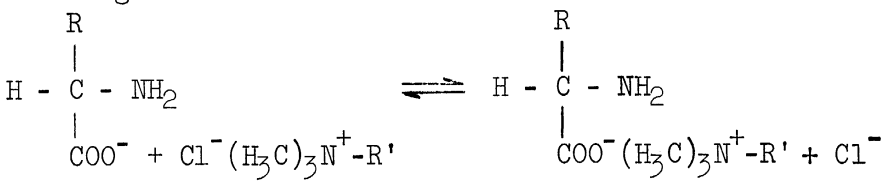
iv. Ionic form - the anion exchange resin was principally chloride form; acetate, hydroxide, and nitrate forms were also tested. The cation exchange resin was principally hydrogen form; sodium and calcium forms were also used.

v. Crosslinkage - the selectivity and the swelling character of a resin are known to be a function of the amount of crosslinking in the resin.

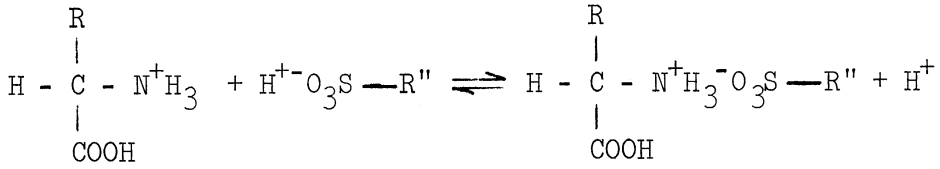
C. Elucidation of Mechanism and Model

The adsorption of bacterial cells of net positive or negative electrical charge onto the surfaces of the resin is considered to be a stoichiometric process that is accompanied by the simultaneous release of other ions exchanged from the resin matrix.

Anion exchange



Cation



The complex surface structure of a bacterial cell is represented by the radical, R ; the large polymeric structures of the anion and cation exchange resins are represented, respectively, by the radicals, R' and R" . The terminal groups characterizing the bacterial cell wall are idealized and may be multiplied by a factor of 10^8, the approximate number of active exchange sites per bacterial cell. The experimental results that can be considered as evidence in support of these postulated mechinisms can be enumerated:

i. Bacterial cells are negatively charged at pH values above their isoelectric points and are capable of being exchanged as anions; conversely, cells are positively charged at pH values below their isoelectric points and are capable of being exchanged as cations.

ii. Ions are released from the resins simultaneously with the adsorption of bacterial cells onto the resin; these exchanged counter ions can be detected from pH changes in the suspending medium and by specific ion tests.

iii. The adsorption of bacterial cells onto a resin can be reversed by changing the pH and/or the salt concentration of the suspending medium.

iv. Experimental values of the capacity of the resins for adsorption of bacterial cells are in good agreement with values calculated on the basis of the available surface area of the resins assuming a longitudinal orientation of the adsorbed cells.

Limitations in the experimental results with respect to the mechanism can also be enumerated:

i. Quantitative data for release of ions from the resin to the suspending medium are too limited to permit calculation of values of the resin capacity for bacterial cells.

ii. The selectivity for adsorption of bacterial cells by the various ionic forms of the anion and cation exchange resins are limited to qualitative comparisons.

iii. The signs, magnitudes, and distributions of the surface charges of the bacterial cells were not experimentally verified by the electrophoretic or other techniques.

iv. The predominant chemical groups producing the net positive and negative surface charges on the bacterial cells were not specifically identified.

The rates of adsorption and desorption of bacterial cells contacting particles of ion exchange resins has been postulated to be a composite function of the cumulative time and the square root of the cumulative time of agitated contact. The mathematical form of the rate expression is: $f(A/A_0) = kt + k'\sqrt{t}$ where $f(A/A_0) = A/A_0$ for adsorption, $f(A/A_0) = 1-(A/A_0)$ for desorption, k and k' are rate constants, and A_0 and A are absorbances of the suspension containing only unadsorbed cells initially and at some later time. This model is based upon the theory of colloid coagulation advanced by Smoluchowski. The experimental results offered as supporting evidence of this proposed model are:

i. The difference plots of the sorption rate data are linear and the rate constants, k and k', can be easily determined.

ii. Reasonable agreement was demonstrated between the theoretical and experimental values of the ratio of the rate constants, $|k/(k')^2|$ for the case of adsorption.

iii. The diffusion coefficients calculated by several methods for a variety of experimental conditions are also in reasonable agreement. They are of the order of 10^{-7} cm^2/sec.

The limitations of the proposed mathematical model are:

i. The experimental rates of desorption are not as well represented by the model as are the rates of adsorption.

ii. The diffusion coefficients depend upon the concentration of the bacterial cells present in the reacting mixtures.

iii. The effects of pH and salt concentration upon the rates of adsorption and desorption are not adequately defined.

iv. The effects of the degree of agitation and the temperature upon adsorption and desorption were not completely established.

D. Development of Separation Techniques

The distribution of bacterial cells in mixed aqueous suspensions containing several species of microorganisms together with dissolved and particulate matter, can be altered in three ways: (1) purification of the cells by the selective adsorption of contaminanting substances with no attendant concentration of the cells, (2) nonspecific concentration of the cells and the contaminants with little or no purification of the cells, or (3) resolution of the cells themselves into separate fractions, each fraction containing the cells of a single species. The following conclusions are made with regard to the adsorption and desorption of the cells of individual bacterial species from water suspensions:

i. The cells of each of the six bacterial species described earlier can be concentrated by adsorption onto particles of anion exchange resin.

ii. The cells of each of the six bacterial species can be desorbed from the anion exchange resin by lowering the pH, adding solid salt, or both.

iii. The cells of certain of the above species are also capable of adsorbing onto cation exchange resins.

The bacterial species used in these studies were divided into four groups on the basis of their adsorption and desorption with anion exchange resins:

i. Limited adsorption which becomes self-reversing (Escherichia coli).

ii. Strong adsorption, with desorption occurring by charge reversal upon pH reduction (Staphlococcus aureus and Pseudomonas ovalis)

iii. Strong adsorption with desorption promoted by the addition of salt (Proteus vulgaris)

iv. Very strong adsorption with desorption promoted only by the combined actions of low pH and the addition of salt (Bacillus subtilis)

Practical schemes for purification and nonspecific concentration of bacterial cells are subject to the following restrictions in the present work:

i. The pH and salt content of the medium were initially adjusted to permit either anion or cation exchange to occur.

ii. Absorbance was established as a valid index of bacterial cell concentration.

The resolution of a binary mixture can be accomplished in two ways: Type I. Selective adsorption of the cells of one species while those of the other remain unadsorbed, or Type II. Nonselective adsorption of the cells of both species followed by sequential desorption of

cells of each species. The specificity of ion exchange as a technique for resolving mixtures of bacterial cells was demonstrated experimentally. Five binary mixtures were resolved:

i. Escherichia coli - Bacillus subtilis: This mixture of Gram-negative and Gram-positive bacilli was resolved by a Type I anion exchange into fractions containing, respectively, 96.9% and 92.6% of the cells of the two component species.

ii. Escherichia coli - Staphlococcus aureus: This mixture of Gram-negative bacilli and Gram-positive cocci was resolved by a Type I anion exchange into fractions containing, respectively, 100.0% and 100.0% of the cells of the two component species.

iii. Escherichia coli - Pseudomonas ovalis: This mixture of two Gram-negative bacilli was resolved by a Type I anion exchange into fractions containing, respectively, 100.0% and 95.8% of the cells of the two component species.

iv. Staphlococcus aureus - Bacillus vulgaris: This mixture of Gram-positive cocci and Gram-positive bacilli was resolved by a Type II anion exchange into fractions containing, respectively, 95.0% and 91.1% of the cells of the two component species.

v. Bacillus subtilis - Proteus vulgaris: This mixture of Gram-positive and Gram-negative bacilli was resolved by a Type I cation exchange into fractions containing, respectively, 82.0% and 91.1% of the cells of the two component species.

The Type I and Type II schemes of resolution can probably be extended to other mixtures subject to the following limitations:

i. The resin must have sufficient capacity to adsorb all cells of the species to be adsorbed.

ii. The cells of each species present in a mixed suspension must adsorb and desorb independently.

iii. The physical separation of adsorbed cells and resin particles from freely suspended unadsorbed cells should be complete.

iv. Specific tests for the identification of the cells of each component species in a mixture to be resolved should be established; viabilities of the cells should be checked.

v. If resolution is conducted batchwise, care should be taken to avoid chemical precipitation of cells by low pH, high salt,

concentration, etc; if resolution is conducted on a column basis, care should be taken to avoid the complications of attendant filtration of cells.

The resolution of each of ten binary mixtures, has been qualitatively predicted on the basis of the resolutions experimentally verified. A proposed resolution of a quaternary mixture containing cells of Escherichia coli, Pseudomonas ovalis, Staphlococcus aureus, and Bacillus subtilis is also outlined.

APPENDIX A

OPTICAL MEASUREMENT OF BACTERIAL DENSITY

1. Introduction

The quantity of protoplasm or the number of organisms present in a cell suspension must be determined in many bacteriological investigations. The total cell mass can be directly estimated by measuring some parameter of the protoplasm, such as the centrifuged or filtered volume, or the dry weight. Alternatively, the total cell mass can be indirectly determined by measuring the concentration of some component proportional to the amount of protoplasm, e.g. the nitrogen content, certain colored reaction products, radioactivity, etc. Cell mass can also be indirectly determined by measuring metabolite consumption, product accumulation, or stoichiometric uptake of dyes or colloids.

The total number of organisms present in a suspension can be microscopically determined using a counting chamber of known volume, by proportional counting, or by the use of stained smears. The number of viable organisms can be determined by the ability of single organisms to multiply into macroscopic colonies. The most common technique of determining viable cell counts based on this characteristic of reproduction are: the dilution or most probable number method, and various plating methods involving roll tubes, pour plates, drop plates, capillary tubes, and microporous filters.

A rapid, sensitive, and preferably non-destructive technique of measuring total cell counts is required to determine the degree to which bacterial cells adsorb onto a surface. Optical techniques are

applicable provided that a correlation can be developed between the amount of light scattered by or transmitted through a suspension of cells and the concentration of cells in the suspension. The total number of cells is not always a linear function of cell mass, particularly in growing cultures. Number and mass determinations, therefore, must be recognized as independent quantities except for certain limited cases.

A linear relationship between the cell mass, expressed by an absorbance value, and the total cell number, determined by direct counting, may exist over certain ranges of concentration. This requires a stable suspension, in which non-metabolizing, non-reproducing cells are uniformly dispersed in a non-absorptive medium of constant pH, salt content, and temperature. These restrictive conditions have been met by the washed, resuspended, and filtered preparations of cells that were used in the adsorption experiments of this report.

Optical measurements of bacterial suspensions have been periodically reviewed (Angerer, 1924; Mestre, 1935; Longsworth, 1936; Dognon, 1945; Mitchell, 1950; and Gavin, 1957). The growth of cells under different culture conditions have been extensively described (Longsworth, 1936; Gavin, 1957; Harris, 1958; Faguet, 1959; Wilkins, Allen and Alway, 1959; Kurokawa et al. 1962; Wentink and LaRiviere, 1962; and Sevcik, Liska, and Hosek, 1964). Another area of application has been in the investigation of cell agglutination (Lasseur and Renaux, 1934; Häntsch, 1938; Dognon, 1945; Oster, 1946a; Gordon and Price, 1953; Schmidt, 1959; and Ryan and Kolin, 1964).

2. Correlations from the literature

The concentration of a component present in a liquid at which

light beam is directed can be expressed in terms of the relative proportion of the incident light that is either scattered or reflected. A fraction of the incident light from a beam directed at a liquid containing suspended particles that is scattered is a function of the concentration of the particles. The remainder of the light is transmitted unaffected. The light losses due to ordinary absorption are generally assumed to be negligible or compensatable in such systems. The concentration of the species in solution can be determined in certain cases by measuring either the amount of scattered light (nephelometry) or the amount of transmitted light (turbidimetry). The usual approach has been to measure the amount of transmitted light that is detectable over a relatively small solid angle 180° from the incident beam. The optical measurements can then be correlated with total cell counts that have been microscopically determined.

A contribution due to forward scattering is also contained in the total amount of the transmitted light. This contribution is generally assumed negligible for particle concentrations below a certain level. Transmission measurements of suspensions of bacterial cells are assumed to follow Beer's law until scattering becomes appreciable.

The nomenclature as defined in this Appendix is in accord with a compilation published by the editors of Analytical Chemistry (1964). Clarification is necessary because considerable discrepencies in the commonly applied terminology are to be found in the literature. The ratio of the radiant power transmitted by a sample, I , to the radiant power incident on the sample, I_o , is denoted as the transmittance, T . The negative base ten logarithm of the transmittance is further defined

as the absorbance, A , (cf. optical density, absorbance, extinction). Assuming a sample concentration, c , contained in a cell of path length, b , the absorptivity is defined as $a = A/bc$.

According to Beer's law, the absorptivity of a substance is a constant with respect to changes in concentration. The concentration of particles present in a suspension is therefore a linear function of the absorbance as expressed by Equation (A-1).

$$A = -\log(I/I_0) = -\log T = abc = \alpha_0 n \qquad \text{(A-1)}$$

The constants a and b , can be combined as α_0 ; the bacterial cell concentration (number/unit volume) has been denoted as n .

Many attempts have been made to empirically correlate optical measurements with cell densities. The amount of light transmitted through a suspension of cells is the most common optical measurement. Several of the more important correlations will be briefly discussed in the following paragraphs. A new correlation is then developed for specific application to the investigations of bacterial adsorption.

Longsworth (1936) established a parabolic relationship between absorbance and cell density to account for deviations from Beer's law. This correlation can be expressed by Equation (A-2).

$$A = \alpha_1 n - \beta_1^2 n^2 \qquad \text{(A-2)}$$

The constants, α_1 and β_1 , are determined as the slope and intercept, respectively of a plot of A/n versus n . The measured absorbances are not strictly proportional to cell densities. This can be remedied by the use of Equation (A-3) in which the corrected absorbance is denoted as A' .

$$A' = \alpha_1 n \qquad \text{(A-3)}$$

Equation (A-4) is independent of the cell density, and is formed by the elimination between Equations (A-2) and (A-3).

$$A = A' - \gamma_1^2 A'^2 \qquad \text{(A-4)}$$

Non-zero values of the constant, $\gamma_1 = \beta_1/\alpha_1$, are a measure of the deviation from the Beer's law relation. This correlation was developed for white light transmitted through suspensions of cells of Lactobacillus acidophilus suspended in growth media at a constant pH.

Toennies and Gallent (1948, 1949) further developed the correlation of Longsworth to correct for deviations from Beer's law. In their work, the corrected absorbance, A', is expressed as a function of the observed net absorbance, A, by an inversion of Equation (A-4) to form Equation (A-5).

$$A' = (1 - \sqrt{1 - 4\gamma_1^2 A})/2\gamma_1^2 \qquad \text{(A-5)}$$

Two values of ρ_1 were required for $0 \leq A' \leq 0.196$ and $0.197 \leq A' \leq 0.800$. Data for fifteen dilution series were correlated to within 1% of the average of the exact linear values. Data were collected for cell suspensions of seven species of bacteria, including four species of Lactobacillus, one species of Leuconostoc, and two species of Streptococcus. The bacterial cells were suspended in any one of four media. Three different instruments including the Coleman Models 11 and 14 at 675 mμ, and the Klett-Summerson Model 800-3 at 600 mμ were used.

Vas (1955) briefly reviewed the earlier literature. He then proposed a hyperbolic correlation between absorbance and cell density in contrast to the earlier parabolic correlations. This hyperbolic correlation is given by Equation (A-6), where α_2 and β_2 are empirical constants.

$$A = \frac{n}{\alpha_2 n + \beta_2} \qquad \text{(A-6)}$$

Suspensions of spores of Bacillus cereus, and vegetative cells of Bacillus cereus, Corynebacterium michiganese, two varieties of Saccharomyces cerevisiae, and one variety of Torulopsis utilis, were serially diluted in nutrient broth. Absorbances were determined at 665 mμ using a Pulfruch photometer. Absorbance values of suspensions containing cells of Saccharomyces cerevisiae as high as 1.65 were correlated with an average deviation of 2% according to Equation (A-6). The main disadvantage of this correlation is the uneven distribution of experimental points that are derived from the usual series of binary dilutions.

Kurokawa et al. (1962) developed a linear relationship between absorbance and cell density. Transmittance was first defined in terms of the two metameters of Equations (A-7a) and (A-7b).

$$A = -\log T \quad , 0 \leq T \leq 0.5 \qquad \text{(A-7a)}$$

$$A = 0.6 + \log(1-T) \, , 0.5 \leq T \leq 1.0 \qquad \text{(A-7b)}$$

The complete correlation is then given by Equation (A-8), where A, T, and n are previously defined; α_3 and β_3 are empirical constants.

$$A = \alpha_3 \log n + \beta_3 \qquad \text{(A-8)}$$

Suspensions containing cells of Staphlococcus albus and Corynebacterium diptheriae were serially diluted with nutrient broth or buffer solution. Absorbances were measured at a wavelength of 660 mμ. Linear regression values of the empirical constants were calculated. This correlation was found to be applicable over the entire range of transmittance values except at the extreme ends.

Theoretical predictions of light scattering behavior by macromolecules have been discussed by many authors (Hulst, 1957; Tanford, 1961; Koch, 1961; Fikhman, 1963' and Petukhov, 1965). The fraction of the light incident upon a particle of diameter, d , that is scattered by the particle, K , is related to the absorbance, A , through Equation (A-9).

$$A = \frac{\pi d^2 b}{4} Kn = \alpha_4 n \qquad \text{(A-9)}$$

The dimensionless quantity, K , can be further defined in terms of various complex mathematical functions depending upon the theory that is applied. Extensive tables of light scattering functions have been published in the literature (Boll, Leacock, Clark, and Churchill, 1958; Pangonis, Heller, and Jacobson, 1957; and Gumprecht and Sliepcevich, 1951).

The simplest case is Rayleigh scattering. It is applicable only to particles that are very small compared to the wavelength of the impinging light. Koch (1961) developed the expressions given in Equation (A-10a) and (A-10b).

$$K = \frac{8}{3} z \left(\frac{\phi^2 - 1}{\phi^2 + 2}\right)^2 \qquad \text{(A-10a)}$$

$$A = \frac{32\pi^2}{2.303 \cdot 3} \left[\frac{(dn/dc)}{n_0}\right]^2 \frac{W^2 b}{\lambda^4} \qquad \text{(A-10b)}$$

The absorbance, A , is proportional to the square of the anhydrous mass of the particle, W , and inversely proportional to the fourth power of the wavelength of the impinging light, $\lambda = \lambda'/\eta_o$. The remaining terms are: the concentration of particles, c , the wavelength of light in vacuo, λ' , the index of refraction of the particles, η , the index of refraction of the medium, η_o , the ratio of the refractive indices, $\phi = \eta/\eta_o$, the reduced particle diameter, $z = \pi d/\lambda'$, and length of the light path, b . The absorbance is unchanged by swelling or contraction of the particles if W remains unchanged. Equation (A-10a) is applicable regardless of the shape of the particles so long as they are very small and isotropic, and provided the quantity, $\phi - 1$, is also very small.

If the size of the particle is not small with respect to the wavelength of the scattered light and if the indices of refraction of the particle and the medium are not nearly the same, then the more complex theory of Mie (1908) must be applied. The quantity, K , as defined for the Mie theory, is a combination of two complex functions. These functions have been tabulated by Gumprecht and Sliepcevich (1957) and others. Applications of the Mie theory have been practically limited to spherical particles and calculation the associated functions is extremely cumbersome except by electronic means.

Koch (1961) incorporated a modification of the Mie theory due to Jobst (1925) in developing the mathematical forms of Equations (A-11a) and (A-11b).

$$K = 2\left(\frac{\pi d}{\lambda'}\right)^2(\phi - 1)^2 \qquad \text{(A-11a)}$$

$$A = 5.9844\left[\frac{(dn/dc)}{n_o}\right]^2 \frac{W^2 b}{(d/2)^2 \lambda^2} \qquad \text{(A-11b)}$$

These forms are applicable to particles of a size comparable to the wavelength of light that have an index of refraction similar in magnitude to that of the suspending medium. The applicable constants are:

$\phi = n/n_o = 1.03$, $z = \pi d/\lambda' = 8$, and $d/\lambda' = 1.902$.

