THE UNIVERSITY OF MICHIGAN

COLLEGE OF LITERATURE, SCIENCE, AND THE ARTS
Computer and Communication Sciences Department

Technical Report

COMPUTER SIMULATION OF A LIVING CELL: MULTILEVEL CONTROL SYSTEMS

Roger Weinberg
Bernard P. Zeigler

with assistance from:

National Science Foundation
Grant No. GJ-519
Washington, D. C.

and

Department of Health, Education, and Welfare
National Institutes of Health
Grant No. GM-12236
Bethesda, Maryland

and

Department of the Navy
Office of Naval Research
Contract No. N00014-67-A-0181-0011
Washington, D. C.

and

U. S. Army Research Office (Durham)
Grant No. DA-31-124-ARO-D-483
Durham, North Carolina

administered through:

OFFICE OF RESEARCH ADMINISTRATION ANN ARBOR

December 1969

Distribution of This Document is Unlimited

COMPUTER SIMULATION OF A LIVING CELL:

Multilevel Control Systems. by Roger Weinberg and Bernard P. Zeigler

SUMMARY

A simple bacterial cell (Escherichia coli) has been modeled, and the input-output behavior of the model has been simulated as a program in FORTRAN IV for an IBM 360/67 digital computer.

Automata theoretic analysis of the homomorphic model underlying the computer simulation enables us to investigate the information content and complexity of the simulation and of the measurement space representing our data base.

The simulated cell is able to adjust its enzymes and DNA to grow in different chemical environments using allosteric modification of enzymes, and repression of RNA synthesis. It grows at realistic rates and achieves limited metabolic stability.

INTRODUCTION

Availability of experimental and theoretical analyses of the systems operative in living and reproducing organisms, as well as excellent presentations of powerful and convenient computer techniques make a computer simulation of a living cell a logical endeavor at this time.

In particular much is known about the biochemical behavior of bacterial cells. [Biological laboratory studies have been done elucidating control of DNA replication in bacteria (Eberle and Lark, 1969; Clark, 1968). The 1968 Cold

Spring Harbor Symposium in Quantitative Biology was devoted to papers concerning replication of DNA in micro-organisms (edited by Frisch, 1969). Lark has reviewed initiation and control of DNA synthesis (1969), and the subject is covered in books by Mandelstam and McQuillen (1968), Hayes (1968), and Davis et. al. (1968).

Control of Enzyme production by repression of messenger RNA production by the DNA is still being studied (Umbarger, 1969), and original hypotheses modified. Modification of enzymes already present in the cell, as a control mechanism, is reviewed by Umbarger (1969), and Batta (1960), and the relationship of this type of control to energy relationships in the cell has been reviewed by Atkinson (1966), discussed in *Control of Energy Metabolism* edited by Chance et. al. (1965), and investigated by Murray and Atkinson (1968). The importance of the ATP/ADP ratio in energy control relationships is becoming apparent in these works. These subjects, as well as convenient presentation of data on metabolic pathways, appear in books by Bernhard (1968), Westley (1969), Reiner (1968, 1969) and Mahler and Cordes (1966).

Mathematical analyses of enzyme modification utilizing computer techniques have been published by Walter (1969a, 1969b), Cennamo (1969), Griffith (1968), Heinmets (1964), and Yeisley and Pollard (1964). Relationships have been drawn between regulatory mechanisms in microbial cells, and in higher cells by Mitchison (1969), Tsanev and Sendov (1969), Comings and Kakefuda (1968), Britten and Davidson (1969), Gause (1966), and Heinmets (1966), making plausible the extension of computer simulation studies of a bacterial cell to studies of cancer in higher organisms (Tsanev and Sendov, 1969). Weinberg and Berkus (1969), Weinberg (1969a, b, c) and Stahl (1967) have modeled living cells as computer

programs. Formal aspects of self-reproducing systems are described in Waddington (1969), Burks (1969), Codd (1968), Mesarovic (1968), and von Neumann and Burks (1966). Techniques for automata theoretic numerical analysis, and also computer simulation are well described in Gordon (1969), Mize and Cox (1968), IBM Corporation Scientific Subroutine Package (1969), Knuth (1969), Wendroff (1969), Ginzburg (1968), Kalman, et. al. (1969) and Ulam (1966).

The connection between molecular controls and evolutionary mechanisms has been outlined for a computer simulation by Weinberg and Berkus (1969) taking advantage of basic genetic mechanisms (Strickberger, 1968, Kimura, 1964), and theoretical analyses connecting econometric studies (Gale, 1967) to a general theory of adaptive systems (Holland, 1969a, b).]

The present computer simulation of a living cell is the first effort to compare the predictions of hypotheses concerning a complete, functional cell with detailed laboratory data.

In our attempt to model a simple cell at the biochemical level we had to confront the complexity of the metabolic pathways present in even the simplest of cells. More than 3,000 different kinds of molecules are present in a complex spatial and functional relationship. This complexity had to be drastically reduced to permit a simulation involving five hundred instructions in FORTRAN IV for a 360/67 digital computer. To construct our simulation we lumped molecules into pools, and considered 1) metabolic topology, 2) functional relationships among cell structures and chemicals, and 3) type of experimental data available in the literature. This process of lumping is further described and justified from a systems viewpoint in the appendix.

Metabolic topology was considered, and an attempt was made to lump together as a single entity only those molecules which could be drawn adjacent to one

another on the metabolic map (Figures 1, 2). For example, in a pathway $A \rightarrow B \rightarrow C$, chemicals, A, B, C, might be partitioned into A, and BC, but would not be lumped into AC, and B.

Functional relationships between groups of molecules were extremely important, and the molecules lumped together in any one model entity were, in some way, functionally a unit (Figure 3). Thus, all the molecules produced in the breakdown of sugar to CO_2 and water to produce energy were lumped in this model since they could be considered functionally as molecules intermediate in a chemical pathway used to produce energy. Later models will employ more refined partitions, for example to capture the subtle and important relationships between molecules at different points in the glycolytic pathway citric acid cycle and cytochrome system.

Experimental data were often available for large chemical pools, e.g. products of glycolysis and the citric acid cycle, making these separate entities logical candidates for grouping into single entities in this model of the cell.

DESCRIPTION OF THE MODEL

The simulation can be described by its state together with its transition function (Figures 1-5). The state of the cell is described 1) by the concentrations of thirty internal chemical pools, 2) by the genetic apparatus, and 3) by the cell volume. The transition function used to obtain the state of the cell at the next time step of the simulation from a given time step consists of difference equations and Boolean expressions describing 1) enzyme catalyzed chemical reactions, 2) allosteric modification of enzymes, 3) repression of

messenger RNA-production, 4) self-replication of DNA under genetic controls, and 5) permeability of the cell to the chemical pools represented in the simulation (Figure 3). A detailed description of an earlier version of the simulation is available (Weinberg and Berkus, 1969b).

Input to the simulation consists of the concentrations of chemicals in the liquid environment in which the cell is growing. Output from the simulation consists of state descriptions during successive time increments. Comparison between simulation output and experimental data from the real world (Figures 5-15) enables us to judge the validity of the hypotheses used to write the simulation, to modify the hypotheses used to write the simulation in order to make the simulation more realistic, and to suggest critical real world experiments.

The equations in the transition function are general, thus allowing us to simulate cell behavior in many different environments, and in changes from one environment to another. This is especially significant since we wish to test the stability of the model under a variety of conditions.

RESULTS OF THE SIMULATION

The environment simulated was liquid growth medium at a temperature of 37 degrees Centigrade, with an abundance of oxygen. The simulated experiments presented were in 1) minimal medium (medium containing glucose, ammonium salt, and minerals) and 2) broth (a rich medium containing additional amino acids, nucleosides and vitamins (Weinberg and Berkus, 1969b). The simulated cell grew faster in broth than in minimal medium, in agreement with laboratory data. This is a reasonable result, since addition of growth products implies that there are fewer molecules to be synthesized by the cell itself.

The simulated cell produced chemicals and cell mass at a logarithmic rate, but duplicated in a stepwise fashion (Figure 5) just as the real cell does. Since the simulated cell produced these smooth growth curves from a complex interaction of many equations, the growth curves are a good preliminary confirmation of the models used to write the simulation.

The simulated cell employed repression to control the production of its enzymes (Figure 21, Appendix). Repression operated at the DNA level. For example EK2 was the enzyme pool needed for producing amino acids from carbohydrates. EK2 was produced under control of DNA by way of the RNA pools as long as the amino acid pool concentration was below a certain critical level. DNA directed the production of messenger RNA specific for the production of EK2. EK2 was produced by hooking together amino acids attached to transfer RNA. This hooking was done by messenger RNA attached to ribosomes. If the amino acid level rose above the critical level, production by DNA of messenger RNA responsible for EK2 production was sharply curtailed by the nature of the Boolean equations in the transition function. The messenger RNA already present rapidly decayed, and almost no new messenger RNA for EK2 production was formed.

Of course if the amino acid concentration fell too low, insufficient amino acids were available for hooking together into the EK2 enzyme; production of all enzymes was blocked in the event of extreme scarcity of amino acids as a result of the form of the differential equations concerning their production.

The simulated cell employed feedback inhibition to control the activity of the enzymes already present (Weinberg and Berkus, 1969b). For example, EK2,

the enzyme for production of amino acids from carbohydrates, appeared in three different forms: pure enzyme, enzyme with one molecule of amino acid attached to it, and enzyme with two molecules of amino acid attached to it. These three forms of EK2 had different catalytic ability. The relative amount of EK2 in each form determined the activity of the EK2 present in the cell in terms of its efficiency in converting carbohydrate into amino acids. The percentage of EK2 in each of the three forms was determined by the number of amino acid molecules per cell volume unit (one cell volume unit was taken as the volume of a cell growing rapidly in mineral glucose medium with ammonium salt). The higher the amino acid concentration in the simulated cell, the greater was the percentage of EK2 in its low activity form, and the less effective was the EK2 in production of amino acids from carbohydrates.

We tested the ability of the simulated cell to grow in a medium it had never "seen" before by simulating a shift down to low glucose minimal medium with 10^{-4} times the usual glucose concentration found in minimal medium (Figure 15). The simulated cell decreased its growth rate in this shift down, just as the real cell does (Moser, 1958).

The cell could also adjust to shifts up from minimal medium to broth, and back down from broth to minimal medium when it was using its feedback controls. It is significant that without feedback controls the orderly shift up from minimal medium to broth was not possible (Figures 6-10).

The simulation experiments to determine the function of the feedback controls was performed as follows: the simulated cell was shifted down from growth in broth to growth in minimal medium, and growth was followed for ten seconds. The results of the shift were plotted along with a broth control for

the simulated behavior obtained with and without feedback equations. Similarly, shifts up from minimal medium to broth were studied. Each graph represents measurements of some pool (such as ATP) during the shift, and during the corresponding control run.

The simulated data agreed well with laboratory data when feedback controls were present (Figures 6-10) but the simulated cell without its feedback controls was no longer able to realistically handle shifts up from minimal medium to broth (Figures 11-14). This suggested that feedback in the real cell was evolved to handle shift up situations since the normal pathways are not stable in this condition without feedback.

The simulated cell with feedback controls maintained stability through rapid oscillation of concentrations about equilibrium points, a phenomenon well known in the literature on feedback control.

Oscillation of concentration to maintain equilibrium was strikingly illustrated by ATP and ADP concentrations during a shift up from minimal medium to broth (Figures 6, 7). The "restoring force" effected by the feedback equations enabled the cell to maintain equilibrium concentrations of ATP and ADP. In contrast the concentration of ATP was too high and the concentration of ADP was too low after a simulated shift up without feedback controls in the simulated cell.

Similarly an overshoot in ribosomal RNA concentration was quickly corrected by the simulated cell with feedback controls, while a similar simulation experiment on a cell without feedback controls produced so much ribosomal RNA that a real cell would lyse (Figure 10).

CONCLUSIONS

Preliminary conclusions drawn are 1) Shifts from poor to rich medium are more of a challenge to the cell than shifts from rich to poor medium. The shift up to rich medium requires elaborate feedback control mechanisms, whereas the shift down to poor medium does not require feedback control as strongly, but can be handled by metabolic topology. 2) Oscillations occur about equilibrium concentrations; fixed equilibrium concentrations are not maintained in the simulation. However, this agrees with experimental observations which indicate that real cells are constantly oscillating biochemical systems and suggests, indeed, that the oscillating concentrations produced by feedback control systems are necessary for the flexibility characteristic of living systems.

APPENDIX

Formally, in constructing a model on which to base a simulation a homomorphic mapping is often used to reduce a complex system description to a relatively simple one (Figure 17). Such a homomorphism is essentially a partition of the state space of the real system which preserves the transition function of the system just as a group homomorphism preserves the group multiplication. In practice, the mapping between real system and model is never truly a homomorphism--indeed when divergence between the behavior of the partitioned real system and the behavior of the model is detected this may initiate a search for more truly homomorphic and hence more adequate models (Ulam, 1966)

The kind of partition considered may depend on several factors. The measuring instruments with which experimental observations are made impose certain equivalences which cannot be cut across. The partition must relate to experimental data which is actually or potentially available. It must also be fine enough to maintain distinctions between the parts of the system which are of primary interest.

When a simulation by computer is involved, as it is in this paper, additional restrictions are placed on the kind of model that can be considered. The model must not exceed the information processing capabilities of real computers. Suppose for example we momentarily consider describing the state of a biological cell by listing the states of each of the elementary atomic particles of which the cell is composed. The sheer enormity of the number of such particles would relegate such an approach to the realm of wishful thinking in two ways: one, we would hardly have enough data storage capacity to keep track of such a long list, and two, we would not have enough program storage capacity to specify how each atomic state changes as a function of the prior states of the atoms which

influence it. (Moreover, the time required to run such a program would exceed a scientist's patience if not his lifetime).

