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Technical Report

THE REACTION OF LUMINOUS BACTERIA TO MICROWAVE RADIATION EXPOSURES IN THE FREQUENCY RANGE OF 2608.7 TO 3082.3 MEGACYCLES

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ABSTRACT

Experimental results from previous investigations of biological effects resulting from microwave exposure cannot always be fully explained by purely thermal arguments. Temperature measurements during exposure indicated the possible existence of a non-thermal effect. Past attempts to demonstrate unequivocally the existence of a biologically significant, non-thermal effect have failed. The danger through potential exposure to a large number of people makes it imperative to resolve the question of a non-thermal effect. This is the purpose of this investigation.

Suspensions of luminous bacteria, chosen for their short thermal time constant and the convenience of luminescence as an index of biological effect, were exposed under known thermal conditions to continuous wave, microwave radiation at frequencies of between 2608.7 to 3082.3 Mc. Doses of 1.7 to 6.7 x 10^9 ergs/gm were administered to the suspensions circulating through a waveguide water load at power levels of 8.3 to 16.7 watts. Total exposures were equivalent to power densities of about 490 to 990 milliwatts/cm^2 applied for 43 to 86 minutes on a 0.5 duty cycle.

The small differences observed between exposed and control suspensions were found to be independent of frequency and were eventually shown to be due to a system contaminant. Results show that no biologically significant, non-thermal effect resulting from exposure to microwaves in the frequency range of 2608.7 to 3082.3 Mc occurs in this biological system.

CHAPTER I. INTRODUCTION

1.1. The Problem

Little is known concerning the biological effects of microwaves. They are capable of cooking tissue and have produced cataracts, thermal death, and testicular degeneration in laboratory animals. Those who have studied the biological effects of microwave exposure seem to feel that thermal effects, though they may prove to be the major, acute exposure problem, do not constitute a complete explanation of effects observed in laboratory animals to date. It has been suggested that an effect more subtle than the thermal effect may contribute significantly to the total exposure of those who are chronically exposed. The nature of these latter effects is not postulated here in any detail; the purpose of this investigation is to prove or disprove only their existence in the frequency range studied.

1.2. Uses of Microwaves

Applications of energy in the microwave region of the electromagnetic spectrum have served many purposes. These applications include communications systems, aircraft guidance systems, police radar, electronic telescopes, medical diathermy units, food-preparation units, early warning and search radar, television, professional and amateur radio, and special sets

in operation for research, development, and instruction.

The diversity of these applications indicates that a large number of individuals are potentially exposed. It is, therefore, important to determine whether non-thermal effects exist; if they do, they may then be studied further and considered appropriately with respect to other environmental hazards.

1.3. The Microwave Spectrum

Montgomery[1] identifies the word "microwave" with the short-wavelength region of the radio spectrum adjacent to the far infra-red region; the U.S. Army[2] defines microwaves as radio waves having frequencies greater than 1000 Mc/sec. Ginzton[3] prefers that the term "microwave" be defined as "that portion of the electromagnetic spectrum at which it is possible to make the laboratory equipment approximately equal in size to the operating wavelength" and implies a lower frequency limit of 300 Mc. Young and Jones[4] agree with this lower limit of 300 Mc. But Moreno[5] places the lower boundary of the spectrum "somewhere" between 300 and 3000 Mc and the upper boundary in the millimeter wavelength region where radio and infra-red techniques overlap. Practical limitations of size place the high-frequency limit of the microwave portion of the electromagnetic spectrum at about 100 KMc.

Although the microwave region of the electromagnetic spectrum is thus rather arbitrarily and vaguely defined, the consensus is that it is the por-

tion of the spectrum in which circuit components have dimensions that are an appreciable fraction of or greater than one wavelength. However, investigators of the biological effects of microwaves consider frequencies as low as 1 Mc a part of the microwave region. Engineers would very likely argue that frequencies this low would be better termed "radio waves."

The term "radio" is more general than "microwave" or "radar" but is usually restricted to the art of communications by electromagnetic radiation.[4] "Radio" may, therefore, include portions of the microwave spectrum.

Some may consider radar and microwaves as synonomous and to some degree they are; however, radar is a colloquialism coined from the descriptive phrase "radio detection and ranging."[4] The term may be applied to any portion of the radio or microwave region. This may be part of the reason for the vagueness of the definition of the microwave region. Though most radar equipment operates in the microwave region, the term "radar" includes "radio" in its definition. However, the distinction between radar and radio or microwaves is clear. Radar pertains to a specific use of electromagnetic waves, but the term "microwave" is an arbitrary designation.

There is another practical limitation to the microwave region if its application is to be in communications or radar. For frequencies above 15.8 KMc serious absorption occurs in moist atmosphere as a result of a molecular transition in the water vapor.[4] Free water undergoes dielectric dispersion at 20 KMc.[6] Molecular resonances may cause serious absorption at higher frequencies. A number of absorption bands are present in the

region between 30 KMc and 300 KMc.

Figure 1 will assist the reader to visualize the range of the microwave spectrum and to identify that portion of the spectrum covered by this investigation. The figure is also helpful in placing this work in the proper perspective with respect to a few previously observed biological responses to microwaves.

1.4. Evidence of Non-Thermal Effects

1.4.1. INTRODUCTION

Although the concept of purely thermal effects resulting from exposure to microwaves has been popular among investigators, few have stated that the effect is entirely thermal. No experiment reviewed by the author has shown conclusively the absence of a non-thermal effect. In fact, investigators have suggested, and have provided some evidence, that the biological effects produced by microwaves may be other than purely thermal. Evidence has accumulated to the point where one of the staunchest critics of those who suggested the presence of non-thermal now believes that non-thermal effects may occur. Such effects may result from either breakage of molecular bonds in high field strengths or orientation effects resulting from alignment of molecules.[6] The latter effect is referred to as "pearl-chain" formation.

Criticism of the results of the following investigations stems from the investigators' failure to consider localized, selective heating effects

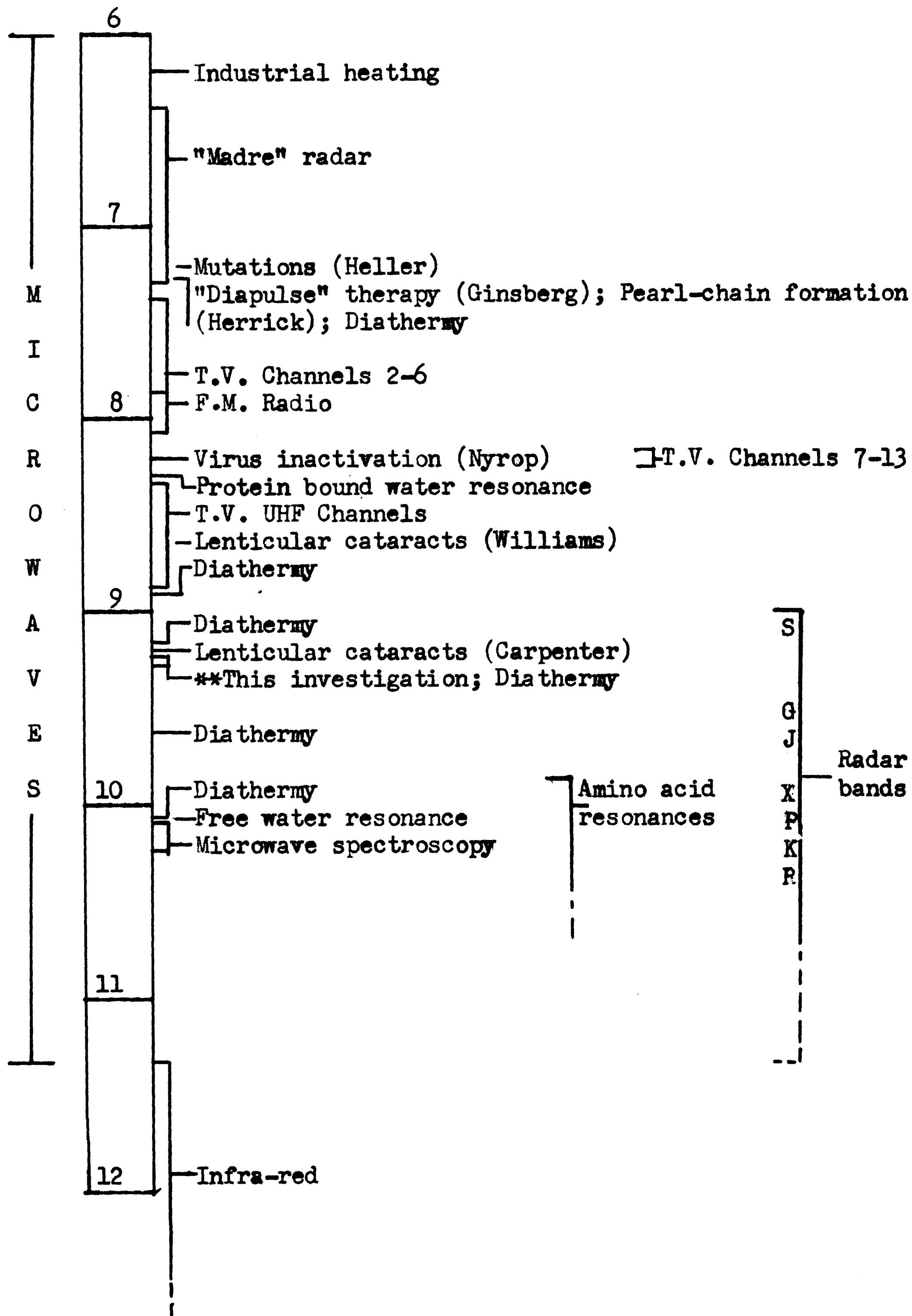


Fig. 1. The microwave spectrum.

and heat distribution throughout the biological system studied. These considerations have been neglected probably because the determination of the temperature distribution in the biological systems used to date (mammals in almost all cases suggesting the existence of non-thermal effects) would be extremely difficult if not impossible without introducing very undesirable, additional variables into the experiments.

In studies with microorganisms, investigators have used field strengths of unknown magnitude or have estimated exposures from the equipment specifications. It develops that the relation between exposure and microwave equipment specifications may vary by orders of magnitude. In any case the temperature to which the microorganisms may have been raised is unknown so it is impossible to know whether non-thermal effects are the cause of the results discussed in this section; however, the work, discussed in Section 1.4.4, presents the strongest evidence for the existence of a non-thermal effect.

1.4.2. THE SAME EFFECT AT DIFFERENT TEMPERATURES

Infra-red and microwave irradiation can produce the same histopathological effect in the testes of albino rats, but a lower temperature is observed to produce the effect when microwave irradiation is used.[7] Temperatures in this experiment were measured with a thermocouple needle placed in the center of one testis irradiated with 2.5-KMc microwaves. The observed differences may have resulted from different temperature distributions within the testis produced by the two sources of radiation. If it

were argued that the dielectric properties of the testis are similar to fat, the inner layers would reach higher temperatures than the outer layers, and the measured temperature would represent a maximum. Localized heating of the temperature-measuring device itself[8] could not represent a critical error since the temperature required to produce the effect by microwave irradiation was lower than that required to produce the same effect by infra-red irradiation. Localized heating about the temperature-measuring device would have caused higher observed temperatures for microwave exposure than for infra-red exposure. Localized heating in this case would strengthen the argument for the existence of a non-thermal effect. However, if the testis were considered as muscle tissue rather than fatty tissue the relative heating in the tissue would decrease with increased depth. The result would be observed lower temperatures for microwave heating.[9]

Keplinger[10] has found that higher rectal temperatures are observed to produce death in rats irradiated with infra-red heat lamps than in rats exposed to 24-KMc microwaves. Observations of this type are relatively common in the literature and it is difficult to decide whether they are evidence for the existence of a non-thermal effect. They may be simply the result of invalid measurements of animal body temperature Of particular interest, however, is the fact that Keplinger observed temperature recovery in rats exposed to infra-red rays, but no such recovery in rats exposed to microwaves.

1.4.3. VIRUS INACTIVATION AND INHIBITORY EFFECTS IN NEUROSPORA

Nyrop[11] has found that virus inactivated by heat can be used as a vaccine, but a virus inactivated with microwaves of 200-Mc frequency has no vaccinating effect. He attributes this difference to different actions of the two radiations upon the virus molecule.

Carpenter[12] has reported that conidiophores (fruiting bodies) of the breadmold Neurospora crassa, in lusteroid test tubes exposed to 2.45-Kmc waves, germinate and grow when subcultured on agar slants but do not form conidiophores. Controls handled similarly but heated in an incubator not only exhibited normal growth but also developed conidiophores. This is an example of interference with cell differentiation without inhibition of growth. Air temperature, not cell temperature, histories were duplicated in this experiment.

1.4.4. INDUCED MUTATIONS

Exposure of living cells to fields of hundreds to thousands of volts per centimeter at frequencies ranging from 1 to 100 Mc and pulse rates of 30 to 10,000 per second result in chromosomal aberrations which appear to be frequency-specific according to Heller.[13] Chromosomal bridging and linear shortening in metaphase were observed in garlic root tip cells 14 to 24 hours after a 5-minute exposure to 13.5- and 22-Mc microwave radiation. Even though the field strengths in the exposure chamber were not well known, Heller estimates that the temperature changes associated with the observed phenomena were of the order of 0.001°C/gm/min.

1.4.5. PEARL-CHAIN FORMATION

Herrick states that "The cellular constituents of blood and lymph assume a pearl-chain formation *in vitro* when alternating currents are applied properly."[14] She found pearl-chain formations in milk and blood provided the power was low enough to cause no heating effect. The effect is demonstrable by passing alternating current through water containing randomly distributed oil droplets. The current aligns the droplets so that the water appears to be permeated by many oversized neutron tracks. No conjectures have been advanced about what effect one might expect pearl-chain formation to have upon a biological system.

The word "properly" in the quotation above is important. The effect is obtained only at certain low power levels not specified by Herrick. However, Herrick indicates that the machine used was one designed by Dr. Ginsberg. It can be deduced that these power levels were less than the maximum peak power output (1333 watts) from a special 27-Mc, therapeutic, "Diapulse machine" used by Dr. Ginsberg of New York.[15,16]

1.4.6. CATARACT FORMATION

Williams[17] has shown that exposures to microwaves of 600-Mc frequency, which could not have raised the lens of the eye to a damaging temperature for any single exposure, have caused lens cataracts in anesthetized rabbits. This suggests that the changes observed in the lens were not the result of simple heating.

Carpenter[18] exposed the eyes of rabbits and reported that the threshold

time for opacity formation coincides with the time of exposure necessary to elevate the vitreous temperature to 50°C. This at first glance suggests a thermal effect for acute exposures of 0.12 to 0.40 watts/sq cm for intervals of 5- to 55-minutes duration. However, he emphasizes that "opacities may develop as a cumulative effect when the eye is repeatedly exposed to amounts of microwave radiation which singly exert no harmful effect." Furthermore, experiments with 2.45-KMc, pulsed radiation "strongly suggest that the cataractogenic effect of microwave radiation at this frequency is not primarily a thermal effect." His basis for this statement was the observation that exposures to 0.28 w/sq cm peak power, pulsed on a 0.5 duty cycle for 20 minutes, did produce opacities, but exposure to 0.14 w/sq cm continuous power (c.w.) for 20 minutes did not produce opacities. This observation may or may not be indicative of a non-thermal effect. It may be that a localized high temperature of short duration was associated with the peak power. It is also possible that average temperatures were different even though average powers were the same in either case. This would occur if the rate of cooling were slower than the rate of heating. Furthermore, the heating equivalence of two different power densities assumes a linear relation between power density and temperature. But his experiments also indicate that opacities occur at lower temperatures for 0.14 w/sq cm, c.w. and 0.14 w/sq cm average power than for 0.28 w/sq cm c.w. This latter observation is consistent with what has been said concerning pearl-chain formation, and suggests the possibility of a non-thermal effect.

1.5. NON-THERMAL VS. THERMAL EFFECTS

The absorption of microwave energy in living tissue can elevate the tissue temperature to the point where injury is produced. Such injury is properly described as a thermal effect. The question this study attempts to answer is whether microwave energy absorbed in amounts, or at rates, which produce no injurious temperature elevation can produce biological effects.

Heat is a form of energy. The mechanical equivalent of heat, 4.185 joules per calorie, and the law of conservation of energy allow one to express energy of any form as an equivalent amount of heat energy. This equivalence applies, for example, to electrical energy where the product of volts, amperes, and seconds represents the electrical energy in joules.[19] This does not mean, however, that energy and heat are identical in a given system.

Energy absorption may result in several reactions, among which are:

1. molecular and/or atomic excitation;
2. molecular and/or atomic polarization;
3. random motion of molecules and/or atoms;
4. directed motion of molecules and/or atoms;
5. molecular and/or atomic orientation.

These reactions are not necessarily mutually exclusive; however, the response of the absorbing system to the absorbed energy may be affected more by the physical nature of the absorption process than by the thermal energy which

accompanies it. An example of this is the interaction of X-rays and living tissue. A dose of X-rays, lethal to human beings, causes the body temperature to rise only a few thousandths of a degree centigrade. This temperature rise is biologically insignificant, yet the exposed individual may die in a relatively short period of time as a result of the exposure. This response is the result of absorption reactions other than number 3 in the list above. It is an example of a non-thermal effect. The possibility exists that an interaction analogous to this occurs as a result of exposure to microwaves.

A thermal effect is one attributable to heat. The effect of number 3 in a given volume of material is called heat energy. The calorie, or quantity of heat, is measured in terms of temperature change in a given volume of material. Temperature is an average measurement that cannot distinguish the source of heat. It is a measure of the average energy of the random motion per particle of the molecules in the absorbing system. The point of reference is important here, for one could theoretically consider heat energy localized in a volume with dimensions much smaller than those of the over-all system being considered, as did Dessaur in his heat-point theory.[20] To consider point sources of heat resulting from concentration of energy in a small volume is not realistic since energy absorption occurs throughout at least several atomic layers of an absorber. The reaction of a system to "heat" localized in volumes which are small compared to the total system cannot be termed a thermal effect.

The rate of absorption of energy in a dynamic system is also important.

If the heat loss from a system is equal to the rate of energy absorption, no temperature rise will be observed. Effects accompanying temperature changes which alone would produce no change in a system must be termed non-thermal effects. Similarly, if two radiations are absorbed at an equal rate and produce the same temperatures in matched systems, differences in the responses of the two systems must be the result of a non-thermal effect.

Susskind and Vogelhut[21] refer to thermal effects as indirect effects which result from degradation of energy to a form that increases the random motions of molecules of a system. They refer to non-thermal effects as direct effects which are conditions of resonance or discrete changes in energy levels within molecules. This definition is consistent with the foregoing arguments.

Non-thermal effects, then, are those which cannot be attributed to temperature and result from direct, discrete structural reorganization or changes within or between molecules. Non-thermal effects, like thermal effects may be expected to be dependent upon exposure rate.

CHAPTER II. BIOLOGICAL SYSTEM

2.1. Choice of Organism

The biological organism chosen for this work is the bacterium *Photobacterium fischeri* (*Achromobacter fischeri*), the general properties of which are well known.[22] The organism is non-pathogenic to white rats and has never infected man even though ingested.[22,23] The organism, therefore, may be handled without fear of serious consequences. The luminescence of this organism provides a convenient index of biological effect produced by exposure to a foreign agent with minimum disruption of the organism. That the luminescence may be used as an index of biological effect produced by ionizing radiations has been shown by Whipple.[24] His observations make it possible to determine microwave exposure doses required to produce a significant, non-thermal biological response if such an effect exists.

Whipple[24] has provided a thorough and convenient summary of the environmental factors which need to be taken into consideration in using *P. fischeri* as a radiobiological test organism. Except as noted below, it has been assumed that optimum conditions found for bacteria he employed, which were originally from the same source as the bacteria used in this investigation, apply.

Merchant[25] maintains The University of Michigan culture of *P. fischeri*,

originally obtained from F. H. Johnson of Princeton University in 1957, on media having the following composition.

Difco - Bacto nutrient agar	23 gm
Glycerol	5 ml
Sodium Chloride	30 gm
Calcium Carbonate	5 gm
Distilled Water	1000 ml

This medium differs only slightly from Whipple's[24] and was used to grow the cells in this experiment. The principal advantage of this medium compared to Whipple's is the absence of ground fish as an ingredient.

To determine whether a change resulting from exposure to microwaves is indeed a non-thermal effect, it is necessary to know the temperature of the organism being exposed and the relation of this temperature to the biological response exhibited by the organism. Heretofore, microwave exposures have been performed with large laboratory animals such as dogs, rabbits, rats, and mice. It is virtually impossible either to measure or to control temperatures accurately in animals of this size. In this respect the bacteria are ideal; if one assumes the worst conditions concerning heat transmission in suspensions of the bacteria, it is shown below that thermal time constants of the order of 10^{-5} seconds are involved.

2.1.1. THERMAL TIME CONSTANT OF SUSPENDED CELLS

Kreezer and Kreezer[26] provide a method of determining (a) the rate of heat transfer through the cell membrane and (b) a thermal time constant

by computing the ratio of thermal capacitance to thermal conductance. The thermal capacitance of the cell may be determined from the mass of the bacterial cell and its specific heat. The area, thickness, and thermal conductivity of the membrane surrounding the cell permit an estimate of the thermal conductance or rate of transmission of heat through the membrane. The thermal time constant of the bacterial cell will then be given by the ratio of thermal capacitance to thermal conductance.

Individual cells of P. fischeri are short, thick rods, slightly curved,[22,27] with rounded ends and dimensions of approximately 0.8 by 2.5μ. The cells are, therefore, more cylindrical than spherical.

In the case of radial conduction of heat through cylindrical shells, the thermal conductance or heat leaving the cylindrical surface under equilibrium conditions is given by:[19]

$$\frac{dQ}{dt} = \frac{2 \pi k L (T_1 - T_2)}{\ln (r_2/r_1)} \qquad (1)$$

where

$\frac{dQ}{dt}$ = calories/sec

L = cylinder length (cm)

T_1 = inside temperature (°C)

T_2 = outside temperature (°C)

r_2 = outside radius (cm)

r_1 = inside radius (cm)

k = thermal conductivity in cal/(cm^2)(sec)(°C/cm)

The temperature increment in the bacterial suspension as it passes through the wave guide at 20 watts of power with a flow rate of 40 cc/min is about 7.3°C if it is assumed that the specific heat and the density of the suspension are 1.0 and that all the power is absorbed. If it is further assumed that either the suspending solution alone is heated or the bacteria alone are heated, then $T_1 - T_2$ in Eq. (1) is 7.3°C.

The best heat-insulating material listed among either organic or inorganic materials in the Handbook of Chemistry and Physics[28] is felted cotton with a thermal conductivity of 3.3×10^{-5}. It is assumed that this is the conductivity of the bacterial membrane.

The following, then, are considered to be conservative values which may be used in Eq. (1) for the bacterium *P. fischeri*.

$$k = 3.3 \times 10^{-5}\ (\text{cal})/(\text{cm}^2)(\text{sec})(°\text{C}/\text{cm})$$

$$L = 2.5 \times 10^{-4}\ \text{cm}$$

$$T_1 - T_2 = 7.3°\text{C}$$

$$r_2 = 0.8 \times 10^{-4}\ \text{cm}$$

$$r_1 = 0.4 \times 10^{-4}\ \text{cm}$$

Substitution of these values into Eq. (1) yields a rate of heat transfer through the cell wall of 5.5×10^{-7} cal/sec.

A cylindrical cell of the size described above will occupy a volume of 5×10^{-12} cm^3. The density of bacterial cells is estimated to range from 1.07 to 1.19 gm/cm^3.[29] If the specific heat as well as the density of the cell are assumed to be 1.0, then the cell will have a mass of

5×10^{-12} gm and a heat capacity of 5×10^{-12} cal/°C. For T_1-T_2 = 7.3°C this becomes 3.7×10^{-11} calories.

The time required for a suspended cell to attain 63% of temperature equilibrium with the suspending medium is, then, given by the ratio (3.7×10^{-11} cal) over (5.5×10^{-7} cal/sec) or 6.5×10^{-5} sec. Hence, in the exposure system the temperature of the cells is the same as the temperature of the suspension.

2.2. Bioluminescence

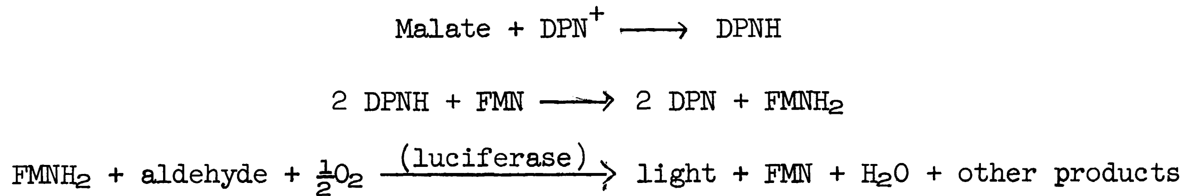
2.2.1. MECHANISM OF BACTERIAL LUMINESCENCE

Although the exact mechanism of light production in P. fischeri is still unknown, numerous theories have been postulated. The reaction is believed to be similar to chemiluminescent reactions in which the light comes from either an excitation process or the actual decomposition of a substrate molecule or complex rather than from a catalyst.[30]

It has been common to refer to the compounds which provide the energy for the light as "luciferin." The compound(s) which catalyze the light emitting reaction(s) have been termed "luciferase."[30]

The identity of luciferase in bacteria is unknown. Evidence suggests that at least one compound in bacterial luciferin is probably reduced flavin mononucleotide ($FMNH_2$). It is believed that DPN acts as a hydrogen carrier to FMN and that the oxidation of $FMNH_2$ in the presence of an aldehyde is the light-emitting reaction.[30,31]

The following simplified scheme of reactions has been postulated by McElroy and Strehler[31,32] to explain the luminescence observed in bacterial extracts.



$$\text{Malate} + \text{DPN}^{+} \longrightarrow \text{DPNH}$$

$$2\ \text{DPNH} + \text{FMN} \longrightarrow 2\ \text{DPN} + \text{FMNH}_2$$

$$\text{FMNH}_2 + \text{aldehyde} + \tfrac{1}{2}\text{O}_2 \xrightarrow{\text{(luciferase)}} \text{light} + \text{FMN} + \text{H}_2\text{O} + \text{other products}$$

The rate of the luminescent reaction, i.e., the luminous intensity, depends upon the concentration of the enzyme (luciferase) just as in any other enzymatic reaction, provided the substrate concentration (luciferin) is not limiting. As in all enzymatic reactions, water is required for the reaction and the rate of luminescence is affected by many factors which may affect the enzyme such as pH, temperature, and heavy metals.