Koch (1961) developed a still more general equation by applying the Rayleigh-Gans method to correct for the interference of wavelengths scattered in the direction θ of propagation from the incident beam. This correction was incorporated into a factor $P(\theta)$ that is rather complicated for the various shapes of interest, such as shells, oriented rods and disks, etc. For random ellipses of semi-axes, a' and b', this correlation is of the form of Equation (A-12).

$$P(\theta) = \sum_{i=0}^{\infty} (-1)^i 3.4! \frac{(2i+2)(2i+5)}{(2i+6)!} \cdot 2^{2i} x^{2i} \sum_{h=0}^{\infty} \frac{i!}{h!(i-h)!} \cdot \frac{\rho^h}{2h+1} \qquad \text{(A-12)}$$

$$\rho = (b'^2 - a'^2)/a'^2 \;, \quad x = (4\pi a'/\lambda)\sin(\theta/2)$$

Additional developments of this type have been reported by Doty and Edsall (1951), Doty and Steiner (1950), and Butler (1962).

The absorbance of a suspension of particles can thus be expressed by Equation (A-13).

$$A = \frac{4\pi^3}{2.303 \cdot 9}\left[\frac{(dn/dc)}{n_o}\right]^2 \frac{W^2 b}{\lambda^4} \int_0^{\pi} P(\theta)(1+\cos^2\theta)\sin\theta \, d\theta \qquad \text{(A-13)}$$

Numerical evaluations of this integal were compiled by Koch (1961). Results were presented assuming the typical values of $a' = 0.5\ \mu$, $b' = 2\ \mu$, $\lambda = 400$ mμ, $n = 1.0442$, and $n_o = 1.33$. At a wavelength of $\lambda = 400$ mμ, the coefficient, α_4, defined in Equation (A-9) is equal to 0.613×10^{-8}

for spheres, 1.491×10^{-8} for shells, and 0.518×10^{-8} for 4:1 ellipsoids. The validity of Beer's law is tacitly assumed in this treatment. Similar derivations have been reported by Duysens (1956) and by Volker and Bücker (1963).

Hulst (1957) developed a theory of anomalous diffraction to describe situations in which both reflection and refraction are negligible and the refractive indices of the particles and the medium are of comparable magnitude. The applicable definition of the quantity, K, is presented as Equation (A-14).

$$K = 2 - \frac{4}{\rho_1} \sin \rho_1 + \frac{4}{\rho_1^2} (1 - \cos \rho_1) \qquad \text{(A-14)}$$

$$\rho_1 = 2\pi(\phi - 1)/\lambda_0$$

Oriel (1967) evaluated the flocculation of Gram positive bacteria using the above model. An electronic computer was used to calculate values of K from turbidimetric measurements collected as a function of wavelength.

The various correlations summarized in this section are attempts to analytically define total cell mass or total cell number in terms of optical parameters of a cell suspension. Application of these correlations to the current work is restricted by any one of several factors. There may be regions of nonlinear fit of the data to the defining equation. Multiple sets of empirical constants may be required. Extensive calculations or tabulations of functions may be necessary. The mathematical forms may be incompatible with the kinetic model derived in Section III-C of this report. The latter factor is the most severe restriction. A more easily applied correlation that is compatible with the kinetic model has therefore been developed.

3. Experimental correlation

Suspensions of bacterial cells were prepared according to the procedures described in Section IV-A of this report. The cultured cells were washed free of soluble nutrients and salts, resuspended in distilled water, and filtered through paper to remove any large clumps. A series of binary dilutions, each of 50 ml total volume, were then prepared. Absorbances for this series were measured using a colorimeter (Klett-Summerson Model 900-3) with a blue, 420 mμ filter with a bandwith of 400-450 mμ. The 40 mm path of a 20 x 40 mm rectangular cuvette (Klett No. 901) was used.

A depth of suspension in the sample cuvette of at least 5 cm (40 ml) is recommended by the manufacturer. The ratio of the absorbance measured for any depth to the absorbance measured for a depth of 6.25 cm (50 ml) is plotted as a function of the volume of suspension in Figure A-1. The absorbance of the suspension is twice affected by the rising meniscus as the total volume of suspension is increased. Approximately 95% of the absorbance measured at the 50 ml volume is measured at the 30 ml volume.

A dimensionless plot of absorbance as a function of cell density is presented in Figure A-2. The logarithm to the base ten of the absorbance is plotted against the logarithm to the base two of the dilution ratio. A binary dilution series of 1, 1/2, 1/4, ... $1/2^{n/n_o}$, would therefore be plotted as 0, 1, 2, ... n/n_o, with $\log_2(n/n_o) = 3.322 \log_{10}(n/n_o)$. The data of Figure A-2 can be expressed in terms of total cell counts per unit volume for given absorbance values and fitted to Equation (A-14), where α_o and β_o are empirical constants.

$$A = \alpha_o n^{\beta_o} \qquad \text{(A-14)}$$

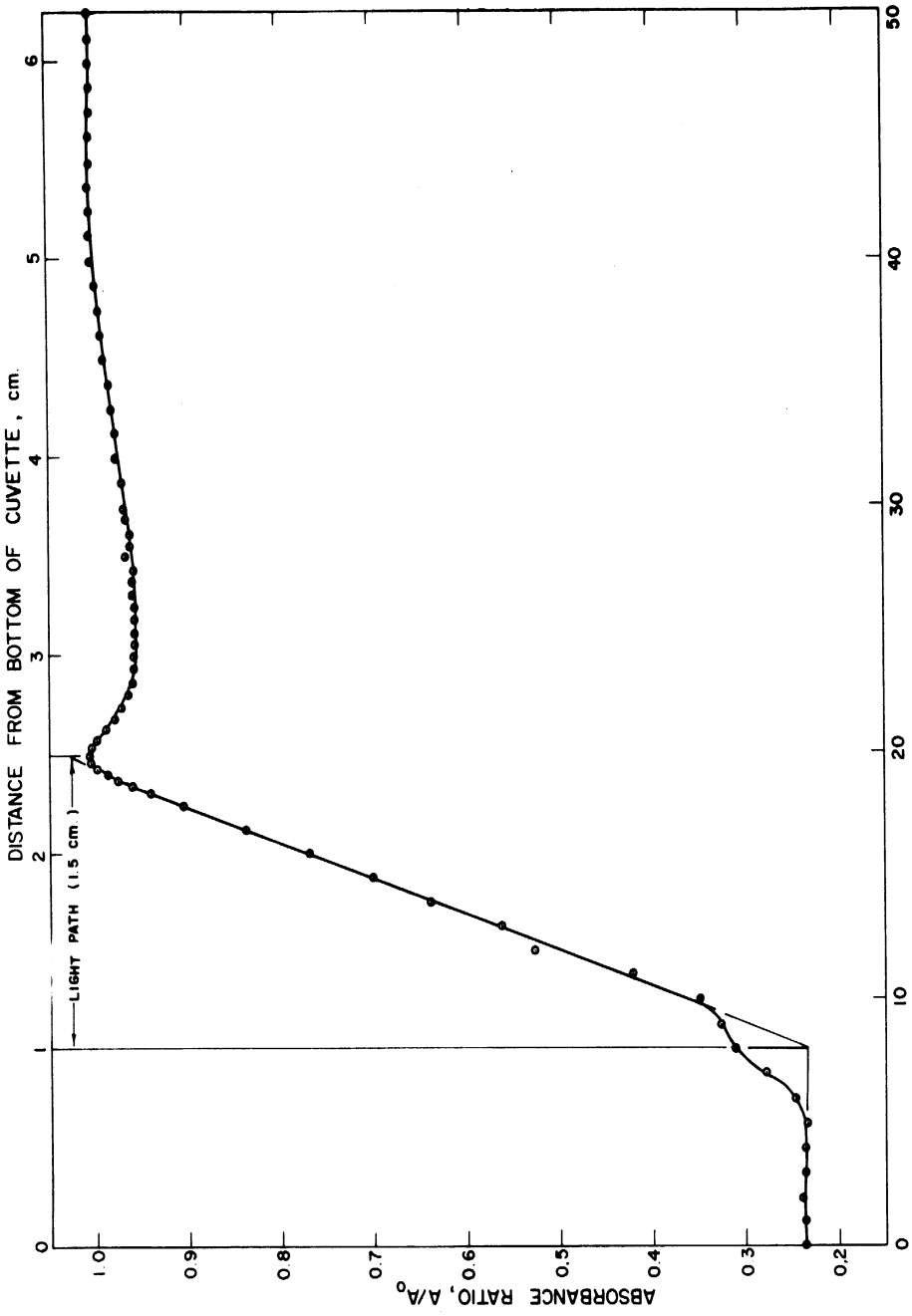


Figure A-1. Absorbance as a Function of the Depth of a Suspension of Bacterial Cells (Bacterial species: Bacillus subtilis, A_o = 0.600, pH 7, 50 ml volume; instrumental system: Klett-Summerson Model 900-3 photoelectric colorimeter, 420 mu wavelength, 20 x 40 mm rectangular absorption cell, 40 mm light path used).

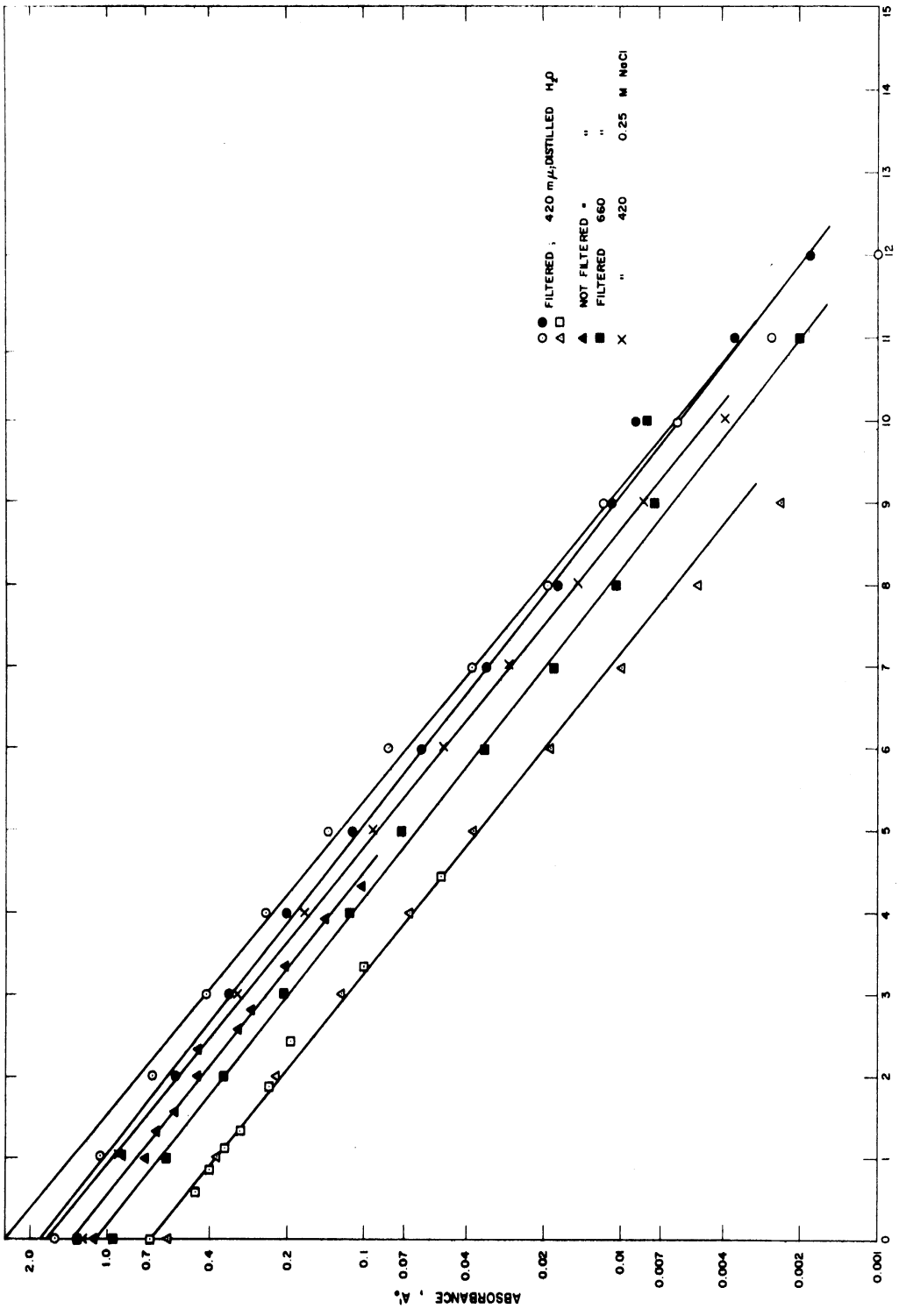


Figure A-2. Absorbance as a Function of the Dilution Ratio of Suspensions of Bacterial Cells for Various Conditions of Wavelength and Cell Preparation (Bacterial species: Bacillus subtilis, ph 7).

Equation (A-14) is a modified form of Beer's law for which the exponential constant $\beta_o = 1$. Although this correlation has no theoretical basis, the experimental data are adequately fitted over the ranges of interest. Absorbances and total cell counts were measured for a series of suspensions containing cells of Bacillus subtilis diluted with distilled water and with 0.25 M. sodium chloride solution. These results are presented in Figure A-3. This correlation is linear of slope $\beta_o = 0.83$ for both diluents.

No great improvement in linearity over the previously described correlations was obtained using this correlation. It is suitable for $A \leq 0.6$. Values for β_o and the range of linearity of this modified Beer's law correlation are functions of the bacterial species and the type of suspending media.

The mathematical form of Equation (A-14) is particularly advantageous in the current investigation. A logarithmic ratio can be formed as given in Equation (A-15), where initial reference quantities are denoted by the subscript o.

$$\log(n/n_o) = (1/\beta_o) \cdot \log(A/A_o) \qquad \text{(A-15)}$$

A relatively simple form can therefore be retained upon substitution of the numerical ratio of total bacterial cells per unit volume, n/n_o, by a ratio of absorbances, A/A_o, multiplied by the constant, $1/\beta_o$.

4. Optical behavior of suspended cells

Pronounced effects upon the optical behavior of suspended particles are caused by the conditions existing in the suspending medium. The

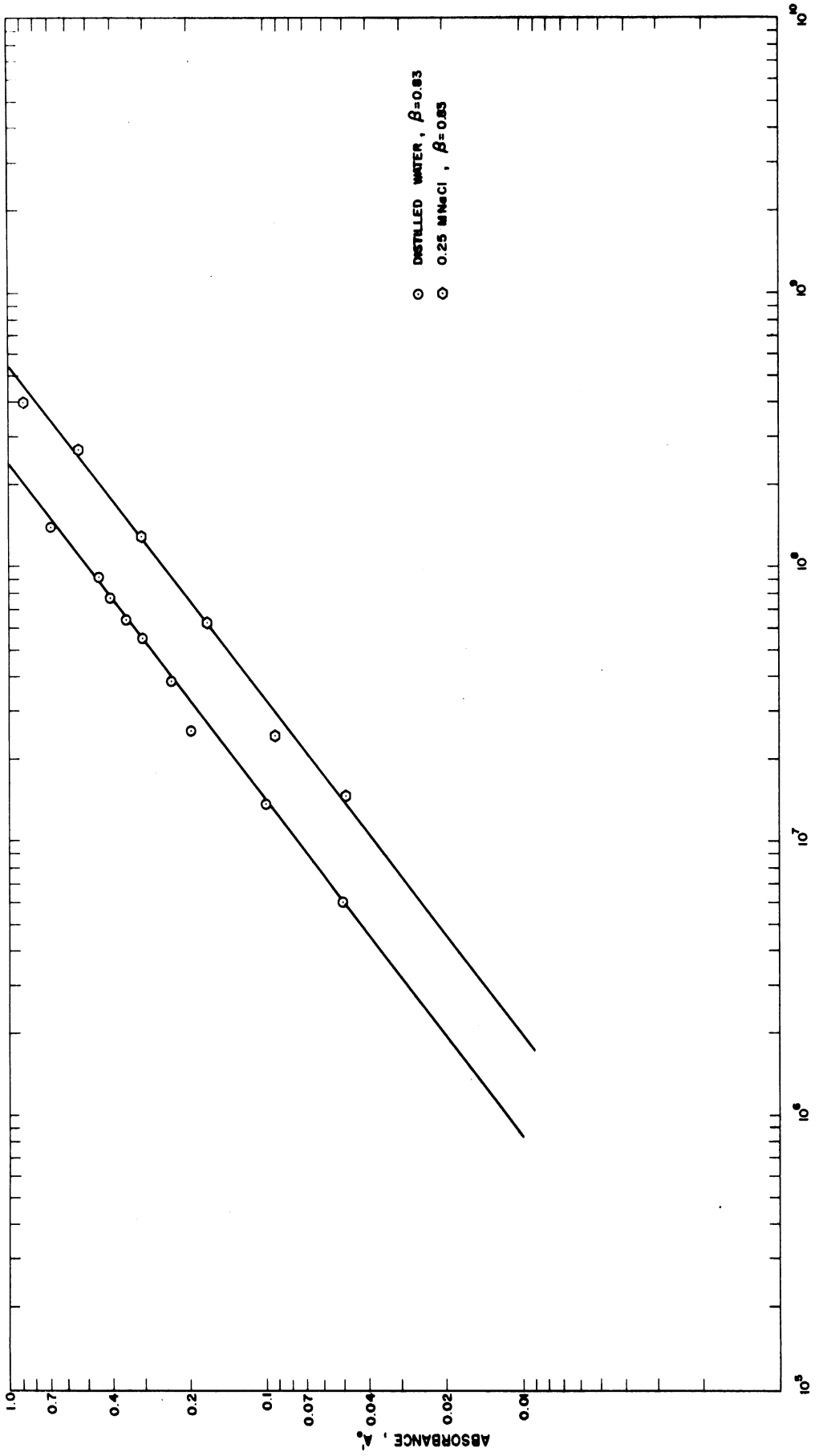


Figure A-3. Absorbance as a Function of the Total Cell Number of a Suspension of Bacterial Cells (Bacterial species: Bacillus subtilis, pH 7).

most important of these conditions are: the hydrogen ion concentration and the concentrations of various other ions. Serious swelling or contraction of bacterial cells can result from changes in osmotic pressure. Effects of pH and salt concentration are evidenced in changes in the optical behavior of the cellular suspension.

Lasseur, Dupaix-Lasseur, and Renaux (1934) observed the effects of pH upon the absorbance of bacterial suspensions. The agglutination of the cells of different bacterial species by various acids was also studied by Lasseur and Renaux (1934). Differences in the pH values at which the maximum absorbances of suspensions of tobacco mosaic virus occurred were determined from acid titration data by Oster (1946, 1951) and later by Gordon and Price (1953). Ryan and Kolin (1964) refined this method and applied it to the continuous turbidimetric analysis of coalescence phenomena occurring in biological systems. The absorbance maxima and apparent isoelectric points of several viruses, and the bacterial species, Bacillus subtilis and Erwinia carotovora, were determined.

The effect of pH upon the absorbances of suspensions containing cells of Bacillus subtilis can be observed by titration with acid or base. An absorption maximum near pH 2.5 is shown in Figure A-4. This pH is considered to be the apparent isoelectric point, pI, of this species. A slightly different maximum of pH 3.5 is observed in similar data collected by Ryan and Kolin (1964). Characteristic maximal absorbances were also experimentally determined in the current work for suspensions of five other bacterial species: Escherichia coli, Staphlococcus aureus, Bacillus cereus, Pseudomonas ovalis, and Proteus vulgaris. Titration curves of cell suspensions of these species are shown in Figure A-5. Apparent isoelectric points are tabulated in Table XXIX.