This paper concerns a model of a bacterial cell (E. coli) which has been constructed to enable computer simulation of the cell behavior in its living form. We believe that our model is best understood as an attempt, operating under the constraints on model making just mentioned, to achieve a truly homorphic mapping of a real world system. (We suggest that it is fruitful to deal with the modelling process in general in this way but we do not further argue this proposition.) Accordingly, we devote some time to an exposition of the system theoretic concepts underlying the idea of models as attempted homomorphisms. This development is briefly sketched here and will be more fully expanded in subsequent publications (Weinberg and Zeigler, to be published).

In its most basic form, a system is defined as a set of states S, together with a transition function $\tau: S \rightarrow S$. τ describes the behavior of the system over time by indicating which next state is to follow the present state. Thus if the state at time t is s(t) then the state at time t+1, $s(t+1) = \tau(s(t))$.

The state space S is usually described as a cartesian product of component state sets i.e. $S = \times_{\alpha \epsilon D} S_\alpha$ where D is called a set of co-ordinates (or entities) and S_α is the state set (or attribute set) of co-ordinate α. In Figure 1 we list a number of possible state spaces and indicate the form a transition function might take in each. In Figures 2 and 3 we specify in more detail the state space and transition function of the present model.

A homomorphism from a system (S, τ) to a system (S', τ') is a map h from S onto S' such that for all $s \epsilon S$

$$h(\tau(s)) = \tau'(h(s))$$

Thus, a homomorphism preserves the transition function and guarantees that every

state trajectory in (S, τ) has a corresponding state trajectory in (S', τ').

As we have stated, model making may be identified as an attempt to obtain a truly homomorphic image of a real system which among other things, is simple enough to implement on a computer. The process of going from lower level to higher level functional units may be viewed in this light. We cannot implement an atomic state model of an E. coli (even though presumably such a simulation would be maximally informative) because such a model would require an information processing capacity well beyond that possessed by any man made computer. At the molecular and concentration levels of Figure 18 the same thing would be true. Notice that each of these levels arises by grouping together co-ordinates at a lower level to form higher level units. It is only by continuing this process one more step that we are able to arrive at models of a cell simple enough to implement on a computer. It is still possible at this level to construct models which can be meaningfully tested against real cellular behavior as consideration of our present simulation has demonstrated.

Partitioning the co-ordinates of system to achieve a simpler system can be given a mathematical formulation (Zeigler, to be published). We indicate the conditions under which such a partition will yield a homomorphic image system. We also show how the complexity of the system (as determined by measures relevant to computer implementation) can be made to decrease in this way. The point to be made, however, is that such a partitioning can be justified mathematically and experimentally at any level, not only the levels--atomic, molecular, etc.--traditionally accepted.

REFERENCES

Atkinson, D. E. (1966)."Regulation of Enzyme Activity", Annual Rev. Biochem. 35, 85-123.

Bernhard, Sidney (1968). The Structure and Function of Enzymes, New York: Benjamin.

Britten, F. J., and E. H. Davidson (1969). "Gene Regulation for Higher Cells: A Theory", Science, 165, 349-357.

Burks, A. W., editor. (1969, in press). Papers on Cellular Automata, Urbana, Illinois: University of Illinois Press.

Cennamo, C. (1969). "Stead-state Kinetics of One-substrate Enzymic Mechanisms Involving Two Enzyme Conformation II. Kinetic Treatment of a Reversible Mechanism: Effects of Modifiers and Product Inhibition", J. Theoret. Biol. 23, 53-71.

Chance, B., R. W. Estabrook, and J. R. Williamson, editors (1965). Control of Energy Metabolism, New York: Academic Press.

Clark, J. D. (1968). "Regulation of Deosyribonucleic Acid Replication and Cell Division in Escherichia coli B/r", J. Bacteriol., 96, 1214-1224.

Codd, E. F. (1968). Cellular Automata, New York: Academic Press.

Comings, D. E. and T. Kakefuda (1968). "Initiation of Deosyribonucleic Acid Replication at the Nuclear Membrane in Human Cells", J. Mol. Biol., 33, 225-230.

Datta, P. (1969). "Regulation of Branched Biosynthetic Pathways in Bacteria", Science, 165, 556-562.

Davis, B. D., R. Dulbecco, H. N. Eisen, H. S. Ginsberg, and W. B. Wood, (1968) Microbiology, New York: Harper & Row.

Eberle, H. and K. G. Lark (1969). "Relation of the Segregative Origin of Chromosome Replication to the Origin of Replication After Amino Acid Starvation", J. Bacteriol., 98, 536-542.

Fisher, R. A. (1958). The Genetical Theory of Natural Selection, New York: Dover.

Frisch, Leonora, editor. (1969). Replication of DNA in Micro-organisms, Cold Spring Harbor Symposia on Quantitative Biology, vol. 33.

Gale, D. (1967). "A Geometric Duality Theorem with Economic Application", Review of Economic Studies, 32, 19-24.

Garfinkel, D. (1966). "A Simulation Study of Mammalian Phosphofructokinase", J. Biol. Chem., 241, 286-294.

Gause, G. F. (1966). Microbial Models of Cancer Cells, Philadelphia: Saunders.

Ginzburg, A. (1968). Algebraic Theory of Automata, New York: Academic Press.

Gordon, Geoffrey (1969). System Stimulation, Englewood Cliffs, New Jersey: Prentice-Hall.

Griffith, J. S. (1968). "Mathematics of Cellular Control Processes", J. Theoret. Biol., 20, 202-216

Hayes, W. (1968). The Genetics of Bacteria and their Viruses, second edition, New York: Wiley.

Heinmets, F. (1964) "Analog Computer Analysis of a Model-System for the Induced Enzyme Synthesis", J. Theoret. Biol., 6, 69-75.

Heinmets, F. (1966). Analysis of Normal and Abnormal Cell Growth, New York: Plenum.

Holland, J. H. (1969a). "Heirarchical Descriptions, Universal Spaces and Adaptive Systems", in Essays on Cellular Automata (ed. A. W. Burks), Urbana: University of Illinois Press (to appear).

Holland, J. H. (1969b). Adaptive Plans Optimal for Payoff only Environments," in Proceedings of the Second Hawaii Conference on System Sciences.

IBM Corporation (1969). System/360 Scientific Subroutine Package (360-CM-03X) Version III Programmer's Manual, New York: IBM.

Kimura, M. (1965). "Changes of Mean Fitness in Random Mating Populations when Epistasis and Linkage are Present", Genetics, 51, 349-363.

Knuth, Donald E. (1969). The Art of Computer Programming, Volume 2, Seminumerical Algorithms, Massachusetts: Addison-Wesley.

Koch, A. L. (1967). "Metabolic Control Through Reflexive Enzyme Action", J. Theoret. Biol., 15, 75-102.

Lark, K. G. (1969). "Initiation and Control of DNA Synthesis", Annual Rev. Biochem., 38, 549-604.

Maaløe, O. and N. O. Kjeldgaard (1966). Control of Macromolecular Systhesis, New York: Benjamin.

Mahler, H. R. and E. H. Cordes (1966). Biological Chemistry, New York: Harper & Row.

Mandelstam, J., and K. McQuillen, editors (1968). Biochemistry of Bacterial Growth, New York: Wiley.

Mesarovic, M. D., editor (1968). Systems Theory and Biology, New York: Springer-Verlag.

Mitchison, J. M. (1969). "Enzyme Synthesis in Synchronous Cultures", Science 165, 657-663.

Mize, J. H., and J. G. Cox (1968). Essentials of Simulation, Englewood Cliffs, New Jersey: Prentice-Hall.

Moser, H. (1958). The Dynamics of Bacterial Populations Maintained in the Chemostat, Washington, D. C: Carnegie Institution of Washington Publication 614.

Murray, A. W. and M. R. Atkinson (1968). "Adosine 5' Phosphorothioate. A Nucleotide Analog that is a Substrate, Competitive Inhibitor, or Regulator of Some Enzymes that Interact with Adenosine 5'-Phosphate", Biochemistry, 7, 4023-4029.

Reiner, J. M. (1968). The Organism as an Adaptive Control System, Englewood Cliffs, N. J.: Prentice-Hall.

Reiner, J. M. (1969). Behavior of Enzyme Systems, New York: Reinhold.

Stahl, W. R. (1967). "A Computer Model of Cellular Self-Reproduction", J. Theoret. Biol., 14, 187-205.

Strickberger, M. W. (1968). Genetics, New York: Macmillan.

Sugita, M., and N. Fukuda (1968). "Functional Analysis of Chemical Systems in vivo Using a Logical Circuit Equivalent", J. Theoret. Biol., 5, 412-425.

Tsanev, R. and B. Sendov (1969). "A Model of Cancer Studied by a Computer", J. Theoret. Biol., 23, 124-234.

Ulam, S. M. (1967). in Prospects for Simulation and Simulators of Dynamic Systems, edited by G. Shapiro and M. Rogers, New York: Spartan.

Umbarger, H. E. (1969). "Regulation of Amino Acid Metabolism", Annual Rev. Biochem., 38, 323-370.

von Neumann, J. (1966). Theorey of Self-Reproducing Automata (edited by A. W. Burks), Urbana: University of Illinois.

Waddington, C. H. (1969). Towards a Theoretical Biology, Birmingham, Great Britain: Kynoch.

Wallace, B. (1968). Topics in Population Genetics, New York: W. W. Norton.

Walter, Charles (1969a). "Stability of Controlled Biological Systems", J. Theoret. Biol., 23, 23-38.

Walter, Charles (1969b). "The Absolute Stability of Certain Types of Controlled Biological Systems", J. Theoret. Biol., 23, 39-52.

Weinberg, R. (1968a). "Analytic and Logical Equations in a Computer Simulation of Cell Metabolism and Replication", Sixth Annual Symposium on Biomathematics and Computer Science in the Life Sciences, The University of Texas, pp. 102-103.

Weinberg, R. (1968b). "Computer Simulation of a Living Cell", Bacteriological Proceedings, G114.

Weinberg, R. (1968c). "Computer Simulation of Self-Reproduction by a Living Cell", Genetics, 60, 235.

Weinberg, R. and M. Berkus (1969a). "Computer Simulation of Evolving DNA", Abstract to be published in Biometrics.

Weinberg, R. and M. Berkus, (1969b). Computer Simulation of a Living Cell, Ann Arbor, Michigan: University of Michigan Technical Report 01252-2-T.

Wendroff, B. (1969). First Principles of Numerical Analysis, Massachusetts: Addison-Wesley.

Westley, John (1969). Enzymic Catalysis. New York: Harper & Row.

Yeisley, W. G. and E. C. Pollard (1964). "An Analog Computer Study of Differential Equations Concerned with Bacterial Cell Synthesis", J. Theoret. Biol. 7, 485-501.

Figure 1. *Natural Groupings. Chemical species in one large block were represented in the simulation as one pool.*

Figure 2. *Flow of Materials.*

Figure 3. *Model of a Living Cell Used for the Computer Simulation.*

Figure 4. *Differential Equations. Quantity to the left of = is the change in amount of the substance; e. g., DDNA represents the change in the amount of DNA in one time increment DT. The differential equation underlying the first equation is*

*DDNA = K(6)*NUC*EK(6)*ATP*DT for a discrete time interval DT. As DT approaches 0, we get the underlying continuous differential equation*

$$\lim_{DT \to 0} D(DNA)/DT = d(DNA)/dt = K(6)*NUC*EK(6)*ATP$$

Figure 5. *Logarithm of Various Quantities in a Growing Culture. Logarithmic Increase in Cell Mass over Time, Stepwise Increase in Number of Cell. Comparative magnitude of various quantities are a function of the scaling factors used in order to plot all quantities on one graph. A cell doubles after a DNA replication cycle. The doubling takes a relatively short time, as indicated by the sudden, stepwise increase in "TOTAL NUMBER OF CELLS", where as "TOTAL DNA" increases throughout the replication cycle.*

Figure 6. *ATP during Simulated Shift Up.*

Figure 7. *ADP during Simulated Shift Up.*

Figure 8. *DNA during Shift Up. Well aerated liquid cultures at 37°C were used for simulated and laboratory data. Symbols: □○ simulated shift from minimal glucose to broth at time zero; ○ simulated minimal · X laboratory shift up; X laboratory minimal glucose.*

Figure 9. *Protein during Shift Up. Well aerated liquid cultures were used for laboratory and experimental data. Symbols: □ ○ simulated shift from minimal glucose to broth at time zero; ○ simulated minimal glucose control; X laboratory shift up; + laboratory minimal.*

Figure 10. *Ribosomal RNA during Shift Up. Well aerated liquid cultures at 37°C were used for laboratory and simulated data. Symbols: ○ simulated minimal glucose liquid medium; □ ○ simulated shift from broth to minimal glucose at time zero; X laboratory shift up; + laboratory minimal glucose.*

Figure 11. *ATP during Simulated Shift Down.*

Figure 12. *DNA during Shift Down. Well aerated liquid cultures at 37°C were used for laboratory and experimental data. Symbols: ○ simulated shift down from broth to minimal glucose at time zero; ○ broth control; X laboratory shift down; X laboratory control.*

Figure 13. *Ribosomal RNA during Shift Down from Broth to Minimal Glucose. Well aerated liquid cultures at 37°C were used for simulated and laboratory data. Symbols: ○ nutrient broth; ○ shifted to minimal glucose from nutrient borth at time zero; X laboratory shift down; X laboratory control.*

Figure 14. *Protein/Cell during Shift Down. Well aerated liquid cultures at 37°C were used for laboratory and simulated data. Symbols: ○ simulated shift from broth to minimal glucose at time zero; ○ ◇ simulated broth; + laboratory shift down; X laboratory broth.*

Figure 15. *Simulated Growth in Low Glucose Concentration. Well aerated liquid cultures at 37°C were used for simulated and laboratory data. Symbols: all cultures were grown in minimal medium; □ laboratory, 40 mg glucose per liter; △ laboratory, 4.10^{-4} mg glucose per liter; ○ simulated 4 mg glucose per liter to 4.10^{-4} mg glucose per liter at time zero; ○ simulater HIGH.*