2.2.2. REACTION TO DEXTROSE

The response of bacterial luminescence to additions of an oxidizable substance such as dextrose has been attributed to the accumulation of luciferin ($FMNH_2$) or luciferin precursor (FMN) during rapid growth in an adequate medium. If dextrose is added to suspensions of resting cells (i.e., cells in a nitrogen-free medium) the intensity of luminescence will rapidly increase many fold. The explanation for this is believed to be reduction of accumulated luciferin or its precursor by hydrogen derived from the dextrose in the presence of dehydrogenases.[30] If the precursor accumulates, it may be assumed that DPNH is not present in excess but

is limited in quantity by the reaction rate of the reaction which produces DPNH. If $FMNH_2$ is indeed luciferin, the molecule is already reduced and additions of dextrose would make no difference in the luminescence. It is, therefore, more reasonable to assume that reduction of a precursor is the explanation for the increased luminescence following additions of dextrose; but it may also be that luciferin is not simply $FMNH_2$.

Synthesis of luciferin or its precursor cannot occur in the absence of nitrogenous nutrient; consequently, the luminescence of nitrogen-free suspensions of bacteria must decrease with time.[33]

2.2.3. CHANGE IN TOTAL LIGHT OUTPUT AS A MEASURE OF BIOLOGICAL EFFECT

The concentration of luciferin does not affect the rate of the luminous reaction, but it does place a limit on the total amount of light which can be produced. Giese[30] states it thus: "The same amount of light will be produced from the same amount of luciferin whether little or much luciferase is present, but the reaction will be completed much more rapidly if luciferase is abundant. It is evident that any agent which removes or inactivates luciferin will decrease the total amount of light produced, although if it does not affect luciferase, it will not alter the rate of reaction."

Since the reaction rate is a function of the concentration of luciferase, which has the characteristic behavior of an enzyme, differences between luminous intensities of two suspensions of organisms may vary in magnitude and direction depending upon the time of the observation and

the environment to which the suspensions have been subjected. Even if intensity differences were in one direction, the investigator would not know whether the difference was caused by inactivation of luciferase or luciferin for if substrate concentration is limiting, the luminous intensity will also be affected.[30]

If one integrates luminous intensity with respect to time to obtain total light, differences will always be in the same direction and may be attributed to a single variable—luciferin concentration. Total light may also be considered as an indication of the total work done by the luminescent system.

The measurement of total light is particularly applicable in this investigation since at least one of the constituents of luciferin is suspected of having a resonant absorption frequency in the microwave frequency range studied (see Section 3.7).

2.2.4. LUMINESCENCE VS. EFFECT AND LIFE

One of the questions confronting the investigator of biological responses of living systems is the choice of an index of an effect and the determination of the significance of the observed effect to the test organism. The purpose of this investigation is to demonstrate whether a non-thermal effect is induced in P. fischeri as a result of microwave irradiation without regard to whether this effect is beneficial or deleterious to the bacterium. However, if an effect were observed, it would be interesting to know whether the effect was beneficial or harmful to the or-

ganism. This section is devoted to the argument that diminution of luminescence in P. fischeri is an indication of some undesirable interaction in suspensions of the organism.

In 1957 the classification of the test organism was changed from Achromobacter fischeri to Photobacterium fischeri.[22] There is no requirement that a bacterium be luminescent to be classified in the genus Achromobacter, whereas an organism must be luminescent to be classified in the genus Photobacterium. This is hardly a strong argument but it does indicate that the luminescence of the organism is its recognized, natural state.

It has been demonstrated that the respiration, luminescence, and division mechanisms of this particular organism are increasingly sensitive to ultra-violet radiation in the order named.[34] If cell division is more sensitive than luminescence for a given radiation exposure, the depression of luminescence by that radiation could be considered to be a deleterious effect since cell division, a very fundamental requirement for survival, will also be affected.

Another argument which supports the belief that luminescence is directly related to important life processes of the bacterium is provided by Baylor,[35] who found a positive correlation between the rate of growth and the total light emitted by P. fischeri. His data indicate that changes in the physiology of the culture are seen immediately in the luminous intensity but are reflected one generation time later on the growth curve.

These arguments suggest that the luminescence is an excellent choice as an index of biological effect in the organism and is directly related to the physiological state of the organism.

CHAPTER III. MICROWAVES

3.1. Generation and Nature of Microwaves

3.1.1. INTRODUCTION

Microwaves are electromagnetic radiation generated as a result of energy coupling between electrons and electromagnetic fields. This energy coupling causes the electrons to give up energy in the form of electromagnetic radiation.

No attempt will be made here to discuss the generation and transmission of microwaves in any detail. Only the fundamental theory of microwave transmission and the components directly associated with the generation or detection of microwaves in this investigation are discussed in the following paragraphs.

3.1.2. THE MAGNETRON

Fundamentally, the magnetron is a type of diode in which a magnetic field is applied perpendicularly to an electric field between the cathode and the plate.[36] If a source of electrons is provided at the cathode, they will be accelerated toward the plate (anode) in curved paths and U.H.F. oscillations will be produced as a result of the currents induced by the moving electrons.[36] The field between the anode and cathode consists of a direct electric field upon which is imposed an alternating electric field generated by the oscillations of the

electrons in the magnetic field. If the magnetic flux density is high enough, for a given anode voltage, the electrons do not strike the anode but return to the cathode and the anode current is cut off. Magnetrons are operated in magnetic fields which are higher than this critical value.

A traveling-wave type of magnetron is the most commonly used today. The cathode is surrounded by a cylindrical anode containing a number of equally spaced cavity resonators having slots into the interelectrode space—the space between the cathode and the anode structure. Resonators are coupled to each other by the magnetic flux that links them and by capacitance between the segments that separate the slots.[37] The alternating field extends into the interelectrode space from the slots. Energy is transferred to the resonator system of the magnetron from a d-c high-voltage power supply which supplies the anode voltage, by virtue of the interaction of electrons with the radial, direct electric field and the fringing rf field. In practice the cathode is usually driven negative and the anode is kept at ground potential. Oscillations are started by random phenomena in the interelectrode space and in the resonators. The magnetic field gives the electrons a circular component of motion about the cathode.

The capacitive and magnetic coupling between the resonators enables power to be extracted from a single resonator of the system by means of a coupling loop or waveguide adaptation. Choice of coaxial line or waveguide output is governed by considerations of mechanical

strength, ease of construction, size, power-carrying capabilities, and operating frequency.[37]

Magnetrons may be tuned by variation of resonator capacitance or inductance or by an auxiliary resonator coupled to the resonator system.

3.1.3. MODES OF TRANSMISSION

Electromagnetic waves may be guided to points of interest or transmitted into free space by waveguides of various configurations and compositions. The configuration and composition of the guiding structure is a function of frequency and mode of transmission desired.

An infinite number of types of traveling waves, or "modes," can exist in a waveguide but they are separable into three classes:

1. principal mode;
2. transverse electric modes;
3. transverse magnetic modes.

The principal mode consists of electric and magnetic fields that are solely transverse to the direction of energy flow. It is referred to as the TEM mode. All other modes have field components in the direction of energy flow. Waves with no electric field in the direction of energy flow are transverse electric (TE) waves. Waves with no magnetic component in the direction of energy flow are transverse magnetic (TM) waves.

Two numerical subscripts are used to indicate the field distribution of the mode in rectangular wave guides. If "b" and "a" are the

shortest and longest transverse dimensions of a rectangular waveguide, the first subscript of a mode designation gives the number of half-period variations of the field along "a" and the second subscript, the number of half-period variations along "b." Hence the $TE_{1,0}$ mode used in this investigation has one half-period variation of the electric field in the "a" dimension, none in the "b" dimension, and its electric field component is entirely transverse to the direction of energy flow.[5]

The $TE_{1,0}$ mode is usually used in the transmission of microwave power.[37] It has the simplest field configuration and the lowest cut-off frequency, and is called the dominant mode.[37] The dominant mode has lower power dissipation and requires smaller, lighter, and cheaper guiding structures for a given excitation frequency. It also requires simpler components for coupling power into and out of guides.[37]

3.1.4. CUTOFF WAVELENGTH

The cutoff wavelength is the wavelength above which the guide will not transmit the microwave energy. There is the problem of being certain the wavelength in the waveguide is not above cutoff for the guide. This cutoff wavelength is a function of the physical dimensions of the guide and the mode of transmission. The cutoff wavelength is given by Reich as:[37]

$$\lambda_c = 2/\sqrt{(m/a)^2 + (n/b)^2}$$

where

a = long transverse dimension of guide

b = short transverse dimension of guide

m = number of electric field variations in a

n = number of electric field variations in b

For the $TE_{1,0}$ mode, m = 1 and n = 0 and the formula collapses so that the cutoff wavelength becomes simply, $\lambda_c = 2a$. S-band frequency waveguide has inside dimensions of 3.4 x 7.2 cm. In the $TE_{1,0}$ mode, therefore, the wavelength above which the guide will not propagate energy is 14.4 cm (i.e., twice the longer transverse dimension of the guide).

The cutoff frequency is the quotient of the velocity of light divided by the cutoff wave length. For the above waveguide this is 2.08 KMc. Exposures made in this waveguide at less than 2 KMc are not exposures at all since the guide simply will not propagate lower frequencies. The wave is "cut off." Experiments conducted in a microwave engineering laboratory verified this.

3.1.5. VOLTAGE STANDING-WAVE RATIO (VSWR), POWER STANDING-WAVE RATIO (PSWR), AND REFLECTION COEFFICIENT

The extent to which the transmission of microwave energy is disrupted in a waveguide is largely dependent upon discontinuities along the line and load mismatch which introduce reflections into the system. These reflections produce a standing wave in the line, the amplitude of which is dependent upon the degree of mismatch in the line. This relation is valid provided attenuation of traveling waves in the line

is sufficiently low so that the standing-wave pattern is independent of position along the line.[5]

The VSWR is defined as the ratio of maximum field strength to minimum field strength along the line over a distance of at least a half wavelength.[5] The standing wave is produced by waves traveling in opposite directions through the waveguide. Each traveling wave has an electric field strength associated with it. If the field strengths at any point in the transmitted or reflected wave are designated E_t and E_r, respectively, then the maximum and minimum field strengths observed in the standing wave may be designated:[5]

$$E_{max} = E_t + E_r$$

$$E_{min} = E_t - E_r$$

By definition the VSWR is equal to E_{max}/E_{min}, or

$$VSWR = \frac{E_t + E_r}{E_t - E_r} = \frac{1 + E_r/E_t}{1 - E_r/E_t}$$

Now E_r/E_t represents the fraction of the transmitted field strength which is reflected and is termed the reflection coefficient. Rewriting the above formula for VSWR and substitution of the symbol Γ for the reflection coefficient yields:

$$\Gamma = \frac{VSWR - 1}{VSWR + 1}$$

The VSWR may be determined by introducing a movable probe into the microwave field. The signal induced in the probe is rectified by a crystal rectifier and measured by a microammeter. Crystals are not linear devices. Their output is directly proportional to the square of the input. What is measured, then, is really proportional to power and not voltage since the power transmitted through the guide is directly proportional to the square of the electric field strength. The meter, therefore, measures PSWR.[5]

$$\text{PSWR} = \frac{1 + E_r^2/E_t^2}{1 - E_r^2/E_t^2}$$

$$= \frac{1 + \Gamma^2}{1 - \Gamma^2}$$

where Γ^2 = fraction of power reflected

or $\Gamma^2 = \dfrac{\text{PSWR} - 1}{\text{PSWR} + 1}$

and, percent of power reflected $= 100\Gamma^2 = 100 \left(\dfrac{\text{VSWR} - 1}{\text{VSWR} + 1}\right)^2$ (2)

3.1.6. FREQUENCY PULLING

The output frequency of a microwave generator may be affected by the load characteristics of the line into which the energy is fed. This phenomenon, called frequency pulling, is of importance when the two-way transit time of the line is short compared to the pulse length. The effect has, therefore, been referred to as the "short-line" effect but

more frequently the term "long-line effect" has been used because frequency pulling disappears when the line is made very long.[38] In the case of continuous waves the transit time of the line is small compared with the length of the "pulse" since the "pulse" is continuous. Frequency pulling, therefore, must be taken into consideration in this investigation.

The pulling figure of a magnetron or klystron oscillator is defined for a particular VSWR at its output as the maximum frequency excursion found for loads which have this VSWR.[4] Pulling figures which have been accepted as reasonable for unstabilized tubes are 10 Mc at 3 KMc, 15 Mc at 10 KMc and 30 Mc at 30 KMc.[38]

3.2. Frequency Measurement

Microwave frequency may be determined by a wavemeter which consists of some form of calibrated, tunable cavity. The cavity is tuned to resonance mechanically and accuracies are only as good as the machinist who made the cavity. Accuracy of the cavity is also affected by temperature and humidity.

A closed cavity with conducting walls has associated with it an infinite number of discrete resonant frequencies, each corresponding to a different configuration of electromagnetic fields. For a given cavity there is a unique value of resonant frequency for each mode of resonance. For a given mode and cavity shape, this resonant frequency

depends only upon the size of the cavity.[5] The frequency or wavelength may then be determined by tuning the cavity to resonance and observing the size of the cavity from a vernier which has been mechanically calibrated in frequency or wavelength.

3.3. Power Measurement

3.3.1. CALORIMETRIC LOADS

A calorimetric load is a microwave absorber designed to measure the temperature rise resulting from energy dissipation in the load. Calorimetric loads may be of many different designs and may use either a static or circulating fluid such as water which is a good absorber of microwave energy. Only one specific type will be discussed here, namely, a waveguide, circulating water load of simple design.

A glass tube is inserted through the waveguide along a tapered wedge. The taper concentrates the electric field in the water and also helps to broaden the frequency range over which the load is useful. If the tube is tapered itself toward the input end of the load, it is possible to flatten and broaden the frequency response of the load and at the same time to distribute the power along the length of the water tube.[3] The advantages of this type of load are simplicity, low-heat capacity, and excellent microwave matching.

Microwave power is absorbed directly in the flowing fluid. Measurement of the temperature rise produced in the fluid in passing through

the load enables computation of the average power in watts (P) from the relation:[3]

$$P = 4.187 \ vdc\Delta T$$

where

v = rate of flow (cc/sec),

d = specific gravity of fluid (gm/cc),

c = specific heat of fluid (cal/gm-degree),

ΔT = temperature increment (°C),

4.187 = Joule's equivalent of heat (watts/cal).

The formula neglects heat transfer between various parts of the load, heat which escapes from the water tubing at the ends of the load, and heat losses through connecting tubing and temperature-measuring devices. Such losses are a function of flow rate and can be detected by measuring power at different flow rates.

Uniformity of fluid flow is important to avoid nonuniformity of heating; furthermore, turbulence should be maintained in the tubing to eliminate the possibility of laminar flow. "Flow of fluid will be turbulent if the Reynold's number (R_n) exceeds 2000."[3] R_n for water is given by[3]

$$R_n = 56 \ vD \ (1 + 0.033T + 0.0002T^2)$$

where

v = velocity of water flow (cm/sec),

D = tubing diameter (cm),

T = average water temperature (°C).

This is not to say, however, that insuring turbulence will insure uniform heating; when turbulent flow occurs over smooth, solid boundaries, a thin film of laminar flow will still exist at the boundary.[39] It is possible to eliminate this laminar film by making the boundary surfaces rough. But roughness would introduce undesirable microwave reflections into the line.

One is also faced with the problem of providing a flow rate which is fast enough to insure turbulence and avoid high temperature losses and yet is slow enough to yield a reasonably accurate temperature reading.

3.3.2. BOLOMETERS AND BOLOMETER MOUNTS

The principal source for this and the next succeeding paragraphs is Ref. 37.

Devices which undergo a change of resistance due to absorption of radiant energy are called bolometers. Short pieces of fine metal wire having positive temperature coefficients of resistivity and enough total resistance to enable matching to the system for efficient absorption of the microwave energy may be used as bolometers.[37] Bolometers of this type are called "barretters."

Barretters burn out easily but have thermal time constants of the order of 300 μsec. Ambient temperature changes do not appreciably affect barretter characteristics since they are usually operated at high temperatures.

To obtain maximum available power, the barretter should be matched to the line impedance. This may be achieved through the use of a tuning element in a suitable mounting system which transforms the impedance of the barretter to match the impedance of the line.

Bolometers and bolometer mounts are used in conjunction with power meters.

3.3.3. POWER METERS

Power meters are resistance bridges which convert the change in resistance produced in a bolometer as a result of microwave heating into a meter reading. In self-balancing bridges of the type used in this investigation, a high-gain amplifier is used across the bridge both as a detector and a driving source for the bridge. The circuit oscillates at an amplitude that keeps the bridge normally balanced. When microwave energy is applied to the bolometer, which is one of the bridge arms, the amplitude of the oscillation decreases by an amount sufficient to maintain the bolometer resistance constant. The power decrease is equal to the power added by the microwave source and can be read on a voltmeter which is calibrated in watts. "Equivalence of microwave power and low-frequency heating allows microwave power to be determined."[37]

3.3.4. DIRECTIONAL COUPLERS

Directional couplers are devices which consist of overlaid waveguide sections mutually coupled by holes 1/4 wavelength apart so that a small fraction of the power in the main guide is coupled into a secondary guide. This coupling provides a very useful tool for monitoring the power level in the primary guide.

The wave coupled into the secondary guide will travel in both directions in the secondary guide, but the spacing of the coupling holes introduces a phase shift in the waves traveling in one direction in the secondary guide so that the waves traveling in that direction are self-cancelling. As a consequence of this phenomenon, the coupler is directional. A matched termination is used in one arm of the secondary guide in power sampling couplers (a) to absorb reflected power coupled from the main guide and (b) to absorb the small amount of power associated with imperfect directivity in the coupler.[37] The response of such a directional coupler is, therefore, independent of the VSWR in the main guide.

Two quantities which specify the performance of a directional coupler are the "coupling factor" and the "directivity." The coupling factor is a measure of the percentage of power traveling in one direction in the main guide which is coupled into the secondary guide. The directivity is a measure of the quality of the coupler. Well-designed couplers have directivities of the order of 30 db, which is sufficiently high to make practical measurements possible.[37] A unit having 30-db

directivity is effectively 1000 times as efficient for sampling power traveling in one direction as it is for sampling power traveling in the opposite direction.

3.4. Microwave Absorption Mechanisms

3.4.1. ENERGY AND MOTION

A comparison of the energy levels associated with various types of molecular motion provides insight into the type of interactions which may occur in the microwave region.

Electronic motion, vibrational or rubber-band-like oscillations, rotational motion, and translational motion have certain discrete energies associated with them. The energies associated with these types of motion in the order mentioned are of the order of 10^{-11}, 10^{-13}, 10^{-14}, and 10^{-28} ergs.[40] However, translational energy levels are so close that they may be considered to have a continuous spectrum. These energies correspond to wavelengths in the visible, infra-red, microwave, and very-low-frequency portion of the electromagnetic spectrum. One would, therefore, expect only translational or rotational types of molecular reactions in the microwave region if combinations of the several types of motion were not possible. But this is not so; for example, dipole orientation may occur not only by rotation of the entire molecule but by rotation of intramolecular structures about carbon-carbon bonds.[41] Furthermore, there may be various types of rotational motion which may require less energy than end-over-end rotations

which brings the possibility of effects well within the microwave-frequency range. It has also been suggested that absorption may occur as a result of transitions of hydroxyl groups from one hydrogen-bonded position to another.[42]

3.4.2. DIELECTRIC ABSORPTION

The mechanisms of microwave absorption are not well understood but one of the mechanisms is certainly dielectric absorption. Dielectric absorption refers to the absorption of energy which accompanies induced rotation of a dipole.

Where an oscillation frequency is associated with dipoles, there exists the possibility of resonances in applied microwave fields. Most of this microwave energy will be dissipated as a friction type of heat resulting from dielectric absorption. There is the possibility that resonances may be strong enough to displace pieces of molecules in a biological system and thereby disrupt the processes of that system.

Before one can understand the arguments concerning the dielectric absorption of microwave energy, certain fundamentals must be explained.

3.4.2.1. Definition of Symbols.—The following symbols and definitions are used throughout Sections 3.4. and 3.7.

ϵ = absolute dielectric constant = $\epsilon' \epsilon_s$

ϵ' = dielectric constant relative to vacuum = ϵ / ϵ_s

ϵ'' = dielectric loss factor

ϵ_s = dielectric constant of vacuum

= 8.854 s 10^{-12} farad per meter in mks units

= 1.0 erg in esu units

ϵ^* = complex dielectric constant

ϵ_o = dielectric constant at "zero" frequency

ϵ_∞ = dielectric constant at "infinite" frequency

tan δ = dielectric loss tangent = ϵ''/ϵ' = power dissipated/power stored

u_o = vapor dipole moment = order of 10^{-18} esu

C = M/d = molar volume = gm-molecular weight/density

N = Avogadro's number

T = absolute temperature (°K)

k = molecular gas constant = 1.38 x 10^{-16} ergs/°C = Boltzman constant

τ = relaxation time in seconds

ω = $2\pi f$

f = frequency in cps

σ = conductivity in mhos per meter

3.4.2.2. Dielectric Constant.—A dipole moment is the product of charge and distance between two points or "poles" which are charged relative to each other. Dipole moments may be permanent or may be induced by external fields. Induced dipole moments result from shifts of the electron cloud relative to the nucleus.[43] It is the magnitude of the relative shift (electronic polarization) between the nucleus and the electron cloud together with the number of atoms per unit volume which

determines the relative dielectric constant. The dielectric constant is directly proportional to the degree of polarization and the number of atoms present per unit volume.[43] This is true for rare gases but dipole interactions and ionic and orientational polarizations complicate the relationship for polyatomic materials and molecules.[43] In liquids, for example, the relative dielectric constant decreases with increasing temperature as a result of reduction in orientational polarization of molecular dipoles. If, however, no permanent dipoles are present, the relative dielectric constant is nearly independent of temperature.[43]

In the visible part of the spectrum, ions are too heavy to follow the rapid field variations; hence, in that region one is concerned only with electronic polarizations. Since natural frequencies of ionic vibrations lie in the infra-red part of the spectrum, electrical engineers are more concerned with orientational polarizations which are the result of alignment of permanent, molecular dipoles by an externally applied electric field.[43]

The relative dielectric constant is, then, the result of contributions from several molecular mechanisms, namely:

1. electronic and ionic polarization;
2. orientation of permanent dipoles;
3. ionic or electronic conduction.

The first of these effects contributes a frequency-independent factor to the dielectric constant but no loss.[5] The contribution of the other

two mechanisms is frequency-dependent and affects the dielectric constant. Therefore these quantities often have values at microwave frequencies different from their low-frequency values, which may also be temperature-sensitive.[5]

3.4.2.3. <u>Complex Dielectric Constant</u>.—When an electrical engineer speaks of "loss" in a dielectric material, he is referring to that portion of the microwave energy absorbed in the media through which the wave passes. "This loss may be taken into account by considering the dielectric constant as complex and of the form:

$$\epsilon^* = \epsilon' - j\,\epsilon''$$

where ϵ' is known as the real part and ϵ'' the complex part of the dielectric constant."[5] The complex notation is introduced as a convenient means of solving the motion equations applicable to electronic and ionic polarizations; it develops that the absorbed energy is proportional to the imaginary part of the complex dielectric constant.[43] Ramo and Whinnery[44] write the dielectric constant as:

$$\epsilon^* = \epsilon' (1 + \sigma/j\omega\epsilon') \text{ where, } j = \sqrt{-1}\ ,$$

which shows the dependence of the loss factor (ϵ'') of the dielectric constant upon frequency.

From the above one can conclude that loss tangents and loss factors mean the same thing in terms of energy absorption. Hence, materials having highest loss factors or loss tangents will be the most effective

absorbers of microwave energy. The ratio ϵ''/ϵ' (or loss tangent) is a common constant for dielectrics since it is a direct measure of the ratio of conduction current to displacement current in the dielectric.