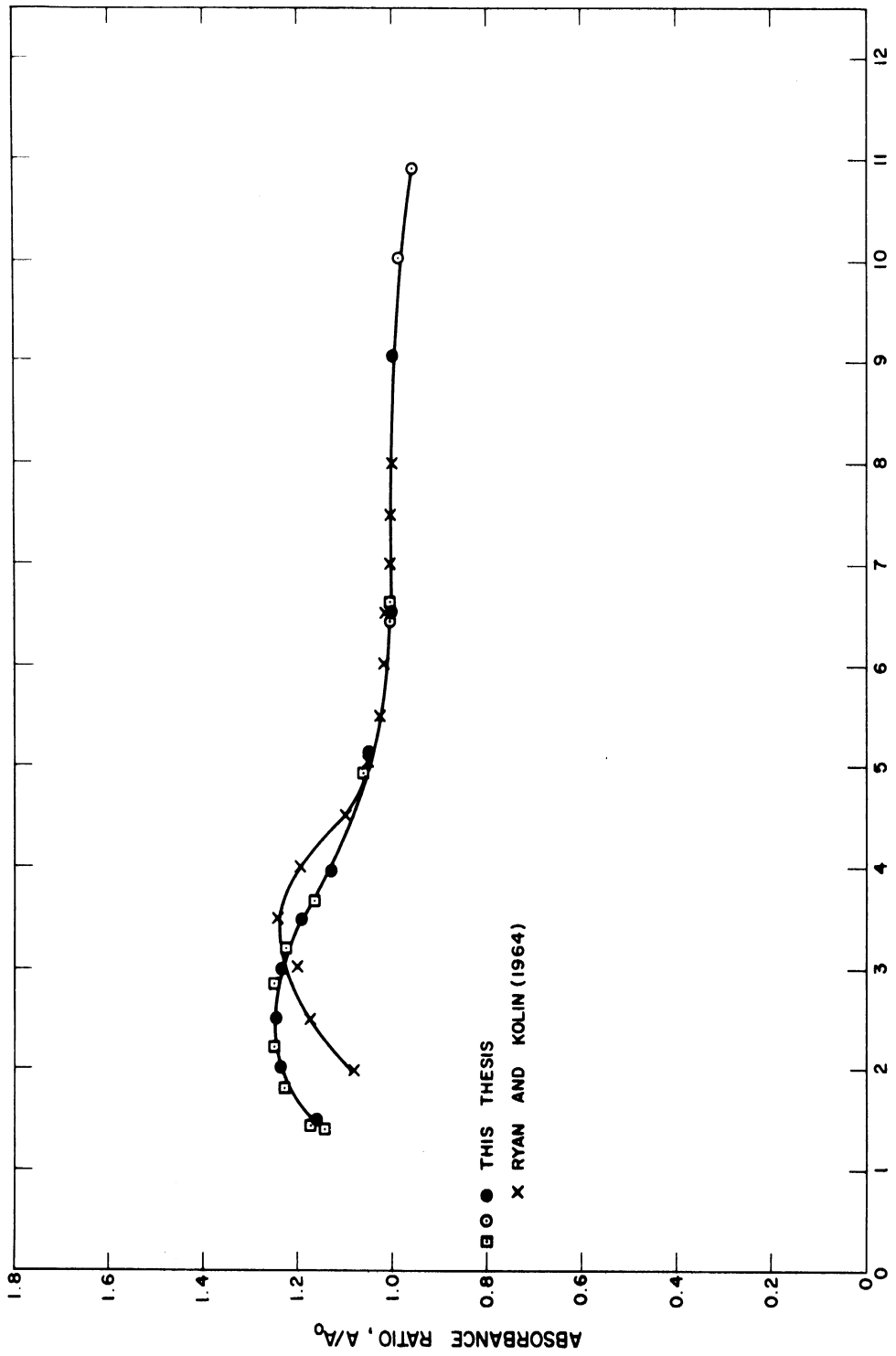


Figure A-4. Absorbance as a Function of pH of Suspensions of Bacterial Cells (Bacterial species: Bacillus subtilis).

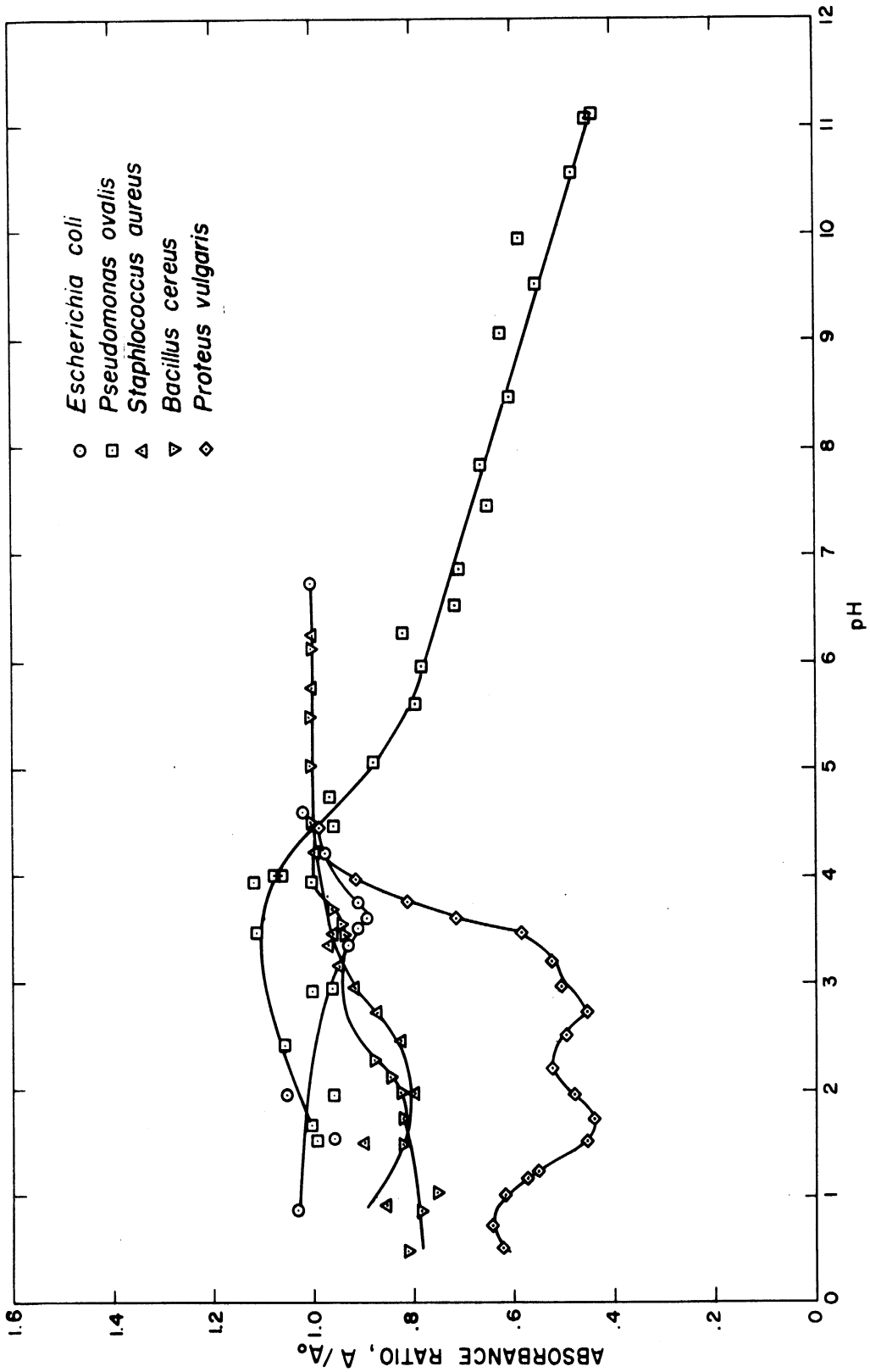


Figure A-5. Absorbances as Functions of pH of Suspensions of Five Different Bacterial Species.

The effects of salt concentration upon the absorbance of a bacterial suspension may also be significant. Esche (1953a, 1953b) observed changes in the size, charge, and heat resistance of bacteria treated with solutions of various electrolytes. Mager et al. (1956) observed that the absorbances of suspensions of various Gram-negative bacteria were affected by the ionic strength of the suspending medium. Maximal increases ranging from 30-140% were observed for different bacterial suspensions upon increasing the concentration of salt. This effect was dependent upon the pH and composition of the medium, and the age of the culture. Maximum absorbances were noted at salt concentrations of about 0.2 M.

Avi-Dor et al. (1956) studied the kinetics of changes in absorbance of bacterial suspensions as a function of the osmotic pressure. A rapid increase in the absorbance of a cell suspension was observed within 10 minutes after the addition of salt. This increase was followed by a gradual decrease which continued for at least one hour. Kuczynski-Halmann and Avi-Dor (1958) conducted similar studies using cells of the Gram-positive bacterium, Bacillus megaterium. The results were compared with those collected using cells of the Gram-negative bacterium, Escherichia coli. The pattern of an initial rapid increase in absorbance followed by a gradual decrease in absorbance was observed for suspensions of cells of both species. The absorbances of suspensions of both organisms were increased by the addition of sodium chloride. Very little changes in absorbances were produced by the addition of urea. An increase in the absorbance of a suspension containing cells of Escherichia coli and a decrease in the absorbance of a suspension containing cells of Bacillus megaterium was produced by the addition of sucrose.

Hodge and Metcalfe (1958) compared the flocculation of bacteria by several hydrophilic colloids on the basis of changes in the amount of light transmitted by a suspension of cells. Christian and Ingram (1959) concluded that the osmotic status of organisms was chiefly controlled by small ions and molecules that rapidly leak from the cells upon transfer to more dilute environments. The changes in absorbances of cell suspensions produced upon decreasing the concentrations of several chloride salts having monovalent cations were studied. Salton and Pavlik (1960) studied the lysis of bacterial cells by measuring reductions in the absorbances of suspensions of cells treated with lysozyme.

Increases in the absorbances of suspensions and morphological changes in the cells of red halophilic bacteria were produced by increased salt concentrations according to Abram and Gibbons (1960). These authors observed the previously described rise and fall of absorbance with time using cell suspensions to which salt had been added. Powell and Stoward (1962) determined changes in the average length of bacterial cells suspended in a flowing liquid by measuring the amount of scattered light. Transition of the organisms from spherical to rod shapes were noted upon increasing the salt concentration of suspensions containing cells of Halobacterium cutirubrum. In a later paper Abram and Gibbons (1961) described the effects of salts, urea, detergents, and heat upon the morphology and turbidity of suspensions of halophilic bacteria. Increased absorbances were observed for preparations of cells suspended in solutions of increased salt concentration. The extent of this increase was a function of the type of monovalent cation present in the suspending medium.

Packer and Perry (1961) observed decreases in the amount of light scattered by suspensions containing cells of Escherichia coli upon addition of various organic carbon compounds. Bovell, Packer, and Helgerson (1963) found that the optical behavior of cells in suspension was greatly affected by the addition of metabolizable and non-metabolizable compounds. Changes in the amount of light scattered by cells in suspension due to the presence of several metabolizable substances were correlated with their oxygen consumption. Suspensions containing cells of Escherichia coli were also titrated with various inorganic salts and the changes in absorbance were measured.

Bernheim (1963b) studied the factors affecting the sizes of cells of two bacterial species and the absorbances of their suspensions. The absorbances were initially increased at a rapid rate upon the addition of salt. This was assumed to be due to a decrease in the size of the organisms as a result of the loss of bound water. The rate of decrease in absorbance was changed if the metabolic rates of the organisms were altered. Bernheim (1964) measured the rate of decrease in absorbance of various buffer solutions containing cells of Pseudomonas aeruginosa. The rates of decrease in absorbance was a function of both the pH value and the osmotic pressure.

The effects of adding solid sodium chloride to suspensions of bacterial cells were also studied in the current research. The absorbances of suspensions containing cells of Bacillus subtilis are presented as a function of the salt concentration in Figure A-6. Maximum absorbances were reached within one minute after addition of solid sodium chloride to make a final concentration of 1.5 M. Similar data of absorbance as a

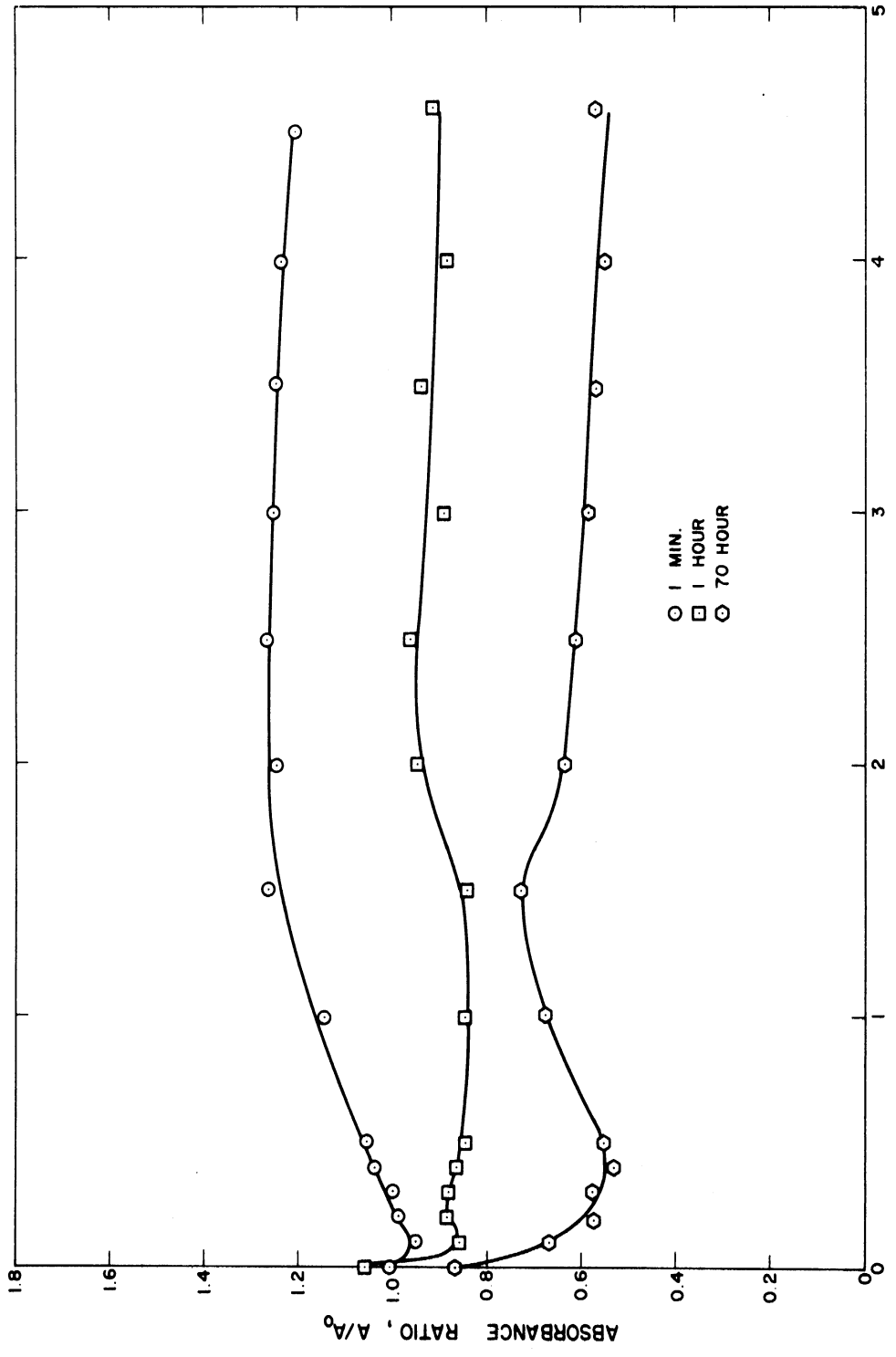


Figure A-6. Absorbance as a Function of the Concentration of Salt (Bacterial species: Bacillus subtilis, pH 7).

function of salt concentration taken from the literature are plotted in Figure A-7. The optical changes of suspensions containing cells of Bacillus subtilis are relatively small compared to those containing cells of Escherichia coli, Bacillus megaterium, or Halobacterium cutirubrum.

The variations with time of the absorbances of suspensions containing bacterial cells and a constant concentration of sodium chloride are shown in Figure A-8. The absorbances of suspensions containing cells of Pasturella tularensis and Pseudomonas aeruginosa are more variable. The typical pattern is again a rapid initial change in absorbance followed by a gradual decline. The absorbance of a suspension containing cells of Bacillus subtilis and a concentration of 0.2 M sodium chloride is reduced only 10-15% after one hour.

The wavelength of the incident light is particularly important when measuring the amount of light transmitted through a suspension of bacterial cells. Most investigators have used a wavelength of 600 mμ or greater for turbidimetric measurements. Most of their determinations were conducted using growing cultures that contained many substances that absorb considerable amounts of light at the lower wavelengths. Absorbances can be measured at the lower wavelengths if the cells are first washed, resuspended, and then filtered free of suspended contaminats.

The adsorption spectra of suspensions containing the cells of living microorganisms have been extensively investigated. Shibata, Benson, and Calvin (1954) have presented some of the best spectral data for suspensions containing cells of algae, yeasts, bacteria, viruses, and plant tissues, and have tabulated absorption maxima. A strong adsorption band at about 260 mμ was observed for suspensions of most microorganisms.

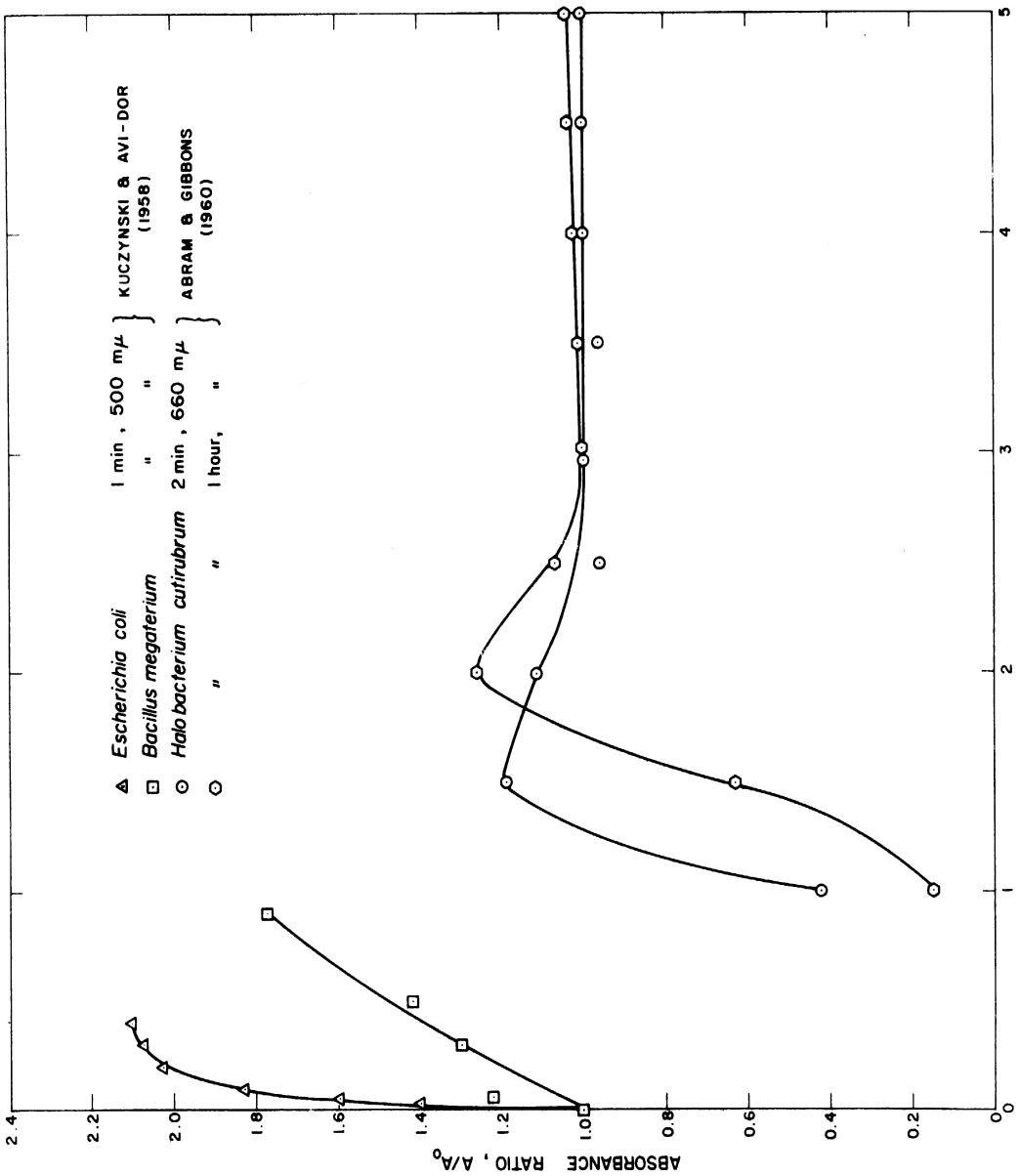


Figure A-7. Absorbances as Functions of the Concentration of Salt of Suspensions of Three Different Bacterial Species.