Figure 16. *Formal Definitions.*

Figure 17. *Useful Homomorphisms.*

Figure 18. *Summary of Program.*

Figure 19. *Growth Cycle.*

Figure 20. *Repression and Allosteric Inhibition. Repression is obtained by adjustment of KK8K(INTGR). Allosteric inhibition is obtained through adjustment of C(INTGR).*

Table 1. *Variables in Program.*

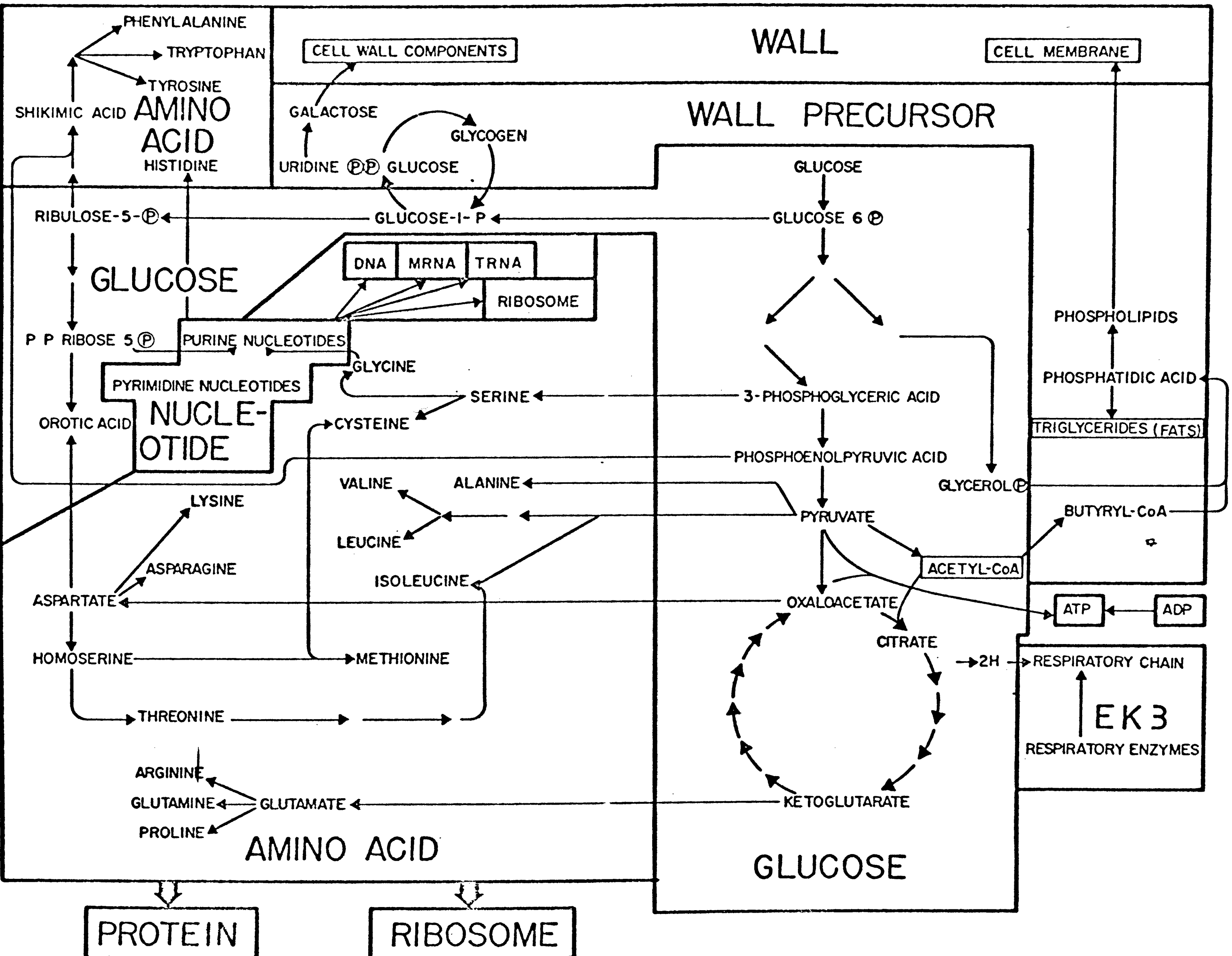

figure 1

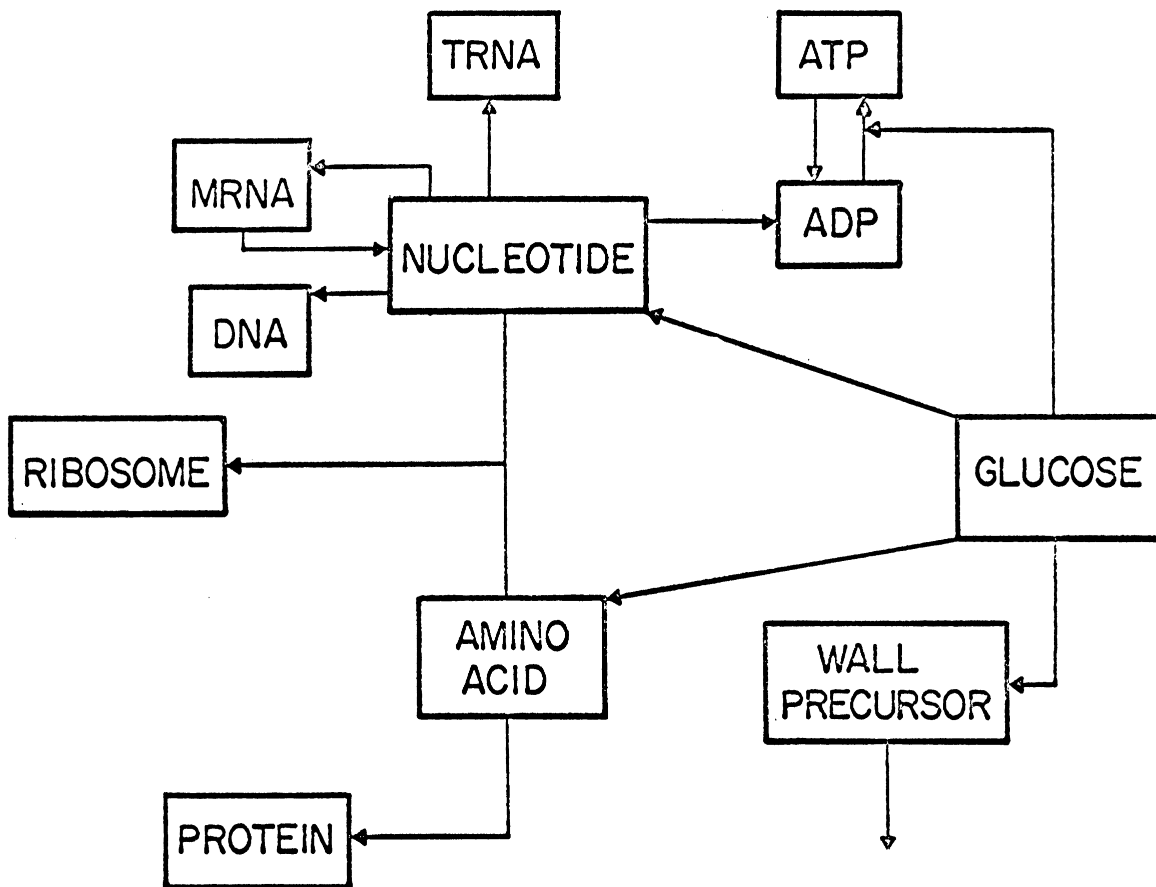

figure 2

STATE

COORDINATE = ENTITY	RANGE OF VALUE OF COORDINATES = ATTRIBUTE OF ENTITY
Pools of Chemicals, PRDC(1),..., PRDC(10)	Concentration of pool
Enzymes, EK(1),...,EK(10)	Concentration of enzyme
Messenger RNA, RNK(1),..., RNK(10)	Concentration of RNA
Genetic Apparatus	Amount of DNA, site of replication, number of genes in cell for producing sites for replication
Cell Volume	Total volume of the cell
Cell Number	Number of cells represented in the culture

TRANSITION FUNCTION
(for calculating the state of the system in the next time step from the present state)

A. *The differential and boolean equations* relating concentrations of variables at a given time to the concentrations of those variables DT seconds later. e.g. for AA, the amino acid pool, one needs enzyme EK(2) to catalyze the production of AA from glucose, and one uses ATP as an energy source. At the same time, AA is lost as it is used for the production of RIB and PRTN.

1. DAA = K(2)*GLUC*DT*EK(2)*ATP - 1.E6/102.*DRIB - (4.E4/102.)*DPRTN
   - K(2)*GLUC*DT*EK(2)*ATP: production of AA from GLUC
   - 1.E6/102.*DRIB: loss of AA to RIB
   - (4.E4/102.)*DPRTN: loss of AA to PRTN

2. RNK(2) produced EK(2) from AA under the direction of DNA, using ATP for energy.

3. DEK(2) = K(7)*AA(RNK(2)/MRNAO)*DT*EK(7)*ATP

4. RNK(2) itself was produced from NUC under the direction of DNA, catalyzed by EK8, using ATP for energy. RNK(2) decayed spontaneously at the same time, producing some loss of RNK(2) already present.

5. DRNK(2) = (K8K(2)*NUC*DNA*EK(8)*ATP - KDRNK*RNK(2))*DT
   - K8K(2)*NUC*DNA*EK(8)*ATP: production of RNK(2)
   - KDRNK*RNK(2): decay of RNK(2)

B. *Allosteric modification* of enzymes simulated by modifying the rate constant which characterizes all different forms of any enzyme associated with a particular reaction.

C. *Repression* of messenger RNA directing the production of a particular enzyme

D. *Genetic behavior* of DNA in response to the state of the cell

E. *Permeability*

figure 3

```
   91  DDNA = K(6)*NUC*DT*EK(6)*ATP
       DDNA1 = K(6)*(T/DBLE)*.5*IN1*NUC*DT*EK(6)*ATP
       DDNA2 = K(6)*(T/DBLE)*.5*IN2*NUC*DT*EK(6)*ATP
       DDNA3 = K(6)*(T/DBLE)*.5*IN3*NUC*DT*EK(6)*ATP
C****      40 MINUTES TO REPLICATE .5 * DNA
       DO 100 I = 1,10
  100  DRNK(I) = (K8K(I)*NUC*DNA*EK(8)*ATP - KDRNK*RNK(I))*DT
C****
       DMRNA = DRNK(1) + DRNK(2) + DRNK(3) + DRNK(4) + DRNK(5) + DRNK(6)
     1 + DRNK(8) + DRNK(9) + DRNK(10) + DRNK(7)
C****
       DTRNA = K(10)*NUC*DT*EK(10)*ATP
       DRIB = K(9)*NUC*AA*DT*EK(9)*ATP
       DRNA = DMRNA + .25*DTRNA + .75*DRIB
       DWALL = K(4)*GLUC*DT*EK(4)*ATP
C****
       DO 101 I = 1,10
  101  DEK(I)= K(7)*AA*(RNK(I)/MRNA0)*DT*EK(7)*ATP
C****
       DPRTN = DEK(1) + DEK(2) + DEK(3) + DEK(4) + DEK(5) + DEK(6)
     1 + DEK(8) + DEK(9) + DEK(10) + DEK(7)
C****
       DNUC=           -(2.5E9/660.)*DDNA - (1.E6/660.)*DMRNA
     1 +K(1)*GLUC*DT*EK(1)*ATP - (2.5E4/660.)*DTRNA
     2  -(2.E6/660.)*DRIB - K(5)*NUC*DT*EK(5)*ATP
C****
       DAA=K(2)*GLUC*DT*EK(2)*ATP - 1.E6/102.*DRIB - (4.E4/102.)*DPRTN
       DATP = K(3)*GLUC*DT *ATP*EK(3) - DNAP*DDNA - MRNAP*DMRNA
     1  - TRNAP*DTRNA - RIBP*DRIB - PRTNP*DPRTN-WALLP*DWALL
     2 - (AAP*K(2)*GLUC*EK(2)*ATP + NUCP*K(1)*GLUC*EK(1)*ATP +2*K(5)*NUC
     3 *EK(5)*ATP )*DT
C****
       DADP = -DATP + K(5)*NUC*DT*EK(5)*ATP
       DVOL = K(14)*WALL*DT
C****      INCREASE IN VOLUME PER UNIT INCREASE IN CELL WALL
```

Figure 4

Differential Equations: quantity to the left of = is the change in amount of the substance; e.g., DDNA represents the change in the amount of DNA in one time increment DT. The differential equation underlying the first equation is