3.4.3. ABSORPTION COEFFICIENT

For the $TE_{1,0}$ mode of transmission Whiffen and Thompson[45] have defined an attenuation coefficient, ψ, in terms of power transmitted through a waveguide cell which could be filled with various solutions. They provide the relation:

$$P = P_o e^{-\psi L} \tag{3}$$

where

P = transmitted power

P_o = incident power

L = length of the cell

ψ = attenuation coefficient in nepers/unit length

One neper is equivalent to 8.69 db.[4]

For the $TE_{1,0}$ mode the attenuation coefficient is related to the loss tangent by:[45]

$$\tan \delta = \frac{\beta\psi}{(\pi/b)^2 + \beta^2}$$

or

$$\psi = \frac{\epsilon''}{\epsilon'} \left[\left(\frac{\pi}{b}\right)^2 + \epsilon' \left(\frac{2\pi}{\lambda_o}\right)^2 - \left(\frac{\pi}{a}\right)^2 \right] \tag{4}$$

where

$$\beta^2 = \epsilon' \left(\frac{2\pi}{\lambda_o}\right)^2 - \left(\frac{\pi}{a}\right)^2$$

λ_o = free space wavelength

β = propagation constant

b = longer transverse dimension of guide

a = shorter transverse dimension of guide

ψ = attenuation coefficient

The above formulas neglect the internal reflections within the cell and assume a perfect match of the cell to the line. Powles[46] has further developed Eq. (3) to include a factor of $(1 - \Gamma^2 e^{-2\psi L})$ to allow for the effect of internal reflections in the absorption cell and a factor $(1 - \Gamma^2)$ to correct for mismatch of the cell at the power input side of the cell. Applying these factors to Eq. (3), one obtains the absorption relationship:

$$P = P_o \frac{(1 - \Gamma^2)\ e^{-\psi L}}{(1 - \Gamma^2 e^{-2\psi L})} \qquad (5)$$

where Γ is the reflection coefficient at the power input to the cell. Note that for a good match to the line Eq. (5) collapses to Eq. (3). For the purposes of the present investigation, Eq. (3) represents a reasonable description of the absorption relation.

Hence the only information necessary to compute an absorption coefficient is the loss factor, dielectric constant, wavelength, and guide dimensions. But the effects of dielectric dispersion will make the attenuation coefficient dependent on both frequency and temperature.[45]

The absorption will also be a function of the form of the absorbing material. For example, free water has an absorption peak at about 20 KMc and bound water in protein is estimated to have its maximum absorption at about 300 Mc, while ice shows an absorption below the microwave-frequency range.[6]

3.4.4. ANOMALOUS DISPERSION

Were it not for the effects of anomalous dispersion, the fall of dielectric constant with increasing frequency, computation of an absorption coefficient would be a simple matter. The resistance of molecular inertia and viscous forces to the orientation of molecular dipoles by an applied electromagnetic field results in a lag between the application of the field and the rotation of the dipole.[45] This phenomenon leads to anomalous dispersion which is attributed to dielectric relaxation, i.e., the exponential decay with time of the polarization in a dielectric when an externally applied field is removed.[47] The relaxation time is the time in which this polarization is reduced to 1/e times its original value.[47]

One would like to calculate the frequency at which the loss tangent or loss factor is a maximum for various molecules or solutions of materials. Relationships between dipole moment (u_o) and dielectric constant have been developed by Debye, Onsager, and Kirkwood,[47] which enable one to do this with reasonable accuracy for very simple molecules provided a number of other dependent variables are known. The equations

of Onsager and Kirkwood are essentially refinements of the Debye theory. Smyth[47] compares values of dipole moment calculated using the several equations and tabulates these values in Table 16.2, p. 33. Values of u_o agree within 10 to 30% of each other. Since the Debye equation is the simplest of the three and the results from all three agree fairly well, it has been chosen for presentation here. The equation may be put in the form:[48]

$$u_o^2 = \frac{27\ k\ T\ \epsilon''(1 + \omega^2\tau^2)}{4(\epsilon' + 2)^2\ NC\ \pi\ \omega\ \tau} \tag{6}$$

Smyth[47] develops the following relationships between dielectric constants, frequency, and relaxation time from the Debye equation.

$$\epsilon' = \epsilon_\infty + \frac{\epsilon_o - \epsilon_\infty}{1 + \omega^2\tau^2} \tag{7}$$

$$\epsilon'' = (\epsilon_o - \epsilon_\infty)\ \omega\tau/(1 + \omega^2\tau^2) \tag{8}$$

The latter expression approaches zero for both large and small values of $\omega\tau$ and has a maximum for $\omega\tau = 1$. The frequency at which ϵ'' is a maximum is therefore given by:

$$f_m = 1/2\pi\tau \tag{9}$$

and

$$\epsilon''_m = (\epsilon_o - \epsilon_\infty)/2 \tag{10}$$

Figure 2 is a general plot of Eqs. (7) and (8).

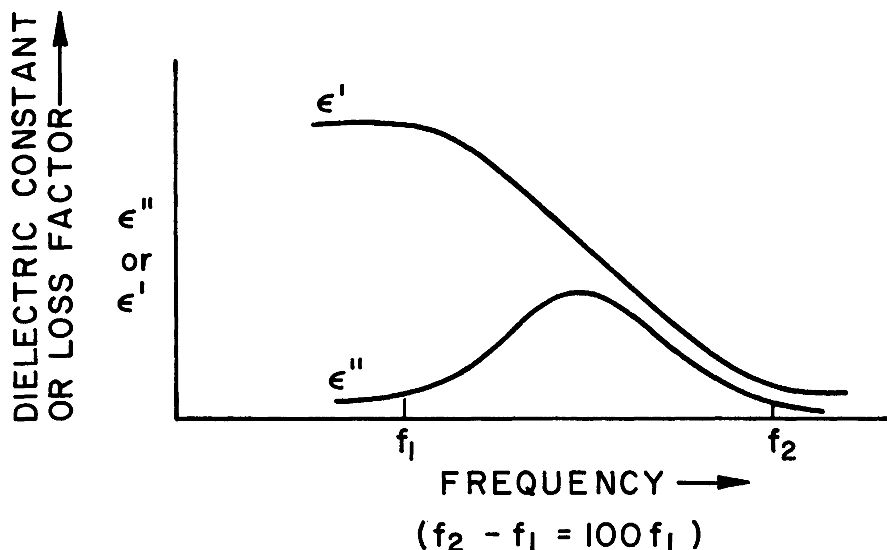


Fig. 2. Anomalous dispersion.

Anomalous dispersion commonly occurs over a wider frequency range with a maximum value of ϵ'' lower than predicted by theory. This is attributed to a distribution of relaxation times.[47] Dielectric absorption, then, is not a single frequency event but occurs to some extent over a fairly wide range for any given material.

With the above equations it would be possible to take the data of various workers and reduce them to common values of u_o, ϵ''_m, or τ, and calculate optimum absorption frequencies for various molecules. This would be an immense task. Furthermore, the Debye theory is limited in its scope. The theory does not hold for pure polar liquids due to dipolar interactions,[45] and the application of the theory will yield only approximate results which are sometimes, but not always good for dilute solutions.[47]

In view of these considerations, the chosen course is that of es-

timating from the experimental data of others the most effective absorption frequency for some of the constituents of the bacterial system.

3.5. Relative Biological Effectiveness of Radiation

3.5.1. GENERAL

The relative biological effectiveness of a radiation, based upon effects produced by a given dose of x-radiation, is defined by the relation:[49]

$$\text{RBE} = \frac{\text{Dose from X-rays to produce an effect}}{\text{Dose from given radiation to produce the same effect}}$$

The concept is of limited usefulness because the biological effectiveness of any radiation may depend on many factors such as the type and degree of biological damage, the absorbed dose rate, fractionation of the dose, oxygen tension, pH, and temperature.[50]

The physical parameter underlying different biological effectiveness is believed to be the linear energy transfer (LET) of secondary, charged particles.[51]

The above considerations require that considerable care be exercised in determining the relative biological effectiveness of radiations. Best comparisons will result from exposures which are alike in all respects save for the physical nature of the radiations.

3.5.2. FLUX DOSE RBE OF MICROWAVES

According to Carpenter,[12] an exposure dose of 1500 roentgens of

2 Mev X-rays given at a rate of 100 roentgens per minute produces lens cataracts in rabbit eyes 35 days after irradiation. Microwaves of 2450-Mc frequency (continuous wave) produce cataracts in rabbit eyes 1 to 6 days following irradiation for 15 minutes to a power density of about 175 milliwatts/cm^2.[18] Temperature of the rabbit eye rises during such an irradiation from a normal body temperature of about 38°C to about 48°C. This information provides the basis for the calculation of the RBE which follows.

One roentgen (r) is equal to 0.107 ergs/cc air energy deposition. The linear absorption coefficient for 2-Mev X-rays in air is 3.0×10^{-5} cm^{-1}, which is very close to that of 200-kev X-rays. The quotient of ergs/cc-r divided by the absorption coefficient provides the incident, energy flux density, which will result in energy deposition equal to one roentgen. It is possible to convert the flux density to units of mw-hr/cm^2-r, knowing that 1 watt equals 10^7 ergs/sec. One roentgen of x-radiation is, therefore, equivalent to 10^{-4} mw-hr/cm^2. Hence, 1500 r is equivalent to 0.15 mw-hr/cm^2 and the RBE for microwaves of this frequency, in cataract production, is:

$$RBE_f = 0.15/44 = 3.4 \times 10^{-3}$$

This is an RBE based upon flux densities, not absorbed dose.

Carpenter's data[12] show that, as in ionizing radiation, recovery is involved in microwave exposures; higher total exposure is required at lower power densities to produce a given effect. This is demonstrated

by Fig. 3. Cataract production resulting from microwave exposure therefore depends on the dose rate. Now the exposure rates for the above comparisons were not equal. X-rays applied at a rate of 100 r/min are equivalent to a flux density of 0.60 mw/cm^2, but the exposure rate in the case of the microwaves was about 300 times this. The difference in dose rates may make the eye appear comparatively more sensitive to microwave irradiation, in which case the determined RBE_f would represent something greater than the true value. This can be seen from Fig. 3. Were the microwave exposure rate reduced to 0.60 mw/cm^2, the total exposure to produce cataracts, if it were possible to do so at this level, would be exceedingly large and the RBE would be extremely small.

Temperatures associated with a 1500-r exposure are negligible but the temperature during a microwave exposure to 175 mw/cm^2 is significantly elevated. Radiation sensitivity may be directly proportional to temperature.[49] If elevated temperatures increase sensitivity to radiation, estimates of the RBE without compensation for temperature differences will be high.

3.5.3. ABSORBED DOSE RBE OF MICROWAVES

In determining the RBE of radiations one is really interested in absorbed doses rather than in flux comparisons. For example, if microwaves are more efficiently absorbed than X-rays, the energy absorbed for a given flux density of X-rays and microwaves will be higher in the case

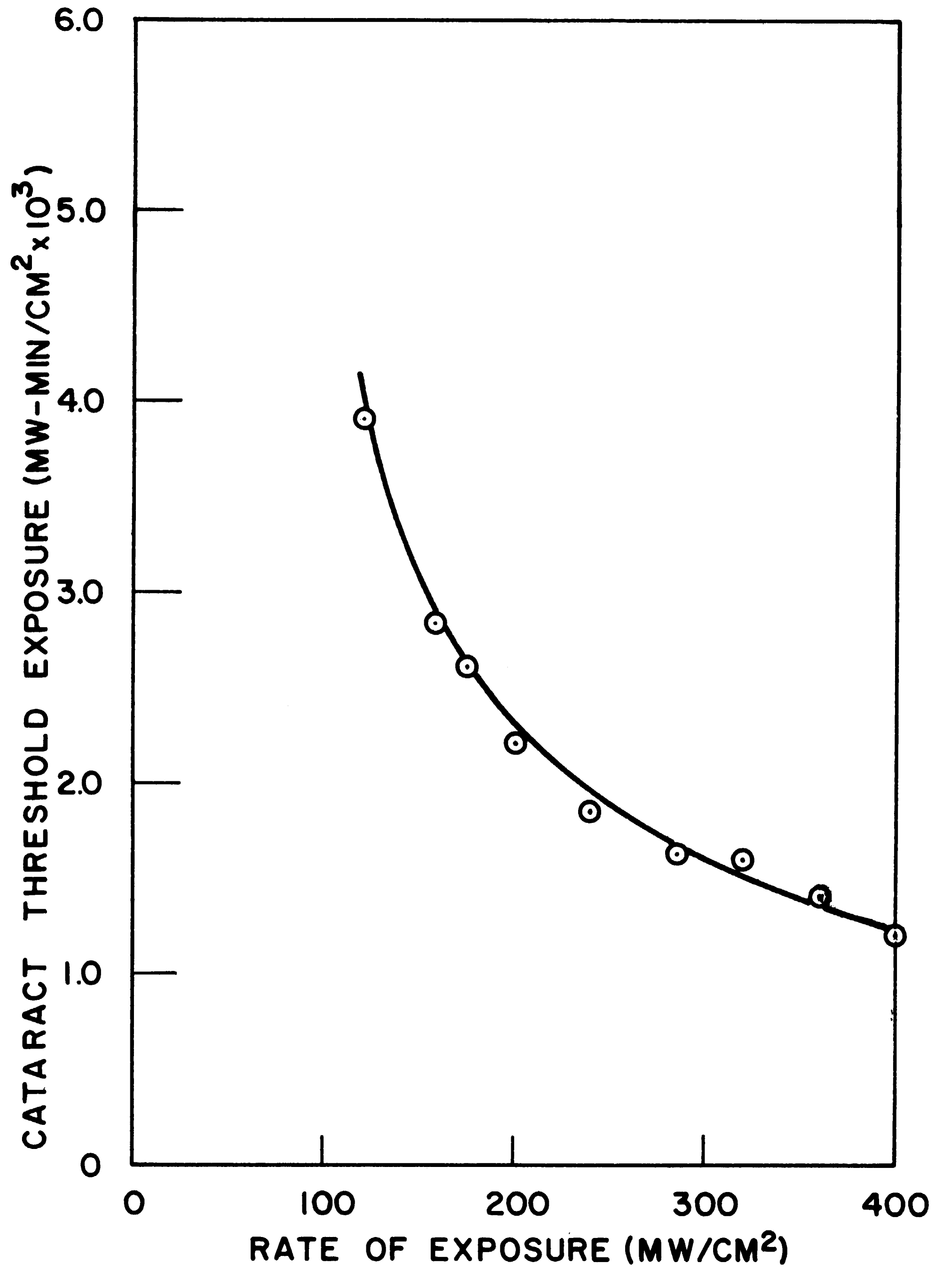


Fig. 3. Dose to produce lenticular cataracts in rabbits vs. microwave exposure rate.[12]

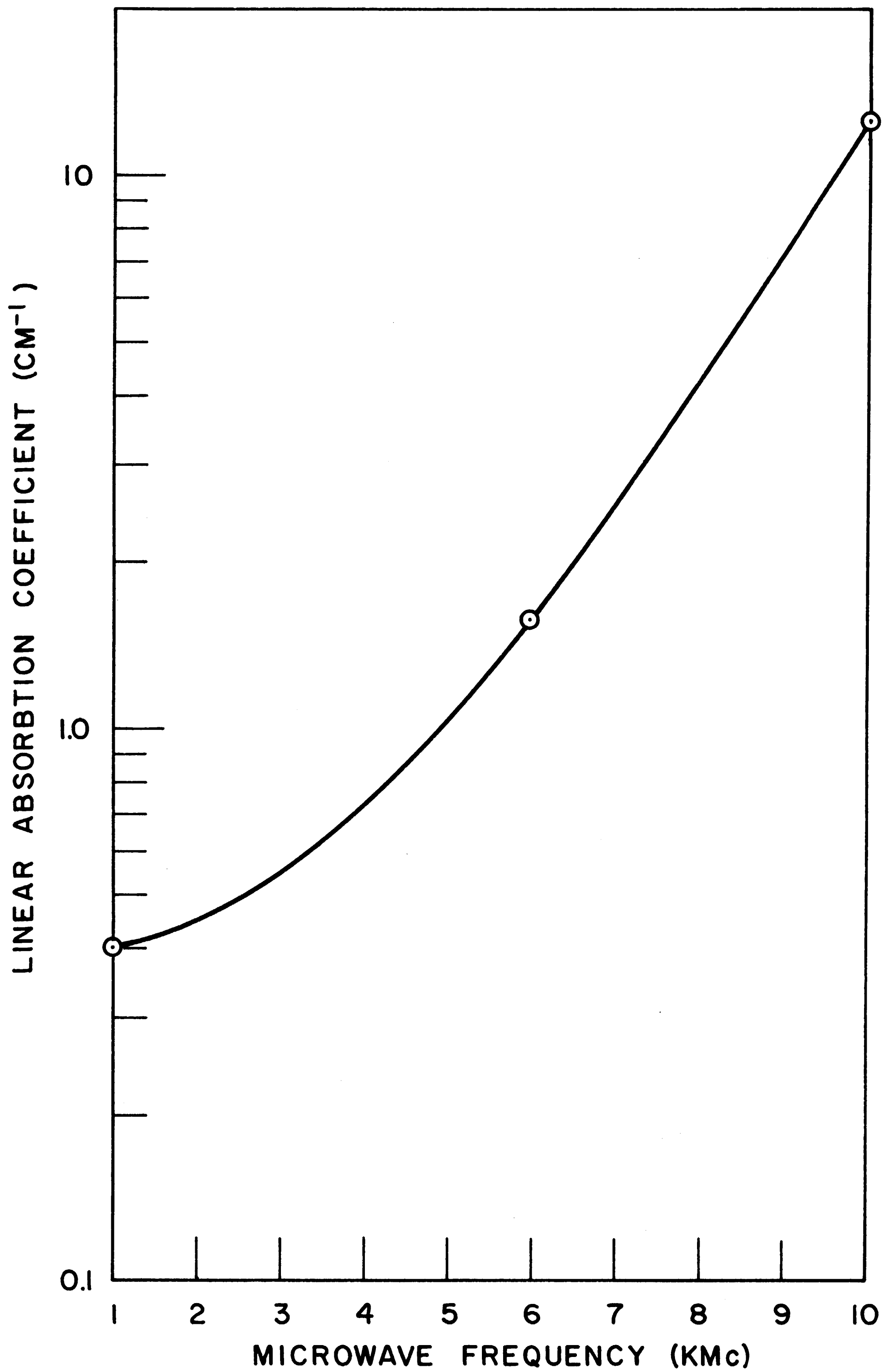


Fig. 4. Microwave absorption coefficient in muscle and similar tissue as a function of frequency.[9]

of the microwaves and the RBE will be further reduced.

Schwan[9] provides data for the absorption of microwave energy in muscle and similar tissue over a wide frequency range. Figure 4 is a plot of absorption coefficient as a function of microwave frequency in the range of interest. The absorption coefficient for muscle at 3 KMc is about 0.6 cm^{-1}. The true, linear absorption coefficient in tissue for 0.2-Mev X-rays is approximately 0.03 cm^{-1},[20] about 1/20 of the 3-KMc coefficient.

These absorption coefficients allow modification of the flux dose RBE to an absorbed dose RBE in tissue of high water content in the following way:

$$RBE_{aw} = \frac{0.15 \times 0.03}{44 \times 0.6}$$

$$= 1.7 \times 10^{-4}$$

The microwave absorption coefficients for fatty tissues or tissues of low water content are approximately 15% lower than those for muscle and similar tissues.[9] Consequently, the absorption coefficient of tissues having low water content such as the lens of the eye is about 0.09 cm^{-1} at 3 KMc. This leads to an RBE for microwaves in "dry" tissues (which is probably the best estimate since the criterion of effect used here is cataract formation in the lens of the eye) of:

$$RBE_{ad} = \frac{0.15 \times 0.03}{44 \times 0.09}$$

$$= 1.3 \times 10^{-3}$$

An additional comparison may be made if one looks at the absorption of energy from the calorimetric viewpoint. A 1500-r exposure to the eye would increase the eye temperature by about 3.3×10^{-3} °C. The temperature rise associated with the microwave cataract formation was about 10°C in the vitreous humour of the eye. On the basis of these temperatures the absorbed dose RBE is approximately 3.3×10^{-4}.

3.5.4. RBE OF HEAT

It has been found that the fatality rate of heatstroke victims is about 40% if the body temperature does not reach 43.3°C.[52] This constitutes a temperature increase of 6.3°C above normal body temperature.

The dose of ionizing radiation which will kill 50% of the humans exposed is about 400-500 roentgens.[53] A dose of 450 r will raise the temperature of the average human body by only about 0.001°C.[54] From this one may estimate an RBE_h for heat to be about 1.6×10^{-4}.

3.5.5. CONCLUSIONS

From the above comparisons it seems reasonable to place the RBE for microwaves absorbed in tissues of high water content (e.g., muscle) at about 10^{-4} and the RBE in tissues of low water content (e.g., fat, bone, or the lens of the eye) at about 10^{-3}. If the RBE for heat is 1.6×10^{-4}, it appears impossible for non-thermal effects to exist in tissues of high water content. There is a small margin of difference in the RBE of microwaves and heat in "dry" tissue which may be attributable to non-thermal effects.

The error associated with the RBE estimates made above is believed to be no less than 10% nor greater than 30%.

3.6. Significant Microwave Dose

Whipple[24] has observed 50% reduction in the total light from suspensions of P. *fischeri* 24 hours after exposure to 1.6×10^5 ergs/gm of ionizing radiation. His suspending solution (artificial sea water) for the cells was the same as that used here.

If the RBE_{aw} is indeed 1.7×10^{-4} or the same as that for heat (see Sections 3.5.3. and 3.5.4.), then the microwave dose to reduce the total light of a bacterial suspension to 50% of that of controls should be 10^9 ergs/gm.

Microwave exposures here were all greater than 3.3×10^9 ergs/gm except for a single exposure of 1.7×10^9 ergs/gm.

A check of the response to X-ray exposure of the suspensions used in this investigation confirmed Whipple's results and demonstrated the reproducibility of results using P. *fischeri* as a radiobiological test organism.

3.7. Frequency Bands of Interest

Smyth[47] has defined a critical wavelenght (λ_m) as:

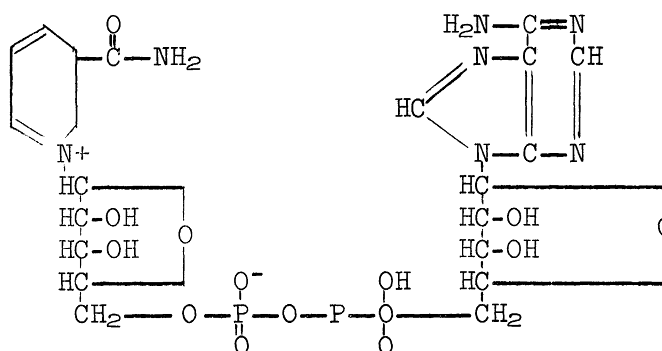
$$\lambda_m = 2 \pi c \tau \qquad (11)$$

That is the wavelength at which ϵ'' is a maximum. He also provides a plot of λ_m versus number of carbon atoms in n-alkyl bromides at 25°C in the pure liquid state (Fig. 5). The relationship probably explains in part the higher frequencies at which microwave spectroscopists work.

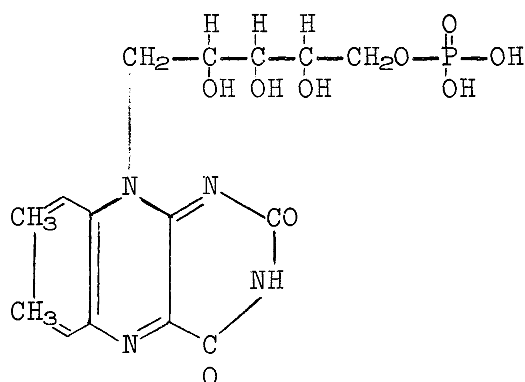
Figure 6 demonstrates the dependence of critical wavelength upon temperature.

Giese[30] suggests that it is likely that DPN and FMN play a role in the luminescent system of luminous bacteria. The chemical formulas and configurations for these two compounds are as follows.