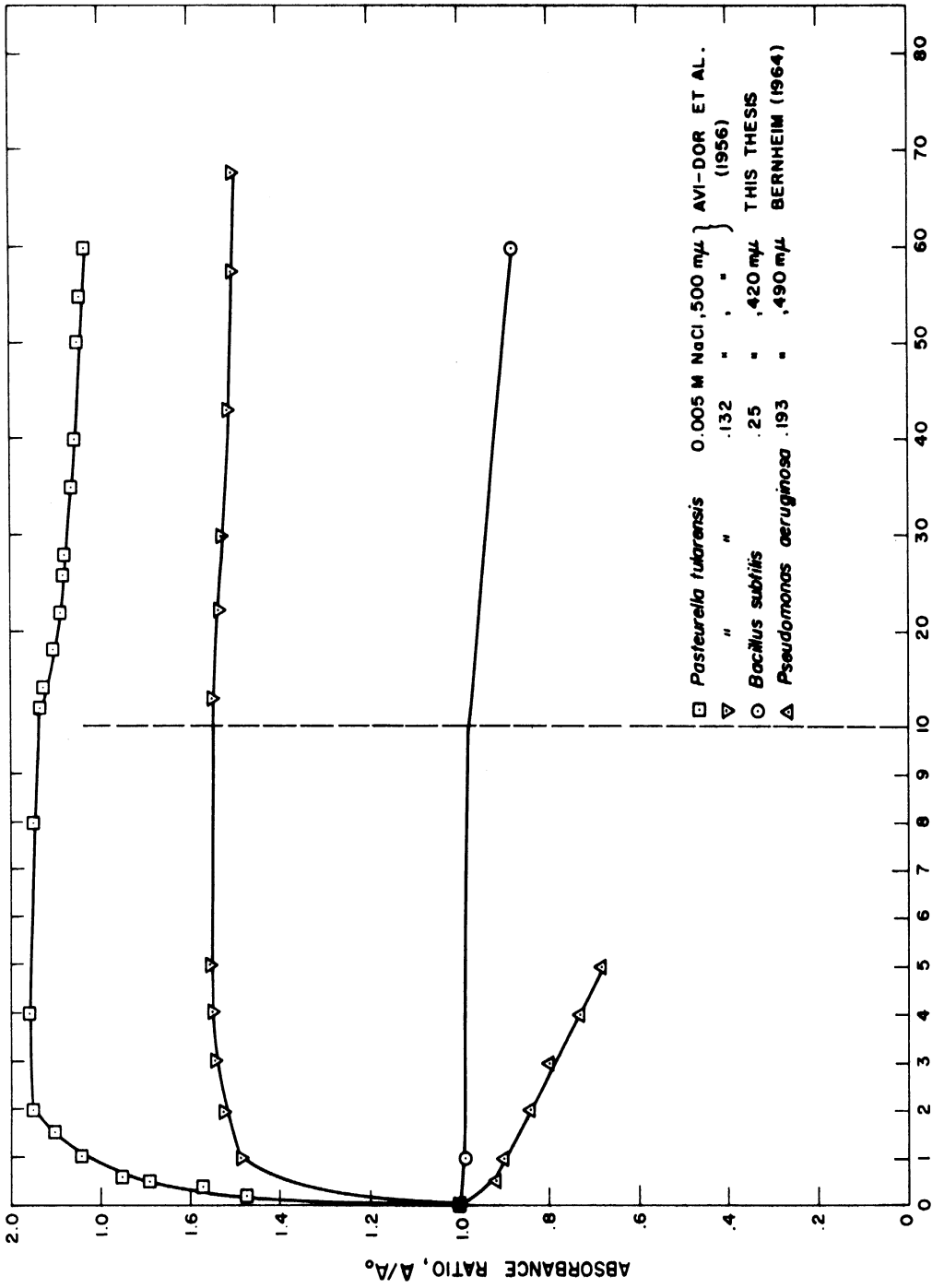


Figure A-8. Absorbances as Functions of Time After the Additional of Salt to Suspensions of Three Different Bacterial Species.

This band was assumed to be primarily due to the absorption of light by nucleic acid present in the cells. Absorbance values were decreased sharply as the wavelength was increased beyond 260 mμ. Secondary peaks were observed near 420 and 600 mμ; the latter peak is probably characteristic only of algal pigments. Bacterial cells generally absorb strongly in the 180 to 400 mμ range.

Mitchell (1950) obtained spectra over the range of 200 to 350 mμ of suspensions containing cells of Staphlococcus aureus. Very little light was absorbed by the constituents of the cells over this range of wavelengths and the spectra were therefore considered to be primarily due to scattered light. The variation of absorbance between 350 and 1000 mμ was represented by the relationship given in Equation (A-16) where A and λ are the absorbance and wavelength, respectively, α_5 is a constant, and c is the bacterial concentration (μ grams/ml).

$$A = \alpha_5 c / \lambda \qquad \text{(A-16)}$$

This relationship is to be contrasted with the Jobst approximation in which the absorbance is proportional to the inverse square of the wavelength and the Rayleigh treatment in which the absorbance is proportional to the inverse fourth power of the wavelength. The exponent of the wavelength may be of the order of one to slightly over two (Koch, 1961; Volker and Bucker, 1963; Fikhman, 1963a, Fikhman, 1963b). Other aspects of light measurements of turbid media have also been considered (Doty and Steiner 1950; Kronman et al. 1960; and Butler, 1962). Standardization of techniques and materials have been discussed (Hallinan, 1943; Shibata, 1959; Fikhman, 1962).

Mitchell (1950) correctly stated that spectra due to scattering, as opposed to absorption, are dependent upon the aperture of the light sensitive cell of the photometer. Part of the forward-scattered light is collected along with the transmitted part of the incident beam. Koch (1961) also discussed the influence of aperture size upon turbidimetric measurements. He stated the importance of applying a sufficiently small aperture to achieve optimum sensitivity by viewing the primary beam with very little scattered light. Long narrow vertical slits were employed by Kurozumi et al. (1965). The scattered light was eliminated almost completely and the rectilinear attenuation of light through the suspension was then proportional to the sum of the light-scattering cross-sectional, or projected, areas of the cells.

Shibata et al. (1954) successfully separated the absorption and scattered light contributions and obtained the sharply defined spectra previously described. The difficulty of resolving these contributions was overcome by placing oiled paper or opalescent glass between the solution cells and their detectors. Both the specularly transmitted light and the scattered emergent light then uniformly diffused regardless of incident direction. More details are given in an excellent work by Shibata (1959).

APPENDIX B

CALIBRATION OF THE MAGNETIC STIRRING UNIT

The magnetic stirring unit used in the current experiment work was calibrated for three different fluids: distilled water, water containing 10^8 cells of Bacillus subtilis per milliliter, and cell suspension containing 2 to 8 grams of Dowex 1 x 8, 100/200 mesh resin in chloride form per 50 ml. The test fluid was introduced into a 150 ml glass beaker of 2 inch inside diameter which served as the reaction vessel in the adsorption studies. Visual or stroboscopic calibrations were made at 25 C. Agitation of the test fluids was provided by one of a series of Teflon-coated stirring bars. The diameters of these bars were 3/8 inches; lengths of 1/2, 3/4, 1-1/4, and 1-3/4 inches were available. One end of each bar was marked with waterproof black ink so that it could be distinguished from the unmarked end during calibration.

The beaker containing the test fluid was suspended in either of two positions. It was either placed directly over the magnetic drive unit and separated only by a 1/8 inch asbestos square for insulating purposes, or suspended in the constant temperature bath which was placed on the drive unit. The actual adsorption experiments were conducted with the beaker suspended in the latter position. The distance between the botton of the beaker and the top of the drive unit was about 2 cm in this position.

The speed of the magnetic drive unit (Magnetstir Cat. No. 1250, Labline, Inc., Chicago, Ill.) was controlled by a potentiometer which was indexed over a 180° arc in seven major divisions on an

arbitrary scale. Agitation speeds below 200 rpm were visually calibrated by first adjusting the setting of the potentiometer to a given position and then measuring the time required for 100 complete revolutions of the black end of the stirring bar. Readings were taken in quintuplicate at each setting and then averaged. Each speed setting was reset three times yielding a total of three average values.

Stirring speeds above 200 rpm were calibrated using a stroboscope (Strobotac Type 1531-A, General Radio Co., Concord Mass.) that was capable of measuring in the range 110 to 2500 rpm. Three readings were taken at each speed: the fundamental, and the first and the second harmonics. These readings were observed as stationary images of the stirring bar possessing, respectively, one, two, or four black ends. The first and second harmonic values were then corrected to the fundamental and the resulting three values thus obtained were averaged. Each whole and half index number was reset and measured a total of three times thereby yielding three average values. Single calibration measurements were also made at the 1/4 settings.

The correlation of the arbitrary stirrer index numbers with the actual rotaional speed (rpm) of the stirring bar is shown in Figure B-1. The test system that is reported in this figure is 50 ml of water at 25 C., agitated with a 3/8 x 1-3/4 inch stirring bar in a 150 ml beaker placed directly over the drive unit. Data points obtained for the bacterial suspension and for the cell suspension plus varying amounts of ion exchange resin were indistinguishable from those of pure water. No differences were observed upon raising the reaction vessel 2 cm above the unit; there were no differences between stirring bars of different length. Although the rotational speed of the stirring

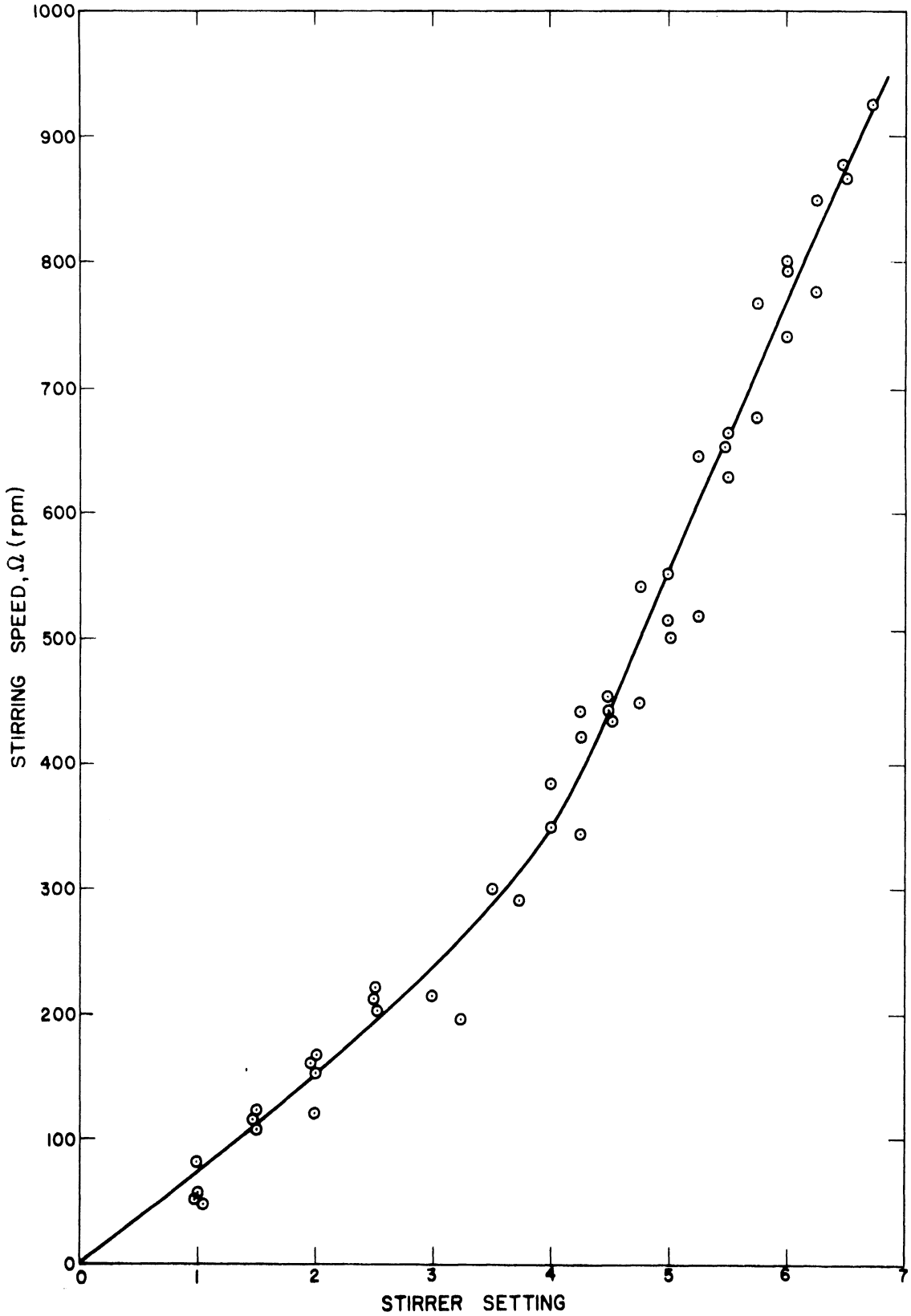


Figure B-1. Agitation Speed as a Function of Stirrer Setting (System: 50 ml distilled water at 25 C. in a 150 ml beaker agitated by a 3/8 x 1-3/4 inch magnetic stirring bar driven by a Magnetstir Cat. No. 1250, Labline, Inc.)

bar was found to be fairly constant at one setting, the degree of mixing will probably be a function of the particle size of the resin, the quantity of resin per unit volume of suspension, and the extent of flocculation of the resin with the bacterial cells.

APPENDIX C

SAMPLING OF MIXED SUSPENSIONS

A knowledge of the relative proportions of freely-suspended and adsorbed bacterial cells present in a cell adsorbent mixture is necessary to determine the rate and degree of adsorption. A suitable technique is required to rapidly separate a sample os suspension containing the freely-dispersed (unadsorbed) cells from the reaction mixture since particles of the adsorbent loaded with adsorbed cells are also contained in the mixture. Some techniques that have been described in the literature are: sedimentation, filtration, centrifugation, and direct microscopic enumeration. The disadvantages of these techniques when applied to the current inbestigation are briefly reviewed in this Appendix.

The heavier particles of the adsorbent loaded with the adsorbed cells can be allowed to settle to the bottom of the container while the unadsorbed cells remain in suspension. This technique was used during much of the early work (Krüger, 1889; Dianova and Voroschilova, 1925; Khudiakov, 1926; Karpinskaya, 1926; Minenkov, 1929; Rubentschik et al., 1936; Peele, 1936; Novogrudskii, 1936b; Waksman and Vartiovaara, 1938; Tschapek and Garbosky, 1950, 1950b, 1951; Puck and Sagik, 1953; and Barr, 1957). The disadvantage of this technique is the necessity for extended periods of settling which are not suitable for the rapid determination of rates of adsorption. Furthermore, complete separation of fine particles can not always be assured. This may be the case if the density differences between the fluid and the suspended particles is small, and the particles of the adsorbent settle very slowly or float upon the surface of the suspending fluid.

Filtration was occasionally used to remove the particles of adsorbent. (Bechhold, 1918; Eisenberg, 1918; Michaelis, 1918; Gunnison and Marshall, 1937; and Adamov, 1959). The removal of cells by filtration in certain cases can exceed removal by adsorption. The serious error introduced by filtration of a bacterial suspension during a study of adsorption was recognized by Bechhold (1918). Bacteria were found to strongly adsorb to filter paper according to Friedberger (1919), Putter (1920), Frei and Erissman (1922), and Lasseur, Dombray, and Palgen (1934). Removal of cells by filtration was greatly influenced by the pH and the salt content of the suspending medium. Maruyama and Yanagita (1956), and later Helmstetter and Cummings (1963), purposely used filter paper and other adsorbent surfaces to obtain synchronous growth by selective retention of the older cells.

Several researchers used a column technique to adsorb cells from suspension (Ham and Barnes, 1947a, 1947b; Barnes, 1952a, 1952b; Puck and Sagik, 1953; Gillissen, 1960; Gillissen et al., 1961; and Schwartz and Mayer, 1963). Removal of cells by filtration may also be significant in columns containing fine particles of an adsorbent. Kurozumi et al. (1965) circumvented this problem by inserting a square rod through the center of the adsorbent bed. The particles were slowly agitated by the rotation of this rod and filtration was minimized. Cells that were subsequently eluted from the adsorbent were collected as they passed through a narrow opening between the rotating rod and the base of the column. The larger adsorbent particles were not allowed to pass. Some abrasion of the resin was observed, however, using this procedure.

Centrifugation was used by other researchers to remove the particles of adsorbent from suspension (Gunnison and Marshall, 1937; Weiss, 1951; Estermann and McLaren, 1959; and Lahav, 1962). Centrifugation is more rapid than natural sedimentation but has the disadvantage of being too slow for rate studies since additional adsorption of cells can occur during this separation. Unadsorbed cells may also be swept out of suspension into the rapidly settling cell-adsorbent complex.

Zvyagintsev (1959b) determined the degree of adsorption that had occurred in a cell-adsorbent mixture by counting the numbers of free and adsorbed cells using the technique of fluorescent microscopy. Although this technique is sufficiently accurate for equilibrium studies it is too time-consuming for rate studies. Artifacts such as adsorption to the glass microscope slides, convection currents existing in hanging drops, interaction with dyes, etc., can also appear.

It is difficult to conduct accurate and rapid rate studies by any of the techniques described in the above paragraphs. A simple, rapid technique is therefore mandatory in order to separate the two reactants, cells, and adsorbent particles, at intervals throughout the duration of the reaction. Ideally, the total reaction volume should not be appreciably reduced during the experiment by the periodic withdrawl of samples for analysis. Alternatively, the samples should not be destroyed by the measuring technique and be amenable to return unaltered to the mixture. The latter conditions are possible if the reaction can be momentarily suspended or be induced to continue at a much reduced rate during the sampling period.

Dranoff (1960) devised an ingenous device to study the kinetics of rapid ion exchange reactions. He was able to instantaneously separate particles of an ion exchange resin from a contacting solution by conducting the reaction in a perforated thimble that was enclosed within a second solid tight-fitting thimble. At a predetermined time the outer thimble was removed and the liquid rapidly drained from the inner thimble leaving the resin particles high and dry. The reaction times for Dranoff's experiments were of the order of a few seconds. Only one datum point could be determined for each experiment.

The adsorption of bacterial cells onto particles of an ion exchange resin in an agitated suspension can be studied by a modification of this technique. A sample of the suspension containing only the freely suspended cells can be separated from the reaction mixture while agitation is halted by retaining the ion exchange resin particles, with adsorbed cells on their surfaces, on a finely-meshed wire screen. The total concentration of unadsorbed cells in the filtered sample can then be determined by optical means. The sample can be returned unchanged to the bulk suspension and the reaction continued with resumed agitation. A more complete description of this technique is presented in Section IV-D of this report.

APPENDIX D

VERIFICATION OF BACTERIAL SPECIES

The following morphological and physiological tests were performed using cells of the six bacterial species studied in this report. The results of these tests are summarized in Table XXV of Section III. Each bacterial species is classified according to the nomenclature used in Bergey's Manual of Determinative Bacteriology, 7th Edition (Breed, Murray, and Smith, 1957).

1. Size and shape

The descriptions of the sizes and shapes of the cells of the bacterial species reported in Table XXV were taken from Bergey's Manual, 7th Edition (Breed, Murray, and Smith, 1957) with the exception of values for Pseudomonas ovalis that were reported by Bennett (1963). These descriptions were verified by experimental observations.

2. Growth in nutrient broth

The six bacterial species studied in this report were cultured in trypticase soy broth of the composition given in Table XXVI. The descriptions in Table XXV are applicable for 24 hour-old cells grown in unagitated cultures in tubes incubated at 30°C.

3. Action upon milk

A description of the litmus milk medium is given in Difco (1953, p.192). Acid is produced by bacterial fermentation of the lactose fraction of the mild; the initial blue litmus is turned to red and the casein fraction is precipitated. A clear purplish when and a

precipitate of calcium salts if produced by some bacteria which hydrolyze casein.

Tubes of media were inoculated from 24 hour broth cultures of each of the six species to be evaluated, incubated at 30°C. for 48 hours, and inspected. Acid production was observed in the culture of Escherichia coli. The other five tubes showed no activity. Cultures of both Escherichia coli and Staphlococcus aureus had produced acid and contained curdy precipitations by the end of 72 hours. The remaining four tubes were still alkaline. Sediment was observed for the cultures of Bacillus cereus and Bacillus subtilis.

4. Gram reaction

The Gram staining character of the cells of the six species was frequently determined in the current investigation. The staining procedure was described by Salle (1961a, p.14). The reagents are listed in the first appendix of Salle (1961): Hucker's ammonium oxalate crystal violet stain (#21, p.180), acetone alcohol (#1, p. 177), and safranine staining solution (#51, p.185).

5. Motility

The motilities of the test species were not experimentally evaluated. Descriptions from the literature are given for comparison in Table XXV.

6. Carbohydrate fermentations

The abilities of the cells of certain bacterial species to produce acid, or gas, or both, or neither, from carbohydrates is of considerable value in identification. Solutions of 0.5% lactose,

mannitol, glucose, and sucrose containing bromothymol blue indicator were prepared in test tubes containing small inverted vials for gas collection. The production of acid is indicated by a color change from blue to yellow; gas formation is directly observed in the inverted vials. The results from 48 hour cultures are summarized in Table XXV and are in good agreement with the published descriptions for the six species. The action of the cells of Escherichia coli upon sucrose is often found to be variable. The production of acid by the cells of Proteus vulgaris was somewhat questionable after 48 hours. Acid production was better defined after 72 hours. Gas production was very slight, however, compared to the strong activity shown by the cells of Escherichia coli.

7. Citrate utilization

Media containing inorganic ammonium salts as the sole source of nitrogen, and citrate as the sole source of carbon, can be used to differentiate certain bacterial species, particularly Escherichia coli and Aerobacter aerogenes, and certain members of the genus Salmonella. Simmons citrate agar, as described by Difco (1953, p.182), is turned from green to deep blue by the production of alkali. A very positive test was given by the culture of Bacillus subtilis; slight changes in color were produced by the cultures of both Proteus vulgaris and Bacillus cereus.