DDNA = K(6)*NUC*EK(6)*ATP*DT for a discrete time interval DT. As DT approaches 0, we get the underlying continuous differential equation

$$\lim_{DT \to 0} D(DNA)/DT = d(DNA)/dt = K(6)*NUC*EK(6)*ATP$$

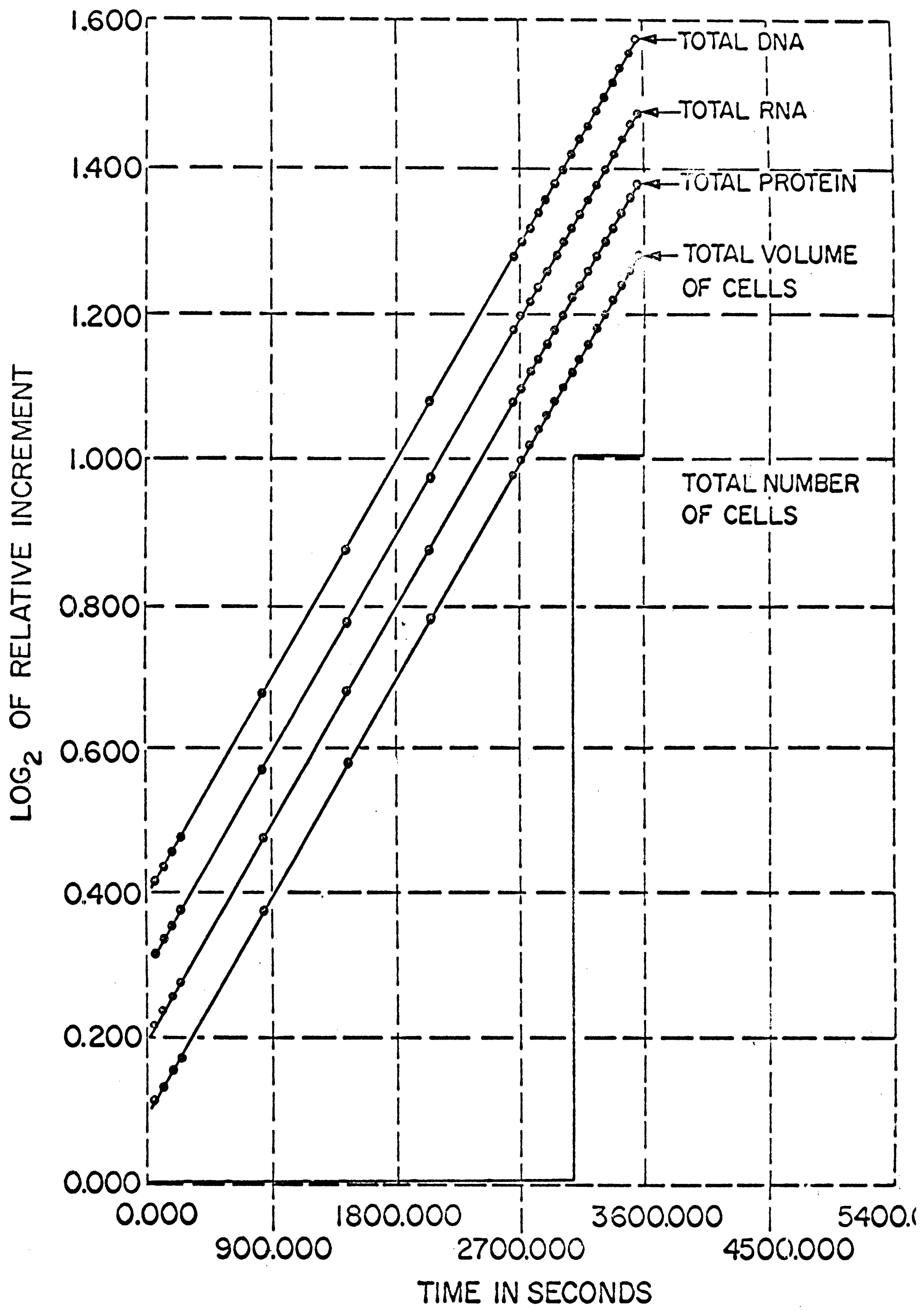

figure 5

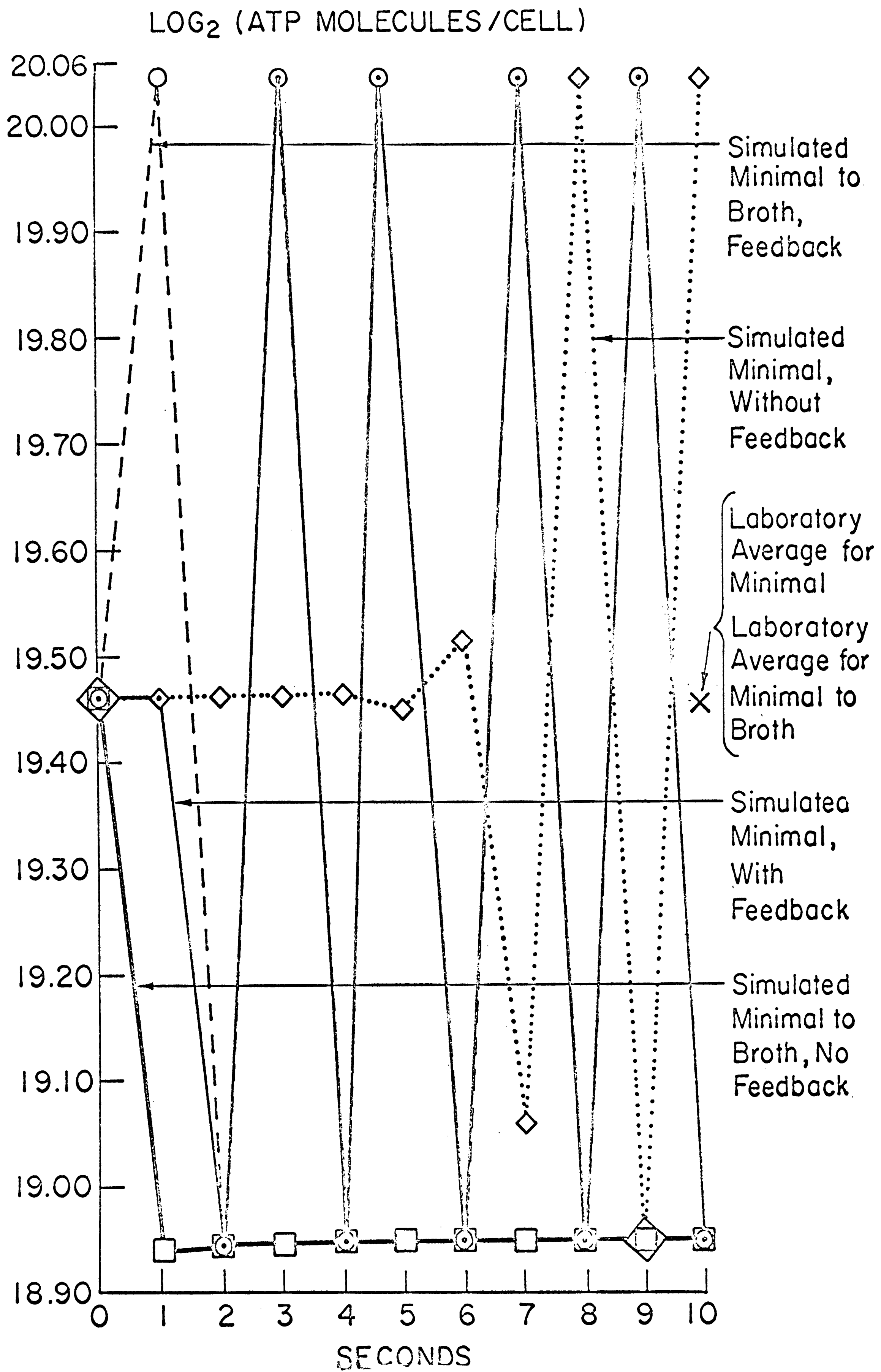

Figure 6. *ATP during Simulated Shift Up.*