Diphosphopyridine nucleotide (DPN):



Flavin mononucleotide (FMN):



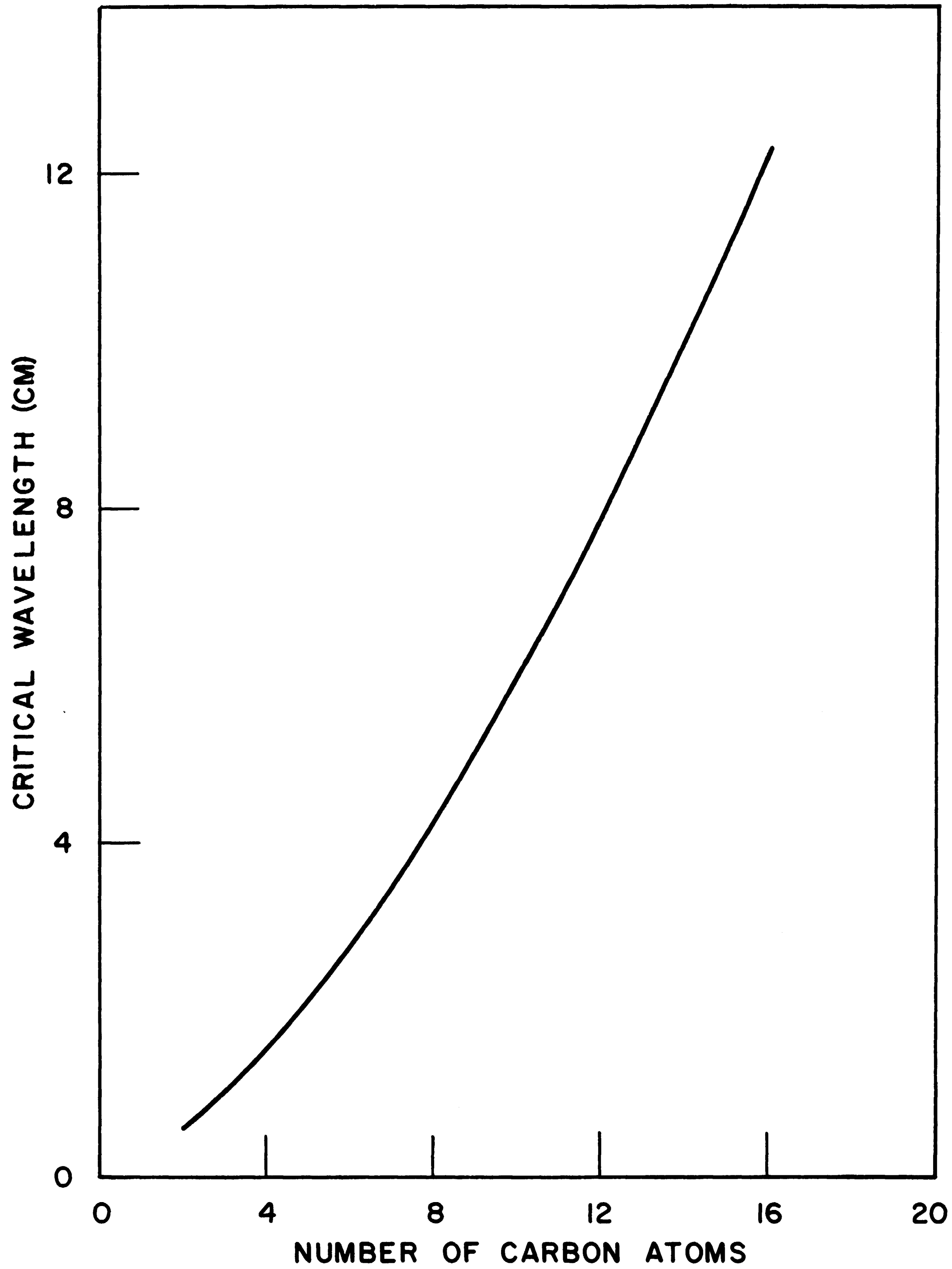


Fig. 5. Critical wavelength vs. molecular chain length for N-alkyl bromides at 25°C.[47]

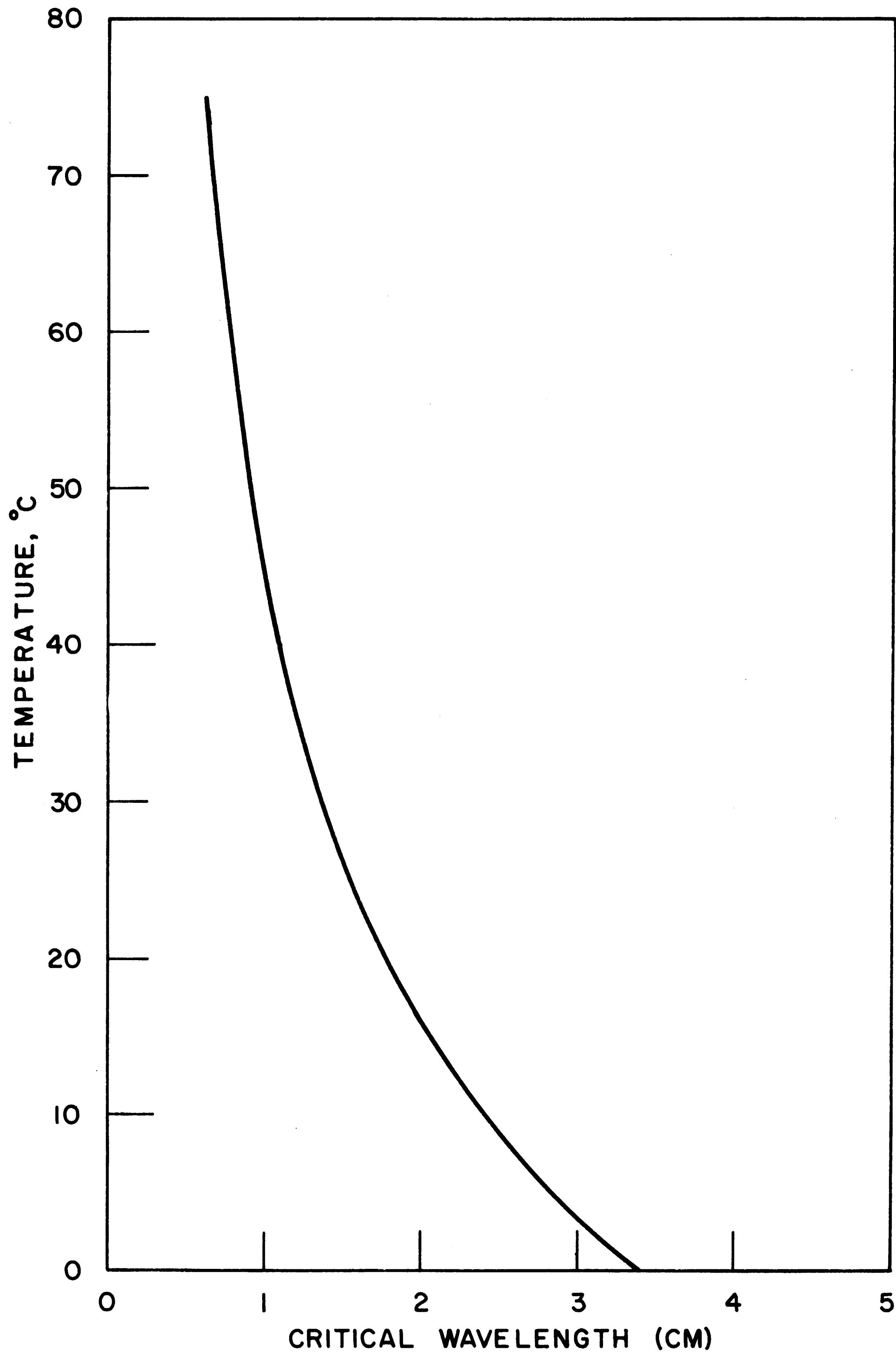
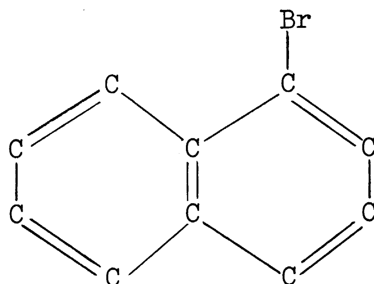


Fig. 6. Critical wavelength vs. temperature for water.[47]

Assuming that pieces of molecules may act as dipoles in themselves, one might expect, from Fig. 5, a critical wavelength of 2.1 cm for FMN and a critical wavelength of 2.1 cm or possibly 4.3 cm for DPN. These wavelengths are in the frequency range of 7-14 KMc. However, ring formation increases the critical wavelength.[47] This fact, coupled with the wide frequency range over which anomalous dispersion occurs, suggests that studies in the S-band range (2-4 KMc) are not at all unreasonable. As an example of the increase of critical wavelength associated with ring formation, there is alpha-bromonaphthalene ($C_{10}H_7Br$):



Smyth[47] found this compound to have a critical wavelength of 16.2 cm at 25°C, which is about three times the critical wavelength of a 10-carbon chain as determined from Fig. 5. Similarly, FMN and DPN may have peak dielectric absorption frequencies of 2-5 KMc, which is exactly the range in which exposures in this investigation have been made, but they, particularly DPN, may also have maximum absorption frequencies much lower than this depending upon whether orientation is molecular or intramolecular.

More practical considerations such as the availability of S-band

equipment played a major role in the choice of frequencies studies, but this is also the frequency range in which Carpenter[18] believes cataract formation in rabbits to result from a non-thermal effect.

CHAPTER IV. EXPERIMENTAL PROCEDURES

4.1. Biological System

4.1.1. BACTERIAL GROWTH AND SUSPENSION PREPARATION

The bacteria were grown on media described in Section 2.1. Thickness of the media was found to be of some importance. The bacteria were grown in 100 x 15 mm petri dishes filled to approximately 5 mm. Determination of the visual brightness of bacterial growth on both thick and thin media showed that the bacteria preferred an intermediate thickness. Thick media (3/4 full petri dish) produce greater and brighter growth faster than thinner media, but the bacteria dim out faster. Colonies develop more slowly on media of medium thickness (approx. 5 mm) but sustain a high-level light output longer than on thick media. Very thin media (just enough to cover the bottom of the petri dish) support growth less effectively than do media of medium thickness.

Cultures were incubated at 19.6 ± 0.6°C for 32.0 - 37.1 hr, at which time they were removed from the incubator and stored at 6.5 - 9.5°C until a total of 48 ± 1 hr had elapsed. This pattern was followed except for three batches. Cells used at 2608.7 Mc were grown at incubator temperature for 48.3 hr and the harvested. Cells used for 2839.1 - 2954.3 Mc were grown at incubator temperature for 35.5 hr and the harvested. Cells used for 2992.7 - 3031.1 Mc were grown at incubator temperature for 35.3 hr

and then harvested. Analysis shows that the differences between light curves are independent of culture age.

The incubation time used in these experiments was far from convenient, but visual observations of plate cultures indicated that the time used provided optimum growth and brightness. This incubation time also produced cells which resuspended most readily. Twenty-four-hour cultures grown at 19.6 ± 0.6°C provided neither sufficient light nor growth. An attempt to use 24-hr cultures grown at 25°C failed due to clumping difficulties.

It was necessary to use three culture plates to obtain 480 cc of bacterial suspension standardized to a concentration of 5×10^8 bacteria per cc. This volume was required to make four exposures.

The suspending solution described and designated by Whipple[24] as BS-2 solution was used for the preparation of nutrient-free suspension of the bacteria. Twelve liters of BS-2 solution were prepared at one time according to the recipe given in Table I.

TABLE I

BS-2 SOLUTION

Compound	Weight (gm)	Moles
NaCl	15.00	0.256
Na_2HPO_4	14.20	0.10
$NaH_2PO_4 \cdot H_2O$	3.45	0.025
$CaCl_2 \cdot 2H_2O$	0.40	0.0027
Distilled H_2O	to make 1000 cc	

The precipitate was filtered off and the pH checked at 7.1. The solution was stored in two 6-liter storage flasks covered tightly with aluminum foil. No evaporation was observed. If evaporation sufficient to produce a significant change in the concentration had occurred, a change in total light output with time might have been expected. No such pattern occurred in the experimental results.

The bacteria were harvested from each plate using about 5 cc of chilled BS-2 solution and a sterilized inoculating loop. A very light touch was used to avoid chunks of media in the resultant suspension. The suspensions from each plate were then combined in a 50-cc beaker. Each plate was rinsed with about 5 cc fo BS-2 solution and the rinse was added to the 50-cc beaker. The combined suspensions were divided between two 25-cc Lusteroid centrifuge tubes. The beaker was rinsed and the rinse divided. Three 8-min centrifugations at 6400 g at a centrifuge bowl temperature of -1 ± 1°C were performed. After each centrifugation, cells were resuspended in 10 cc of BS-2 solution. Agitation was provided by a glass rod rotated by an air-driven laboratory stirrer which, because of its low stirring power, was considered gentle. Only a slight pressure applied to the stirring rod stopped the pump.

Following the third resuspension of the cells, the contents of the two centrifuge tubes were poured into a 250-cc beaker. Rinses of the centrifuge tubes were also poured into this beaker. BS-2 solution was then added to make a total volume of approximately 200 cc. At this point uniform suspension of organisms was almost immediate. (Older cultures re-

quired several minutes of stirring to suspend the cells uniformily.) This volume was stirred by the air-driven pump until an aliquot had been taken with a 1:20 Trenner, automatic blood diluting pipette. The stirrer was then stopped until the counting of the cells in the Petroff-Hausser counting chamber had been completed and the standardization computations made. The stirring was resumed and continued throughout the pipetting of fractions of the suspension into 3-oz, clear, Boston bottles. Dilutions were made directly in the 3-oz storage bottles to make 60 cc of suspension having a concentration of 5×10^8 b/cc.

Following the completion of dilutions, the bottles were capped with "Poly-Seal" screw caps and packed in ice in a polystyrene bucket for transport to the East Engineering building. The time required for the preparation of the standardized suspension was about 1-1/2 hours. Each suspension was labeled with the numbers of the culture plates from which the bacteria were harvested, plus the suffix "S" to indicate that the suspending solution was BS-2 solution, and a number to identify the particular suspension volume. For example, the light curve labeled 395-397 BS 16 was run using the bacteria taken from plates 395B through 397B, put into suspension with BS-2 solution and stored in bottle number 16.

4.1.2. BACTERIAL COUNTING

Bacterial concentrations may be determined by cell counting using a Petroff-Hausser (abbreviated P-H) bacterial counting chamber. The chamber consists of a grid of 25 large squares each of which is divided into 16

smaller squares. Each of the smaller squares with the chamber cover slip in place represents a volume of 5.0×10^{-8} cc.

Instructions supplied with each chamber suggest that 9 out of 16 smaller squares in 10 larger squares be used as a counting pattern (i.e., a total of 90 smaller squares should be counted). If the chamber is filled from a 1:20 diluting pipette and the above counting pattern is observed, the bacterial concentration of the suspension from which the aliquot was drawn is determined by multiplying the total count by the factor 4.44×10^{6}.

The counting of bacterial cells in a counting chamber such as the P-H chamber is known to follow a Poisson distribution.[55] This has been confirmed in this study by comparison of the distribution of $S^2/\overline{X}$ values, calculated from the counting data, with the X^2/d.f. distribution which is Poisson-distributed.[56] The $S^2/\overline{X}$ values fell within the 90% confidence limits for the X^2/d.f. distribution given by Ref. 57. The counting data are, therefore, Poisson-distributed. This simplifies computation of the counting error for in this distribution the mean of the sample count is also the variance of the count.[55]

For a single count, the mean and the total are equal and the standard deviation becomes merely the square root of the total count. No total count less than 100 bacteria was used. Hence, the error associated with any total count never exceeded 20% at the 90% confidence level.

All concentration standardizations were performed on the basis of

the cell count in a P-H bacterial counting chamber. The variation of these counts from day to day was small as shown by the good agreement between concentration determinations made by counting and the concentration determinations made on residual stock suspensions by a laboratory technician. The latter determinations were completely independent of the former, and were made using a Beckman Spectrophotometer, Mod. 2400. Stock BS-2 solution was used to zero the instrument after which the absorbence of the suspension was determined at a wavelength of 420 millimicrons. This involved the use of the ultra-violet lamp and the blue sensitive phototube in the instrument. The instrument was allowed to warm up for 30 or more minutes and suspensions were at room temperature at the time absorbence determinations were made. The agreement between the concentrations determined from the Spectrophotometer and those calculated from the P-H chamber counts is shown in Table II. In no case does the comparative error exceed 15%.

The average percentage difference of the above values is ± 4.6%. On the average, the concentration determination error from day to day is less than ± 10%.

TABLE II

COMPARISON OF SUSPENSION-CONCENTRATION DETERMINATIONS

Date	Petroff-Hausser b/cc x 10^9	Beckman Spec. b/cc x 10^9	Concentration Difference	%* Difference
8-26-60	1.28	1.22	.06	4.7
8-29-60	1.61	1.38	.23	14.3
8-31-60	1.63	1.39	.24	14.2
9-2-60	1.42	1.59	-.17	-12.0
9-7-60	1.55	1.40	.15	9.7
9-9-60	1.58	1.54	.04	2.5
9-12-60	1.47	1.49	-.02	- 1.4

$$\text{*Percentage Difference} = \frac{\text{Concentration Difference}}{\text{P-H Concentration}}$$

4.1.3. LUMINESCENCE VS. TEMPERATURE

Figure 7 is plot of luminous intensity vs. temperature, obtained by using a control suspension which had run 156 minutes after dextrose had been added. The values for Fig. 7 were obtained in 9 minutes to avoid correction for time decay of the luminescence. The graph, plotted in degree Kelvin vs. luminous intensity as a matter of convention, shows the temperature of optimum luminescence for this suspension to be 27-29°C.

Other checks for the temperature of optimum luminescence were performed on control suspensions with no previous running time and without dextrose added. On one occasion, the control-system suspension showed a maximum luminous intensity at 26.8-28.1°C. But the maximum intensity of

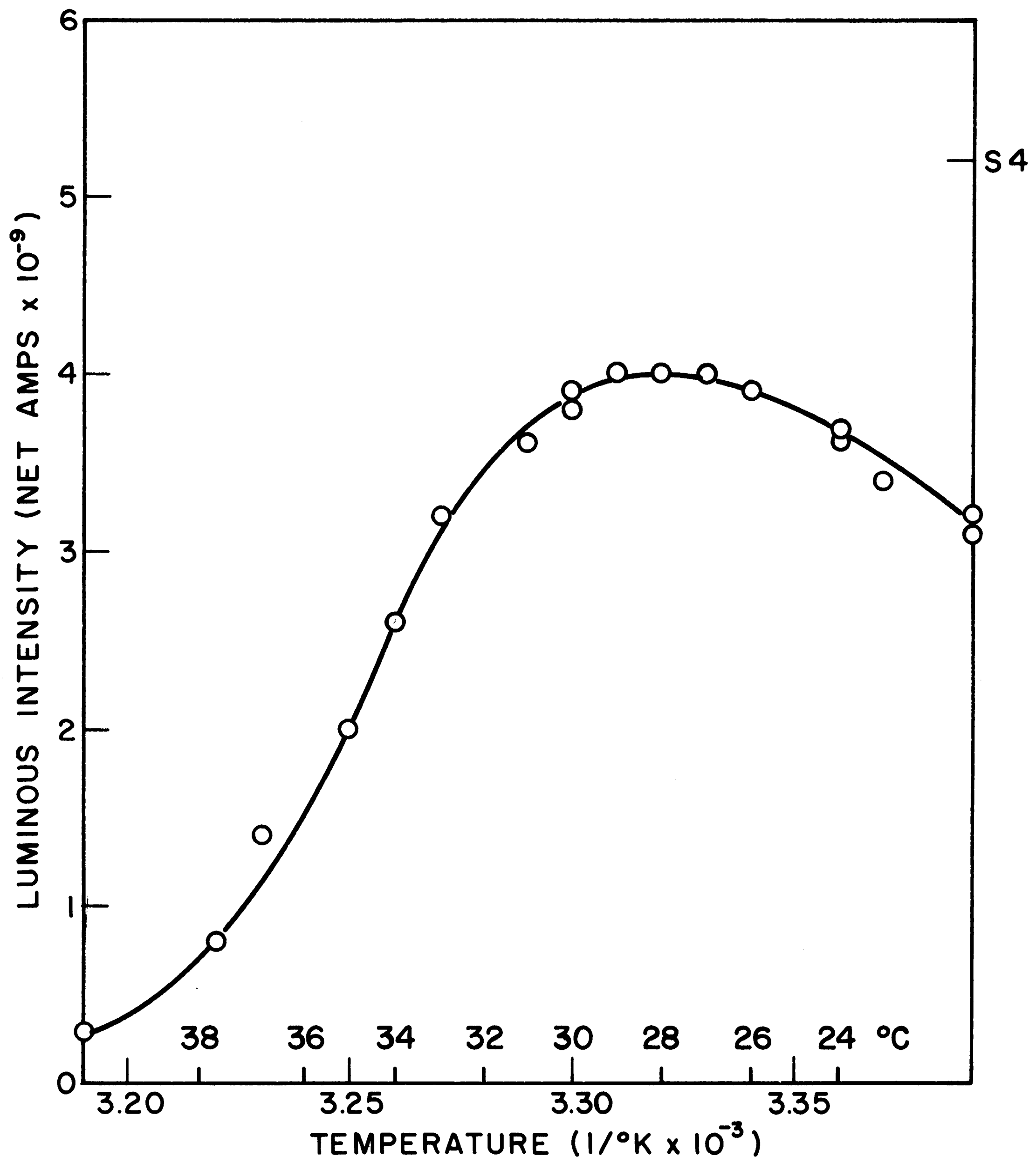


Fig. 7. Luminous intensity vs. temperature.

the suspension in the microwave system (no previous irradiation) occurred at 26.4-27.7°C when heated by microwaves. This difference is small and could be produced by temperature-equilibrium differences since these determinations were performed quite rapidly.

Figure 8 compares the temperatures of optimum luminescence for heating with the control heater and microwaves with and without dextrose added for fresh suspensions. Luminous intensity values with dextrose were determined at the peak of luminescence following the addition of dextrose. The graphs show optimum luminescence in all cases between 25 and 28°C. With dextrose added the center of the peak of the microwave heated suspension is about 1°C lower than the center of the peak for the suspension heated with heating tape. Without dextrose the reverse seemed to apply. That is, the microwave temperature of optimum luminescence is slightly higher than that for the controls. The data for each curve without dextrose were obtained in less than 10 minutes. Data for the curves with dextrose were obtained in 3 minutes. Note that the optimum luminescence temperature with or without dextrose for microwave heating is the same—about 27°C. In the case of the controls, however, the optimum luminescence temperature without dextrose is about 26°C but with dextrose it is about 27°C. Some of this difference may be attributed to the heating of the control suspension beyond the temperature of optimum luminescence. The microwave suspensions were run only slightly beyond the peak before the temperature was reduced and the dextrose added. However, the small variations are considered insignificant and are attributed to the lack of temperature equi-

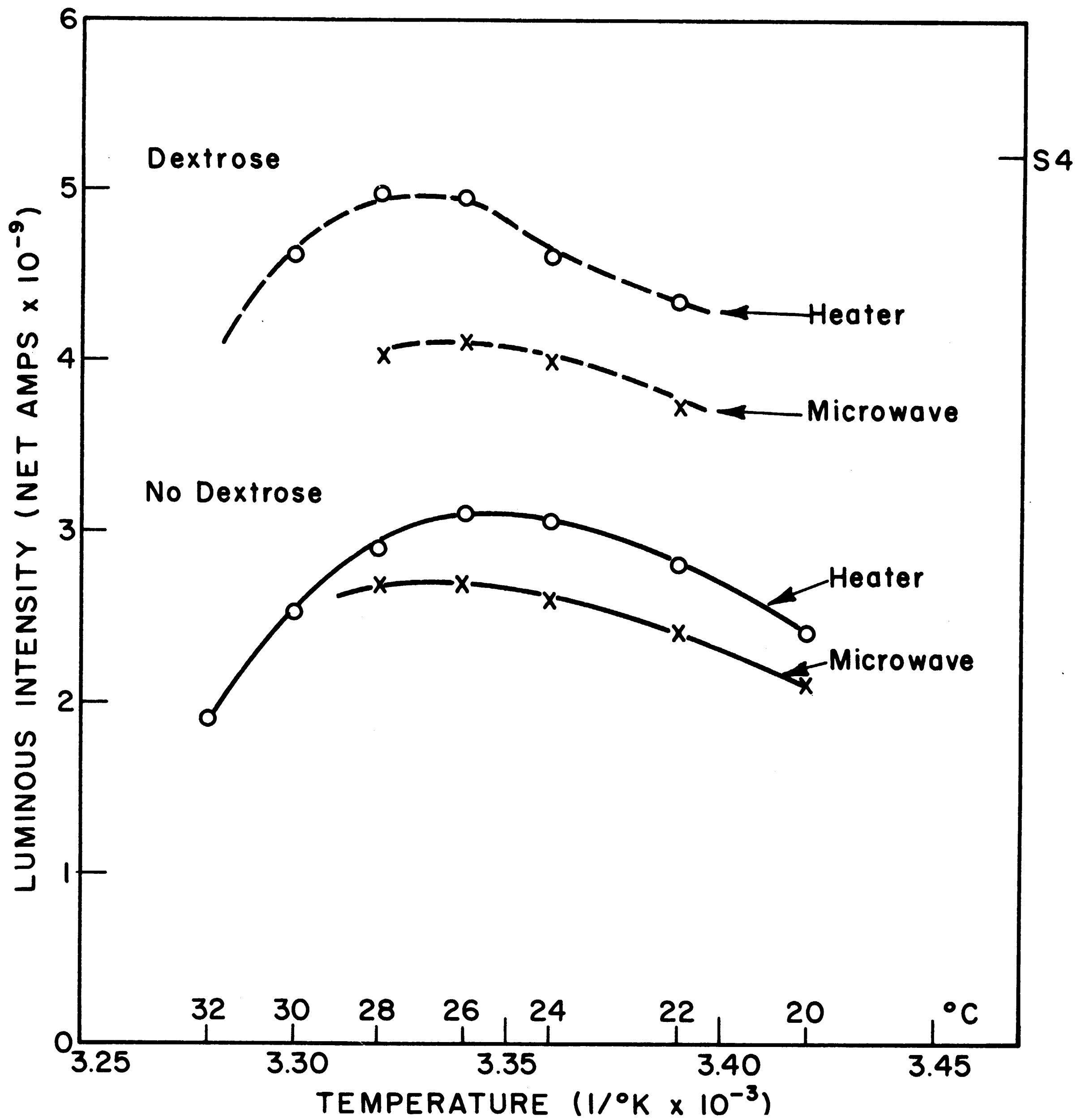


Fig. 8. Temperature of optimum luminescence vs. type of heating.

librium associated with the very rapid temperature measurements. The shift in the control curves is indicative of a difference in suspension heating between microwaves and the heating tape used to heat the controls. For the short times involved, the microwave system would be closer to an equilibrium temperature than the controls at the time of each measurement.

These observations suggest that there is a slight shift upward in the temperature of optimum luminescence as the suspension ages. The optimum for older suspensions with dextrose (Fig. 7) appears to be 28 ± 1°C.