8. Hydrolysis of urea

The ability of certain bacteria to hydrolyze urea is another criterion for differentiation of species. Carbon dioxide and ammonia

are produced by the action of urease. A positive test is observed by the development of a red color due to phenol red dye contained in the medium (Difco, 1953, p.171). This test is particularly suitable for the detection of members of the genus Proteus (with the exception of P. inconstans). A positive test was observed in the present work for the cultures of Proteus vulgaris and Staphlococcus aureus.

9. Liquefication of gelatin

Gelatinase, an extracellular enzyme capable of liquefying gelatin, is secreted by the cells of several bacterial species. The presence of this enzyme was demonstrated in the current work by inoculating the tubes of gelatin from 24 hour cultures and incubating them for 24 hours at 30°C. Tubes of gelatin usually remain liquid at this temperature but solidfy after a brief period of refrigeration if gelatinase is not present. Gelatin was liquified within 48 hours by the cells of Proteus vulgaris, Staphlococcus aureus, Bacillus cereus and Bacillus subtilis. No liquifaction was observed even after 96 hours of incubation of the cultures of Pseudomonas ovalis and Escherichia coli.

10. Reduction of nitrate

The chemical reduction of nitrate to nitrate can be evaluated using medium containing 0.1% potassium nitrate prepared according to Difco (1954, p.184). Two tubes are inoculated from 24 hour broth cultures of each of the six species to be evaluated and incubated at 30°C. Nitrate is detected by placing a few drops from a culture on a spot plate, adding one drop of sulfanilic acid solution and one drop of

α-naphthylamine solution, and observing the formation of a pink-red color. The presence of nitrate is checked if the nitrate test is negative by placing a few drops from a culture on a spot plate and adding one drop of diphenylamine reagent. A blue color is indicative of a positive test.

The chemical spot tests were conducted daily for one week on the six species under investigation. Uninoculated tubes of medium were used as controls. Nitrate was converted to nitrate by the cells of all but one species within one week after incubation. The culture of Pseudomonas ovalis was the exception. The conversion to ammonia or nitrogen was not determined for any species.

11. Hydrolysis of starch

The presence of amylase, an extracellular enzyme, produced by certain bacterial species is indicated by the hydrolysis of starch. Soluble starch was added to trypticase soy broth to make a 0.2% w/v solution, heated to boiling, filtered through glass wool, and dispensed into test tubes, that were sterilized in an autoclave for 15 minutes at 15 lbs pressure. Duplicate tubes were inoculated from 24 hour broth cultures of the six bacterial species. One set of tubes was incubated at 30°C for 24 hours, the other for 48 hours. At the end of each time period five drops of iodine solution were added to each tube of the series and the resulting color observed.

The presence of starch is indicated by the development of a deep blue-black iodine-starch complex. The negative result was observed for 24 hour cultures of Pseudomonas ovalis, Escherichia coli, Proteus vulgaris, and Staphlococcus aureus. The complete hydrolysis of starch

is indicated by the absenco of a blue color. This positive result was observed for 24 hour cultures of both Bacillus subtilis and Bacillus cereus. Identical results were obtained for 48 hour cultures of these species.

12. Growth in strong saline (7%)

A series of tubes containing trypticase broth and 7% NaCl w/v were inoculated with 24 hour cultures grown in the usual 0.5% NaCl w/v broth and incubated for 24 hours. Positive growth was observed within 24 hours in inocula of Bacillus subtilis, Escherichia coli, and Staphlococcus aureus. Growth of inocula of Pseudomonas ovalis, Proteus vulgaris, and Bacillus cereus was not observed after 24 hours. Slight growth developed in the latter inocula after 96 hours. Gram stains of these three species was considered inconclusive.

13. Production of lecithinase

The ability to produce lecithinase has been used as a criterion for differentiating members of the genus Bacillus. The test has been described by Colmer (1948), and Knight and Proom (1950). Details of many other physiological characterizations of this genus are included in the latter reference. The production of lecithinase is observed in nutrient broth containing egg-yolk by the formation of heavy turbidity after 8-10 hours incubation. A thick curd usually appears on the top of the medium after 16-24 hours. A positive test is also observed by the development of a zone of precipitation surrounding colonies grown on egg-yolk agar plates. This test is positive for cultures of Bacillus cereus and to a much lesser extent for cultures of the closely related

B. cereus var. mycoides and B. anthracis. A negative reaction is predicted for cultures of B. subtilis.

Egg-yolk agar plates are prepared by transferring the yolk of one egg to 100 ml or 0.85% saline and passing the mixture through a Seitz filter. One part of the sterile opalescent filtrate is combined with nine parts of 1% trypticase soy or nutrient agar at 45°C with sterile precautions and the mixture immediately poured into sterile Petri dishes. The resulting plates are celar and resemble ordinary agar plates. They can be easily stored under refrigeration for extended periods.

A yolk-agar plate was inoculated from 24-hour nutrient broth cultures of the six experimental cultures. A heavy growth and a very definite zone of precipitation approximately twice the radius of the colony was produced within 24 hours by the cells of Bacillus cereus. The growth of cells of B. subtilis was much less spectacular and a precipitation zone was not produced. Negative results were observed for the other four species.

14. Production of coagulase

The production of coagulase, an enzyme capable of clotting plasma, is generally accepted as the best criterion for determining the potential pathogenicity of members of the genus Staphlococcus (Difco, 1953, p.330). A standard 100 mg ampule of Bacto-Coagulase Plasma (B286) was dissolved in 3 ml of sterile distilled water. A 0.5 ml volume of the plasma solution was combined with 0.1 ml of a 24 hour trypticase soy culture of Staphlococcus aureus. A positive coagulase

test was observed after incubation in a water bath at 37°C for 2 hours. This test was not made on the other five experimental species.

15. Qualitative observations

Several qualitative features were also noted for the six experimental species. An additional odor was superimposed over the odor of fresh trypticase soy broth after growth by several species. Odors were also described for the prepared cell suspensions after separation from the growth media. Descriptions of such odors to a degree are dependent upon the observer. The physical appearances of the cell suspensions during centrifugation and filtration are also distinctive. The degree of flocculation of the cell-resin complex listed in Table XXIX can also be considered as a qualitative test.

Centrifugation was judged by the ability of the cells of a 24 hour broth culture to completely settle after 20 minutes at 1500 rpm. The appearance of the supernatant liquid and the centrifuge cake can be characteristics of a particular bacterial species. The filtration of the washed, resuspended cells was compared using the arbitrary rate of approximately 3 ml/min. Cells of the three Gram-negative species filtered more slowly than the cells of the three Gram-positive species. The generalization of this criterion is not warranted at present, since it may be peculiar to the filtration of the cells of these six species through paper (#4 Whatman). The appearance of the flocculated cell-resin complex is a function of pH, salt content, and many other factors, but can be observed to be very spectacular as shown in Figure 9 of Section V.

16. General conclusions

The following conclusions were based upon the various morphological and physiological tests performed on the six species considered in the current investigations.

(a) Pseudomonas ovalis: This species was verified by its negative Gram reaction, growth in broth, inability to ferment lactose, mannitol or sucrose, acid production only in glucose, and failure to liquefy gelatin. The utilization of citrate is considered variable and urease is usually absent for this species.

(b) Escherichia coli: This species was verified by its negative Gram reaction, growth in broth, rapid production of acid in litmus milk, formation of acid and gas in lactose, mannitol and glucose, and its inability to utilize citrate or urea, liquefy gelatin, or hydrolyze starch. The variable fermentation of sucrose was expected and was not used for confirmation.

(c) Proteus vulgaris: This species was verified by its negative Gram reaction, formation of acid and gas in glucose and sucrose, but not in lactose or mannitol, hydrolysis of urea, and liquefaction of gelatin. The utilization of citrate was considered to be variable. The fermentation of glucose and sucrose with accompanying acid and gas production was very weak. The usually spectacular ability of this species to hydrolyze urea was not observed. This strain, therefore, may be exceedingly weak or the inocula were not truly representative. Growth in shake flasks, however, during preparation of test suspensions was always very substantial.

(d) Staphlococcus aureus: This species was verified by its positive Gram reaction, spherical shape, growth in broth and milk, formation of acid but no gas in lactose, glucose, sucrose, and particularly mannitol, and by its ability to liquefy gelatin and coagulate plasma.

(e) Bacillus cereus and Bacillus subtilis: These species are very similar in their physical appearances and in their reactions toward many of the common differential media. Both species were verified to be Gram-positive. Both produced acid, but no gas, from glucose and sucrose, did not ferment lactose or mannitol, did not hydrolyze urea, but did hydrolyze both gelatin and starch. Citrate was utilized by cultures of Bacillus subtilis but its use by cultures of Bacillus cereus was questionable. The distinguishing test for these species, however, was the ability to elaborate lecithinase and form a zone of precipitation on egg-yolk agar. This test was very positive for cultures of Bacillus cereus; it was negative for cultures of Bacillus subtilis.

APPENDIX E

EXAMPLE CALCULATIONS

Example calculations are presented in this Appendix for the case of the adsorption and desorption of cells of Bacillus subtilis in a suspension contacted with particles of the anion exchange resin, Dowex 1 x 8. The conditions of this experiment are presented in Table E-I. Absorbance and pH values were collected as a function of the square root of the cumulative time of agitated contact between the bacterial cells and the resin particles. The data of the adsorption and desorption are presented, respectively, in Tables E-II and E-III. Only a few scattered Gram-positive bacilli were observed in a Gram stain made of the suspension after 49 minutes ($\sqrt{t} = 7$ min$^{\frac{1}{2}}$) of cumulative adsorption. Large numbers of Gram-positive bacilli, some Gram-variable and Gram-negative bacilli, and scattered cellular debris were evident in a Gram stain made of the suspension after 49 minutes ($\sqrt{t'} = 7$ min$^{\frac{1}{2}}$) of cumulative desorption.

The data obtained during adsorption and desorption were then differenced according to the procedure discussed in Section III-C-3 using the form of Equation (III-30).

$$\frac{\Delta \log f(A/A_o)}{2\Delta\sqrt{t}} = k\sqrt{t} + \frac{k'}{2} \qquad \text{(III-30)}$$

The calculated differences of the adsorption and desorption data are also tabulated in Tables E-II and E-III and plotted, respectively, in Figure 27 and 29 of Section V. The curve for adsorption was plotted in Figure 28 as $f(A/A_o) = \log(A/A_o)$ versus $\sqrt{t}$. The curve for desorption was

TABLE E-I

DATA FOR THE SORPTION OF Bacillus subtilis WITH DOWEX 1 x 8 AT pH 3.5

Bacterium: Bacillus subtilis, 24-hour culture in trypticase soy broth at 30 C., harvested by centrifugation, washed, filtered, and resuspended in distilled water.

A_o' = initial absorbance of suspension = 0.598 = 1.25×10^8 cells/ml (pH 6.60)

A_o = adjusted absorbance of suspension = 0.794 (pH 3.50)

V = volume of suspension = 50 ml

Ion Exchange Resin: Dowex 1 x 8, 100/200 mesh, chloride form, Type I anion exchange resin.

m = mass of resin = 4.00 grams

$2R$ = average particle diameter = 0.972×10^{-2} cm

N = number of particles/unit volume of suspension = 1.00×10^5/ml

Environment: Initial suspension of distilled water, pH adjusted with concentrated hydrochloric acid, regenerated with 1 M potassium chloride added as the solid salt.

T' = temperature = 30 C.

$\curvearrowright$ = agitation rate = 445 rpm

initial pH = 6.60

adjusted pH = 3.50

TABLE E-II

EXPERIMENTAL AND CALCULATED RATE DATA FOR THE ADSORPTION OF *Bacillus subtilis* ONTO DOWEX 1 x 8 AT pH 3.5

$\sqrt{t}$, $min^{\frac{1}{2}}$	A	A/A_o	pH	$-\log(A/A_o)$	$\frac{\Delta \log(A/A_o)}{2\Delta\sqrt{t}}$	$k_a' \sqrt{t}$ [1]	$k_a t$ [1]	$-\log(A/A_o)$	A/A_o	$\sqrt{t}$, $min^{\frac{1}{2}}$
0	0.794	1.0	3.50	0.0		0.0	0.0	0.0	1.0	0
					0.0465					
	.714	.899	3.46	.0465		.045	.00875	.05375	.885	
					.0950					
1	.574	.723	3.52	.1415		.090	.035	.125	.7505	1
					.0920					
	.464	.585	3.49	.2335		.135	.07875	.21375	.6115	
					.0950					
2	.373	.470	3.50	.3285		.180	.140	.320	.475	2
					.1315					
	.275	.347	3.50	.460		.225	.21875	.44375	.360	
					.135					
3	.202	.2545	3.51	.595		.270	.315	.585	.260	3
					.1385					
	.147	.185	3152	.7335		.315	.42875	.74375	.1805	
					.1885					
4	.095	.1197	3.53	.922		.360	.560	.920	.1203	4
					.1715					
	.064	.0807	3.54	1.0935		.405	.70875	1.11375	.0770	
					.2385					
5	.037	.0466	3.55	1.332		.450	.875	1.325	.0473	5
					.1525					
	.026	.0328	3.55	1.4845		.495	1.05875	1.55375	.0280	
					-					
6	.026	.0328	3.56	-		.540	1.260	1.800	.01586	6
					-					
	.027	.0340	3.56	-		.585	1.47875	2.06375	.00864	
					-					
7	.026	.0328	3.56	-		.630	1.715	2.345	.00452	7

1 $k_a = -0.035\ min^{-1}$, $k_a' = -0.090\ min^{-\frac{1}{2}}$ from Figure 27

TABLE E-III

EXPERIMENTAL AND CALCULATED RATE DATA FOR THE DESORPTION OF
Bacillus subtilis FROM DOWEX 1 x 8 AT pH 3.5

$\sqrt{t'}$, $min^{\frac{1}{2}}$	A	A/A_o	pH	$-\log(1-A/A_o)$	$\frac{\Delta \log(1-A/A_o)}{2\Delta\sqrt{t}}$	$k_d' \sqrt{t'}$ [1]	$k_d t$ [1]	$-\log(1-A/A_o)$	A/A_o	$\sqrt{t'}$, $min^{\frac{1}{2}}$
0	0.026	0.0328	3.56	0.0145		0.0	0.0	0.0	0.0	0
					0.0196					
	.060	.0756	3.93	.0341		.004	.00141	.00541	.012	
					.0054					
1	.069	.0869	4.03	.0395		.008	.00564	.01364	.031	1
					.0103					
	.086	.1084	4.07	.0498		.012	.01269	.02469	.055	
					.0118					
2	.105	.132	4.08	.0616		.016	.02256	.03856	.0845	2
					.0147					
	.128	.161	4.10	.0763		.020	.03525	.05525	.119	
					.0167					
3	.153	.193	4.12	.0930		.024	.05076	.07476	.158	3
					.0265					
	.181	.241	4.15	.1195		.028	.06909	.09709	.200	
					.0260					
4	.226	.285	4.17	.1455		.032	.09024	.12224	.245	4
					.0302					
	.264	.333	4.18	.1757		.036	.11421	.15021	.292	
					.0304					
5	.300	.378	4.22	.2061		.040	.14100	.18100	.3405	5
					.0332					
	.336	.423	4.23	.2393		.044	.17061	.21461	.3895	
					.0417					
6	.378	.476	4.26	.2810		.048	.20304	.25104	.439	6
					.0460					
	.420	.529	4.28	.3270		.052	.23829	.29029	.4875	
					.0390					
7	.452	.570	4.35	.3660		.056	.27636	.33236	.535	7

[1] $k_d = -0.00564\ min^{-1}$, $k_d' = -0.0080\ min^{-\frac{1}{2}}$ from Figure 29

similarly plotted in Figure 30 as $f(A/A_0) = \log(1-A/A_0)$ versus $\sqrt{t}$. These original data were directly differenced. Alternatively, plots of $f(A/A_0)$ versus $\sqrt{t}$ could have been constructed, smooth curves drawn through the data points, and difference plots then constructed from the smoothed values.

Values of the slope, k, and intercept, $k'/2$, were determined from Figures 27 and 29 of Section V. The following values of the rate constants were obtained from the difference plot of the adsorption data.

$$k_a = \frac{-0.238 - (-0.045)}{5.5} = -0.035 \text{ min}^{-1}$$

$$k'_a = (2)(-0.045) = -0.090 \text{ min}^{-1/2}$$

The following values of the rate constants were obtained from the difference plot of the desorption data.

$$k_d = \frac{-0.0435 - (-0.0040)}{7.0} = -0.00564 \text{ min}^{-1}$$

$$k'_d = (2)(-0.0040) = -0.0080 \text{ min}^{-1/2}$$

Adsorption and desorption values are denoted, respectively, by the subscripts, a and d. It should be noted that these rate constants are based upon differences calculated using logarithms to the base ten.

The calculated rate constants were then used to calculate idealized adsorption and desorption curves according to the model described by Equation (III-27).

$$f(A/A_0) = kt + k'\sqrt{t} \qquad \text{(III-27)}$$

Calculated values of $f(A/A_o)$ are also included in Tables E-II and E-III. The calculated curves of adsorption and desorption, respectively, are compared with the actual data in Figures 28 and 30 of Section V. The actual values of A/A_o obtained during the desorption have been shifted by a factor of 0.0328 to be fully comparable with the calculated desorption curve for which the condition, $A/A_o = 0$ at $\sqrt{t'} = 0$, is met. The significance of these results is discussed in Section V.

Numerical values of the diffusion coefficients associated with the previously described case of adsorption and desorption were determined by two methods. According to Case B to Section III-C-2, the ratio of the rate constants can be calculated from Equation (III-34a).

$$\left| k/k'^2 \right| = 1/16\,\beta_o R^3 N \qquad \text{(III-34a)}$$

$$= \frac{(50)(8)}{(16)(0.83)(0.972\times10^{-2})^3(1.25\times10^6)(4)} = 6.56$$

The actual values of the ratios of rate constants of adsorption and of desorption that were previously determined can also be calculated.

$$\left| k_a/k_a'^2 \right| = \frac{-0.035}{(-0.090)^2(2.303)} = 1.88$$

$$\left| k_d/k_d'^2 \right| = \frac{-0.00564}{(-0.0080)^2(2.303)} = 38.3$$

The diffusion constants for adsorption and desorption can be determined from Equations (III-35a) and (III-35b).

$$D_k = -k / 4\pi\beta_o R N \qquad \text{(III-35a)}$$

$$D_{k'} = (1/\pi)[-k'/8\beta_o R^2 N]^2 \qquad \text{(III-35b)}$$

$$D_{k_a} = \frac{-(-0.035)(50)(2.303)}{(4\pi)(0.83)(0.972\times10^{-2}/2)(1.25\times10^{6})(4)}$$

$$= 1.59\times10^{-5}\ cm^2/min = 2.65\times10^{-7}\ cm^2/sec$$

$$D_{k'_a} = \frac{1}{\pi}\cdot\left[\frac{(-0.090)(50)(2.303)}{(8)(0.83)(0.972\times10^{-2}/2)^2(1.25\times10^{6})(4)}\right]^2$$

$$= 5.56\times10^{-5}\ cm^2/min = 9.28\times10^{-7}\ cm^2/sec$$

$$D_{k_d} = \frac{-(-0.00564)(50)(2.303)}{(4\pi)(0.83)(0.972\times10^{-2}/2)(1.25\times10^{6})(4)}$$

$$= 2.56\times10^{-6}\ cm^2/min = 0.427\times10^{-7}\ cm^2/sec$$

$$D_{k'_d} = \frac{1}{\pi}\cdot\left[\frac{(-0.0080)(50)(2.303)}{(8)(0.83)(0.972\times10^{-2}/2)^2(1.25\times10^{6})(4)}\right]^2$$

$$= 4.40\times10^{-6}\ cm^2/min = 0.733\times10^{-7}\ cm^2/sec$$

The factor 2.303 is required for conversion from base ten logarithms to natural logarithms. The significance of these calculations is discussed in Section V.