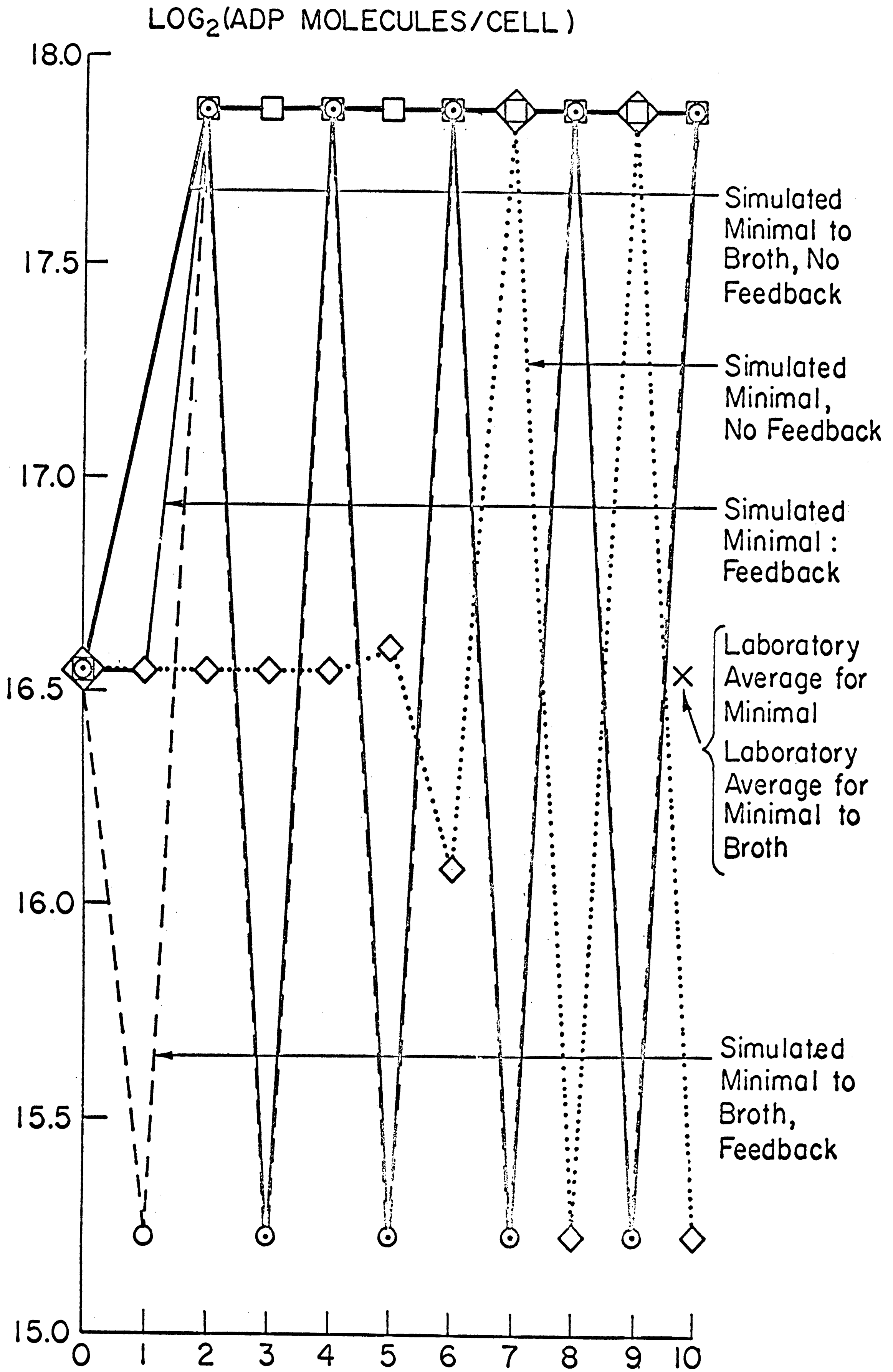

Figure 7. *ADP during Simulated Shift Up.*

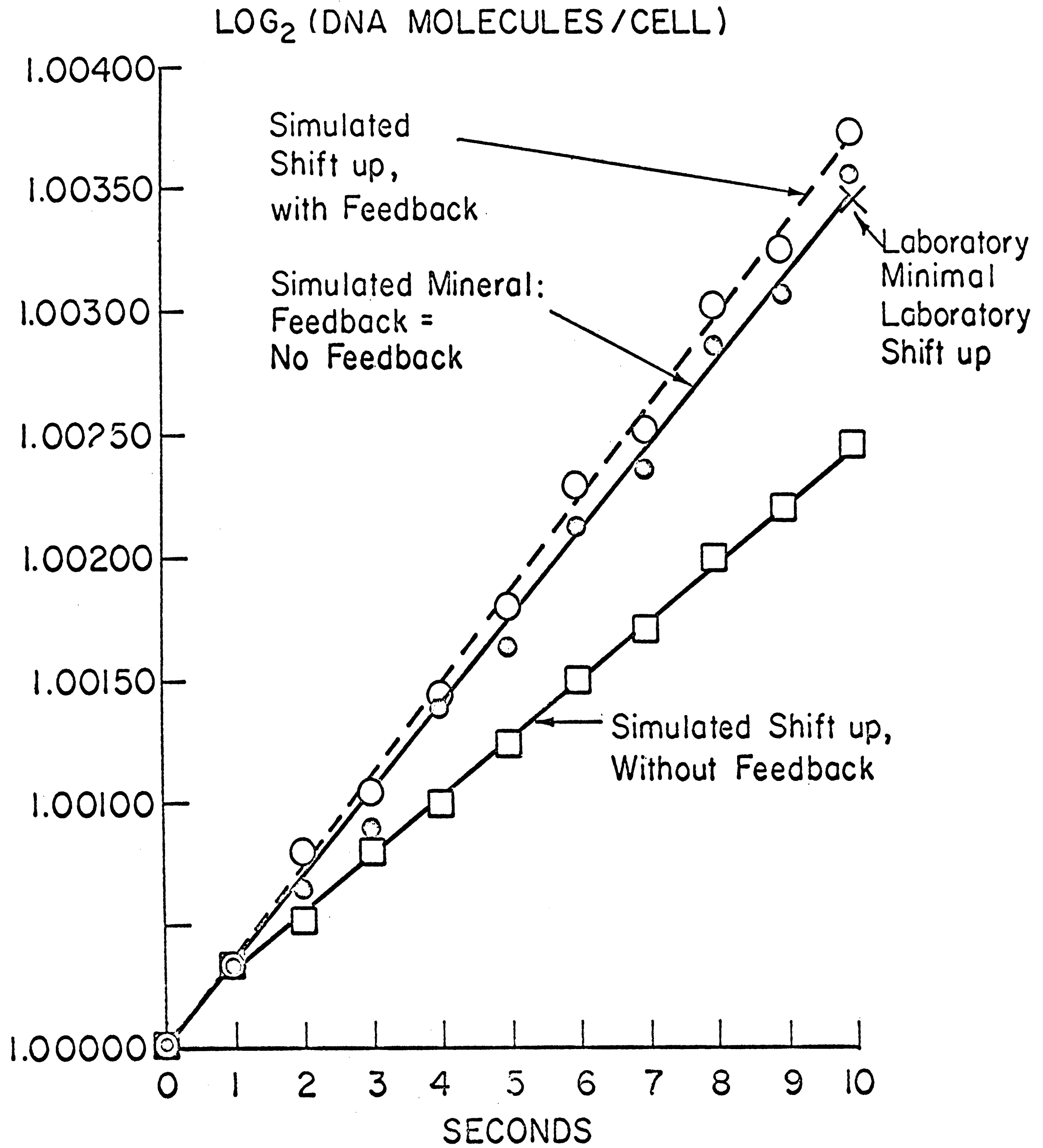

figure 8

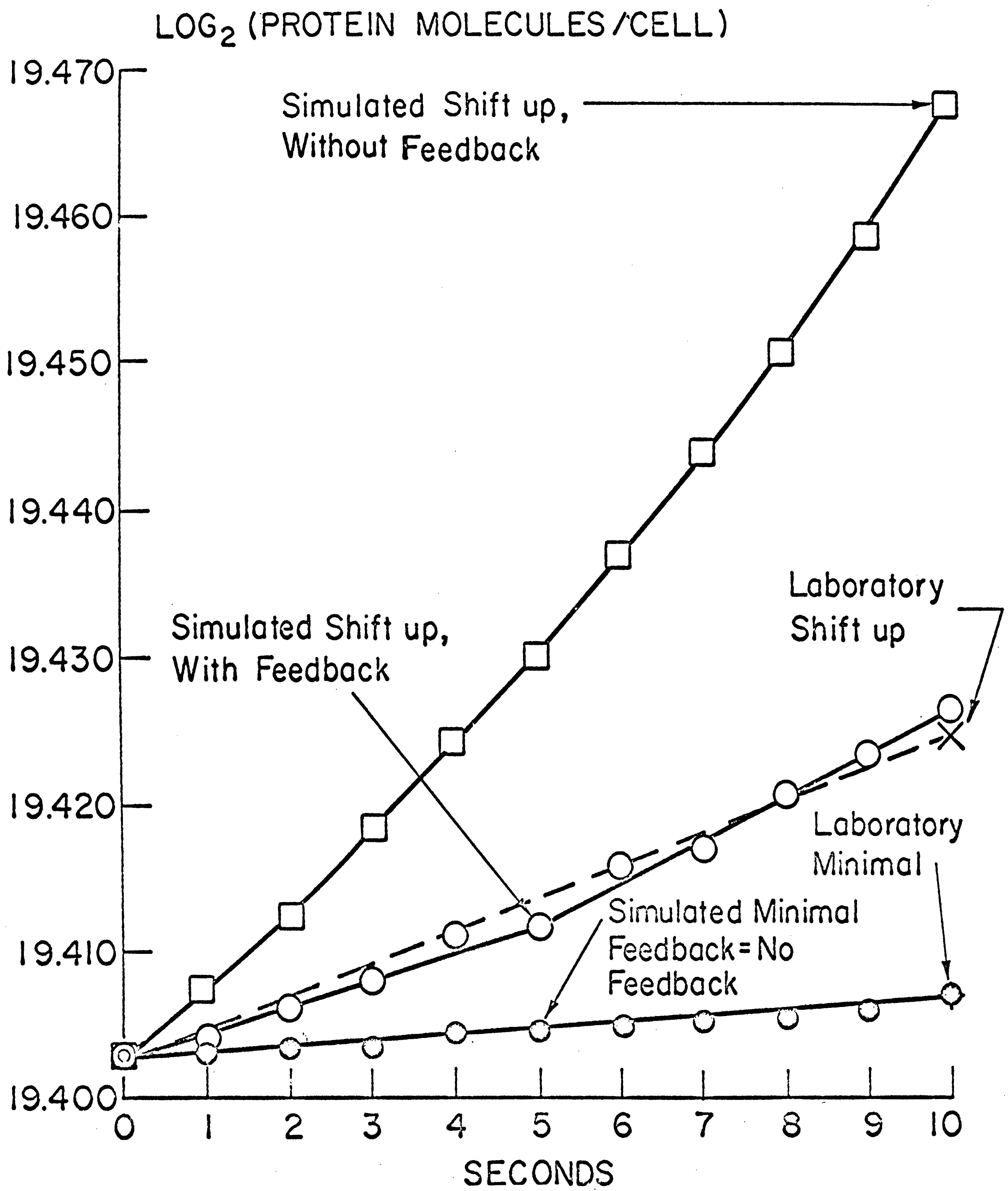

figure 9

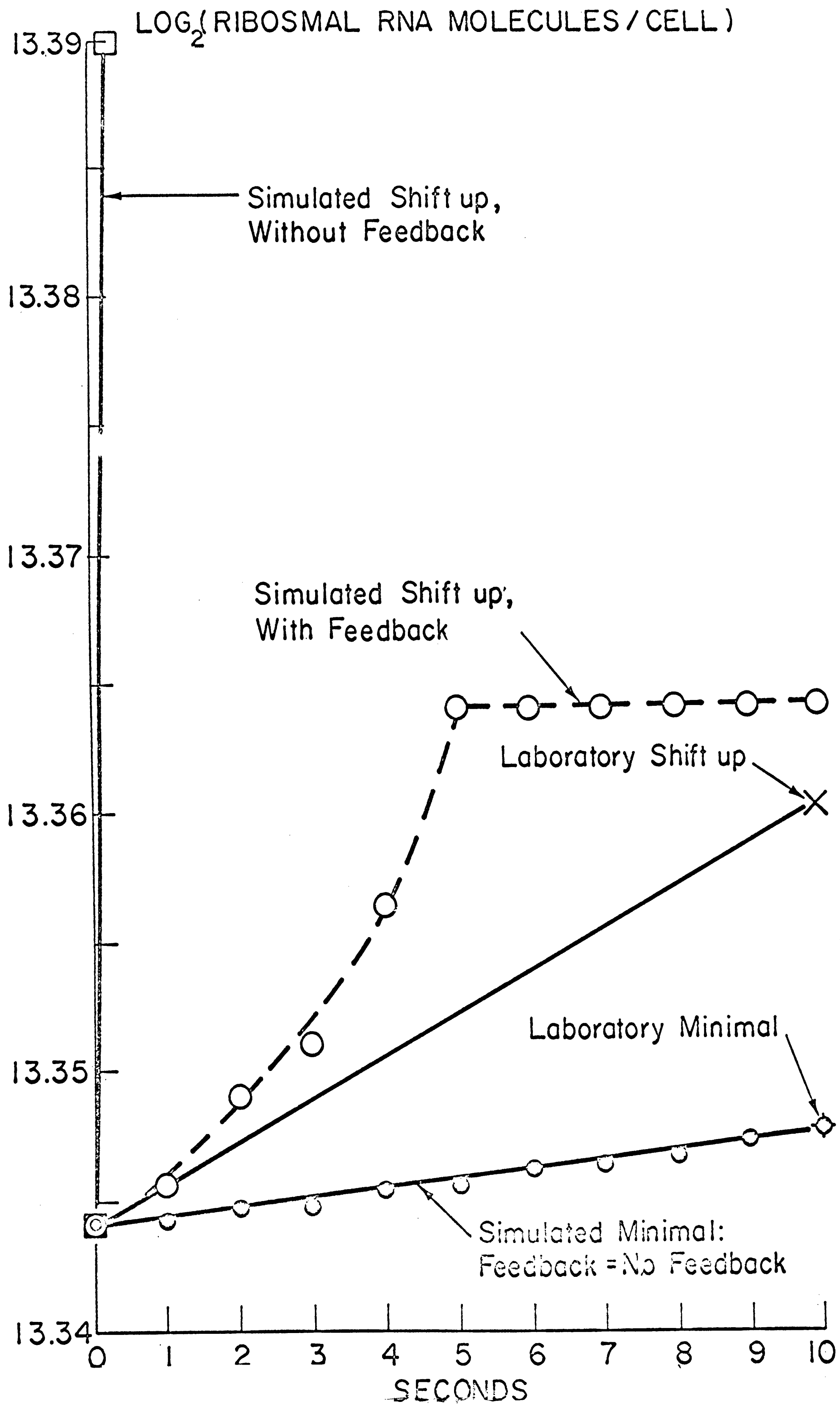

Figure 10

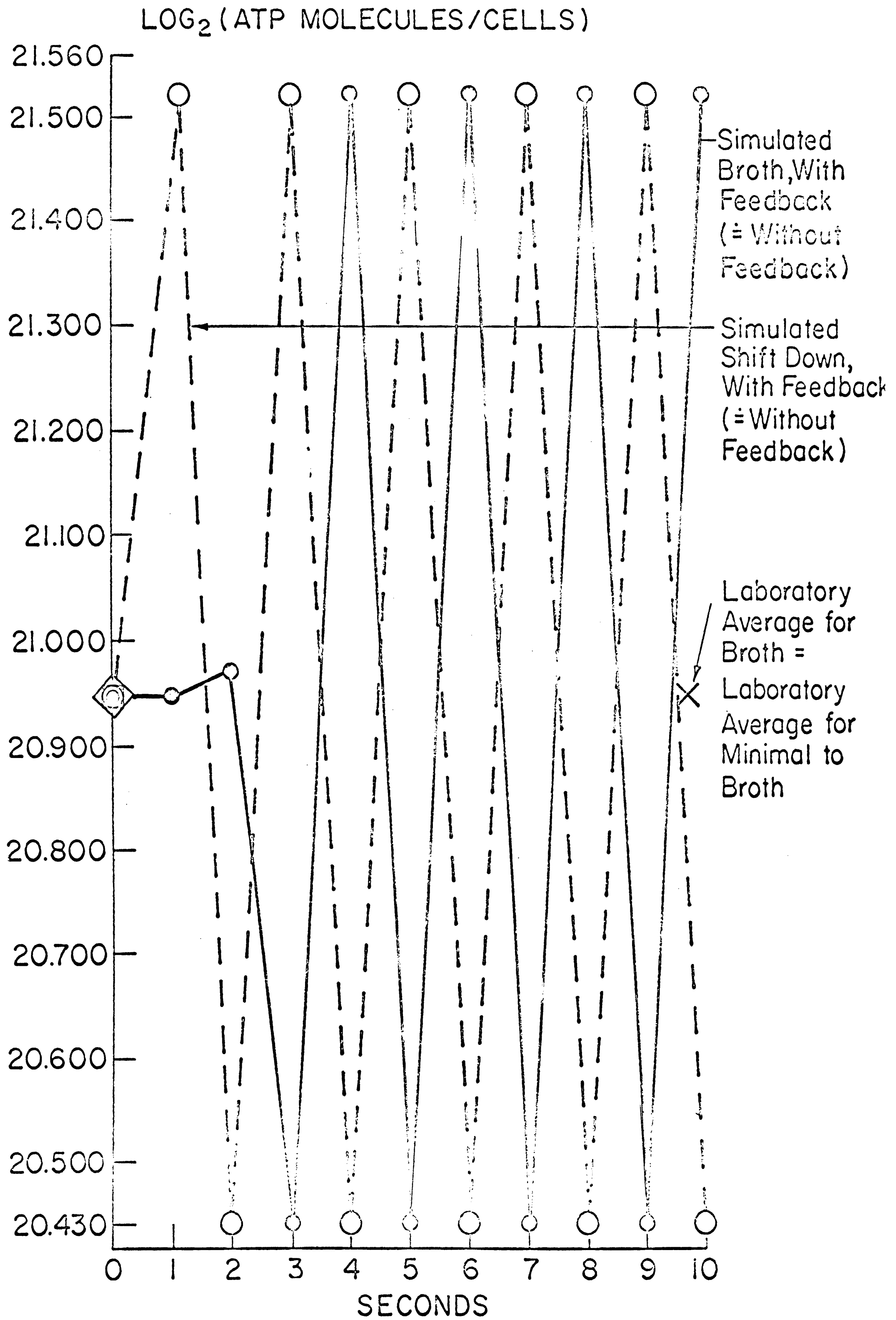

Figure 11.