4.1.4. GROWTH CHECKS

Four bottles of suspensions, prepared and standardized to a concentration of 10^9 b/cc on July 14, 1960, were stored approximately 24 hours in a refrigerator at about 6°C. A recount of the contents of each bottle on July 15 showed that the concentrations in all but one bottle were less than 10^9 b/cc. The one exception did not change in concentration. Another check on July 28 and 29 showed that three bottles of suspensions, standardized to 10^9 b/cc, showed no significant change in concentration after 28 hours of storage in the polystyrene bucket at less than 8°C. Hence, suspensions packed in ice and stored in the polystyrene bucket for up to 28 hours show no increase in bacterial concentration. Light curves for suspensions stored in this way for as long as 33 hours show no indication of growth.

4.1.5. CLUMPING DIFFICULTIES

Clumping of cells in the circulating system reservoirs did not present a problem at concentrations of 10^9 b/cc until duplication of control and irradiated suspension temperature histories was first attempted. Heating to the equivalent of about 20 watts input power caused serious clumping in both control and irradiated suspensions. Accordingly, the standard concentration was reduced to 5×10^8 b/cc. Some clumping still occurred in both controls and irradiated suspensions near the end of the dextrose runs, but control data showed that this was not a source of error. Further reduction in concentration was considered inadvisable since the light intensity from lower concentrations would have been too close to the minimum sensitivity of the photometer equipment after the suspensions had been circulated through the exposure and control systems.

4.2. Light-Measuring Equipment

The photometer assembly used to measure the luminous intensity of the bacterial suspensions consisted of a combination of several instruments which enabled light measurements to be performed during and after irradiations. The component parts of the assembly were: a regulated high-voltage supply, a micromicroammeter, and a photomultiplier tube in a housing designed for the purpose.

4.2.1. HIGH-VOLTAGE SUPPLY

The high-voltage supply chosen, the Model N-401 of Hamner Electronics

Co., provides either negative or positive output voltages in the range of 500 to 1800 volts at 5 milliamperes. The output is continuously adjustable through the range. Its regulation according to the manufacturer is 2.5 parts per million per ma, which is equivalent to 0.25% at 1000 volts and 1 ma.

4.2.2. MICROMICROAMMETER

This instrument was a Keithley Instruments, Inc., Model 410, having full scale ranges of 3×10^{-13} to 10×10^{-4} amperes, with ten scales having ten units full scale and the other ten scales having 3 units full scale. This arrangement provided overlapping of the scales. The zero setting was surprisingly stable for any single scale and remained so for changes of scale, provided the changes were to higher or lower scales having the same full-scale units. For changes between scales of three and ten units full scale, a small zero adjustment was required.

For all light measurements reported in this investigation, only the 10×10^{-9} ampere scale of the instrument was used. According to the manufacturer, the absolute accuracy of this scale is better than ± 4%. However, as will be noted later, one can do much better than this where differences are the important observation.

4.2.3. PHOTOMULTIPLIER TUBE

The *in vivo* emission spectrum of *P. fischeri* ranges from 4000 to 7000Å with a peak at 5000Å.[33] It is, therefore, required that light measurements be made with an instrument having good sensitivity in this range.

The most commonly used photocathode surface is designated S-11.[58] This surface has a spectral response range of 3000-7000Å with a broad peak from about 3800-4800Å. A photomultiplier tube having an S-11 response would, then, perfectly cover the emission spectrum of the bacteria and provide good sensitivity to the bacterial luminescence. The DuMont Model 6291 photomultiplier tube was selected for this reason.

4.2.4. PHOTOMULTIPLIER-TUBE HOUSING

The housing for the photomultiplier was made according to the specifications of the author. Figures 9 and 10 show a composite and disassembled view of the photomultiplier-tube housing and sample observation track.

The housing is made of black, anodized (a nonconducting surface) aluminum except for the screws. It features a slide which enables the tube to be darkened and removed from its carriage without the need for turning off the high voltage applied to the tube. The tube rides on a track through which three sample ports are bored, one of which is always covered by the tube portion of the unit. The tube may be moved to and fro on this track, thus enabling one to observe, in the time that it takes to move the tube, the light from two specimens and a light standard. The pin which serves to center the tube portion with the track sample holes is installed in such a way that the tube cannot be moved without first closing the dark slide on the tube portion.

The unit was satisfactory in performance, but a light leak on the

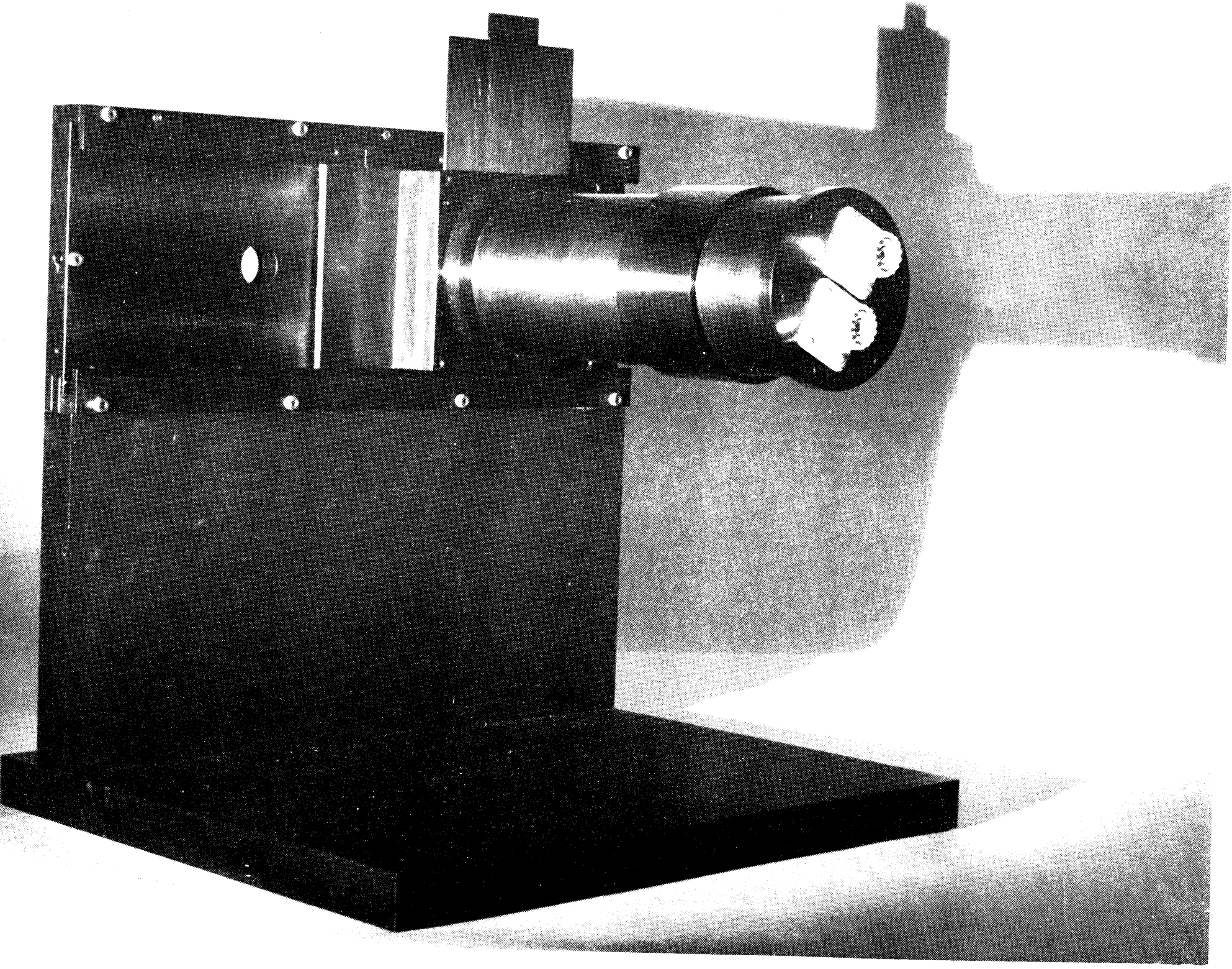


Fig. 9. Photomultiplier tube housing (assembled).

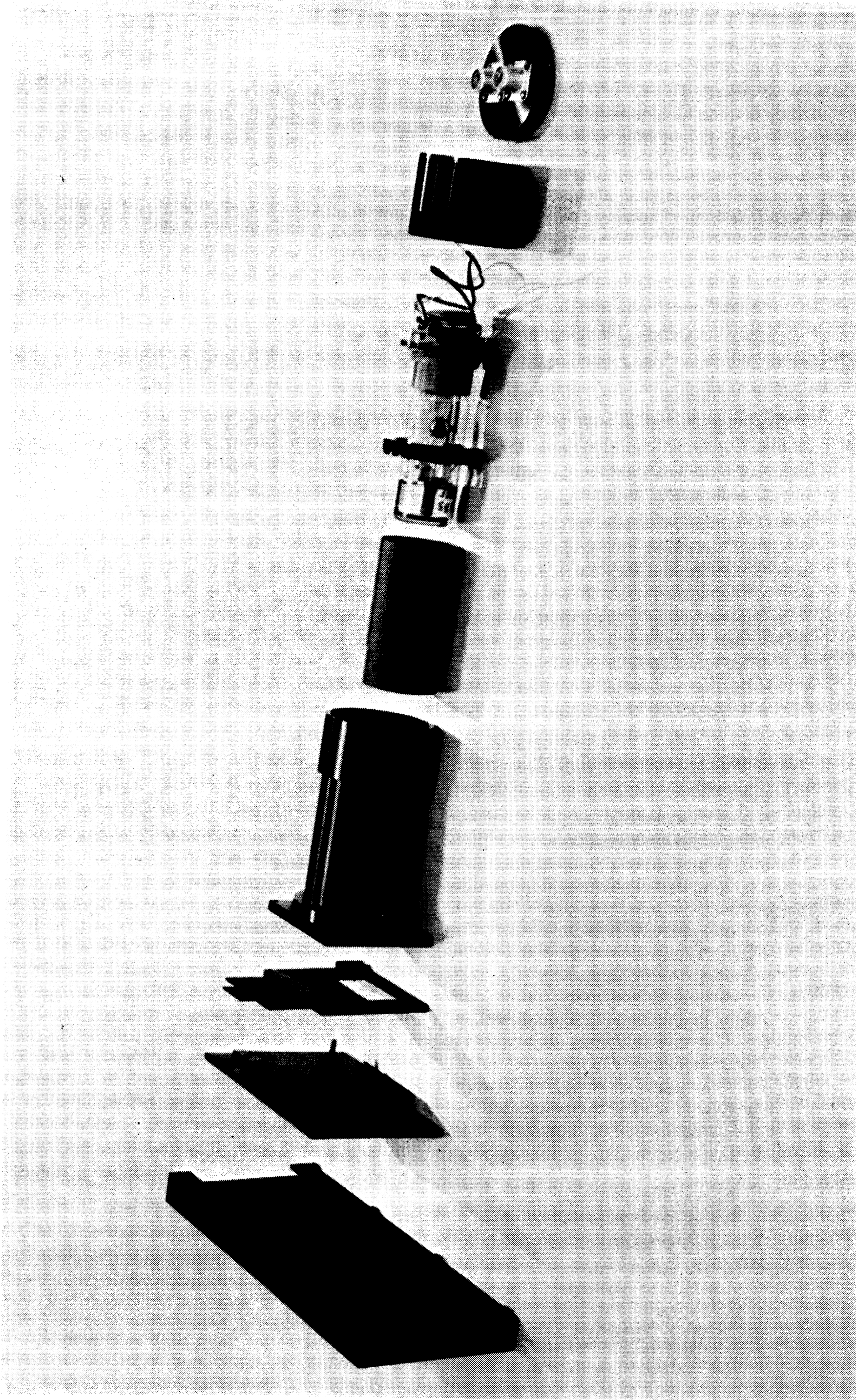


Fig. 10. Photomultiplier tube housing (dis-assembled)

photomultiplier tube side of the dark slide was observed when the slide was in the "down" position. Light leakage was evident for light beams parallel to the slide only. A flashlight beam shone on the point of leakage at any angle less than parallel to the slide gave no indication of a light leak. This leakage was eliminated by applying strips of black tape to the top of the slide.

4.2.5. OVER-ALL PERFORMANCE

Before each light measurement, the photometer dark current was zeroed out so that each reading represented a net intensity. The instrument was also standardized before each reading using a strontium-90 activated luminous standard as a one-point check on the proper operation of the photometer. Standardization adjustments were made to make the instrument read 5.2×10^{-9} amperes when the photomultiplier tube was exposed to the standard, using the fine high-voltage adjustment of the photomultiplier high-voltage supply, but these adjustments were rarely necessary.

Several light standards of different intensities were used to check the linearity of the photometer. Figure 11 shows plots of the log of net amperes versus voltage applied to the photomultiplier tube. The light standards used were labeled S2, S3, S4 in the reverse order of their brightness. Relative brightnesses for the standards were determined by Whipple in 1953.[59] These are:

S2/S3 = 4.70

S2/S4 = 10.9

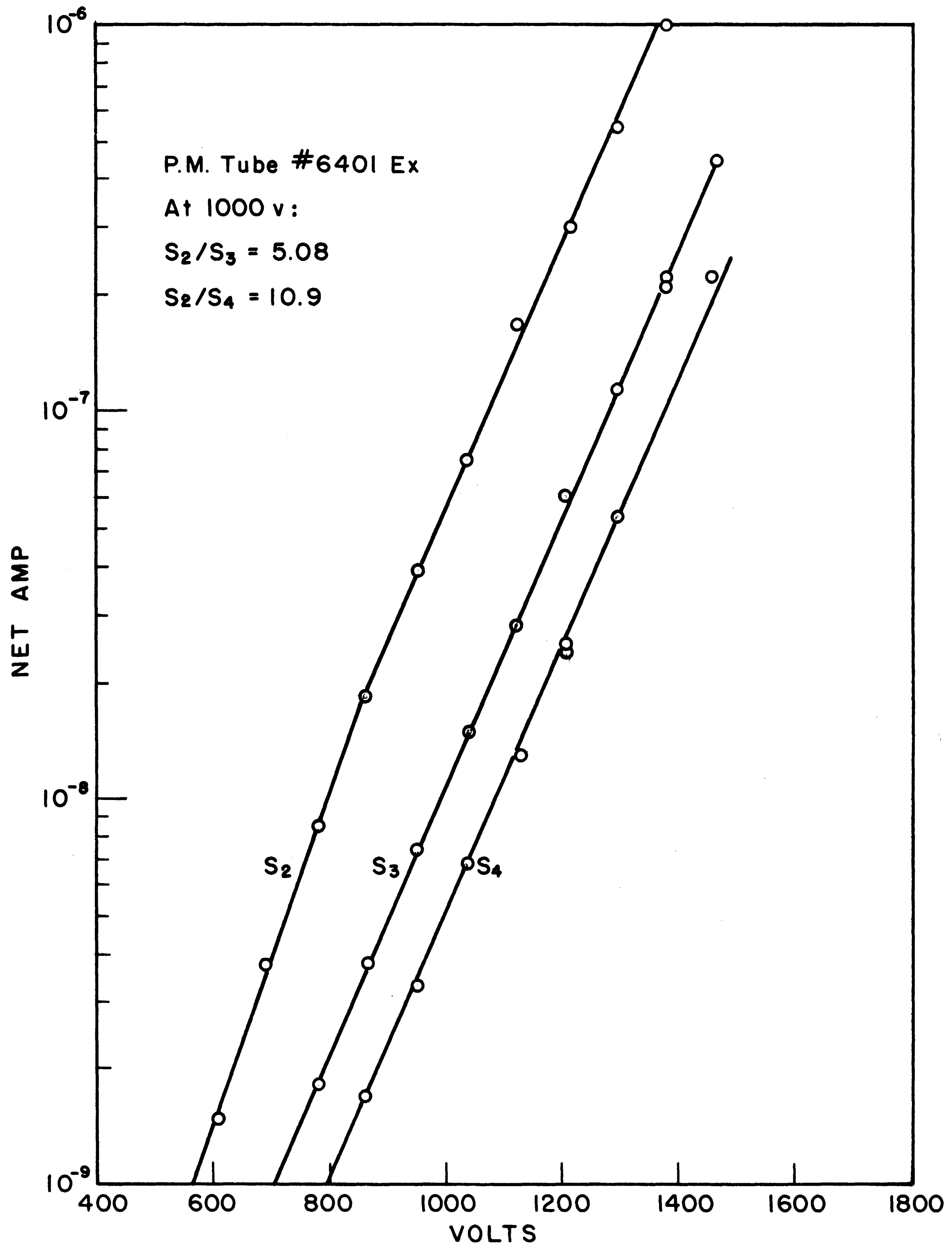


Fig. 11. Photometer linearity-voltage vs. current.

Note that the ratio of the intensities of these same sources in Fig. 11 agrees well with the above calibration values for voltages higher than 900. The ratio of S2/S4 is exactly as it should be and S2/S3 is only 8.1% higher than expected. The photometer can be said to be linear provided the high voltage applied to the photomultiplier tube is greater than 900 volts. The photometer was operated at about 1000 volts. The S4 source was used for routine standardizations of the photometer inasmuch as the light from the suspensions of bacteria was in this same range.

The over-all accuracy of the photometer assembly was estimated to be better than ± 4%.

4.3. Microwave Exposures

4.3.1. MICROWAVE EQUIPMENT

Table III lists the microwave equipment used for the exposures and pertinent specifications.

TABLE III

MICROWAVE EQUIPMENT

Equipment	Specifications	
1. Universal Klystron Power Supply FXR Machine Works, Inc. Type Z815B	Beam Voltage: Beam Current: Voltage Regulation:	200-2000 V 1800-3600 V 0-125 ma ± 3%
2. G. E. Precision Wavemeter Cat. 9007530G1 No. 2645574	Wavelength: Accuracy: Precision:	8-12 cm ±0.1% ±0.02%

TABLE III (Concluded)

Equipment	Specifications
3. Directional Coupler Cu 198/U C.S. Shutter Mfg. Co.	Coupling: 20 db Waveguide coupler
4. Waveguide Dry Load	Max. Rating: 100 watts
5. Waveguide Water Load Rad. Lab. Type 5119 Humble Oil, Ser. No. 1168	————
6. Tunable Bolometer Mount Mod. 475B Hewlett-Packard Co.	————
7. Power Meter Mod. 430B Hewlett-Packard Co.	Accuracy: ±5% of full scale
8. Attenuation Pad Polytechnic Research and Dev. Co. Mod. 130C	Attenuation: 10 db Calibration: ±0.5 db = ±5% Frequency: 2-4 KMc
9. Tunable Detector Mount Hewlett Packard Mod. 440A	————
10. 1N21B Crystal Sylvania	————
11. Two Waveguide Slotted Line Sections	————
12. Microammeter	Current: 0-200 μa
13. Magnetron Filament Supply	Maximum: 6.4 V; 3.4 amp
14. QK 59-62 Magnetron Series Raytheon	Frequency Pulling at VSWR of 1.5: 3.8 Mc Frequency Change at constant temperature over entire tuning range: 0 to +6 Mc, Range: 2.70-3.33 KMc Operating Beam Current: 70-120 ma Operating Beam Voltage: 900-1200 V

Table III is included here only to identify the equipment used and to provide the manufacturer's specifications pertinent to the choice of the frequency intervals of exposures.

4.3.2. CHOICE OF EXPOSURE FREQUENCIES

Table III provides an estimate of the frequency stability one might expect from a microwave generator used in the field, where large numbers of individuals may be routinely exposed. Total error in frequency is based upon the center of the frequency range expected from the QK 59-62 series magnetrons. The center of this range is about 3 KMc. From the above specifications, then, the maximum error is:

Frequency pulling	3.8 Mc
Generator Stability at Constant Temperature	6.0 Mc
Wavemeter Accuracy at 3 KMc	± 3.0 Mc
Maximum Generation and Measurement Error	12.8 Mc

This is probably a low estimate of error since the number is based entirely on manufacturers' specifications. No allowance is made for variations in frequency generation and detection due to thermal expansion and contraction of the magnetron cavities and the wavemeter cavity. Neither is allowance made for the change in frequency with change in magnetron anode voltage which will be a function of the stability of the high-voltage supply. The regulation of the high-voltage supply is ±30 volts at 1000 volts. This is enough to produce a change in frequency of a few

megacycles.

Exposures were made at intervals of 12.8 Mc.

4.3.3. EXPOSURE SET-UP AND PROCEDURE

Figure 12 is a block diagram of the exposure and control experimental arrangements. The numbers in the figure correspond to the numbers in the photograph of the experimental arrangement (Fig. 13).

Standardized suspensions were poured into the reservoirs of the circulating systems and were independently circulated and recirculated by the pumps through the waveguide or heating tape sections, past the photometer, through the coils in the water bath, and back into the reservoirs.

The Tygon tubing through the pumps was replaced at the end of each day and allowed to run overnight to allow the tubing to be "broken in." The pumps were run continuously so that they remained at temperature equilibrium. At the beginning of each run, tubing compressions and rates of flow were matched as closely as possible. In every case differences in flow rates between the two systems was less than 6 cc/min. The oiler pad of each pump was saturated with "3-in-1" machine oil at the end and beginning of each day.

Exposures were made in the following manner. The suspensions were poured into the reservoirs and allowed to circulate until the exit temperatures (temperatures just beyond the microwave or heater sections) were close together. The heater on the controls was then turned on and the powerstat turned to a position which would bring the controls to a tem-

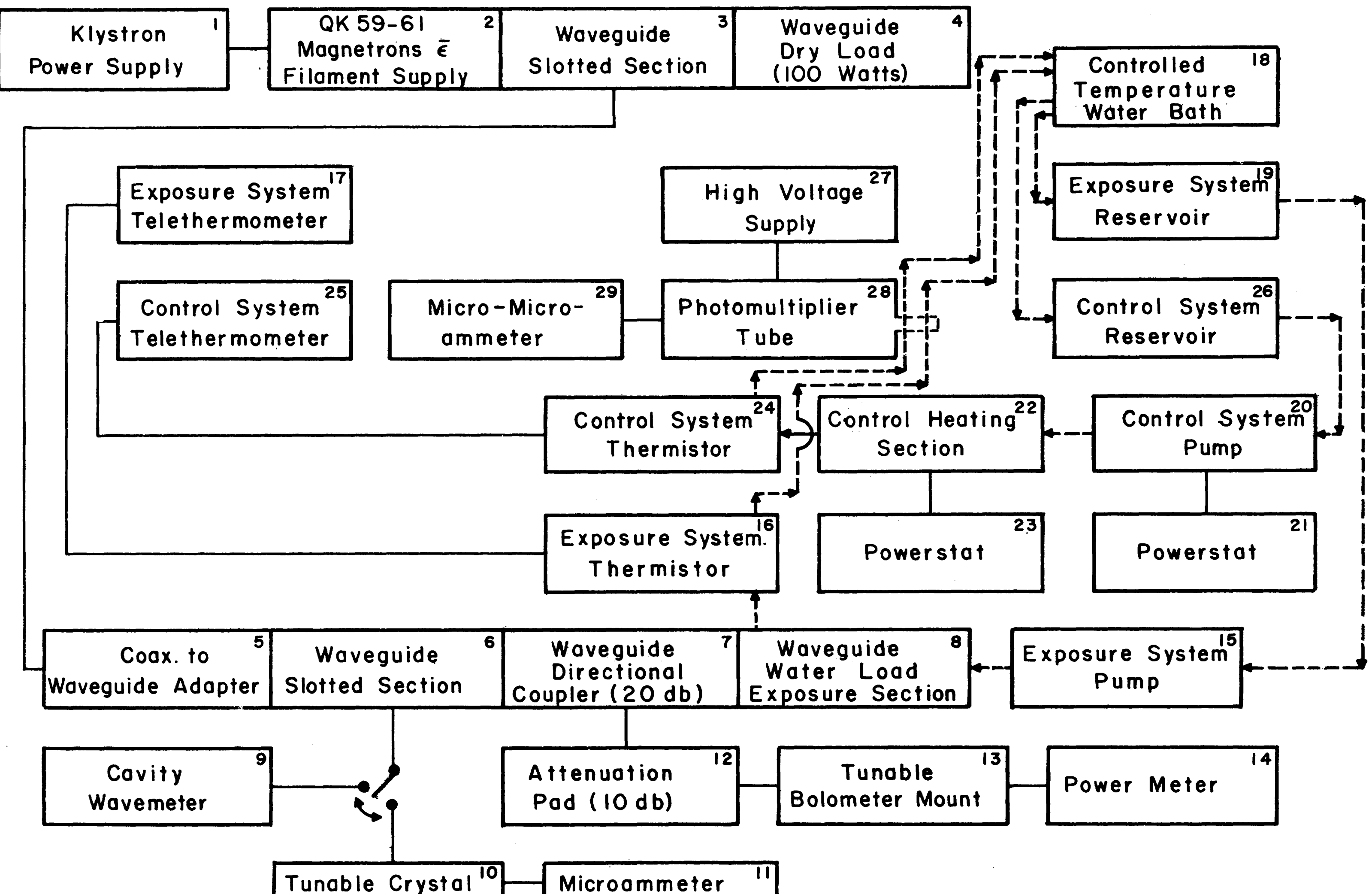


Fig. 12. Diagram of experimental arrangement.