A plot of the theoretical sorption curve, $(1-M_t/M_\infty)=\log(A/A_0)$ versus $\sqrt{\tau}$, as determined from Equations (III-10a-E) and (III-10b-E), is required in the alternative method of calculating the diffusion coefficients, D_a and D_d, based upon Case E of Section III-C-2.

$$(1-M_t/M_\infty) = \sum_{n=1}^{\infty} \frac{6\alpha^*(\alpha^*+1)\exp(-s_n^2\tau)}{9(\alpha^*+1)+\alpha^{*2}s_n^2}\ , \quad \tau \geq 0.1 \qquad \text{(III-10a-E)}$$

$$(1-M_t/M_\infty) = (\alpha^*+1)\left[\frac{\psi_1}{\psi_1+\psi_2}\exp\left(\frac{3\psi_1\sqrt{\tau}}{\alpha^*}\right)^2 \operatorname{erfc}\left(\frac{3\psi_1\sqrt{\tau}}{\alpha^*}\right)\right. \qquad \text{(III-10b-E)}$$

$$\left. + \frac{\psi_2}{\psi_1+\psi_2}\exp\left(-\frac{3\psi_2\sqrt{\tau}}{\alpha^*}\right)^2 \operatorname{erfc}\left(-\frac{3\psi_2\sqrt{\tau}}{\alpha^*}\right)\right] - \alpha^*\ , \quad \tau \leq 0.1$$

A particular value of the volume ratio, α^*, can be calculated form Equation (III-18).

$$\alpha^* = \frac{3V}{4\pi K' R^3 N' m} = \frac{(3)(50)}{(4\pi)(1)(0.972\times10^{-2}/2)^3(1.25\times10^{6})(4)} = 20.797 \qquad \text{(III-18)}$$

Points of the theoretical reduced sorption curve have been tabulated in Table E-IV and are plotted in Figure E-1. The diffusion coefficients,

TABLE E-IV

CALCULATION OF A THEORETICAL REDUCED SORPTION RATE CURVE

τ	$\sqrt{\tau}$	A/A_o [1]	A/A_o [2]
0.7	0.8367	-	0.0005
.6	.7746	-	.0013
.5	.7071	-	.0037
.4	.6225	-	.0102
.36	.6	-	.0153
.3	.5477	0.008	.0281
.25	.5	.020	.0467
.2	.4472	.044	.0777
.180625	.425	.063	.0946
.16	.4	.086	.1168
.140625	.375	.114	.1425
.1225	.35	.146	.1718
.105625	.325	.186	.2048
.1	.3162	.217	.2173
.09	.3	.244	.2418
.08	.2828	.264	.2693
.07	.2646	.292	.3006
.06	.2449	.327	.3364
.05	.2236	.374	.3778
.04	.2	.415	.4264
.03	.1732	.482	.4841
.02	.1414	.569	.5541
.01	.1	.668	.6402
.005625	.075	.749	.6845
.0025	.05	.824	.7191
.000625	.025	.903	.7412
.0	.0	1.0	.7488

$\alpha^* = 20.797$

$\psi_1 = 3.1800$, $\psi_2 = 2.1800$

$S_1 = 3.188$, $S_2 = 6.306$

[1] $\tau \leq 0.1$

$A/A_o = 12.932\ \text{cerfc}(0.45872\sqrt{\tau}) + 8.8652\ \text{cerfc}(-0.31447\sqrt{\tau}) - 20.797$

[2] $\tau \geqslant 0.1$

$A/A_o = 0.59231 \exp(-10.163\tau) + 0.15564 \exp(-39.766\tau)$

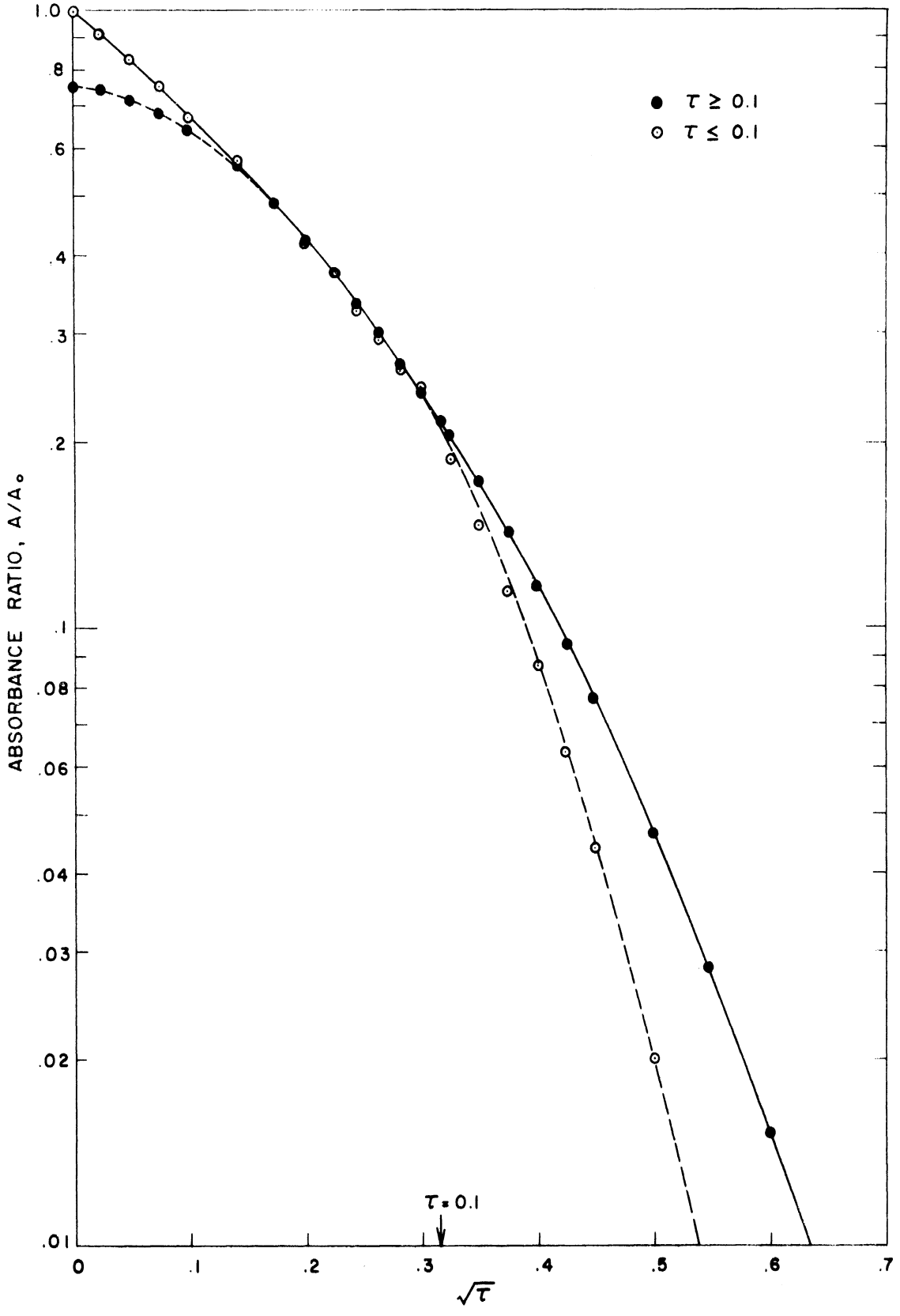


Figure E-1. Theoretical Reduced Sorption Rate Curve Calculated from Equations (III-10a-E) and (III-10b-E) (Conditions: $\alpha^* = 20.797$).

D_a and D_d, can now be determined by selecting values of $\sqrt{\tau}$ and $\sqrt{\tau'}$ from Figure E-2 that correspond, respectively, to the actual values of A/A_o and $1-A/A_o$. These values are tabulated in Table E-V for both adsorption and desorption. Plots of $\sqrt{\tau}$ versus $\sqrt{t}$ and $\sqrt{\tau'}$ versus $\sqrt{t'}$ should be linear of slopes, $\sqrt{D_a}/R$ and $\sqrt{D_d}/R$, respectively, according to Equation (III-19).

$$\sqrt{\tau} = (\sqrt{D}/R)\sqrt{t} \qquad \text{(III-19)}$$

A plot of $\sqrt{\tau}$ versus $\sqrt{t}$ for the adsorption data is presented in Figure 31 of Section V. The following value of D_a is calculated from the slope of this plot.

$$\sqrt{D_a}/R = 0.105$$

$$D_a = [(0.972\times10^{-2}/2)(0.105)]^2/60 = 0.0433\times10^{-7}\ cm^2/sec$$

This value of D_a can now be used to convert the reduced time values, $\sqrt{\tau}$, that were selected from Figure E-1, into actual time values, $\sqrt{t}$.

$$\sqrt{t} = (R/\sqrt{D_a})\sqrt{\tau} = (1/0.105)\sqrt{\tau} = 9.54\sqrt{\tau}$$

The actual adsorption data presented in Table E-II are compared with the calculated adsorption curve in Figure 32 of Section V. A shift in the $\sqrt{t}$ scale is necessary since $\sqrt{t}=0.23$ when $\sqrt{\tau}=0$ in Figure 31.

A plot of $\sqrt{\tau'}$ versus $\sqrt{t'}$ for the desorption data is presented in Figure 33 of Section V. The following value of D_d is calculated from the slope of this plot.

$$\sqrt{D_d}/R = 0.0348$$

$$D_d = [(0.972\times10^{-2}/2)(0.0348)]^2/60 = 0.00477\times10^{-7}\ cm^2/sec$$

TABLE E-V

REDUCED TIME VALUES FOR THE ADSORPTION AND DESORPTION OF *Bacillus subtilis* WITH DOWEX 1 x 8 AT pH 3.5[1]

Adsorption					Desorption				
$\sqrt{t}$	A/A_o	$\sqrt{\tau}$ [2]	$\sqrt{t}$ [3]	$\sqrt{t}+0.23$ [4]	$\sqrt{t'}$	$1-A/A_o$	$\sqrt{\tau'}$ [2]	$\sqrt{t'}$ [3]	$\sqrt{t'}+1.78$ [4]
0	1.0	0.0	0.0	0.23	0	1.0	0.0	0.0	1.78
	.899	.027	.26	.49		.988	.013	.37	2.15
1	.723	.082	.87	1.01	1	.969	.015	.43	2.21
	.585	.133	1.27	1.50		.945	.021	.60	2.38
2	.470	.178	1.70	1.93	2	.9145	.026	.75	2.53
	.347	.239	2.28	2.51		.881	.037	1.07	2.85
3	.2545	.292	2.79	3.02	3	.842	.045	1.30	3.08
	.185	.340	3.25	3.48		.800	.060	1.73	3.51
4	.1197	.397	3.79	4.02	4	.755	.074	2.14	3.92
	.0807	.443	4.23	4.46		.708	.091	2.62	4.40
5	.0466	.500	4.77	5.00	5	.6595	.107	3.08	4.86
	.0328	.533	5.08	5.31		.6105	.124	3.57	5.35
6	.0328				6	.561	.142	4.09	5.87
	.0340					.5125	.165	4.75	6.53
7	.0328				7	.465	.183	5.26	7.04

[1] Data from Table E-III

[2] Determined from Figure E-2 for values of A/A_o or $1-A/A_o$ from this table

[3] Calculated from the relationships: $\sqrt{t} = 9.54\sqrt{\tau}$ or $\sqrt{t'} = 28.8\sqrt{\tau'}$

[4] Translations of 0.23 and 1.78 min necessitated by the failure of the linear fits shown in Figures 31 and 33 to pass through the origins.

This value of D_d can now be used to convert the reduced time values, $\sqrt{\tau'}$, that were selected from Figure E-1, into actual time values, $\sqrt{t'}$.

$$\sqrt{t'} = (R/\sqrt{D_d})\sqrt{\tau'} = (1/0.0348)\sqrt{\tau'} = 28.8\sqrt{\tau'}$$

The actual desorption data presented in Table E-III are compared with the calculated desorption curve in Figure 34 of Section V. A shift in the $\sqrt{t'}$ scale is necessary since $\sqrt{t'} = 1.78$ when $\sqrt{\tau'} = 0$ in Figure 33.

The diffusion coefficient, D, can also be calculated from the Einstein-Stokes Equation (III-32). The value of D can be calculated for a bacterial cell of diameter, $d = 1.0\,\mu$, suspended in water at 25 C.

$$D = \frac{kT'}{3\pi\mu d} = \frac{(1.38\times10^{-16})(298)}{(3\pi)(0.00895)(1.0\times10^{-4})} = 0.0487\times10^{-7}\ cm^2/sec$$

The value of D can also be calculated for a resin particle of diameter $2R = 97.2\,\mu$ suspended in water at 25 C.

$$D = \frac{kT'}{6\pi\mu R} = \frac{(1.38\times10^{-16})(298)}{(6\pi)(0.00895)(97.2\times10^{-4})} = 0.000501\times10^{-7}\ cm^2/sec$$

Therefore, the average diffusion coefficient of the bacterial cell is much larger than that of the resin particles.

The capacity, q_∞, of an ion exchange resin for bacterial cells can be estimated by assuming a resin particle of average diameter to be completely covered with a monolayer of adsorbed cells. A total of 1.25×10^6 particles are contained in one gram of a resin having an average particle diameter of 0.972×10^{-2} cm. The surface area can therefore be calculated.

$$S = 4\pi R^2 N = (\pi)(0.972\times10^{-2})^2(1.25\times10^6) = 371\ cm^2/gram$$

If a single cell of Bacillus subtilis is assumed to have dimensions of $0.75\ \mu \times 2.5\ \mu$, then its longitudinal cross-sectional area is approximately $1.875\ \mu^2$. A fixed number of cells can therefore be accommodated by the above resin.

$$q_\infty = \frac{371}{1.875 \times 10^{-8}} = 1.98 \times 10^{10} \text{ cells/gram}$$

The capacity of the resin can also be calculated from Equation (III-37).

$$\gamma = \frac{1}{1 - A/A_0} \overset{t \to \infty}{=} \frac{n_0 V}{q_\infty m} \qquad \text{(III-37)}$$

The separation ratio, γ_∞, can be calculated from a series of absorbance values obtained at various times. A plot of $\log(\gamma)$ versus $1/\sqrt{t}$ extrapolated to infinite time is shown in Figure 11 of Section V. The extrapolated values of γ_∞ for 1/2 and 1 gram obtained from Figure 11 are 2.02 and 1.04, respectively. The extrapolated values of γ_∞ for 2 and 4 grams were not used for calculating purposes since saturation did not occur in either of these adsorptions. The capacity can then be calculated for the conditions of n_0 = 1.25 x 10^8 cells/ml and V = 50 ml.

$$q_\infty (m=1) = \frac{(1.25 \times 10^8)(50)}{(1.04)(1)} = 0.601 \times 10^{10} \text{ cells/gram}$$

$$q_\infty (m=1/2) = \frac{(1.25 \times 10^8)(50)}{(2.02)(1/2)} = 0.619 \times 10^{10} \text{ cells/gram}$$

$$q_\infty \text{ (avg.)} = 0.610 \times 10^{10} \text{ cells/gram}$$

Saturation has occurred to the extent of 0.610 x10^{10}/1.98 x 10^{10} = 30.8% of the estimated theoretical capacity. Alternatively, 37/x10^8/0.610/10^{10} = 6.08 μ^2 of resin surface is occupied by each bacterial cell. This occupied area is 6.08/1.875 = 3.25 times the longitudinal cross-sectional area of the bacterial cell calculated from measurements obtained from a stained cell preparation.

APPENDIX F

COLUMNAR ADSORPTION OF BACTERIA

1. Introduction

Two techniques have been used in contracting particles of ion exchange resins with suspensions of cells. They are: the batch method and the continuous method. Continuous exchange, as the name implies, is a constant contracting of the exchange resin with fresh solution. It can be considered to be an infinitely large number of batch operations conducted in series. Such a continuous exchange is often referred to as column exchange since the exchange resin is contained in a column through which the contracting solution is passed. Continuous exchange can be subdivided further into fixed-bed exchange and moving-bed exchange depending on the degree of movement and flow pattern allowed in the bed.

In a batch exchange, the solution to be exchanged is introduced into a "holding" tank, the resin is added, and equilibrium is allowed to become established. The solution is then separated from the resin by filtration or some other similar process. The resulting exchange, unlike the continuous exchange, is limited by the selectivity of the resin under equilibrium conditions. Certain components of the solution must be definitely prefered by the solid phase for any separation to occur. Unless this selectivity is quite favorable for exchange of the desired species, only a small percentage of the total resin capacity is exhausted. Regeneration of the resin is usually impractical on a batch basis.

Exceptions to these unfavorable batch exchange conditions are found in those instances in which ions are irreversible removed from the solution during the exchange, e.g. neutralization reactions with the

formation of water, or precipitation of insoluble products such as silver chloride. The problem of poor selectivity is eliminated by continuous exchange on a column basis. Columnar operation, however, is generally associated with additional hydraulic problems such as chanelling of the bed or filtration of suspended particles.

A fixed-bed exchange column is usually operated as a down-flow unit. Both the solution to be exchanged and the regenerating solution pass through the column from top to bottom. Advantage of gravity flow is taken and the need for pumps in the system is eliminated. Resin-solution contact is maximized and mechanical and hydraulic problems are minimized. The pressure drop through the bed may increase and channeling may occur, however, if any insoluble materials are filtered out of the contacting liquid. The flow of the feed and the regenerate are both conducted from the bottom to the top of an up-flow column. The chief disadvantage of this method is that partial fluidization of the bed may occur and the contact efficiency may be reduced. The merits of a completely fluidized bed are that increases in pressure drop and channeling effects are greatly reduced.

A moving-bed exchange is an extended form of a fluidized bed since there is a continuous counter-current contact between the resin and the feed or regenerate streams (two separate stages). One advantage of

this system is the constant production of a uniform effluent. The contacting solution is constantly flowing down the column. The solution of declining solute concentration is always contacting resin of increasing capacity as it flows up the bed. The exhausted resin is then transferred to another unit where it is regenerated in an analgous fashion. The applications of moving-bed systems have been limited by the engineering problems associated with the design of such a unit. The maintenance of a countercurrent flow is dependent upon the density difference between resin and contacting solution which is often quite small. Longitudinal mixing is influenced by both the density and the particle diameters of the particles in the resin bed.

A discussion of the adsorption of bacterial cells onto particles of ion exchange resins contained in columns has been relegated to this Appendix for several reasons. The material (cells) to be adsorbed is in suspension rather than solution. Adsorption of such suspended materials in a fixed column can be obscured by the effects of filtration unless special precautions are taken. The kinetics of adsorption and desorption cannot be adequately described for such a system. The bulk of the experimental work was therefore directed toward investigation of adsorption and desorption rates of batch exchanges. Many of the variables of batch exchange are common to columnar exchange.

2. Description of columnar exchange

The changes in concentration due to adsorption or desorption occurring in columns can be described in terms of loading and regenerating curves. The ordinate of such plots is the ratio of the concentration of the column effluent to that of the influent, A/A_o . The influent

concentration is usually held as constant as possible. The abscissa of such plots is a function of the cumulative liquid volume passed through the column, v , or of the cumulative time if constant flow is maintained.

Portions of interest on the loading curve are: the initial plateau where removal of the adsorable material in the influent may be complete ($A/A_o = 0$), the breakthrough section where a saturation level of the resin capacity is approached as some adsorable material passes through the column unexchanged and ($0 \leq A/A_o \leq 1.0$), and the final plateau where the extent of removal approaches zero and ($A/A_o \rightarrow 1.0$). Similar portions of the desorption curve of interest are: the initial high regeneration where most of the previously adsorbed material is returned to the solution (A/A_o may become greater than unity), a reverse breakthrough where the amount of desorbed material begins to taper off, and a final plateau where no further desorption occurs ($A/A_o \rightarrow 0$).

The area enclosed between the adsorption curve and the unit ratio line during adsorption is a measure of the quantity of material adsorbed by the column. The area enclosed between the desorption curve and the zero ratio line during desorption is a measure of the quantity of material desorbed from the column. Both quantities should be equal if recovery is complete. The efficiency of the column is therefore characterized by a ratio of the respective areas of adsorption and desorption.

3. Procedure for a column exchange

The bacterial suspension and the ion exchange resin were prepared as described in Section IV. A choice to two types of experiment was then made. The column could be loaded with successive, equal volumes of test suspension until saturation occurred, or, alternatively, a limited volume

of suspension could be recycled through the column until an equilibrium adsorption was attained.

Both saturation and equilibrium column experiments commenced with the draining of any excess water from the prepared resin beds. The bacterial suspension was then slowly introduced into the column and the effluent collected in volumetric flasks or test tubes at a flow rate of approximately 3 ml/min. These effluent fractions were analyzed for both pH and absorbance. The effluent from the equilibrium test was collected and analyzed in a fashion similar to that used in the saturation test with one difference. The total volume of suspension contacting the resin bed, except for a small fraction retained in the column, was collected and analyzed for pH and absorbance. The sample was then repeatedly passed through the column. Intermediate analysis were conducted until no changes were observed in the measured parameters of pH and absorbance.

4. Experimental Observations

A typical set of data is presented in Table F-1 for the exchange of cells of Bacillus subtilis onto Dowex 1 x 8 anion exchange resin contained in a column. In Figure F-1 a rather flat loading curve is described if the absorbance ratio is plotted as a function of the effluent volumn. The flow rate was slightly reduced by filtration effects as the experiment progressed. Saturation was not reached in this experiment.