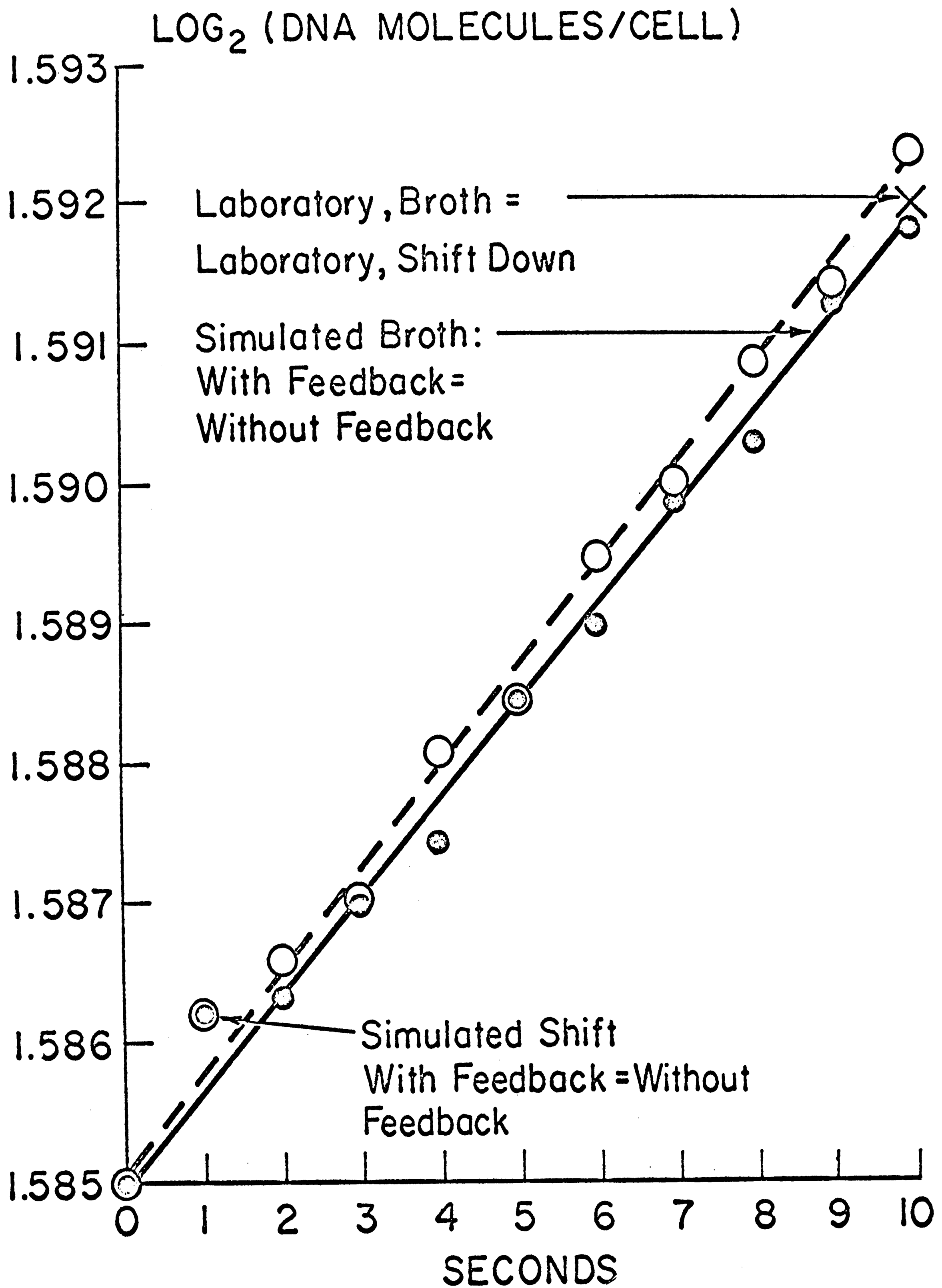

figure 12

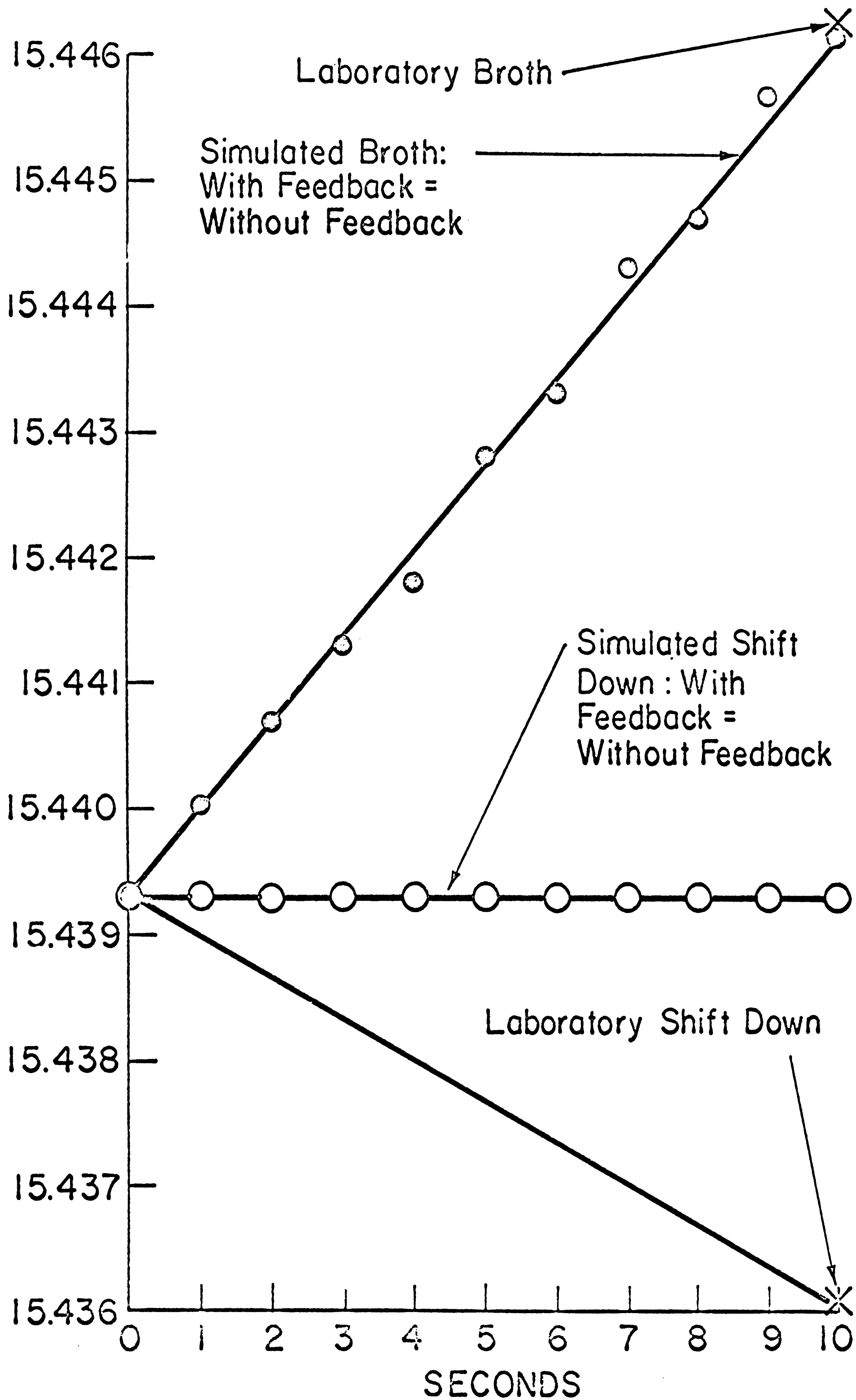

figure 13

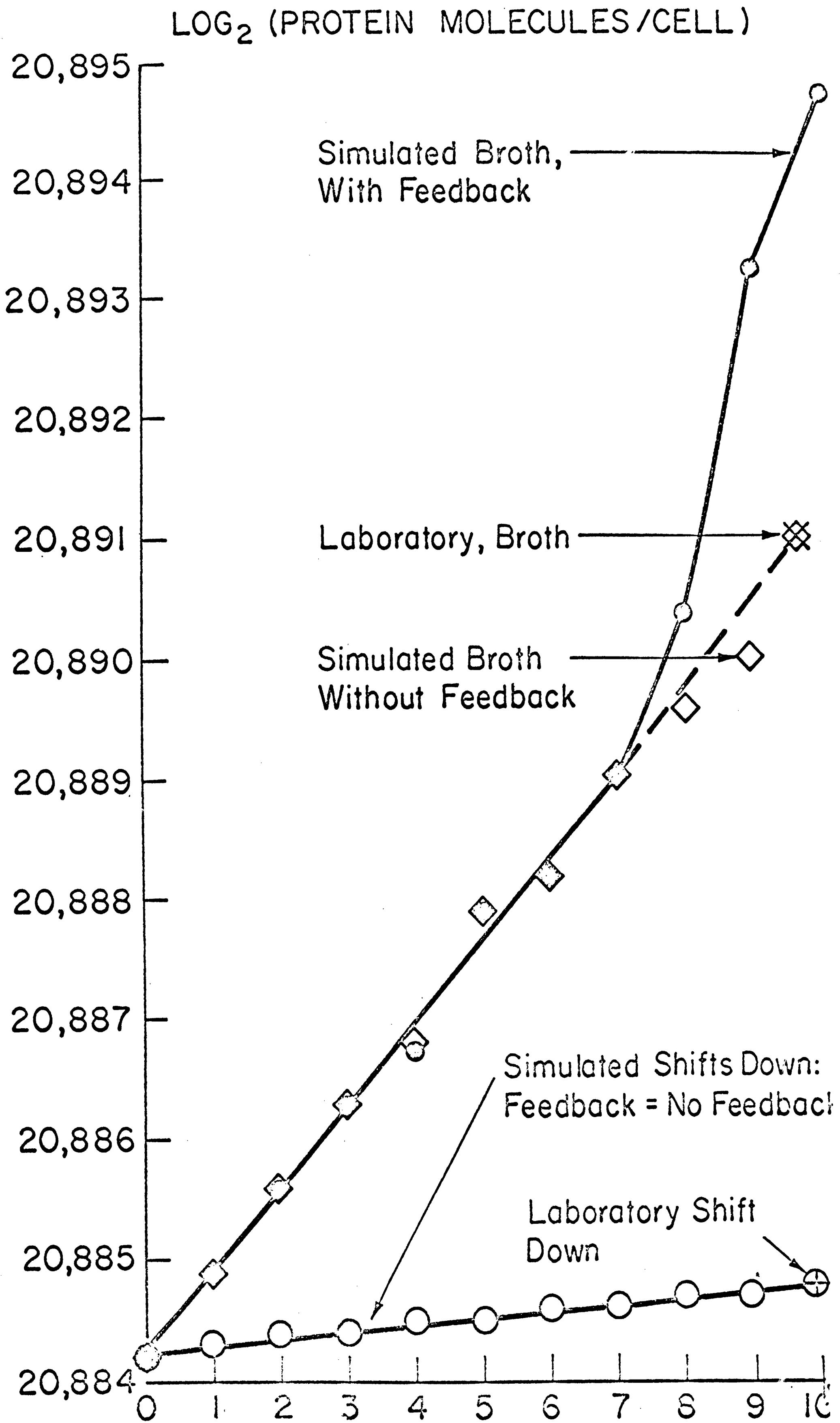

figure 14

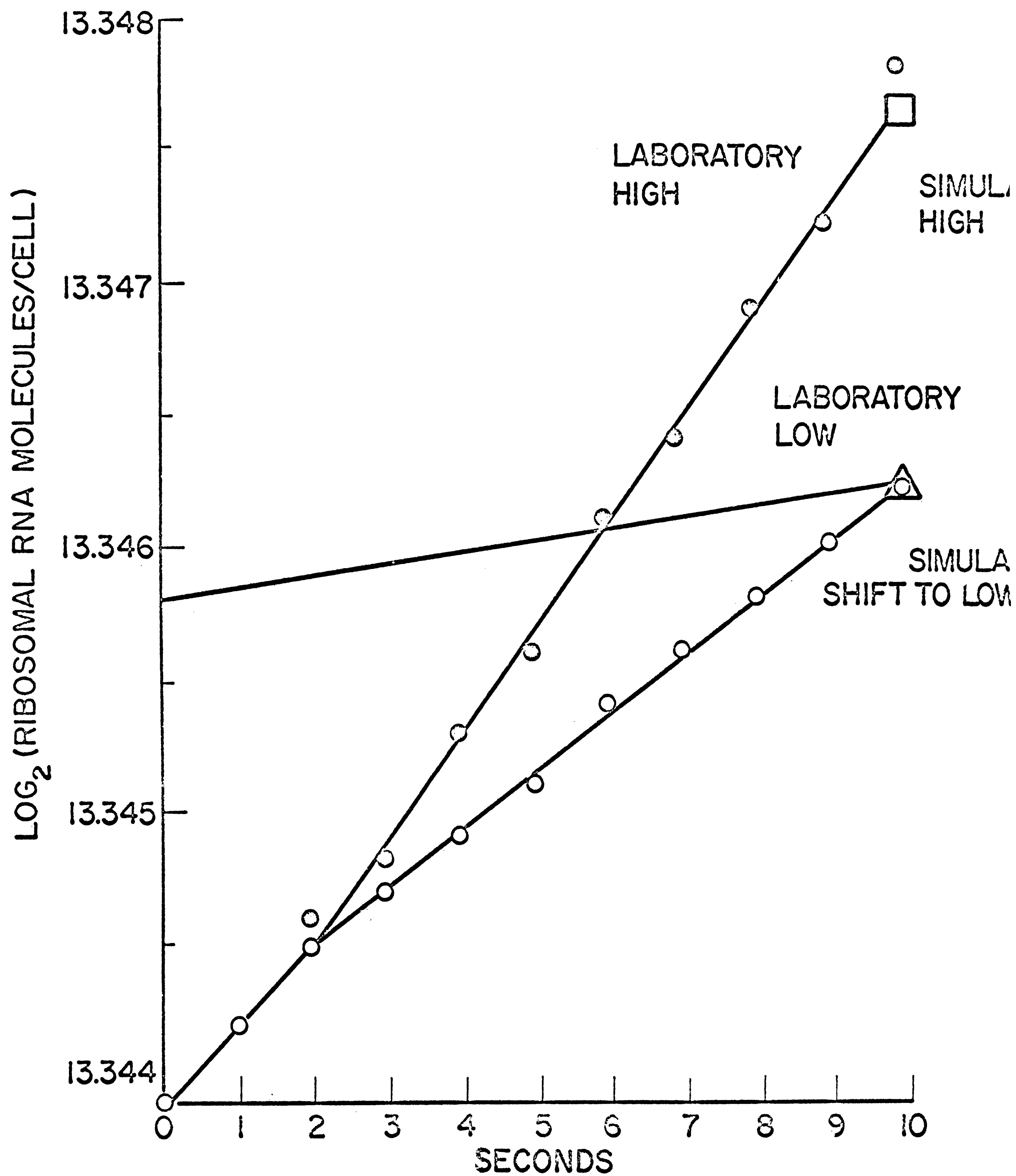

figure 15

(S, τ) is a <u>system</u> where

S - state space

$\tau : S \to S$ (transition function)

$$s_{t+1} = \tau(s_t)$$

S, given as a cartesian product

$$S = \times_{\alpha \in D} S_\alpha$$

where D - set of co-ordinates (entities)

S_α - state set (attribute set) of co-ordinate α

Homomorphism h from (S, τ) to (S', τ') is a map $h: S \to S'$ such that for all $s \in S$

$$h(\tau(s)) = \tau'(h(s))$$

Homomorphisms may be obtained by partitioning co-ordinate set D such that the blocks, or higher level co-ordinates preserve the transition function.

figure 16

STATE SPACE	CO-ORDINATES	CO-ORDINATE SETS	TRANSITION FUNCTION
Atomic	Electrons, nuclei	Position, momentum	Schrodinger's equation (quantum mechanics)
Molecular	Molecules	Shape, active site, energy level	e.g. Koch probabilistic model
Concentration	Molecule type e.g. ADP, DNA	Number of molecules of ADP/cell etc.	Mathematical and logical equations based on chemical kinetics e.g. Chance et al
Higher level groupings	Bio-chemical pools e.g. amino acids	Number of molecules of pool/cell	Mathematics and logical equations based on chemical kinetics and Molecular control mechanisms e.g. repression, allosteric inhibition (Weinberg, Goodwin)

Figure 17.