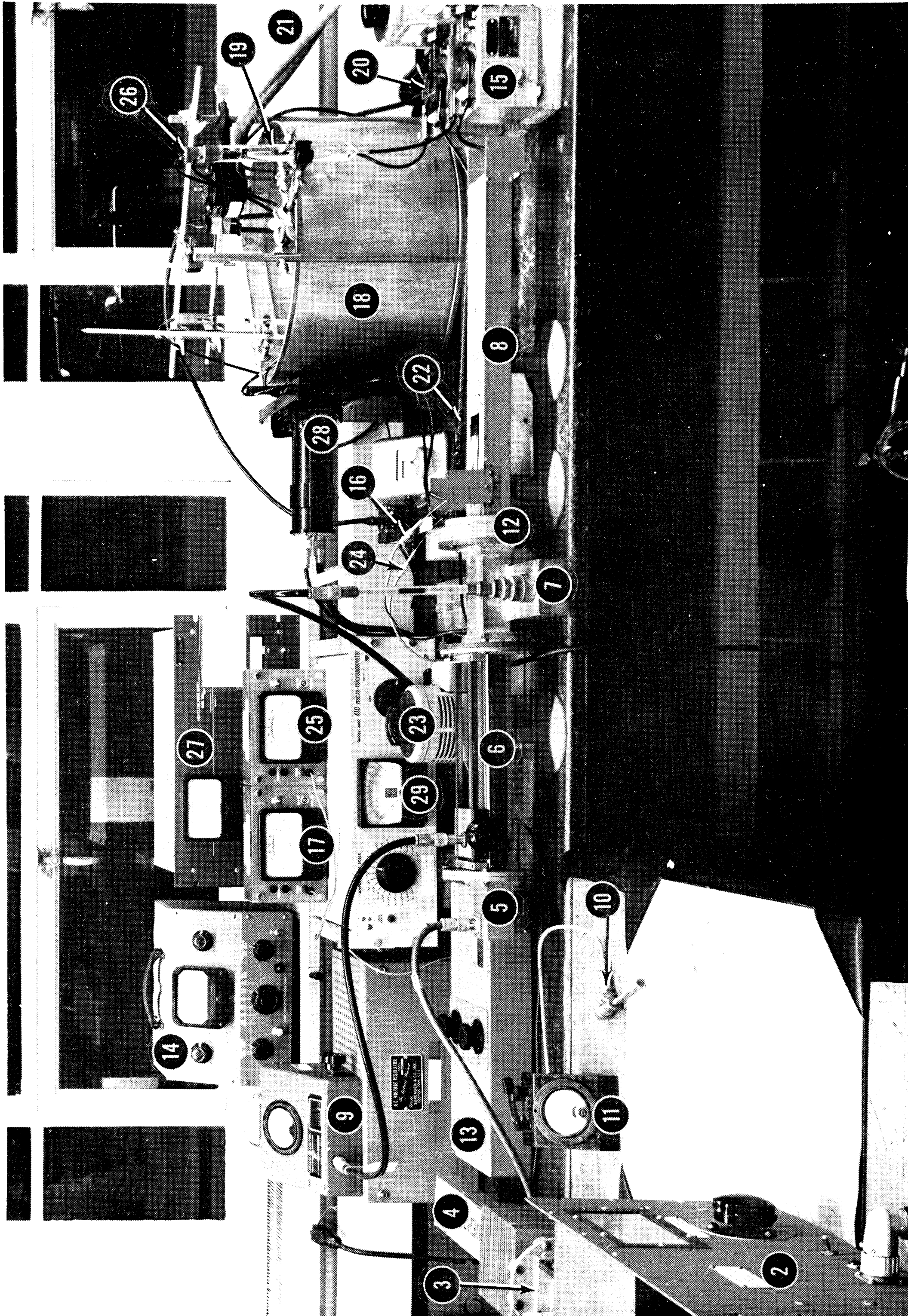


Fig. 13. Experimental apparatus.

perature previously determined by matching the exit temperatures of the two systems using distilled water with microwave power fed into the water load. The temperature of the controls would then increase and this exit temperature was duplicated in the irradiated suspension by slowly increasing the microwave power. The order of heating was important here since the microwave heated the suspension more rapidly than did the heater on the controls. This was to be expected since the microwaves heated the suspension in the load uniformly and directly while the controls were heated by conduction from the heating tape surrounding the control system section. The heater section of the control system was of the same size and configuration as the tube in the microwave water load. Both suspensions were recirculated until the irradiated suspension had been exposed to at least 1.7×10^9 ergs per gram.

Whenever possible, exit temperatures were kept below the temperature of optimum luminescence to avoid suppression of luminescence by enzyme denaturation. This was possible except on a few hot days when it was impossible to keep the exit temperature sufficiently low. Runs made on these days were repeated at lower temperatures. The runs having exit temperatures above the temperature of optimum luminescence were useful in showing, rather obviously, that the microwave heating and the heating tape heating of the controls were different.

After each exposure, the suspensions were drained back into their respective storage bottles and stored in ice until the following day. On

the day following the exposure, the control suspensions were re-introduced into the circulating systems, and allowed to run about 10 to 20 minutes before 4.7 cc of 0.4M dextrose solution were added to each system. The luminous intensity was measured on each suspension just beyond the point at which the temperature of the suspension was measured until approximately 150 minutes had elapsed following the addition of the dextrose. Decay in luminous intensity was noted with increased storage time, but this was unimportant here since each pair of suspensions was stored in the same manner and for the same length of time both before and after exposure.

4.3.4. POWER-METER CALIBRATION

The experimental arrangement enabled the collective calibration of the power meter, attenuator pad, and directional coupler. Hypodermic thermistors were inserted into the microwave exposure system at the entrance and exit of the waterload. Entrance and exit temperatures were recorded for several power levels while distilled water was circulated through the system at known flow rates. The power was computed from the relation given in Section 3.3.1. Figure 14 is a plot of power meter reading as a function of computed power.

The differences observed between the three calibration curves are attributed to radiative heat loss variation with flow rate. Errors due to radiative heat are inversely proportional to flow rate at a given power level. The curve (1), run at the higher flow rate, is the most accurate of the three. It can be seen from Fig. 14 that calculated power for any

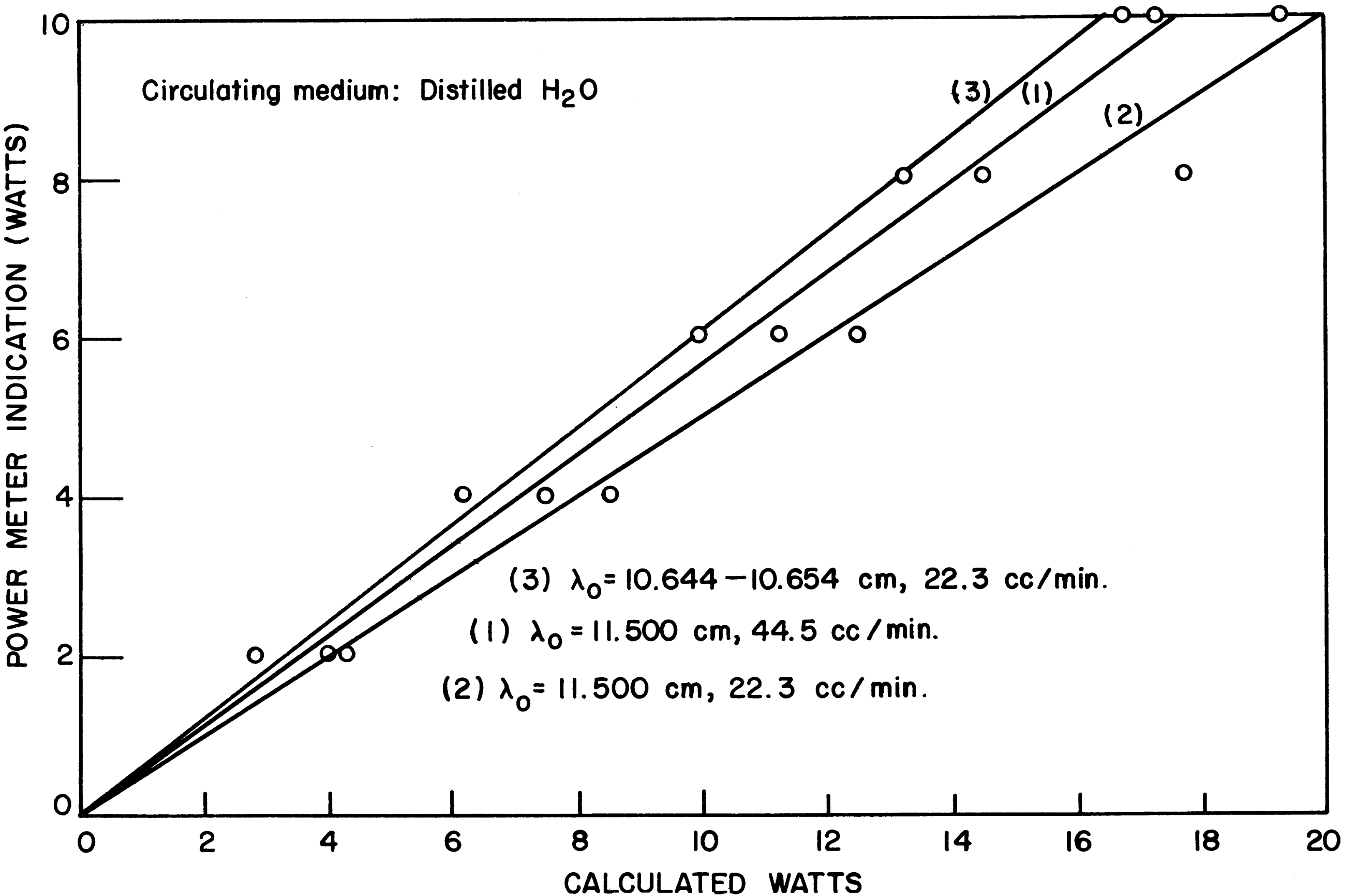


Fig. 14. Power meter, attenuator and directional coupler collective calibration.

given meter reading is within about ±11% of the computed power for curve (1). Hence, flow-rate variations of a factor of 2 make a comparatively small difference in the determination of microwave power.

The curves are numbered in the order in which they were run.

4.3.5. TOTAL DOSE TO THE SUSPENSIONS

The total dose received by each suspension was determined by the following formula:

$$D = tT\,(v/V^2)(1 - \Gamma^2) \qquad (12)$$

where,

D = total dose (ergs/gm)

T = transmitted power (ergs/min)

t = total exposure time (min)

v = volume of guide exposure section (cc)

V = total volume of suspension (cc)

Γ^2 = fraction of power reflected

$= (VSWR - 1)^2/(VSWR + 1)^2$

VSWR = voltage standing-wave ratio

For a VSWR as high as 2.0, the fraction of the transmitted power reflected is only 0.11. Only in rare instances was the VSWR this high and in most of the exposures it was less than 1.5, which resulted in less than 5% of the power being reflected. The reflection coefficient (Γ) therefore has been considered to be zero in all cases and dose computa-

tions were reduced to the nearest 0.1 x 10^9 ergs/gm.

The volume of the exposure section and the heating section of the controls was held constant at 28 ± 0.5 cc. The total volume of the suspension used in each system was held constant at 60 cc. This and the above argument permits the reduction of the dose formula to:

$$D = 4.67 \times 10^6 \, tT \qquad (13)$$

where

D = total dose (ergs/gm)

t = total exposure time (min)

T = transmitted power (watts)

4.4. Circulation of Suspensions

Control and irradiated suspensions were circulated through their respective systems with a pair of peristaltic-action pumps, New Brunswick Scientific Company, Model PA-93. The pump consists of a race of cylindrical bearings mounted on the rotor of a motor, with provision made to compress flexible tubing (in this case 1/4-in. OD x 1/8-in. ID, Tygon, formulation R3400) against the moving bearings by adjusting a movable yoke directly adjacent to the bearing race. Figure 15 is a photograph of the pumps.

A determination of pumping rate for various tubing compressions showed that flow rate goes through a broad maximum as the compression is increased. The height of the maximum is a function of tube running time prior to

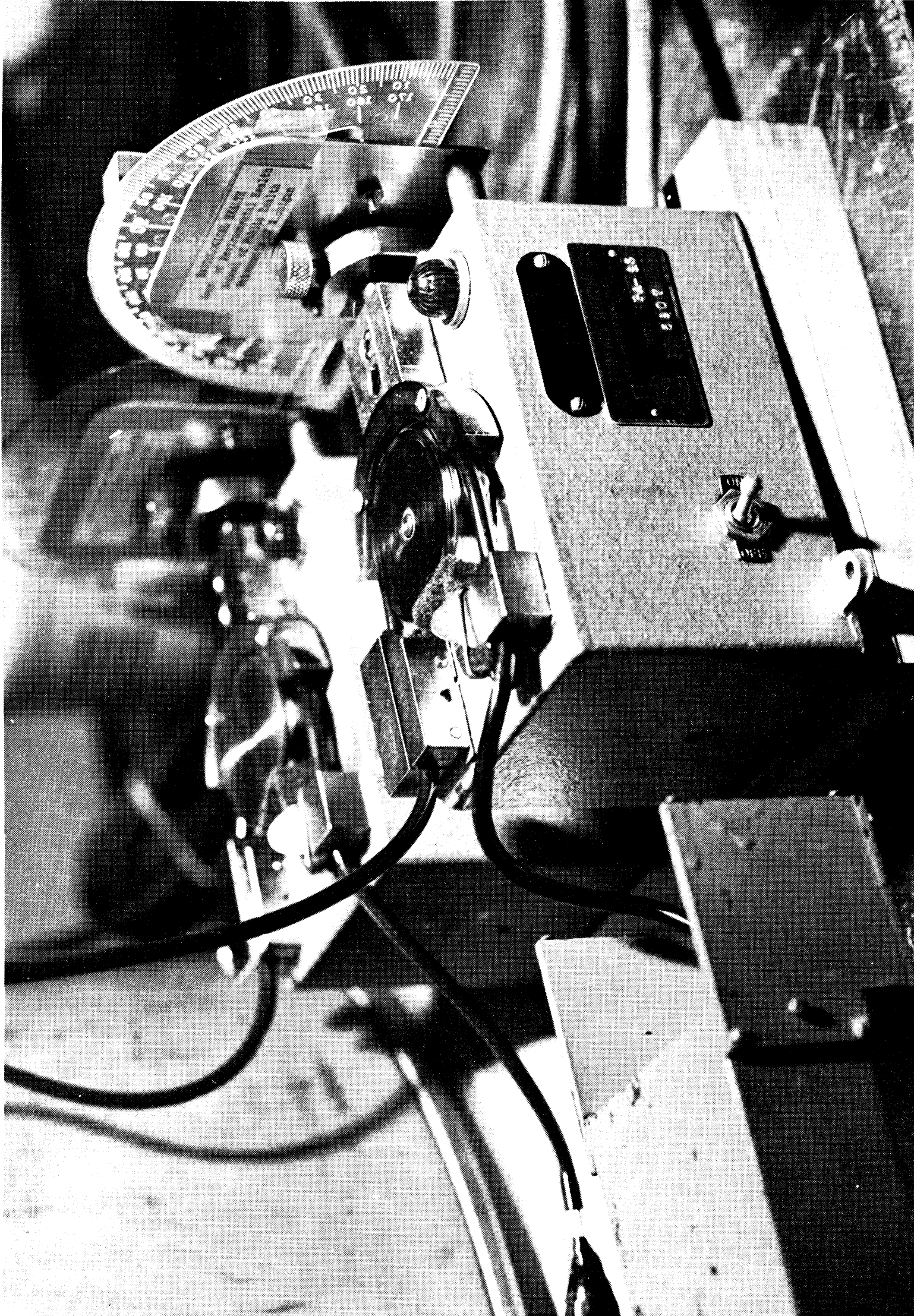


Fig. 15. Peristaltic action pumps.

measurement and "anti-creep" clamp pressure. A fixed clamp setting and pre-running of tubing in the pump is essential to flow-rate reproducibility. The tubing compression at which maximum flow rate occurs is a function of running time and temperature.

The pumps were run continuously at high tubing compression to give maximum flow rate. Oiling of the tubing and the pump rotors with "3-in-1" oil reduced tubing cracking and smoothed out flow rate. A tubing compression experiment, in which the microwave system pump was run at a reduced tubing compression, and the flow rates in the two systems were matched by reducing the rpm of the control pump at high tubing compression, eliminated tubing-compression differences as a source of light-intensity differences in the bacterial suspensions.

Originally, pump flow rates were matched by observing the rate of fall of distilled water in each system prior to each exposure and dextrose run. The match was checked occasionally during each run by visual observation of the drop rate as the suspensions returned to their respective reservoirs. Experience showed that it was possible to observe a difference in flow rate of 6 cc/min or more between the two systems. This turned out to be a saving grace for it was discovered that syphonage did occur from time to time in one or the other of the two systems even though flow rates were set at maximum. This of course would place the matching of flow rates by determination of individual reservoir-level fall rate in error unless the pumps were always required to work against the same head.

The need for a better method of matching the flow rates of the two systems led to the discovery that all that was necessary to match the flow rates was to interchange the lines returning to each system reservoir and adjust the pump tubing compression until the two reservoir levels were steady. Figure 16 is an abbreviated diagram of this method of matching flow rates. This method eliminated much adjusting of tubing compression on the pumps, but it was still necessary to determine the flow rate by observing the rate of fall in one of the reservoirs.

Some variation in uniformity of the Tygon tubing became apparent. It was noted that if the tubing brand mark were placed so that the brand always faced the rollers of the pumps, the tubing offered less resistance to the pumps and slightly higher flow rates could be obtained.

4.5. Suspension-Temperature Measurement

The temperatures of the two suspensions were taken at the point of maximum temperature in each system. Hypodermic thermistors were injected through the tubing in each system at a point just beyond the down-stream end of the exposure section of the microwave system and the heating section of the control system. These temperatures are referred to as "exit temperatures." The temperature was indicated on two "Tele-thermometers" manufactured by the Yellow Springs Instrument Co. for use with the hypodermic thermistor probes.

The tele-thermometers were calibrated in a water bath against a 0-

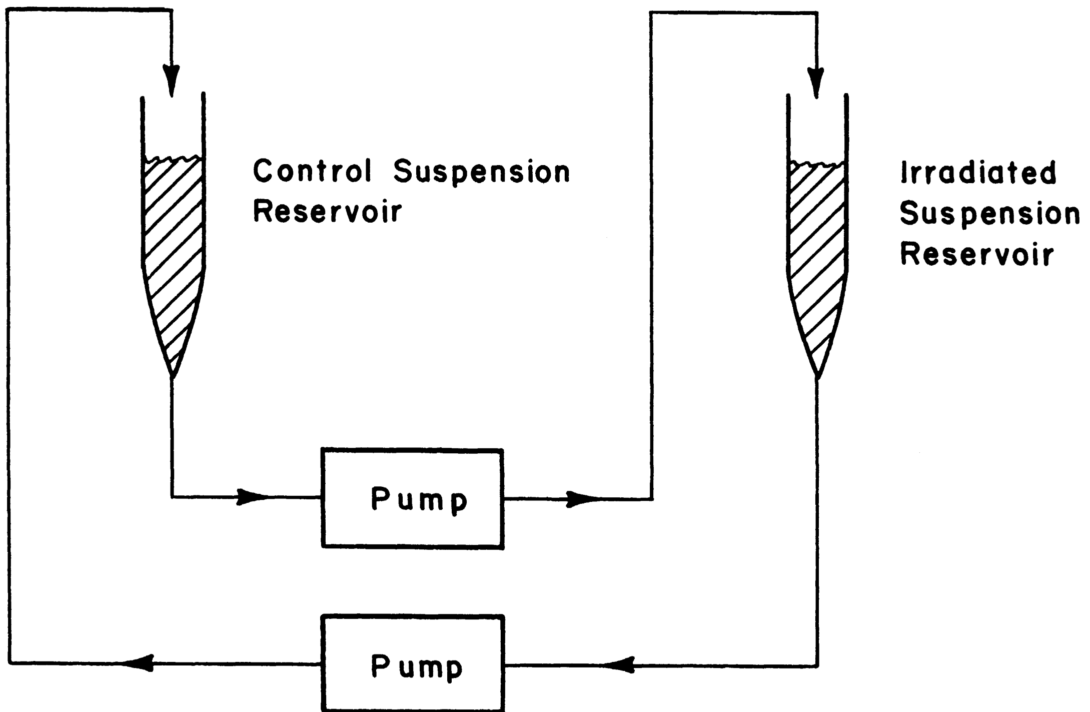


Fig. 16. Configuration of systems for matching flow rates.

50°C, mercury thermometer of ±0.1°C accuracy. The calibration curves for both thermistors were linear, as shown in Fig. 17. All exit-temperature readings were corrected to the equivalent mercury thermometer temperature using the curves of Fig. 17. The accuracy of the tele-thermometers is, according to the manufacturer's specifications, ±0.5°C with a readibility of ±0.2°C. The curves show that they are at least this good. A one-point check of the calibration was made from time to time by immersing the thermistors in the water bath used to cool the circulating suspension. The calibration held very well throughout the experiments.

There was no error introduced into the temperature measurement due to direct microwave pickup by the thermistor probe or leads. This was checked by positioning the probe in the line nearer the waveguide than usual and varying the position of the leads to the meters.

Figure 18 shows a thermistor inserted into the suspension line.

4.6. Data Analysis

4.6.1. EXPERIMENTAL DATA

Typical dextrose-response curves are shown in Fig. 19. The area under each curve from the time of addition of the dextrose to 150 minutes following the addition of dextrose was determined. The average of three determinations made with a planimeter was used. The ratio or difference between the area under each pair of curves was then taken as the statistic to be analyzed. The light-area ratio and difference will be referred to as ΔR

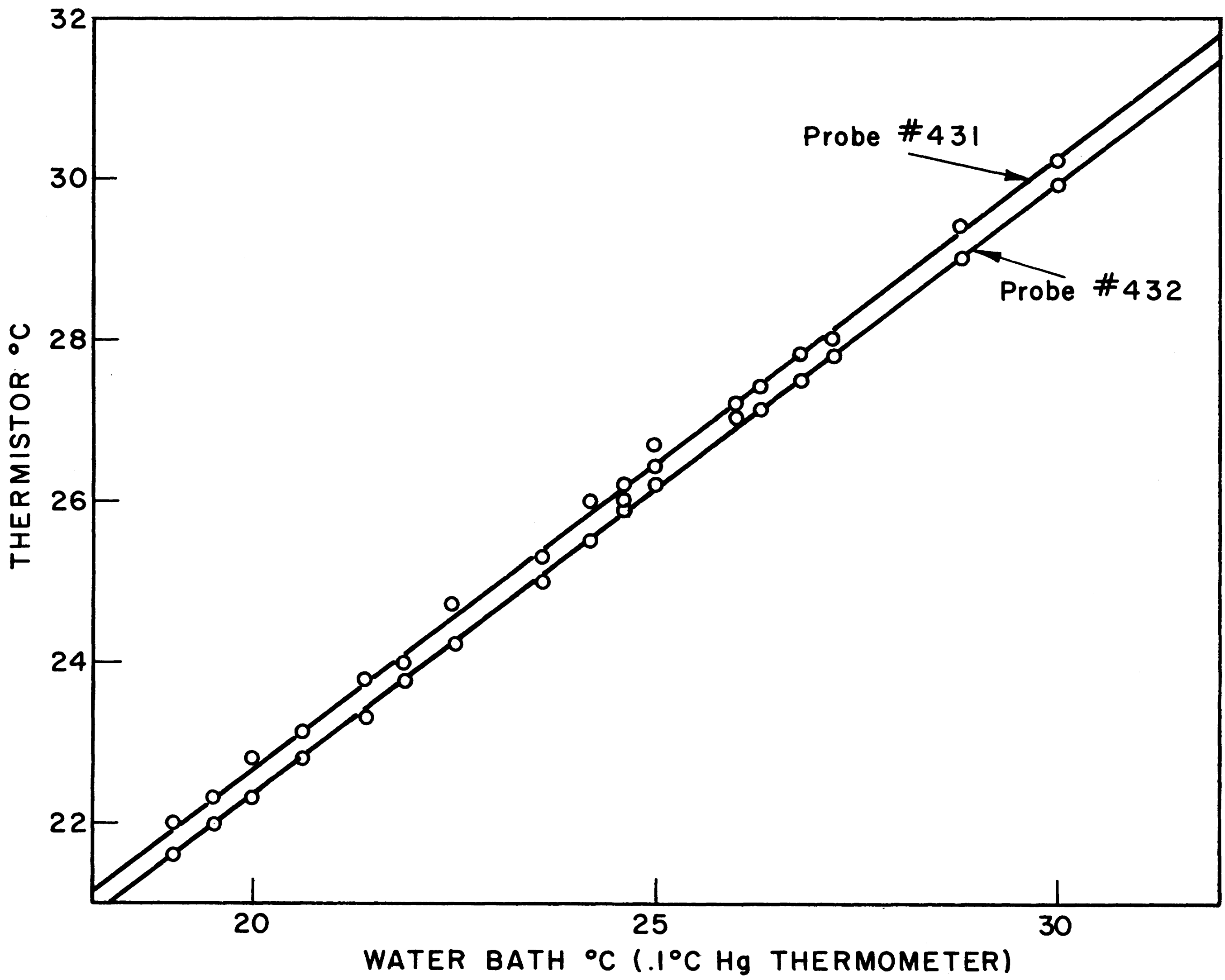


Fig. 17. Thermistor calibration curves.