A more complete loading curve is presented in Figure F-2 for the case of cells of Escherichia coli adsorbed by Dowex 2 x 8 anion exchange resin. This system was buffered with 0.01 M sodium bicarbonate. The adsorbed cells were desorbed by passing a solution 0.137 M sodium hydroxide through the column. The amount of material desorbed from the column determined by comparison of areas is 98.1% of the material initially adsorbed.

TABLE F-I

DATA FOR THE ADSORPTION OF Bacillus subtilis ONTO A COLUMN OF DOWEX 1 x 8

Object: Loading of an anion exchange resin column by Bacillus subtilis, A_o' = 0.176 = 2.8 x 10^7 cells/ml

Column: m = 10 grams Dowex 1 x 8, 20/50 mesh, hydroxide form; flow rate = 5 ml/min, 25 ml fractions

Condition	Effluent fraction	A	A/A_o	pH	Notes
Initial suspension		A_o' = 0.176	1.087	7.22	Washed and filtered suspension diluted 1:5 with 0.1 M NaAc
		.174	1.073	7.92	Concentrated NaOH added at 15 min intervals to determine pH response
		A_o = .162	1.000	9.65	Average pH of prior exchange
Column effluent	1	.015	.093	9.72	pH of initial suspension raised from
	2	.016	.099	9.66	7.22 to 9.72 by release of OH ions
	3	.017	.105	9.74	from resin during exchange
	4	.023	.142	9.67	
	5	.029	.179	9.76	
	6	.036	.253	9.78	
	7	.048	.297	9.74	
	8	.065	.402	9.63	
	9	.073	.451	9.68	
	10	.078	.482	9.66	
	11	.086	.531	9.68	
	12	.096	.593	9.64	
	13	.100	.617	9.62	
	14	.105	.649	9.63	
	15	.111	.685	9.60	
	16	.113	.698	9.65	
	17	.116	.717	9.67	Plateau, some filtration?

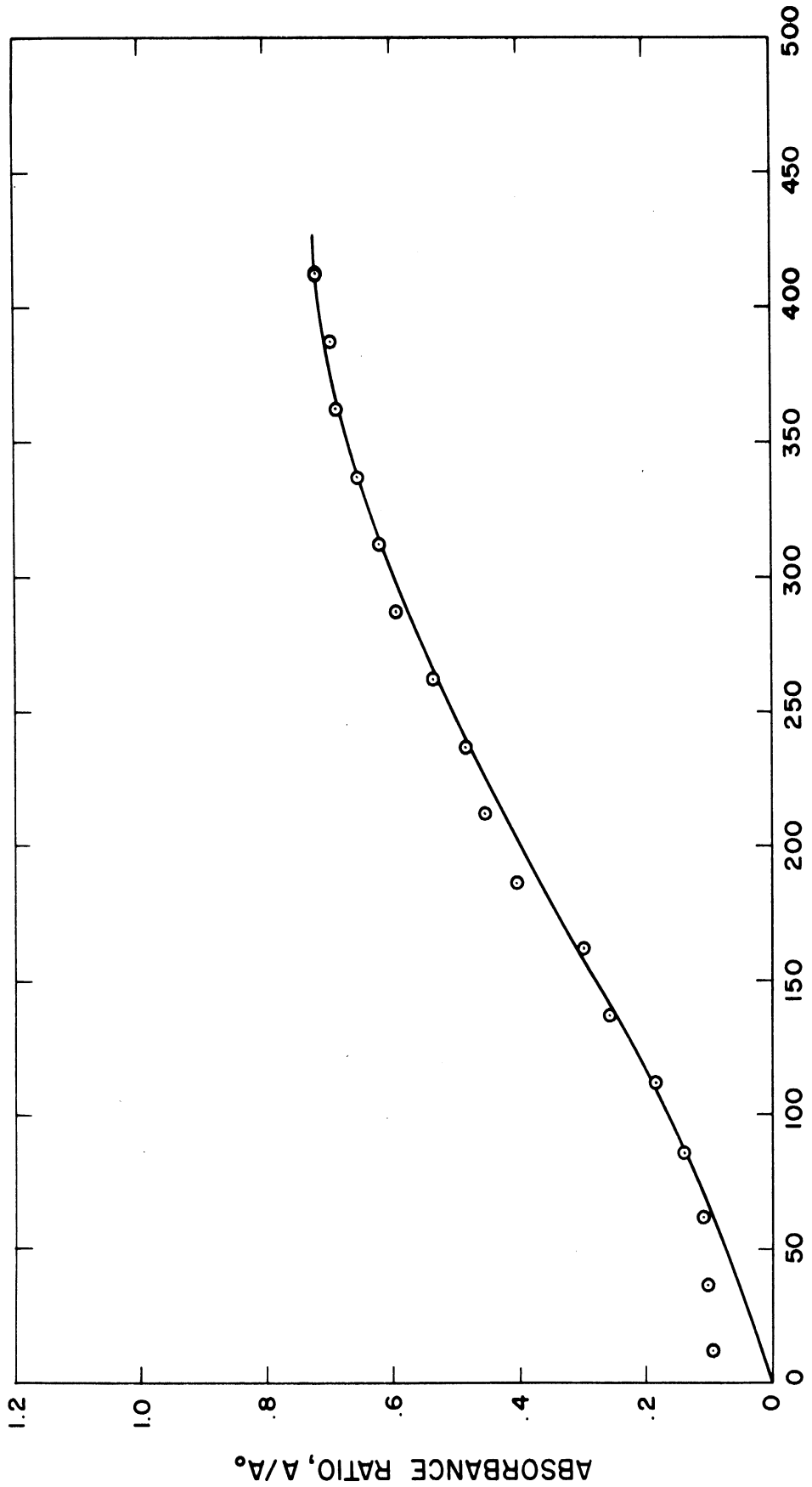


Figure F-1. Adsorption of Bacteria onto an Anion Exchange Resin Contained in a Column (Bacterial species: Bacillus subtilis, A_O = 0.162, pH 3.5; anion exchange resin: Dowex 1 x 8, 20/50 mesh, hydroxide form, 10 grams, 5 ml/min flow rate).

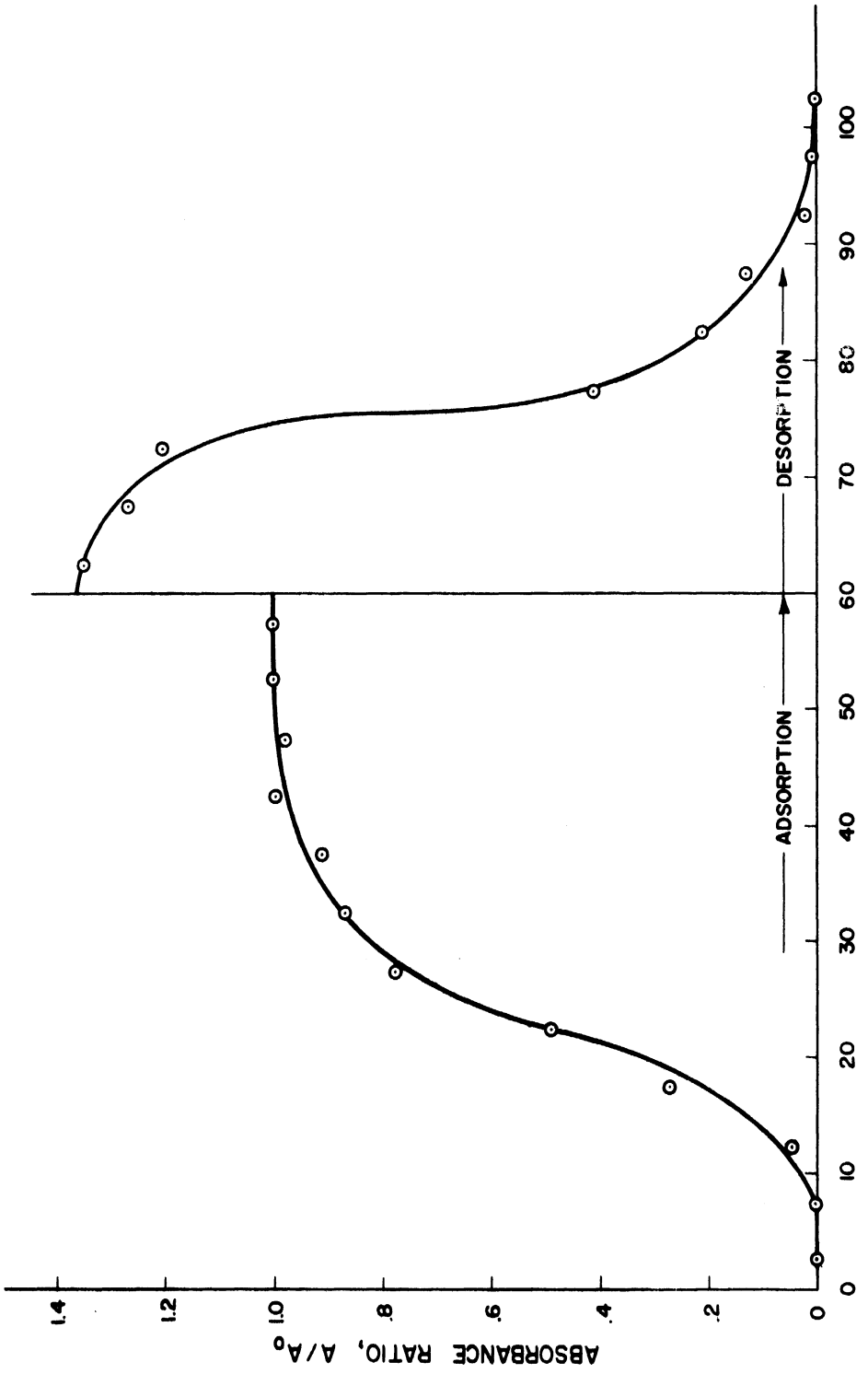


Figure F-2. Sorption of Bacteria by an Anion Exchange Resin Contained in a Column (Bacterial species: Escherichia coli, A_o = 0.600; anion exchange resin: Dowex 2 x 8, 200/400 mesh, chloride form, 1 gram, 2 ml/min flow rate).

The capacity of the anion exchange resin, Dowex 2 x 8, for cells of Escherichia coli is reduced by the presence of a buffer salt. This is illustrated in Figure F-3. The area representing the amount adsorbed for the exchange buffered by 0.01 M sodium bicarbonate is only 54.5% of the corresponding area calculated for the buffered exchange.

The effect of changing the amount of resin in the column upon the quantity of material adsorbed is illustrated in Figure F-4. The cells of Escherichia coli are adsorbed onto particles of Dowex 2 x 8, 50/100 mesh rather than onto the 200/400 mesh particles used in the previous experiments. The quantity of cells that are adsorbed by five grams of the resin is 3.44 times the amount adsorbed by one gram of the same resin. The deviation from the expected factor of five may be due to nonlinear filtration effects, and to a lesser extent upon slight differences in flow rate through the column during the exchange.

The effect of varying the particle size of the resin is illustrated in Figure F-5. Cells of Escherichia coli are adsorbed onto one gram of Dowex 1 x 8, Cl form resin of 50/100, 100/200, and 200/400 mesh size, respectively, contained in three separate columns. The effects of filtration are much in evidenced since the amount adsorbed (area ratios) for the columns containing 50/100, 100/200, and 200/400 mesh particles are, respectively, 1:6.92:7.47. The ideal ratios based on surface areas are 1:2:4 since the average particles diameters are $1:\frac{1}{2}:\frac{1}{4}$.

No significant difference was observed for the adsorption of cells of Escherichia coli by two types of anion exchange resin. The adsorption of the cells of this species in columns containing one gram of Dowex 1 x 8 or Dowex 2 x 8 are shown in Figure F-6. The particle size

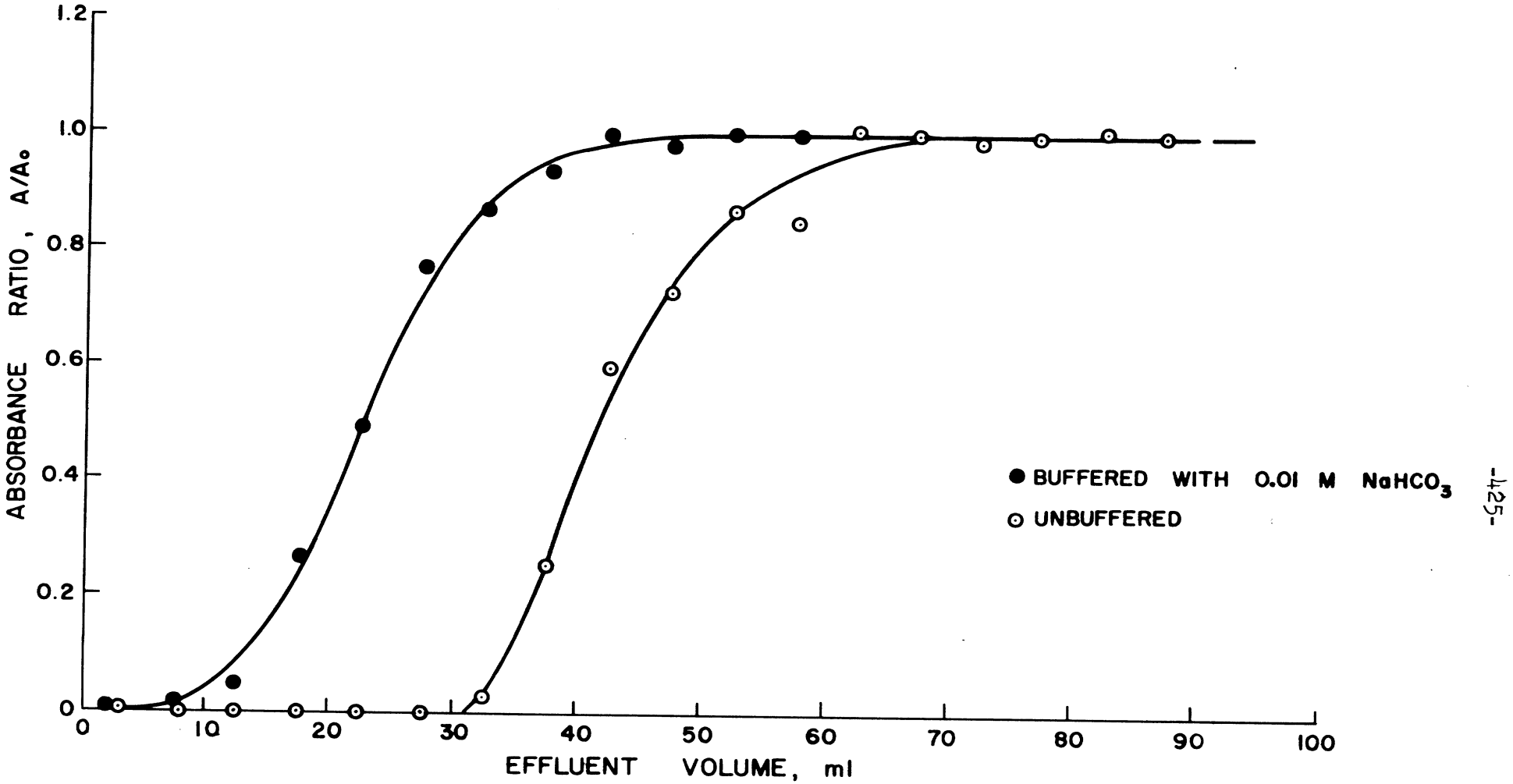


Figure F-3. Adsorption of Bacteria onto an Anion Exchange Resin Contained in a Column as a Function of Buffer Strength (Bacterial species: Escherichia coli, A_o = 0.600; anion exchange resin: Dowex 2 x 8, 200/400 mesh, chloride form, 1 gram, 2 ml/min flow rate).

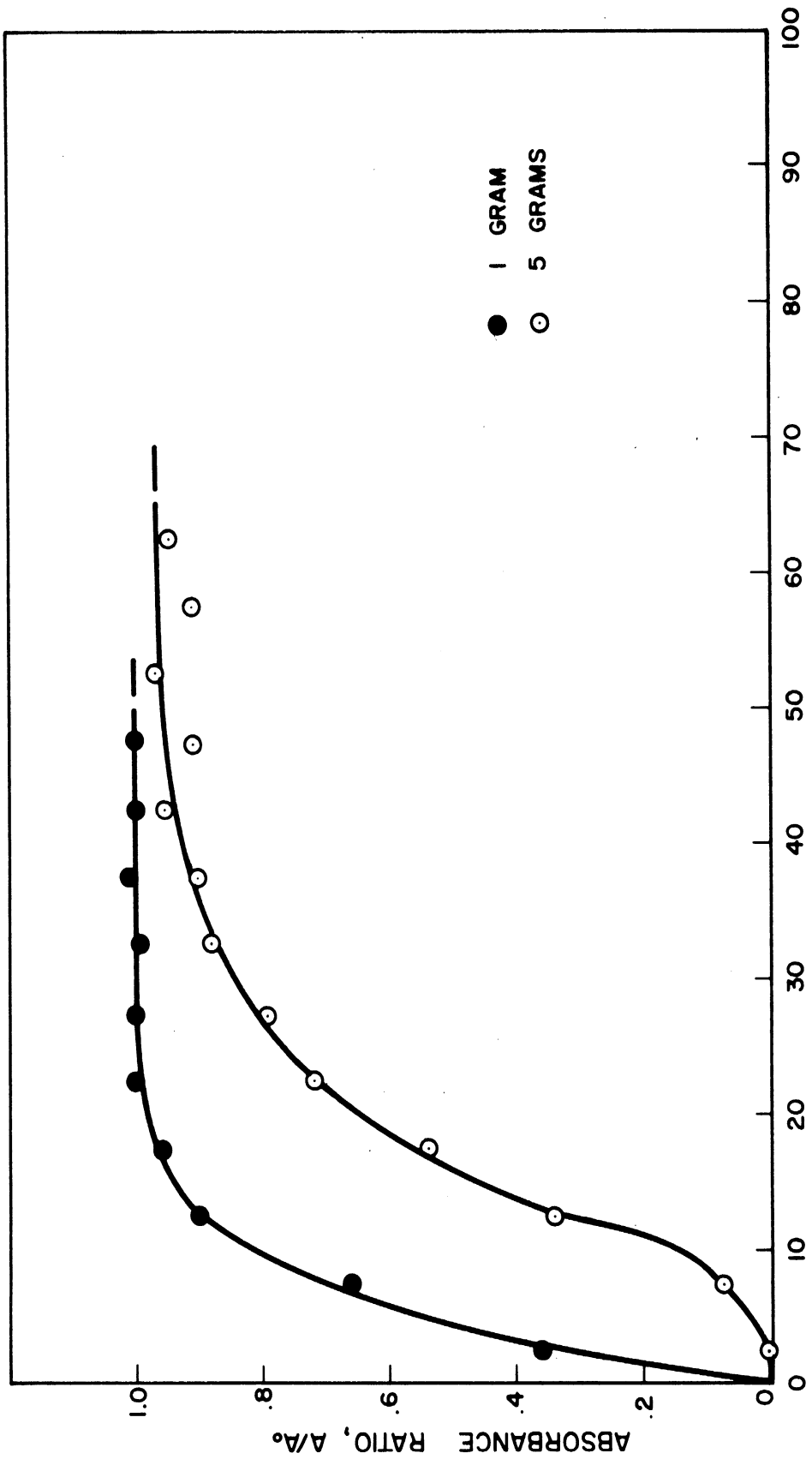


Figure F-4. Adsorption of Bacteria onto an Anion Exchange Resin Contained in a Column as a Function of the Quantity of Resin (Bacterial species: Escherichia coli, A_o = 0.600; anion exchange resin: Dowex 2 x 8, 50/100 mesh, chloride form, 2 ml/min flow rate).