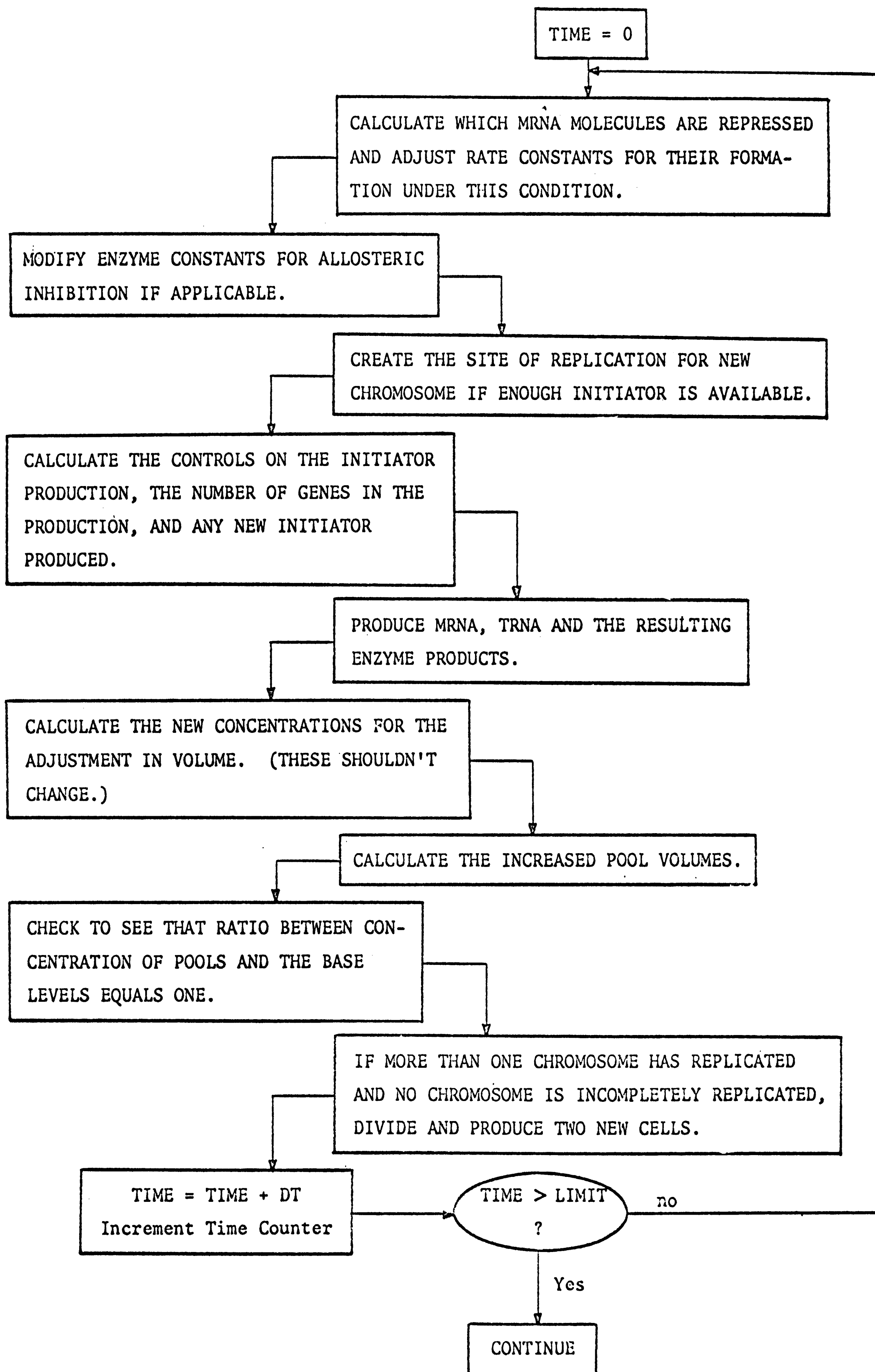

Figure 18. *Summary of Program.*

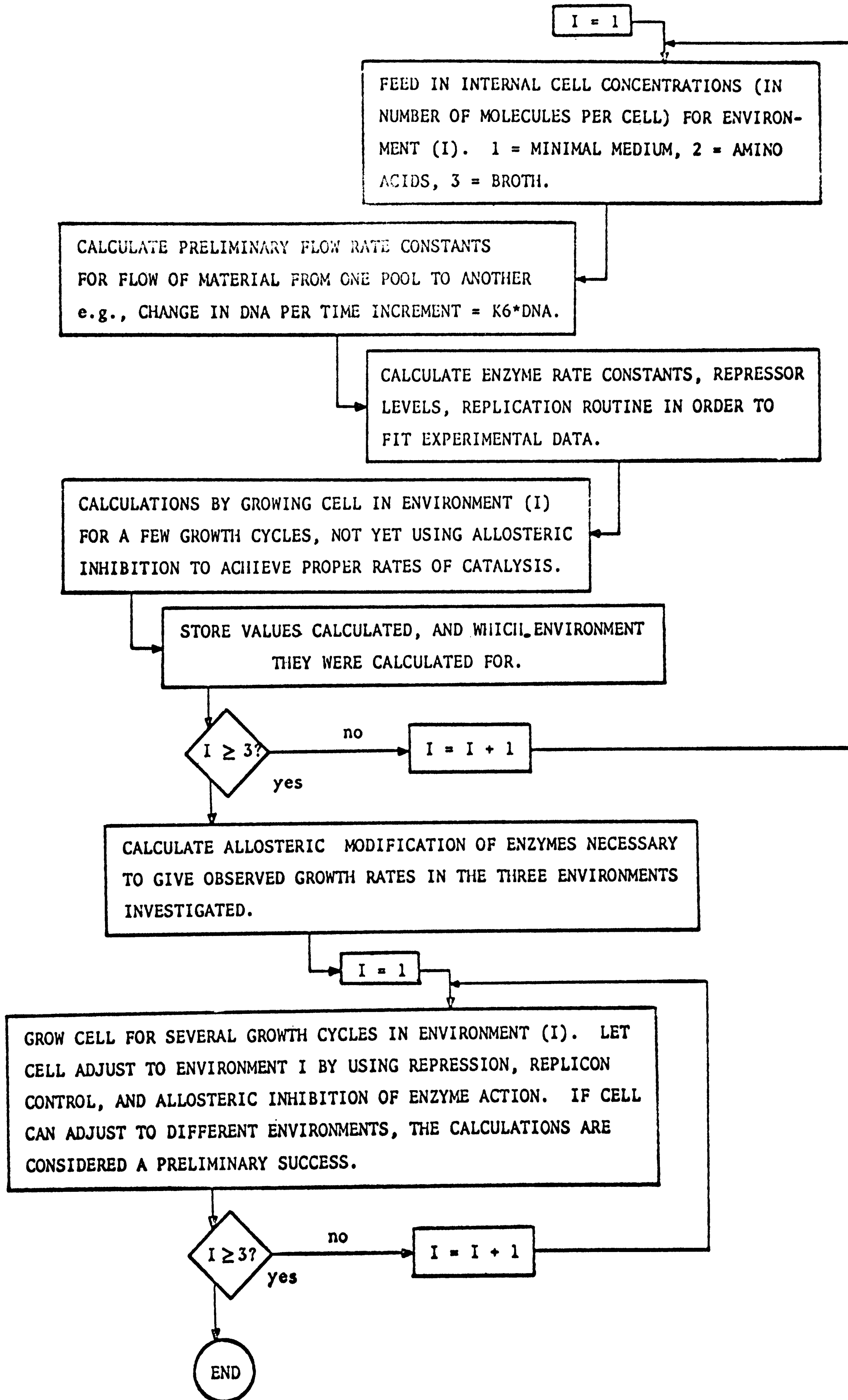

figure 19

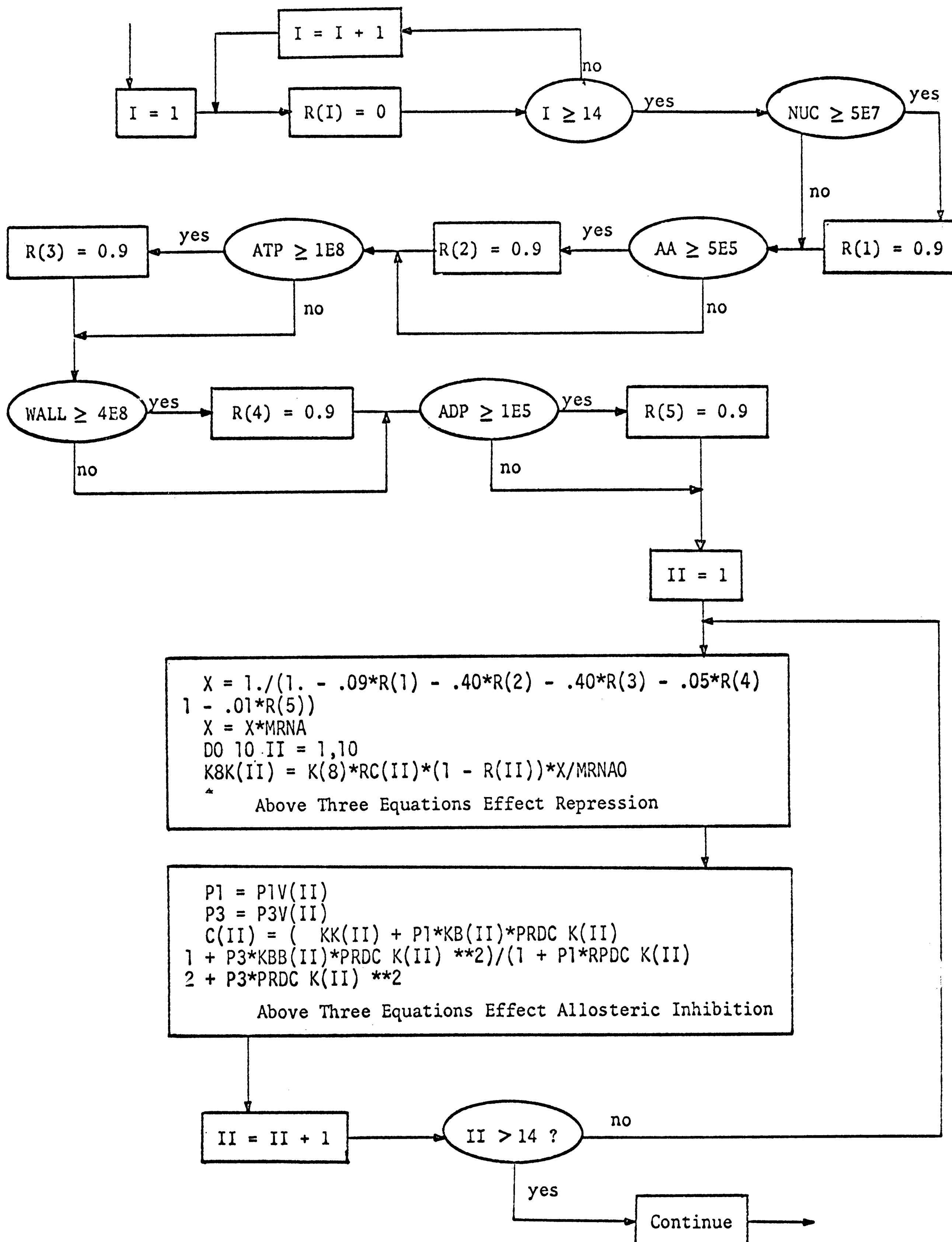

Figure 20 Repression and Allosteric Inhibition.
Repression is obtained by adjustment

TABLE 1

VARIABLES IN PROGRAM: A = array
0 = floating point
1 = integer

Variable	Array	Type	Description
A2	A	0	arrays used in solve function to obtain rate constants used
A3	A	0	in allosteric inhibition
AAO		0	amino acid concentration at time zero
AAP		0	ATP molecules used to make 1 amino acid molecule
AAO		0	amino acid concentration
ADJST		0	adjustment factor for concentrations from volume increase
ADPO		0	ADP concentration at time 0
ADP		0	ADP concentration
ATPO		0	ATP concentration at time zero
ATP		0	ATP concentration
ATPSB	A	0	array to store ATP concentrations in different environments
BROTH		1	equals 1 if cell growing in broth
CAA		1	equals 1 if cell growing in casamino acid
CHRMO		0	number of chromosomes at time 0
CNTRL		1	equals 1 if cell using metabolic controls to adjust growth rate
COUNT		0	number of growth cycles made
CRAZY		1	used as a logical variable
C(I)	A	0	enzyme rate constants
DAA		0	change in amino acid concentration
DADP		0	change in ADP concentration
DAPO2		0	change in ATP concentration from literature
DATP		0	change in ATP concentration calculated from rate constants in one time step
DDNA1		0	change in amount chromosome 1 in one time increment
DDNA2		0	change in amount of chromosome 2 in one time increment
DDNA3		0	change in amount of chromosome 3 in one time increment
DDNA		0	change in total DNA in one time increment
DEK(1)		0	change in enzymes for nucleotide production in one time increment
DEK(2)		0	change in enzymes for amino acid production in one time increment
DEK(3)		0	change in enzymes for glycolysis production in one time increment
DEK(4)		0	change in enzymes for wall production in one time increment
DEK(5)		0	change in enzymes for ADP, ATP synthesis in one time increment
DEK(6)		0	change in enzymes for DNA synthesis in one time increment
DEK(7)		0	change in enzymes for protein production in one time increment
DEK(8)		0	change in enzymes for MRNA synthesis in one time increment
DEK(9)		0	change in enzymes for ribosome synthesis in one time increment