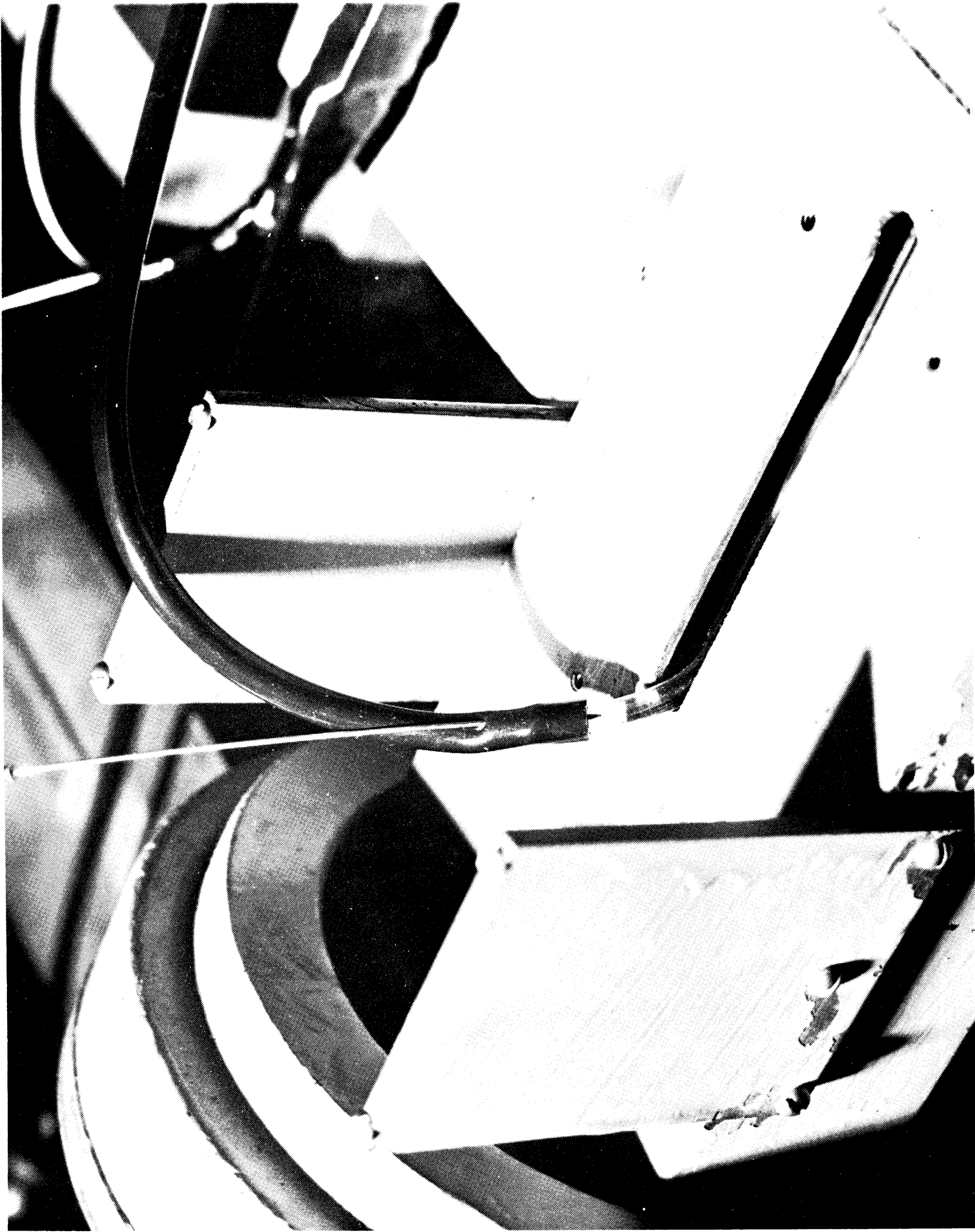


Fig. 18. Hypodermic thermistor.

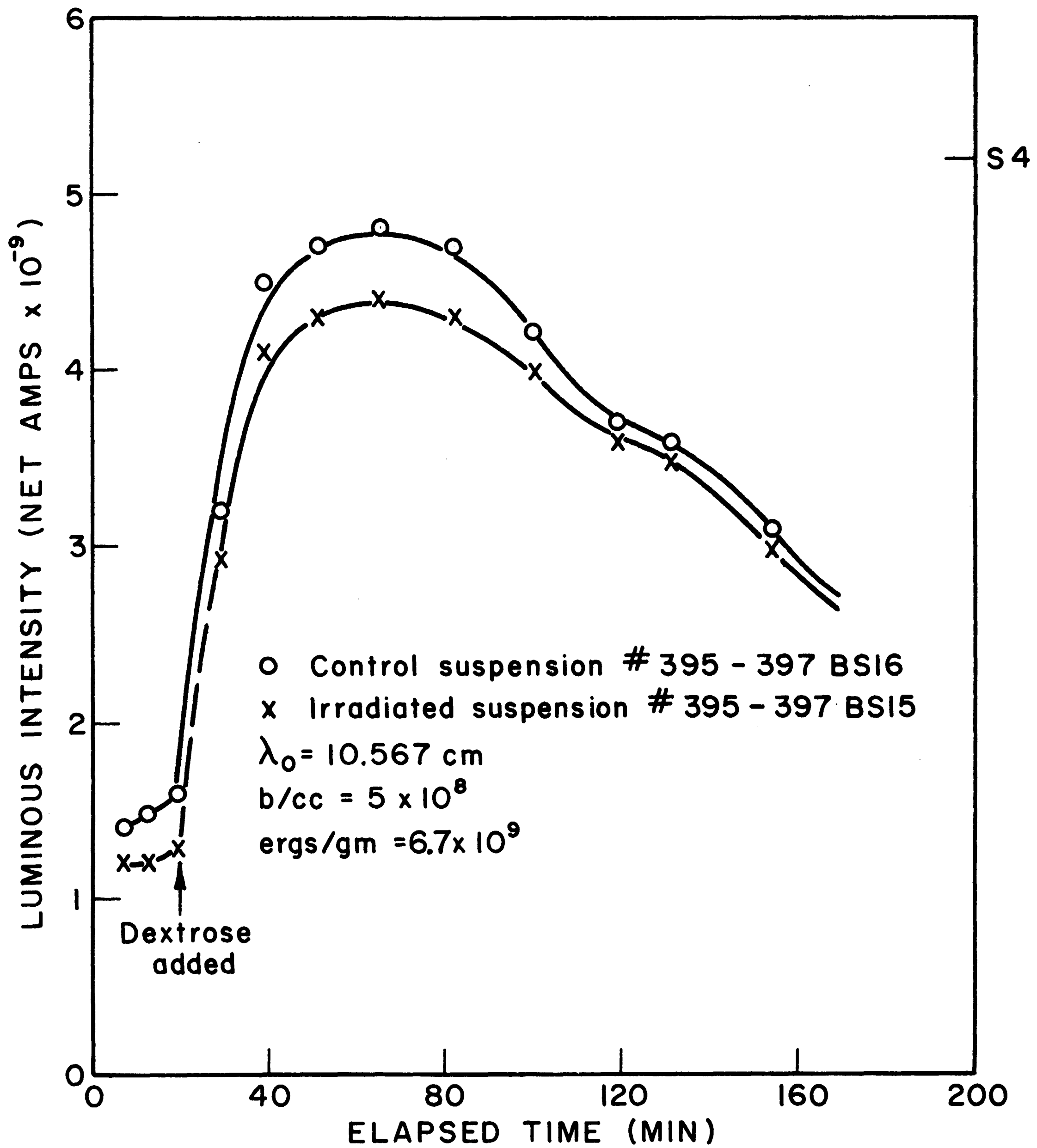


Fig. 19. Luminous intensity vs. time.

and ΔD, respectively.

A summary of the experimental data appears in Tables IV through VI in the Appendix. ΔR's and ΔD's were shown to be normally distributed; hence, familiar statistical analyses were applicable to the data.

Application of the Student t-test to the ΔD's shows that the mean of the differences is significantly nonzero and positive at the 95% confidence level.[57] This same test applied to the ΔR's shows the ratios to be significantly less than 1.00 at the 95% confidence level. This means that if the experimental data were to be collected an infinite number of times, we could expect the mean of the differences and the mean of the ratios to be significantly greater than one and less than one, respectively, in 95% of the repetitions of the experiment.

The correlation coefficients between the ratios or the differences of the light-area data are given in Table VI (Appendix). The correlation coefficients were computed according to the formula provided by Freund.[60]

According to Dixon and Massey,[57] the hypothesis that the correlation coefficient (r), is zero (i.e., no correlation exists) should be rejected at the 1% level of significance if $\underline{r}$ lies outside ±0.33 for an experiment involving 60 observations. The data here involve 63 observations of a single variable, close enough to allow the use of the ±0.33 limit. If $\underline{r}$ lies within these limits, there is no reason to doubt that the two variables involved are independent. In other words, if the experiment were repeated, the same relationships could be expected to occur 99% of the time.

Table VI shows that the following variables are independent:

a. ΔD or ΔR and microwave frequency

b. ΔD and total exposure time

c. ΔD and flow rate during exposure

d. ΔD and exit temperature minus bath temperature

e. ΔR and storage time

f. ΔR and temperature during dextrose runs

g. ΔR and flow rate during dextrose runs

h. ΔR and culture age

i. ΔR and total dose

j. ΔR and exposure power level

k. ΔR and time of addition of dextrose

The fact that there was no correlation between ΔD and exposure time indicates that there is no correlation between ΔD and dose rate since the power level and total dose were constant for 70% of the exposures. The absence of a relationship between dose rate and observed ΔD is substantiated by the absence of correlation between ΔR and power level, as well as the absence of correlation between ΔD and exposure flow rates. This latter observation also shows that the pump differences do not influence the light differences.

A word of caution is necessary about the use of the correlation coefficient as a means of proving the existence or nonexistence of relationships between variables. According to Freund,[60] the correlation coefficient is merely a measure of the goodness of fit of a regression line.

Interpretation of relationships based on the computation of the correlation coefficient are valid only if these relationships are reasonably linear. The analysis does not apply where peaks may be involved such as were expected between ΔD's or ΔR's and microwave frequency at which the suspensions were exposed. The fact that no correlation exists between the exposure frequencies and ΔD or ΔR indicates only that there exists no linear relationship between these two variables.

A plot of ΔR as a function of frequency is given in Figs. 20-22. The mean of the ΔR's is 0.913. It is difficult to place error estimates on the points because of the dependence of ΔD or ΔR upon temperature. But the error estimate for each point on the basis of power and dose estimates, VSWR variation, and photometer errors is ±20%. More exposures were made at different temperatures than were at the same temperatures. But inspection of the figures shows that all points would overlap if an error of ±22% were associated with each. But 87% of the points fall within ±10% of the mean. It is not unreasonable to attribute the remaining points which lie outside these limits to chance.

Additional support of the independence of ΔR and frequency lies in the inability to repeat seemingly large differences at certain frequencies such as at 2.839 KMc.

All but one of the ΔR's greater than one can be explained on the basis of high temperature. If these points are neglected, then 95% of the remaining 57 points lie within ±10% of the mean. There are, then, no

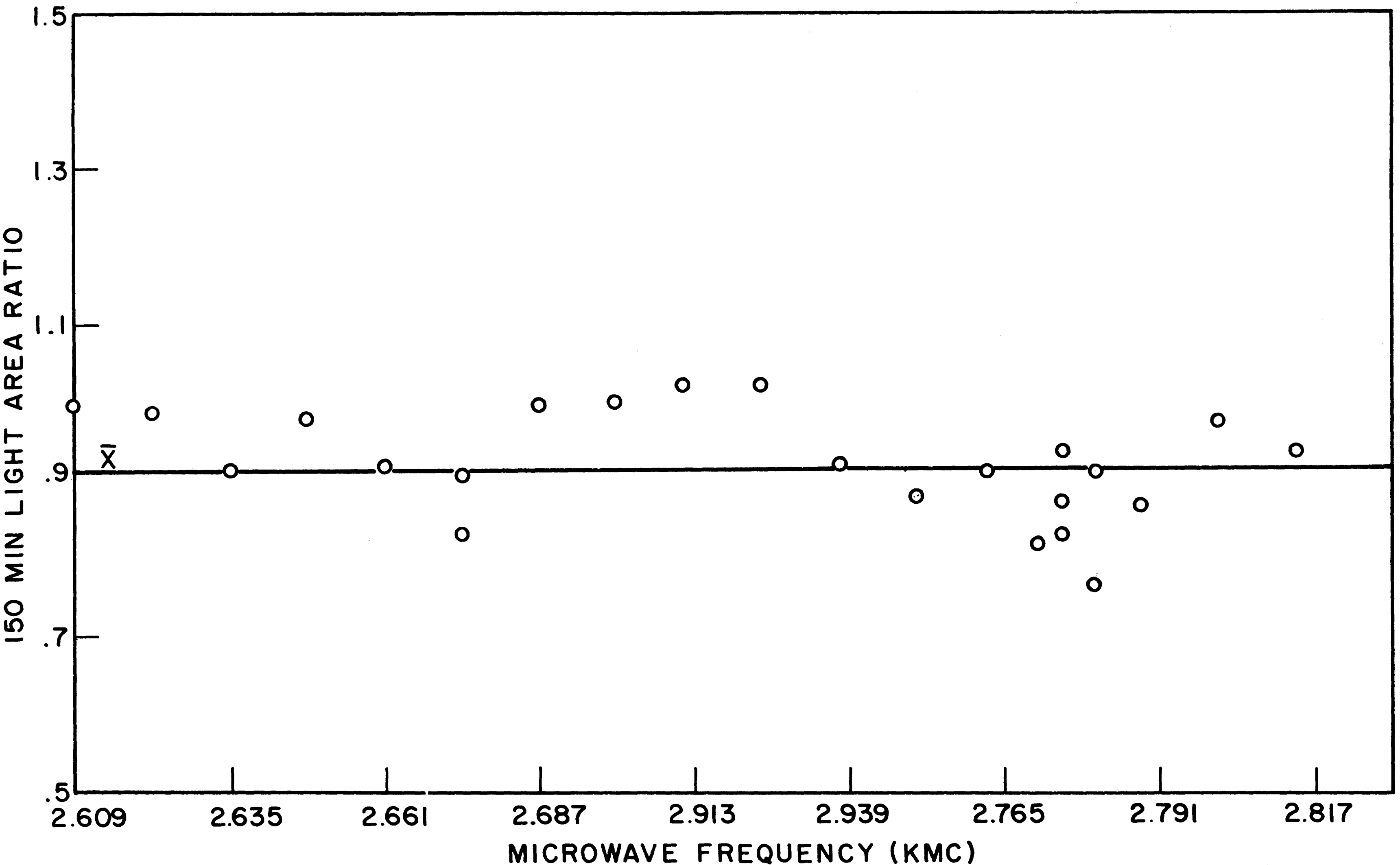


Fig. 20. Light area ratio vs. microwave frequency 2.609-2.817KMc.

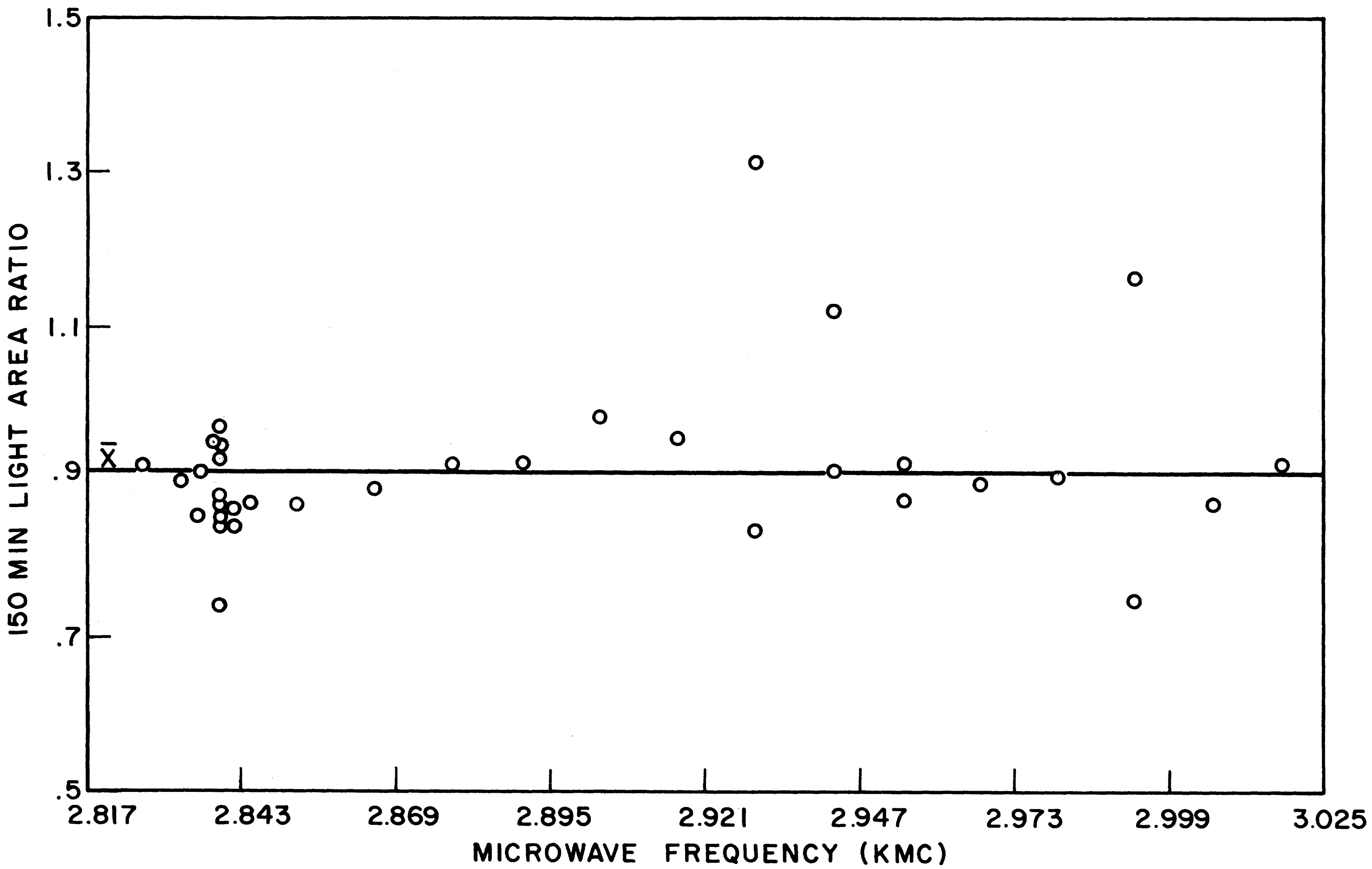


Fig. 21. Light area ratio vs. microwave frequency 2.817-3.025KMc.

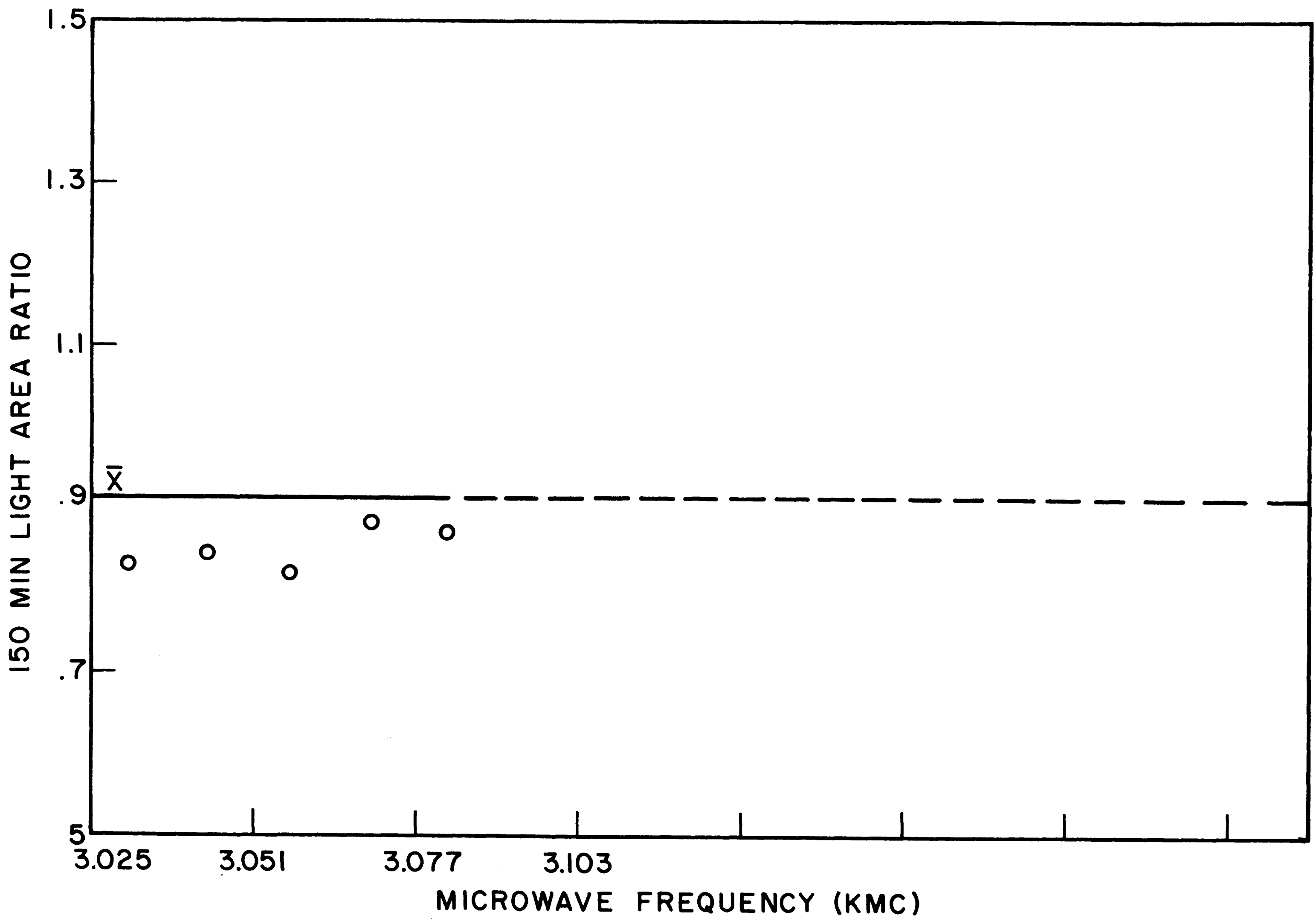


Fig. 22. Light area ratio vs. microwave frequency 3.025-3.082KMc.

obvious peaks in the plots of light ratio versus frequency.

The following variables are not independent:

a. ΔR and ΔD

b. ΔR and intensity difference pre-dextrose

c. ΔR and intensity difference end of irradiation

d. ΔD and irradiated exit temperature

e. ΔD and room temperature during exposure

f. ΔD and bath temperature during exposure

The very high correlation coefficient between ΔD and ΔR indicates that one may use either as the statistic for comparison with another and arrive at comparable answers.

The correlation between the difference in intensities at the end of the irradiations and ΔR the following day indicates an immediate effect which is sustained until the following day. This suggests that addition of dextrose during exposure may prove interesting.

No correlation computation was performed between control exit temperature and irradiated exit temperature during exposure; for, except in two cases when the temperature difference between control and irradiated suspensions varied as much as 1.5°C, temperature differences were never greater than 0.9°C. Furthermore, temperatures of control and irradiated suspensions during the dextrose runs did not differ by more than 0.5°C. This is a negligible variation as indicated in Fig. 7. For temperatures ranging from 25.0 to 30.3°C, a variation of 0.9°C would result in light intensity only 4% below optimum at most. A variation of 0.5°C in this

same range would result in a luminous intensity of only 2.5% below optimum. These errors are small compared to variations in ΔD. Most interesting are the relatively high correlation coefficients between light differences and the several temperatures: irradiation exit temperature, bath temperature, and room temperature during exposures. These three temperatures are all related in that low bath temperatures were impossible without attendant low room temperatures, and the exit temperatures were naturally functions of the bath temperatures. Note that the temperature correlation coefficients are all negative, i.e., the higher the exit temperature, the smaller the difference between the control and irradiated suspensions.

4.6.2. DISCOVERY AND IDENTIFICATION OF A SYSTEM CONTAMINANT

Dissection of the circulating systems revealed a visible contaminant on the walls of the Tygon tubing. The contaminant was cultured on nutrient agar slants. After 24 hours of incubation at 37°C, excellent growth exhibiting a blue-green sheen but no luminescence or fluorescence under ultra-violet light was observed. The unknown also grew at room temperature but less well than at 37°C. It could not be made to grow on media used for the luminous bacteria at 19.5°C, nor would it grow on EMB media (Eosin-methylene-blue media) at 37°C. A broth culture grown for several days at 37°C showed pellicle formation, and slants incubated at room temperature for several days developed a characteristic Pseudomonas odor.[61]

Microscopic examination of the unknown cultures showed the organism to be a small, motile, Gram-negative bacillus which occurred singly, in

pairs, and in clumps.

Nutrient agar slants were inoculated from the distilled water wash bottle used routinely and exclusively for rinsing the suspension-circulating systems between runs. Growth occurred which was in every respect similar to the characteristics of the unknown. The distilled water, therefore, was probably the source of the contaminant.

The following genera and families of bacteria are among those most frequently present in natural water: *Enterobacteriaceae*, *Micrococcus*, *Achromobacterium*, *Bacillus*, *Proteus*, *Leptospira*, *Pseudomonadaceae*, and *Flavobacterium*.[62] Consideration of the characteristics of these from Bergey's Manual[22] and the characteristics of the unknown enabled identification of the unknown by the process of elimination to the genus *Pseudomonas*.

The genus *Pseudomonas* of the family Pseudomonadaceae may or may not produce a water-soluble pigment which is bluish, greenish, or brownish in color. It is a Gram-negative, nonspore-forming bacillus which may or may not be motile. They may be found in soil and water, including sea water or heavy brines. *Pseudomonas* may also attack dextrose oxidatively. There is the possibility, therefore, that such an organism could compete with *P. fischeri* for dextrose in the circulating systems.