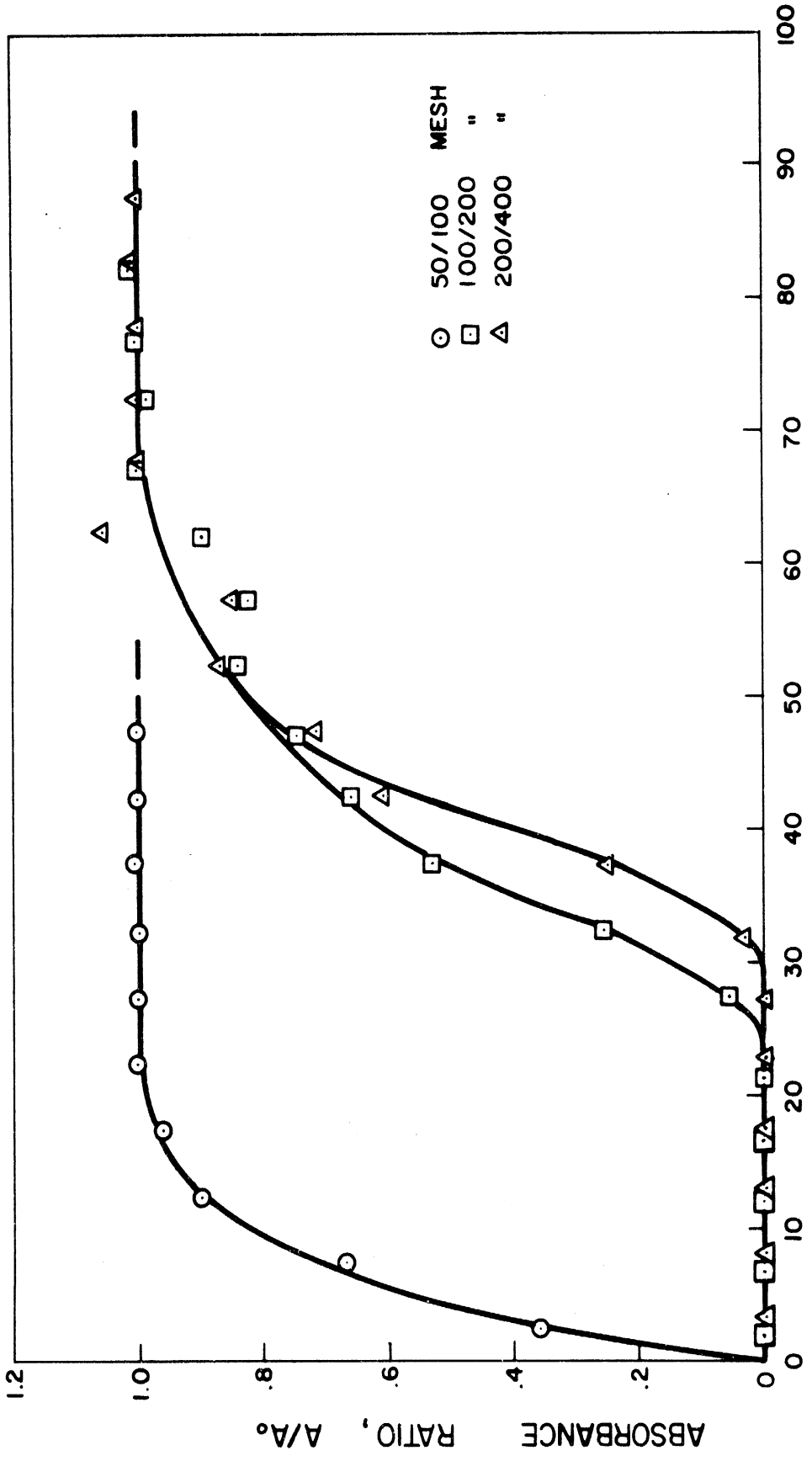


Figure F-5. Adsorption of Bacteria onto an Anion Exchange Resin Contained in a Column as a Function of the Particle Size of the Resin (Bacterial species: Escherichia coli, A_o = 0.600; anion exchange resin: Dowex 2 x 8, chloride form, 1 gram, 2 ml/min flow rate).

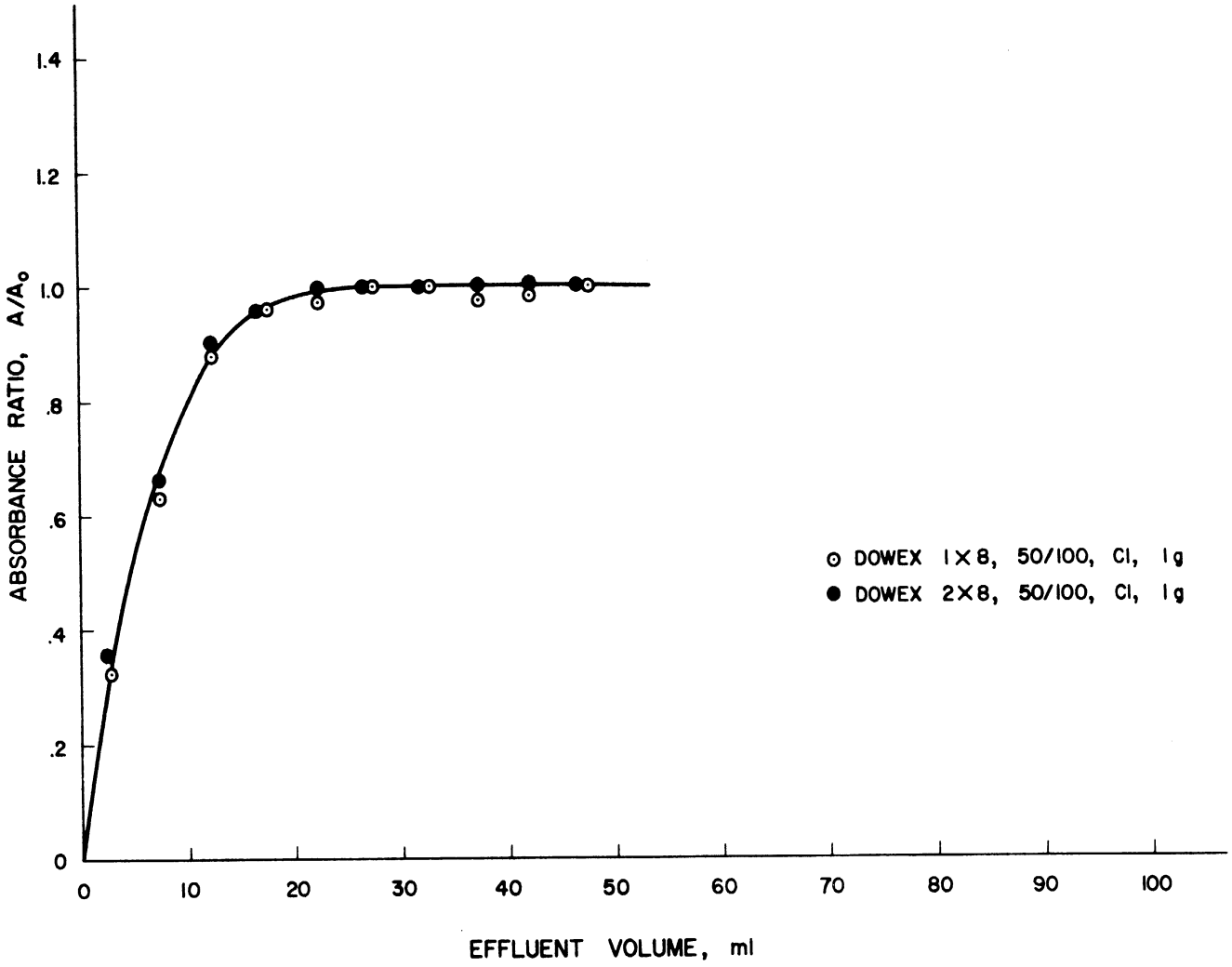


Figure F-6. Adsorption of Bacteria onto an Anion Exchange Resin Contained in a Column as a Function of the Type of Resin (Bacterial species: Escherichia coli, A_o = 0.600; anion exchange resin: Dowex 1 x 8 or 2 x 8, 50/100 mesh, chloride forms, 1 gram, 2 ml/min flow rate).

of both resins is 50/100 mesh; both resins are chloride form. The chemical differences of these resins is discussed in Section II-B-2 of this report.

The separation of mixtures containing several bacterial species by columnar exchange are usually expected to be more efficient than those oftained by the batch techniques discussed in Section V-E of this report. The binary mixture, Escherichia coli (Ec) - Bacillus subtilis (Bs), was previously separated by a batch exchange on the basis of preferential adsorption of the cells of Bs leaving the cells of Ec unadsorbed in the suspension. This separation is shown in Figure 38. Two fractions were obtained containing, respectively, 96.9% Ec and 92.6% Bs. The adsorption of the cells of Escherichia coli onto anion exchange resin contained in a column is more permanent, however, compared to the duration of adsorption observed in the batch exchange (cf. Figures 13 and 25 of Section V). The extent of separation obtained by the column technique may not be as great as that obtained by the batch technique.

In the column study a suspension containing approximately equal proportions of cells of Ec and Bs was passed through a column containing 10 grams of the anion exchange resin, Dowex 1 x 8, 20/50 mesh, acetate form. The absorbance and the pH of the effluent liquid were then measured. The same liquid fraction was recycled through the column and analyses of pH and adsorbance made after each pass until an equilibrium absorbance was reached. A flow rate of 5 ml/min was easily maintained in this column. The pH also remained relatively constant throughout the experiment. The data of this experiment are presented in Table F-II and plotted in Figure F-7. The ratio of the absorbance of the mixture to the absorbance of a pure suspension of component 1 (Ec), A_{1-2}/A_1, was plotted

TABLE F-II

SEPARATION OF Escherichia coli AND Bacillus subtilis BY EXCHANGE ON A COLUMN OF DOWEX 1 x 8

Object: Equilibrium separation of an equal mixture of Escherichia coli (Ec) and Bacillus subtilis (Bs)

Column: 10 grams Dowex 1 x 8, 20/50 mesh, acetate form; flow rate = 5 ml/min, 50 ml mixture recycled

	Pass	A	A_{1-2}/A_0	A_{1-2}/A_1	pH	Notes
Initial suspension Ec		0.600	-	-	7.46	Washed, filtered cells, all G-. resuspended in 0.1 M NaAc
Dilute suspension Ec		.162	0.633	-	7.48	1:5 dilution of initial Ec,
Initial suspension Bs		.600	-	-	7.47	Washed, filtered cells, all G+, resuspended in 0.1 M NaAc
Dilute suspension Bs		.133	.519	-	7.43	1:5 dilution of initial Bs,
Mixture Ec/Bs	0	.256	1.00	1.58	7.46	10 ml initial Ec + 10 ml initial Bs + 30 ml 0.1 M NaAc, G- G+,
	1	.164	.641	1.01	7.31	G- G+
	2	.154	.601	.951	7.28	
	3	.142	.555	.877	7.25	G- G+
	4	.142	.555	.877	7.24	
	5	.142	.555	.877	7.23	G- G+
	6	.140	.547	.865	7.23	

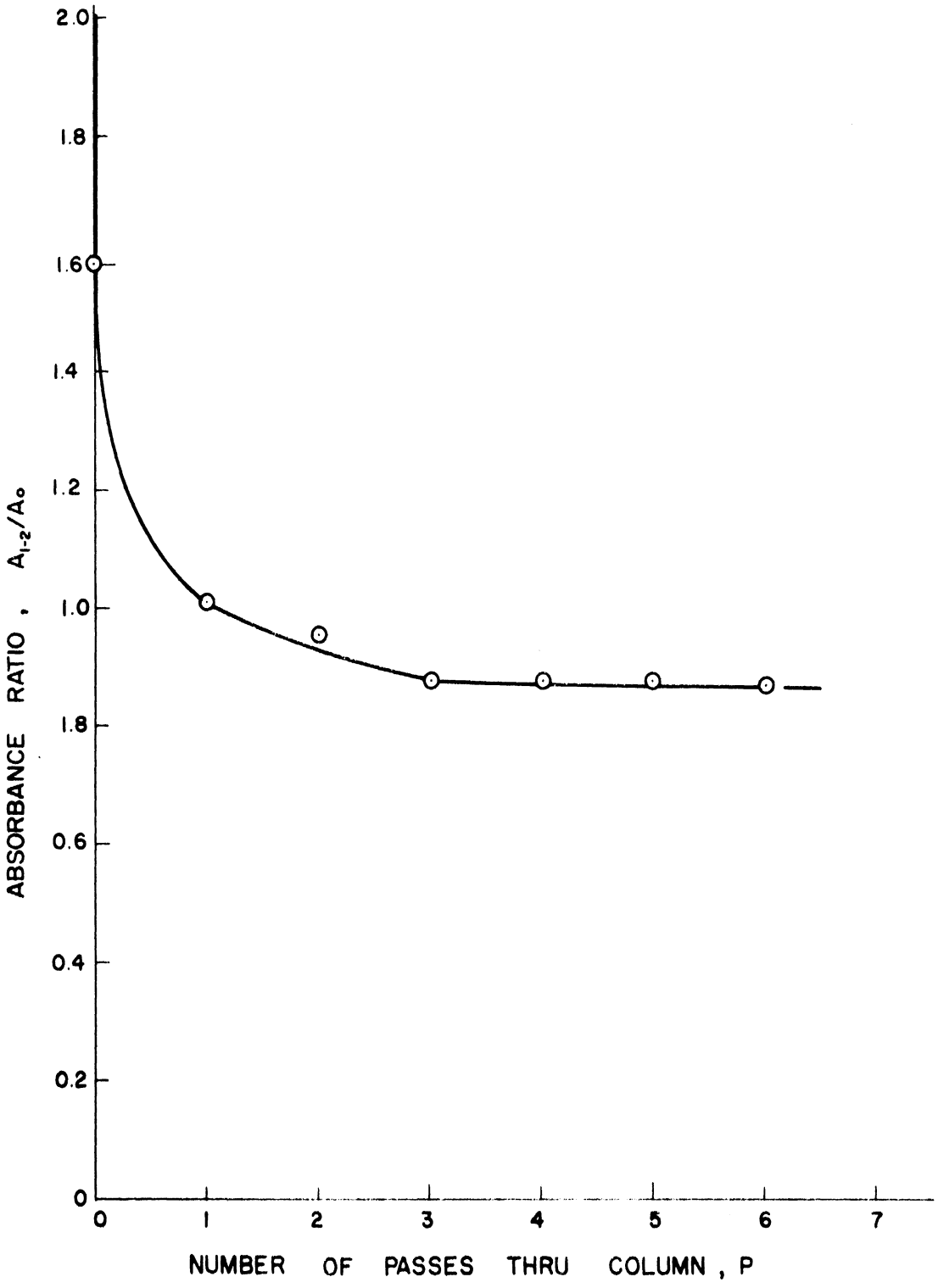


Figure F-7. Resolution of the Binary Mixture: Escherichia coli - Bacillus subtilis (Ec-Bs), by an Anion Exchange Resin Contained in a Column (Bacterial species: Escherichia coli, A_1 = 0.162, Bacillus subtilis, A_2 = 0.133; anion exchange resin: Dowex 1 x 8, 20/50 mesh, acetate form, 10 grams, 5 ml/min flow rate).

as a function of the number of passes through the column. Equilibrium was essentially complete after three passes through the column.

The weakly adsorbing Ec cells were expected to remain in suspension while the strongly adsorbing Bs cells were removed. Some Ec cells were also removed as indicated by the adsorbance ratio ($A_{1-2}/A_1 = 0.865$) falling below unity. Gram stains of the mixture were made initially, and after the first, third, and fifth passes. The ratio of Gram-negative (Ec) to Gram-positive (Bs) cells in the mixture prior to resolution was about equal. As the ratio, A_{1-2}/A_1 ,decreased, the number of Gram-positive cells decreased until the Gram-negative cells were in predominance. A small number of Gram-positive cells remained in suspension even after five passes through the column. A partial separation was thus achieved by the column technique. Further investigations are necessary, however, to establish optimum conditions of flow rate, column size, particle size, ionic form, cell concentration, and pH.

APPENDIX G

NOMENCLATURE

Capitals

A	Absorbance
A'	Adjusted absorbance
A_o	Initial absorbance at adjusted pH of adsorption or desorption
A'_o	Initial absorbance at neutral pH correlated with total cell number
A_i^-	Unspecified anionic species in solution or suspension
$B^{\pm}$	Dipolar bacterial cell in suspension
C_j^+	Unspecified cationic species in solution or suspension
D	Diffusion coefficient, cm^2/sec
I	Transmitted radiant power
I_o	Incident radiant power
J	Mass flux normal to a spherical boundary, Equation (III-9)
K	Ratio of scattered to incident light, Equation (A-9)
K'	Distribution coefficient
M_o	Amount of diffusing substance initially in liquid phase
M_t	Amount of substance diffused after time t
M_∞	Amount of substance diffused after infinite time
N	Number of adsorbing particles per unit volume of suspension, $\#/cm^3$
N'	Number of adsorbing particules per unit weight of adsorbent, #/gram
N_k^o	Uncharged species in solution or in suspension
P	Number of passes through column

$P(\theta)$ Angular correction factor, Equation (A-12)

R Radius of adsorbing particle (ion exchange resin), μ

R' Modified radius, Equation (III-15)

R_I "Radius of influence" = $R + \delta$

S Surface area of adsorbing particle, cm^2

T Transmittance, Equation (A-1)

T' Temperature

U Probability of non-adsorption, Equation (III-11b)

V Volume of suspension, cm^3

W Anhydrous mass of adsorbable particle, Equation (A-10b)

X Probability of adsorption, Equation (III-11a)

Lower case

a Adsorptivity

a' Minor axis of ellipse

b Optical path length, Equation (A-1)

b' Major axis of ellipse

c Concentration of diffusing substance in liquid

c' Concentration of diffusing substance in solid

d Diameter of adsorbable particle (bacterial cell), μ

h Index quantity

i Index quantity

j Mass flux

k Rate constant for t term, min^{-1}

k' Rate constant for $\sqrt{t}$ term, $min^{-1/2}$

m Mass of adsorbing particles, grams

n Concentration of suspended bacterial cells, cells/cm^3

n_o Initial concentration of suspended bacterial cells, cells/cm^3

p Parametric quantity, Equation (A-12)

q Capacity of ion exchange resin for bacterial cells, cells/gram

r Radial distance from center of adsorbing particle

s_n Roots of Equation (III-20)

t Adsorption time, min.

t' Desorption time for first component, min.

t'' Desorption time for second component, min.

v Volume of effluent from column, cm^3

$\bar{v}$ Mass-average velocity

w_i Mass fraction

x Parametric quantity, Equation (A-12)

y Dummy variable, Equation (III-16)

z Reduced radius = $\pi d/\lambda$, Equation (A-10a)

Greek letters

α Probability of adsorption, Equation (III-13)

$\alpha_0, \ldots \alpha_5$ Correlation constants, Appendix A

α^* Volume ratio, liquid/solid, Equation (III-18)

β Reduced radius, Equation (III-12)

$\beta_0, \ldots \beta_3$ Correlation constants, Appendix A

γ Saturation ratio, Equation (III-37)

γ_1 Correlation constant = α_1/β_1 , Equation (A-4)

δ Film thickness

ϵ	Void fraction of packed resin
ξ	Parametric quantity, Equation (III-7b)
η	Index of refraction of suspended particule
η_o	Index of refraction of suspending medium
θ	Angle of scattered light
κ	Specific reaction rate constant, Equation (III-13)
λ	Wavelength of light, mμ
λ'	Wavelength of light <u>in vacuo</u>, mμ
μ	Viscosity of liquid, cp
ν	Reaction rate constant, Equation (III-5-D)
π	Constant = 3.14159...
ρ'	Ratio, mean-squared/mean jump lengths, Equation (III-13)
ρ_1	Parametric quantity, Equation (A-14)
ρ	Density of liquid, Equation (III-1)
σ	Standard deviation
τ	Reduced time variable, Equation (III-19)
ϕ	Reduced index of refraction, Equation (A-10a) = η/η_o
ψ_1, ψ_2	Parametric quantities, Equations (III-21a) and (III-21b)
Ω	Agitation rate, rpm

Subscripts

i	Index quantity
j	Index quantity
k	Index quantity
1	Species one

2 Species two

$1\text{-}2$ Species one and two

a Adsorption

d Desorption

k Derived from rate constant

k' Derived from rate constant

n Index quantity

o Initial quantity

∞ Infinite quantity

Superscripts

(g) Forced diffusion

(p) Pressure diffusion

(T') Thermal diffusion

(x) Concentration diffusion

$—$ Solid phase

$',''$ Desorption when associated with A , or with t or τ

Mathematical operations

∇ "Del" or "nabla" operator

Δ Difference operator

$erf(y)$ Error function of $y = erf(y) = \frac{2}{\sqrt{\pi}} \int_0^y exp(-u^2)\,du$

$erfc(y)$ Complimentary error function of $y = 1 - erf(y)$

$exp(y)$ Exponental function of y

$eerfc(y)$ Combined exponential-complementary error function of y
$= exp(y^2) \cdot erfc(y)$

$f(y)$ Function of y

$ln(y)$ Logarithm of y to the base $e = 2.71828...$

$log(y)$ Logarithm of y to the base ten

Miscellaneous symbols

k	Boltzmann's constant = 1.38×10^{-16} erg/K.
pH	Negative base ten logarithm of $[H^+]$
pI	Isoelectric point
pK'	Negative base ten logarithm of dissociation constant
$[H^+]$	Hydrogen ion concentration
Bc	Bacillus cereus
Bs	Bacillus subtilis
Ec	Escherichia coli
Po	Pseudomonas ovalis
Pv	Proteus vulgaris
Sa	Staphlococcus aureus
R	Radical representing the complex surface structure of a bacterial cell
R'	Radical representing the large polymeric structure of an anion exchange resin
R"	Radical representing the large polymeric structure of a cation exchange resin
ν	Chemical reaction term, Equation (III-1)

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