DEK(10)		0 change in enzymes for TRNA production in one time increment
DIN		0 change in initiator concentration in one time increment
DNAO		0 DNA at time zero
DNA1		0 chromosome 1 "concentration", i.e., amount/volume of cell
DNA1Z		0 chromosome 1 at zero time
DNA1T		0 total chromosome 1
DNA2		0 chromosome 2 "concentration"
DNA2T		0 total chromosome 2
DNA2Z		0 chromosome 2 at zero time
DNA3		0 chromosome 3 "concentration"
DNA3T		0 total chromosome 3
DNA3Z		0 chromosome 3 at zero time
DNAP		0 ATP used per DNA molecule synthesized
DNA		0 DNA
DNASB	A	0 array to save concentrations of DNA in different environments
DNUC		0 change in nucleotide concentration
DBLE		0 time for cell to go through one reproductive cycle
DPRTN		0 change in protein in one time increment
DRIB		0 change in ribosome in one time increment
DMRNA		0 change in MRNA in one time increment
DRNA		0 change in total RNA in one time increment
DRNK(i)	A	0 change in MRNA for enzyme EK(i) in one time increment. i ranges from 1 to 10.
DT		0 length of one time increment, = differential
DUM1		0 dummy variable in solve function
DUM2		0 dummy variable in solve function
DUM3		0 dummy variable in solve function
DVOL		0 change in cell volume in one time increment
DWALL		0 change in cell membrane and cell wall in one time increment
DPRDK	A	0 array of change in product concentration in one time increment
PRD	A	0 the stored array of the previous four product values, for predictor corrector
DPRD	A	0 array of the four previous D(product) values for the predictor corrector
PPRD	A.	0 current array of the predictor values of products
CPRD	A	0 current array of corrector values of products
EK(1)		0 concentration of enzymes for nucleotide production
EKZ(1)		0 concentration of enzymes for nucleotide production at zero time
EK(2)		0 concentration of enzymes for amino acid production
EKZ(2)		0 concentration of enzymes for amino acid production at zero time

where 3 indicates glycolysis
4 indicates cell wall production
5 indicates ADP, ATP production
6 indicates DNA production
7 indicates protein production
8 indicates MRNA production
9 indicates ribosome production
10 indicates TRNA production

FACTR		0	factor by which chromosomes multiply in one reproductive cycle
GLUCO		0	glucose concentration at zero time
GLUC		0	glucose concentration
ID		1	integer variable in RPLACE routine
IN11		0	site for replication of chromosome 11, = 1 if it is present
IN11Z		0	site for replication of chromosome 11 at zero time
IN1		0	site for replication of chromosome 1, = 1 if it is present
IN1Z		0	site for replication of chromosome 1 at zero time
IN21		0	site for replication of chromosome 21
IN21Z		0	site for replication of chromosome 21 at zero time
IN2		0	site for replication of chromosome 2
IN2Z		0	site for replication of chromosome 2 at zero time
IN31		0	site for replication of chromosome 31
IN31Z		0	site for replication of chromosome 31 at zero time
IN3		0	site for replication of chromosome 3
IN3Z		0	site for replication of chromosome 3 at zero time
IN		0	concentration of initiator in cytoplasm
II		1	an integer variable
INZ		0	initiator concentration at zero time
K(1)		0	preliminary rate constant for nucleotide production
K(2)		0	preliminary rate constant for amino acid production
K(3)		0	preliminary rate constant for glycolysis
K(4)		0	preliminary rate constant for cell wall production
K(5)		0	preliminary rate constant for ADP production
K(6)		0	preliminary rate constant for DNA production
K(7)		0	preliminary rate constant for protein production
K(8)		0	preliminary rate constant for MRNA production
K(9)		0	preliminary rate constant for ribosome production
K(10)		0	preliminary rate constant for TRNA production
K(14)		0	preliminary rate constant for volume increase as a function of wall
KDRNK		0	rate constant for MRNA decay
K8K(i)	A	0	rate constant for MRNA EK(i)
K8KZ(i)	A	0	rate constant for MRNA for EKZ(i)
KBB(i)	A	0	rate constant for allosterically inhibited enzyme EK(i) with two molecules of product attached to the enzyme
KB	A	0	array of rate constants of allosterically inhibited enzymes with one molecule of product attached to the enzyme

KIN 0 preliminary rate constant for initiator production
K8K(i) A 0 rate constant for production of EK(i)
KK(i) A 0 rate constant for uninhibited enzyme EK(i)
K(i) A 0 array to store preliminary rate constants, used for each environment
LN2 0 natural logarithm of 2

L 1 integer variable for calling on solve function
M 1 integer variable for printing loop
MRNAO 0 MRNA concentration at time zero
MRNAP 0 ATP per MRNA molecule produced
MRNA 0 MRNA concentration

MULT 0 number of genes producing initiator
NO 0 number of cell in population (doubles when cell divides)
NUCO 0 molecules of nucleotide at zero time
NUCP 0 molecules of ATP to make one nucleotide
NUC 0 concentration of nucleotide

P1 0 rate constant
P1V A 0 array of equilibrium rate constants for enzymes
P3 0 equilibrium rate constant for two molecule allosteric inhibition
P3V A 0 array of equilibrium rate constants for two molecule allosteric inhibition
PRDCO A 0 array equivalenced to products at zero time

PRDCK A 0 array equivalenced to products
PRDC A 0 array for storing concentrations of products in different environments
PRDC(1) 0 NUC
PRDC(2) 0 AA
PRDC(3) 0 ATP

PRDC(4) 0 WALL
PRDC(5) 0 ADP
PRDC(6) 0 DNA
PRDC(7) 0 PRTN
PRDC(8) 0 MRNA

PRDC(9) 0 RIB
PRDC(10) 0 TRNA
PRDC(11) 0 GLUC
PRDC(14) 0 VOL
PRTNO 0 protein concentration at zero time

PRTNP 0 ATP molecules used per protein molecule formed
PRTN 0 protein concentration
RAA 0 ratio of amino acid concentration to a base level
RADP 0 ratio of ADP concentration to a base level
RATP 0 ratio of ATP concentration to a base level

RC	A	0	array of repression constants for MRNA repression
RDNA1		0	ratio of chromosome 1 concentration to a base level
RDNA2		0	ratio of chromosome 2 concentration to a base level
RDNA		0	ratio of DNA concentration to a base level
REK(i)	A	0	ratio of EK(i) concentration to a base level, i = 1,...,10
RIBO		0	ribosome concentration at time zero
RIBP		0	ATP used per ribosome made
RIB		0	ribosome concentration
RNAO		0	RNA concentration at time zero
RNA		0	RNA concentration
TRNAO		0	transfer RNA concentration at time zero
TRNAP		0	ATP per transfer RNA molecule made
TRNA		0	transfer RNA concentration
RNK(i)	A	0	concentration of MRNA for enzyme EK(i), i = 1,...,10
RNKZ(i)	A	0	concentration at zero time of MRNA for EKZ(i), i = 1,...,10
RNUC		0	ratio of nucleotide concentration to a base level
RON		1	used as a logical variable turning repression on
RPRTN		0	ratio of protein concentration to a base level
RRIB		0	ratio of ribosome concentration to a base level
RMRNA		0	ratio of MRNA concentration to a base level
RRNA		0	ratio of RNA concentration to a base level
RTRNA		0	ratio of TRNA concentration to a base level
RRNK(i)	A	0	ratio of RNK(i) concentration to a base level, i = 1,...,10
R	A	0	array for repression constants
RVOL		0	ratio of new volume to old volume at end of one time increment
RWALL		0	ratio of pool for wall to a base level in terms of concentration
SUM	A	0	array used in solve function
T		0	generation time in seconds
VOLO		0	volume of cell at time zero
VOLN		0	volume at end of one time increment
VOL		0	volume
WALLO		0	concentration of pool for wall production at time zero
WALLP		0	ATP molecules used per molecule of cell wall produced
WALL		0	concentration of pool for wall production
X		0	variable used in repression routine
XK(i,j)	A	0	value of K(i) in environment (j)
XEK(i,j)	A	0	value of EK(k) in environment (j)
XK8(i,j)	A	0	value of K8K(i) in environment (j)

ACKNOWLEDGEMENT

This work was supported bya Public Health Service Fellowship 2-F3-CA-6931-03 from the National Cancer Institute awarded to the senior author; Public Health Service Grant GM-12236 from the National Institute of Health, Army Research Office-Durham Contract DA-31-124-APO-D-483 and Office of Naval Research Contract N00014-67-A-0181-0011.

UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) LOGIC OF COMPUTERS GROUP The University of Michigan Ann Arbor, Michigan	2a. REPORT SECURITY CLASSIFICATION Unclassified 2b. GROUP

3. REPORT TITLE

COMPUTER SIMULATION OF A LIVING CELL: MULTILEVEL CONTROL SYSTEMS

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Technical Report

5. AUTHOR(S) (Last name, first name, initial)

Weinberg, Roger
Zeigler, Bernard P.

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
December 1969	48	59

8a. CONTRACT OR GRANT NO. DA-31-124-ARO-D-483 b. PROJECT NO. c. d.	9a. ORIGINATOR'S REPORT NUMBER(S) 9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10. AVAILABILITY/LIMITATION NOTICES

Distribution of This Document is Unlimited

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	U. S. Army Research Office (Durham) Durham, North Carolina

13. ABSTRACT

A simple bacterial cell (Escherichia coli) has been modeled, and the input-output behavior of the model has been simulated as a program in FORTRAN IV for an IBM 360/67 digital computer.

Automata theoretic analysis of the homomorphic model underlying the computer simulation enables us to investigate the information content and complexity of the simulation and of the measurement space representing our data base.

The simulated cell is able to adjust its enzymes and DNA to grow in different chemical environments using allosteric modification of enzymes, and repression of RNA synthesis. It grows at realistic rates and achieves limited metabolic stability.

DD FORM 1 JAN 64 1473

UNCLASSIFIED
Security Classification

UNCLASSIFIED
Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT

INSTRUCTIONS

1. ORIGINATING ACTIVITY: Enter the name and address of the contractor, subcontractor, grantee, Department of Defense activity or other organization *(corporate author)* issuing the report.

2*a*. REPORT SECURITY CLASSIFICATION: Enter the overall security classification of the report. Indicate whether "Restricted Data" is included. Marking is to be in accordance with appropriate security regulations.

2*b*. GROUP: Automatic downgrading is specified in DoD Directive 5200.10 and Armed Forces Industrial Manual. Enter the group number. Also, when applicable, show that optional markings have been used for Group 3 and Group 4 as authorized.

3. REPORT TITLE: Enter the complete report title in all capital letters. Titles in all cases should be unclassified. If a meaningful title cannot be selected without classification, show title classification in all capitals in parenthesis immediately following the title.

4. DESCRIPTIVE NOTES: If appropriate, enter the type of report, e.g., interim, progress, summary, annual, or final. Give the inclusive dates when a specific reporting period is covered.

5. AUTHOR(S): Enter the name(s) of author(s) as shown on or in the report. Enter last name, first name, middle initial. If military, show rank and branch of service. The name of the principal author is an absolute minimum requirement.

6. REPORT DATE: Enter the date of the report as day, month, year; or month, year. If more than one date appears on the report, use date of publication.

7*a*. TOTAL NUMBER OF PAGES: The total page count should follow normal pagination procedures, i.e., enter the number of pages containing information.

7*b*. NUMBER OF REFERENCES: Enter the total number of references cited in the report.

8*a*. CONTRACT OR GRANT NUMBER: If appropriate, enter the applicable number of the contract or grant under which the report was written.

8*b*, 8*c*, & 8*d*. PROJECT NUMBER: Enter the appropriate military department identification, such as project number, subproject number, system numbers, task number, etc.

9*a*. ORIGINATOR'S REPORT NUMBER(S): Enter the official report number by which the document will be identified and controlled by the originating activity. This number must be unique to this report.

9*b*. OTHER REPORT NUMBER(S): If the report has been assigned any other report numbers *(either by the originator or by the sponsor)*, also enter this number(s).

10. AVAILABILITY/LIMITATION NOTICES: Enter any limitations on further dissemination of the report, other than those imposed by security classification, using standard statements such as:

(1) "Qualified requesters may obtain copies of this report from DDC."

(2) "Foreign announcement and dissemination of this report by DDC is not authorized."

(3) "U. S. Government agencies may obtain copies of this report directly from DDC. Other qualified DDC users shall request through
____."

(4) "U. S. military agencies may obtain copies of this report directly from DDC. Other qualified users shall request through
____."

(5) "All distribution of this report is controlled. Qualified DDC users shall request through
____."

If the report has been furnished to the Office of Technical Services, Department of Commerce, for sale to the public, indicate this fact and enter the price, if known.

11. SUPPLEMENTARY NOTES: Use for additional explanatory notes.

12. SPONSORING MILITARY ACTIVITY: Enter the name of the departmental project office or laboratory sponsoring *(paying for)* the research and development. Include address.

13. ABSTRACT: Enter an abstract giving a brief and factual summary of the document indicative of the report, even though it may also appear elsewhere in the body of the technical report. If additional space is required, a continuation sheet shall be attached.

It is highly desirable that the abstract of classified reports be unclassified. Each paragraph of the abstract shall end with an indication of the military security classification of the information in the paragraph, represented as *(TS)*, *(S)*, *(C)*, or *(U)*.

There is no limitation on the length of the abstract. However, the suggested length is from 150 to 225 words.

14. KEY WORDS: Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context. The assignment of links, rules, and weights is optional.

UNCLASSIFIED
Security Classification