CHAPTER V. DISCUSSION AND CONCLUSIONS

5.1. Differences between Pairs of Light Curves

At temperatures less than optimum, the suspensions in the microwave system invariably gave lower light readings than those in the control system, but at exit temperatures greater than optimum, the reverse was true. This is attributed to the existence of a radial, temperature gradient in the controls. At the higher temperatures, a portion of the suspension near the glass wall of the control heater section is presumably heated beyond the temperature of no recovery with the result that the light from the controls is depressed below that of the irradiated suspension.

Similarly, when the exit temperature is less than optimum, a fraction of the controls is heated to a higher temperature than that of the irradiated suspension but a fraction of the suspension near the center of the heating section is at a lower temperature than the controls. In this case, the effects of the two temperatures cancel and the difference between the controls and the irradiated suspensions should remain reasonably constant unless the microwave exposure produces nonthermal effects.

The argument for the influence of a radial temperature gradient in the controls is supported by the observation that the large difference

between control and irradiated suspensions at the end of exposure at high exit temperatures has narrowed by the following day. This suggests that the effect is due to temperature and that the luminescent capability of portions of the suspension do recover when the heat exposure is discontinued.

At first the differences observed between pairs of light curves were thought to be the result of a non-thermal effect produced by the microwave exposure. However, the same magnitude of difference was observed when pairs of controls were run either heated or at room temperature without microwave exposure. The differences, therefore, could not be attributed to microwave exposure.

Reversal of equipment components, one at a time between the twin systems, neither reversed the direction of the difference nor altered its magnitude. Hence, the difference was not due to the system components but was related to the biological system.

Autoclaving of all glass components of the systems and installation of fresh Tygon tubing throughout coupled with the use of sterile, distilled water as the sole flushing agent for the systems between runs eliminated the light difference previously observed. Light curves following this action were identical! Differences, therefore, must have been due to the system contaminant discussed in Section 4.6.2.

5.2. Comparison of Results

To compare the results of this investigation with those of others, the exposures must be converted to exposure time and power density equivalents. It has already been indicated that the microwave power is uniformly distributed along the water load and is absorbed almost entirely by the liquid passing through the load. Knowing the power into the load, the microwave absorption coefficient, water load volume, total volume of suspension, flow rate, and total exposure time, one may determine the effective power densities and exposure times applied.

The water-load volume was 28 ± 0.5 cc. Using the microwave absorption coefficient of 0.6 cm^{-1} from Section 3.5.3 and the exposure power minimum and maximum of 8.3 and 16.7 watts, respectively, one may compute that minimum and maximum exposure power densities were equivalent to approximately 490 mw/cm^2 and 990 mw/cm^2, respectively. The absorption coefficient for muscle and similar tissue has been used since these tissues behave very similarly to water with respect to their dielectric properties.[63] However, at 3 KMc and higher frequencies, the absorption coefficient in tissue is about double that of water, which is an indication of significant ionic conductivity absorption in tissue.[63]

The exposure rate is directly proportional to the power level and inversely proportional to the flow rate. Hence, the highest and lowest exposure rates occurred at frequencies where the power-to-flow-rate ratio was highest and lowest, respectively. Table IV-A shows that the

highest dose rate occurred at 2864.7 Mc at a power level of 16.7 watts and a flow rate of 32.3 cc/min. The exposure at 2685.5 Mc run at 40.0 cc/min and 8.3 watts of power was the lowest dose rate. All dose rates were different by no more than a factor of 2.5.

A single cell required a maximum of 0.87 minutes and a minimum of 0.70 minutes to make a single pass through the waveguide for these two exposure cases while the entire 60 cc of each suspension completed one cycle through the guide only once every 1.9 to 1.5 minutes. The fraction of total running time actually spent in the guide ranged from about 0.87/1.9 to 0.70/1.5 or about 0.5 in either case. The total time for exposure runs ranged from 43-86 minutes. Hence the effective exposure time ranged from 23 to 43 minutes.

Most of the exposures in this investigation may, therefore, be considered to be equivalent to exposures to a power density of approximately 990 mw/cm^2 for about 23 minutes duration. Such a high power density, without temperature regulation, would cause cataracts in the eyes of rabbits following less than three minutes of exposure according to Carpenter's data.[18] His cataract-formation threshold curve shows cataract formation in the eyes of rabbits following 3 minutes of exposure to 400 mw/cm^2 continuous wave irradiation.

Differences between Carpenter's results and those of this study cannot be attributed to either frequency dependence of the absorption coefficient or differences in biological systems since the differences make exposures in this investigation comparatively severe.

The loss tangents for pure water and solutions of sodium chloride up to 0.5 molar concentrations do not differ by more than a factor of 2 in the frequency range of 2-4 KMc.[64] BS-2 solution is about 0.25 molar sodium chloride and concentrations of other compounds are low by comparison. This allows the whole suspension to be considered as the "biological system" rather than the bacteria alone; it cannot be argued that the suspending solution has shielded the organisms.

5.3. Significance of Results

A fundamental question pertinent to investigations of this type is one of the significance of the results to man. Phylogenetically, bacteria are far removed from man; however, the two are not completely unrelated. Man may be infected with symbiotic, communal, or pathogenic organisms. In any case he can act as a host to bacteria and supply the nutrients and environment essential to their growth. This implies that some molecules are common to man and bacteria.

Flavin mononucleotide (FMN) is riboflavin-5-phosphate.[65] Riboflavin is vitamin B_2, contained in minute amounts in every animal and plant cell. Good sources include liver, kidney, and heart tissues. In tissues riboflavin occurs in the form of FMN and riboflavin-adeninedinucleotide (FAD).[66] The presence of riboflavin in man is essential to his good health.[65] Hence, FMN, or molecules very nearly identical to FMN must occur in man as well as in bacteria.

This study has shown that at least for one large molecule, FMN, which is found both in man and bacteria, inactivation of the molecule cannot occur as a result of non-thermal effects produced by microwaves. Since exposures were made at frequencies which could have inactivated this molecule, it is possible to extend the conclusions of this study to other large molecules of similar structure irrespective of their source.

5.4. Conclusions

No significant change in the luminescence of the nutrient-free suspensions of *P. fischeri* has been observed 20 to 24 hours following irradiation by microwaves. No evidence of a non-thermal effect from exposure to microwaves has been observed in this biological system in the frequency range studied. It is, therefore, concluded that biologically significant non-thermal effects do not occur in the test organism and probably not in man in the frequency range of 2608.7 to 3082.3 Mc, for power densities as high as 990 mw/cm^2.

Since a non-thermal effect could not be demonstrated in this frequency range, it may further be concluded that continuous-wave, microwave equipment operating in this frequency range will probably produce no effect other than heating of tissue, and investigations in this frequency range in the future may reasonably be restricted to the study of the thermal response of biological systems.

Some biological effects of microwaves have been shown to be frequency-specific (see Section 1.4.4). Consequently, the conclusions of this investigation cannot be extended to microwave frequencies other than those studied in this investigation.

APPENDIX

EXPERIMENTAL DATA

TABLE IV

SUMMARY OF EXPERIMENTAL DATA TAKEN DURING EXPOSURES

1	2	3	4	5	6
Exposure Frequency (Mc)	Temperature, Avg (°C)				Exposure Exit Temperature Minus Bath Temperature, Avg (°C)
	Exposure Exit	Control Exit	Bath	Room	
2608.7	27.3	27.4	16.8	25.1	10.5
2621.5	27.6	27.7	18.8	28.5	8.8
2634.3	24.8	24.8	15.1	26.2	9.7
2647.1	27.1	27.4	18.9	26.9	8.2
2659.9	25.1	25.1	15.6	26.3	9.5
2672.7	24.7	24.6	15.7	26.3	9.0
2672.7	25.8	25.8	16.2	25.0	9.6
2685.5	26.3	26.6	18.9	28.0	7.4
2698.3	27.5	27.2	18 1	28.2	9.4
2711.1	28.0	28.0	18.5	28.3	9.5
2723.9	26.9	27.1	18.6	28.0	8.3
2736.7	25.9	25.8	16.7	24.9	9.2
2749.5	25.9	26.0	16.8	25.1	9.1
2762.3	26.0	26.0	16.8	25.3	9.2
2770.1	25.9	25.7	15.9	26.3	10.0
2775.1	25.2	25.0	15.2	25.5	10.0
2775.1	25.2	25.2	15.0	25.5	10.2
2775.1	25.4	25.8	15.4	25.8	10.0
2779.6	26.9	26.9	17.1	28.4	9.8
2780.4	25.7	25.7	15.7	26.0	10.0
2787.9	25.5	25.5	16.1	26.1	9.4
2800.7	25.7	25.8	16.7	26.3	9.0
2813.5	25.9	25.9	17.1	25.9	8.8
2826.3	26.6	26.5	17.5	27.8	9.1
2833.7	25.5	25.6	15.4	25.5	10.1
2835.0	25.1	25.1	15.5	25.6	9.6
2836.3	26.4	26.4	18.8	28.6	7.6
2837.7	26.6	26.4	18.4	28.1	8.2
2839.1	27.9	27.8	18.3	28.0	9.6
2839.1	27.6	27.4	19.1	28.4	8.5
2839.1	27.1	27.0	17.4	27.7	9.7
2839.1	25.9	25.9	16.5	26.4	9.4
2839.1	26.4	26.3	17.1	26.3	9.3
2839.1	26.4	26.3	17.2	26.4	9.2
2839.1	27.4	27.4	18.3	26.7	9.1
2839.1	27.3	27.3	18.4	27.3	8.9
2840.4	26.9	26.3	18.2	28.5	8.7
2841.7	25.8	25.9	18.2	28.0	7.6
2843.1	24.2	24.1	14.3	25.3	9.9
2844.4	24.9	24.8	14.9	25.3	10.0
2851.9	26.8	26.9	17.3	27.2	9.5
2864.7	26.3	26.6	16.5	26.8	9.8
2877.5	25.9	26.0	16.0	26.2	9.9
2890.3	25.5	25.6	16.3	26.2	9.2
2903.1	26.4	26.4	16.5	26.4	9.9
2915.9	27.2	27.2	17.2	25.9	10.0
2928.7	29.9	29.9	19.7	29.1	10.2
2928.7	28.4	27.9	19.4	28.8	9.0
2941.5	29.6	30.0	19.8	29.2	9.8
2941.5	28.2	28.3	19.0	28.9	9.2
2954.3	29.3	29.4	19.6	28.9	9.7
2954.3	27.1	27.1	18.2	28.3	8.9
2967.1	27.0	27.1	19.5	29.1	7.5
2979.9	27.3	27.1	16.9	28.6	10.4
2992.7	27.4	27.1	17.0	28.7	10.4
2992.7	24.4	24.4	15.8	26.8	8.6
3005.5	25.6	25.6	16.5	27.5	9.1
3018.3	25.8	25.4	17.0	27.8	8.8
3031.1	25.8	25.8	17.4	28.3	8.4
3043.9	26.0	26.0	16.7	27.7	9.3
3056.7	26.4	26.3	17.1	27.9	9.3
3069.5	26.7	26.6	17.4	28.3	9.3
3082.3	27.0	26.9	17.1	28.5	9.9

TABLE IV-A

SUMMARY OF EXPERIMENTAL DATA TAKEN DURING EXPOSURES

1	2	3	4	5	6	7
Exposure Frequency (Mc)	Exposure Time (min)	Exposure Flow Rate, Avg (cc/min)	Exposure Power (watts)	Total Dose (ergs/gm x 10^9)	Storage Time Pre-Exposure (hours)	Intensity Difference End of Exposure (Control-Exposed Amp x 10^{-9})
2608.7	95	35.7	14.2	6.3	2.8	.3
2621.5	78	31.3	9.2	3.3	2.0	0
2634.3	52	37.0	13.9	3.3	1.0	0
2647.1	61	31.3	11.7	3.3	5.8	0
2659.9	52	37.0	13.9	3.3	2.5	0
2672.7	52	37.0	13.9	3.3	3.8	0
2672.7	86	40.0	16.7	6.7	1.1	0
2685.5	86	40.0	8.3	3.3	1.8	0
2698.3	61	40.0	11.7	3.3	3.5	.1
2711.1	61	42.0	11.7	3.3	4.8	- .5
2723.9	54	45.4	13.4	3.3	6.2	- .1
2736.7	44	40.0	16.7	3.3	2.8	0
2749.5	43	40.0	16.7	3.3	4.0	0
2762.3	43	40.0	16.7	3.3	5.0	.1
2770.1	43	34.5	16.7	3.3	4.8	.2
2775.1	33	40.0	16.7	2.5	1.5	0
2775.1	71	34.5	16.7	5.5	1.3	.1
2775.1	35	34.5	16.7	2.7	2.7	.2
2779.6	86	37.0	16.7	6.7	1.2	.1
2780.4	43	34.5	16.7	3.3	3.8	.1
2787.9	43	40.0	16.7	3.3	2.4	0
2800.7	43	40.0	16.7	3.3	3.4	- .2
2813.5	43	40.0	16.7	3.3	4.5	0
2826.3	48	40.0	15.0	3.3	4.4	.2
2833.7	43	45.4	16.7	3.3	4.6	.1
2835.0	43	41.7	16.7	3.3	3.6	.1
2836.3	62	41.7	11.7	3.3	1.6	.1
2837.7	54	46.5	13.4	3.3	2.9	- .1
2839.1	38	34.5	10.9	2.0	3.9	0
2839.1	43	47.6	16.7	3.3	2.4	0
2839.1	43	35.7	16.7	3.3	3.5	0
2839.1	86	37.0	16.7	6.7	4.4	0
2839.1	43	41.7	16.7	3.3	6.1	.1
2839.1	22	41.7	16.7	1.7	7.0	.1
2839.1	43	41.7	16.7	3.3	1.6	0
2839.1	43	41.7	16.7	3.3	2.6	0
2840.4	54	41.7	13.4	3.3	4.0	0
2841.7	60	41.7	12.0	3.3	5.3	0
2843.1	43	41.7	16.7	3.3	1.7	.2
2844.4	43	40.0	16.7	3.3	2.7	0
2851.9	43	35.7	16.7	3.3	2.4	0
2864.7	43	32.3	16.7	3.3	1.3	0
2877.5	43	34.5	16.7	3.3	4.5	0
2890.3	43	34.5	16.7	3.3	3.5	0
2903.1	43	34.5	16.7	3.3	2.5	- .2
2915.9	43	34.5	16.7	3.3	1.3	- .1
2928.7	43	47.6	16.7	3.3	3.6	-1.2
2928.7	54	50.0	13.4	3.3	3.9	0
2941.5	43	47.6	16.7	3.3	4.5	- .6
2941.5	47	43.5	15.4	3.3	2.8	.1
2954.3	43	47.6	16.7	3.3	5.5	- .1
2954.3	47	43.5	15.4	3.3	1.9	0
2967.1	54	45.4	13.4	3.3	5.1	0
2979.9	43	37.0	16.7	3.3	3.2	.2
2992.7	43	40.0	16.7	3.3	4.2	0
2992.7	43	45.5	16.7	3.3	1.7	0
3005.5	43	45.5	16.7	3.3	2.6	.1
3018.3	43	45.5	16.7	3.3	3.6	0
3031.1	43	45.5	16.7	3.3	4.6	.1
3043.9	43	38.5	16.7	3.3	1.5	0
3056.7	39	41.7	16.7	3.3	2.5	.1
3069.5	43	43.4	16.7	3.3	3.4	- .1
3082.3	43	41.7	16.7	3.3	4.4	0

TABLE V

SUMMARY OF EXPERIMENTAL DATA TAKEN ONE DAY AFTER EXPOSURE

1	2	3	4	5	6	7
Exposure Frequency (Mc)	Temperature, Avg (°C)				Flow Rate, Avg (cc/min)	Intensity Difference Pre-Dextrose (Control-Exposed Amp x 10^{-9})
	Exposure Exit	Control Exit	Bath	Room		
2608.7	24.6	24.3	23.6	23.7	40.0	0
2621.5	26.1	26.0	25.2	26.2	43.5	.1
2634.3	25.4	25.0	24.5	26.0	41.7	.2
2647.1	26.1	26.0	25.3	26.8	43.5	.1
2659.9	25.6	25.4	24.5	26.3	41.7	.1
2672.7	26.0	26.0	24.8	26.4	41.7	.2
2672.7	24.8	24.8	24.3	24.9	40.0	.3
2685.5	26.1	25.9	25.1	25.7	35.7	.1
2698.3	26.6	26.5	25.3	26.7	35.7	0
2711.1	27.1	26.9	25.8	26.7	35.7	0
2723.9	26.7	26.6	24.9	27.3	35.7	- .1
2736.7	25.0	25.0	24.3	25.8	40.0	.1
2749.5	24.9	24.9	24.2	25.9	40.0	.1
2762.3	24.9	24.9	24.3	26.3	40.0	.1
2770.1	24.8	25.1	24.2	25.7	--	.2
2775.1	25.5	25.1	24.5	26.1	43.5	.3
2775.1	24.8	24.8	24.2	25.0	--	.2
2775.1	24.8	25.0	24.3	25.5	--	.3
2779.6	25.0	25.0	24.3	26.0	50.0	.2
2780.4	24.8	24.9	24.3	25.2	--	.4
2787.9	25.8	25.6	24.7	26.3	43.5	.2
2800.7	26.4	26.3	25.3	26.9	43.5	0
2813.5	26.8	26.5	25.8	27.2	43.5	0
2826.3	25.6	25.4	24.2	27.3	47.7	0
2833.7	24.3	24.2	24.3	23.4	45.4	.1
2835.0	24.6	24.6	24.3	24.4	45.4	.2
2836.3	24.9	25.0	24.3	25.5	43.5	.2
2837.7	25.1	25.1	24.2	25.2	43.5	0
2839.1	25.6	25.5	24.3	27.0	45.4	0
2839.1	25.4	25.3	24.3	26.3	45.4	.3
2839.1	25.6	25.4	24.2	28.1	47.7	.4
2839.1	24.9	25.0	24.3	24.9	43.5	.3
2839.1	25.2	25.2	24.2	25.5	44.5	.1
2839.1	25.4	25.3	24.2	26.0	45.5	.2
2839.1	25.5	25.3	24.3	26.3	47.6	.2
2839.1	25.5	25.2	24.2	26.9	43.5	.1
2840.4	25.0	25.0	24.2	25.1	45.4	.1
2841.7	25.2	25.2	24.3	25.1	45.4	.1
2843.1	24.3	24.2	24.3	23.3	45.4	.2
2844.4	24.5	24.5	24.3	23.8	47.7	0
2851.9	25.5	25.4	24.3	28.0	47.7	.2
2864.7	25.7	25.6	24.3	27.1	47.7	.3
2877.5	26.5	26.6	25.5	27.8	43.5	.1
2890.3	26.7	26.7	25.6	27.6	43.5	.1
2903.1	26.7	26.7	25.9	27.4	43.5	.1
2915.9	26.4	26.3	25.7	26.5	43.5	.3
2928.7	25.8	25.9	24.4	27.6	43.4	- .1
2928.7	26.2	26.2	24.2	29.8	47.7	.2
2941.5	25.9	25.9	24.4	28.3	45.4	0
2941.5	26.1	26.2	24.3	31.0	50.0	.2
2954.3	25.9	25.9	24.4	28.5	47.6	.1
2954.3	25.8	26.1	24.2	29.4	50.0	.2
2967.1	26.0	25.7	24.3	28.0	41.7	0
2979.9	25.2	25.2	24.3	27.1	50.0	.2
2992.7	25.3	25.3	24.2	26.9	47.7	- .2
2992.7	25.6	25.6	24.3	26.5	40.0	.2
3005.5	25.8	25.9	24.4	27.2	40.0	0
3018.3	26.0	26.0	24.3	27.9	38.5	0
3031.1	26.0	26.0	24.3	27.8	40.0	.1
3043.9	25.9	26.0	24.3	28.2	41.7	.2
3056.7	26.2	26.2	24.3	29.2	41.7	.1
3069.5	26.5	26.4	24.2	29.9	45.5	.1
3082.3	26.4	26.5	24.2	29.7	40.0	.1

TABLE V-A

SUMMARY OF EXPERIMENTAL DATA TAKEN ONE DAY AFTER EXPOSURE

1	2	3*	4	5	6	7
Exposure Frequency (Mc)	Time Delay in Adding Dextrose (min)	Culture Age (hr in incubator)	150-Min Area Post-Dextrose, (in.^{2})		Area Difference (Control-Exposed)	Area Ratio ± 20% (Exposed ÷ Control)
			Exposed	Control		
2608.7	13	48.3	12.38	12.43	.15	.996
2621.5	9	35.5	8.55	8.67	.12	.986
2634.3	16	32.0	10.81	11.86	1.05	.911
2647.1	11	35.5	9.56	9.78	.22	.976
2659.9	16	32.0	10.86	11.83	.97	.918
2672.7	15	32.0	8.46	10.19	1.73	.830
2672.7	24	32.9	11.79	13.01	1.22	.906
2685.5	16	33.5	15.52	15.65	.13	.992
2698.3	16	33.5	16.33	16.33	.00	1.000
2711.1	16	33.5	13.02	12.75	- .27	1.021
2723.9	16	33.5	13.42	13.13	- .30	1.023
2736.7	16	32.9	12.52	13.63	1.11	.919
2749.5	16	32.9	10.88	12.38	1.50	.879
2762.3	11	32.9	10.49	11.51	1.02	.911
2770.1	15	37.1	8.45	10.33	1.88	.818
2775.1	13	35.2	12.32	14.85	2.53	.830
2775.1	14	37.1	10.92	11.68	.76	.935
2775.1	20	37.1	9.39	10.78	1.39	.871
2779.6	12	36.2	9.73	10.70	.97	.909
2780.4	14	37.1	9.12	11.94	2.82	.764
2787.9	12	35.2	11.96	13.83	1.87	.865
2800.7	12	35.2	11.76	12.09	.33	.973
2813.5	11	35.2	8.59	9.16	.57	.938
2826.3	19	33.3	10.92	11.85	.93	.922
2833.7	12	32.5	10.16	11.28	1.12	.901
2835.0	13	32.5	10.00	11.73	1.73	.853
2836.3	12	32.7	12.44	13.60	1.16	.915
2837.7	17	32.7	12.24	12.88	.64	.950
2839.1	20	32.9	5.19	5.66	.47	.917
2839.1	18	35.5	8.94	10.17	1.23	.879
2839.1	19	33.3	10.38	14.04	3.66	.739
2839.1	20	32.8	13.74	14.76	1.02	.931
2839.1	19	32.8	14.25	15.07	.82	.946
2839.1	18	32.8	11.64	13.66	2.02	.852
2839.1	21	32.9	5.83	6.69	.86	.871
2839.1	16	32.9	5.81	6.89	1.08	.843
2840.4	13	32.7	12.00	12.29	.29	.976
2841.7	13	32.7	9.77	11.30	1.53	.865
2843.1	12	32.5	10.45	12.40	1.95	.843
2844.4	14	32.5	10.52	12.08	1.56	.871
2851.9	20	33.3	13.86	15.92	2.06	.871
2864.7	16	33.3	12.58	14.14	1.56	.890
2877.5	12	32.7	10.94	11.86	.92	.922
2890.3	21	32.7	11.16	12.05	.89	.926
2903.1	14	32.7	13.22	13.41	.19	.986
2915.9	21	32.7	14.30	15.01	.71	.953
2928.7	16	35.5	9.03	6.91	-2.12	1.307
2928.7	35	36.1	4.94	5.89	.95	.839
2941.5	14	35.5	8.05	7.16	- .89	1.124
2941.5	11	36.1	7.36	8.08	.72	.911
2954.3	21	35.5	7.93	8.60	.67	.922
2954.3	16	36.1	7.22	8.26	1.04	.874
2967.1	7	36.1	6.46	7.21	.75	.896
2979.9	12	36.2	9.52	10.51	.99	.906
2992.7	12	36.2	8.57	7.38	-1.19	1.161
2992.7	12	35.3	4.56	6.10	1.54	.748
3005.5	13	35.3	5.65	6.49	.84	.871
3018.3	11	35.3	5.21	5.68	.47	.917
3031.1	8	35.3	4.37	5.26	.89	.831
3043.9	12	32.7	8.23	9.72	1.49	.847
3056.7	11	32.7	7.77	9.47	1.70	.820
3069.5	13	32.7	7.50	8.48	.98	.884
3082.3	12	32.7	6.68	7.68	1.00	.870

*Column 3 contains data which applies before exposures were made.

TABLE VI

SUMMARY OF CORRELATION COEFFICIENTS

	Description of Comparison	Correlation Coefficient
1.	Light Difference vs. Frequency	.011
2.	Light Ratio vs. Frequency	.108
3.	Light Difference vs. Exposure Time	-.251
4.	Light Difference vs. Flow Rate During Exposure	-.242
5.	Light Difference vs. Exit Temperature Minus Bath Temperature During Exposure	.088
6.	Light Ratio vs. Storage Time Pre-Exposure	.161
7.	Light Ratio vs. Temperature During Dextrose Runs	.221
8.	Light Ratio vs. Culture Age	.142
9.	Light Ratio vs. Total Dose	-.077
10.	Light Ratio vs. Time of Addition of Dextrose	-.047
11.	Light Ratio vs. Exposure Power Level	-.225
12.	Light Ratio vs. Light Differences	-.914
13.	Light Ratio vs. Intensity Difference Pre-Dextrose	-.651
14.	Light Ratio vs. Intensity Difference End of Irradiation	-.499
15.	Light Difference vs. Exit Temperature of Irradiated Suspension	-.556
16.	Light Difference vs. Room Temperature During Exposure	-.621
17.	Light Difference vs. Bath Temperature During Exposure	-.504

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