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BEHAVIOR: IMBALANCE IN A NETWORK OF CHEMICAL TRANSFORMATIONS

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Dan F. Bradley and M. Calvin

October 12, 1955

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BEHAVIOR: IMBALANCE IN A CHEMICAL NETWORK

Contents

Abstract 3
Introduction 4
Equilibrium 4
Steady State 7
Network Structure 10
Inflow and Outflow Rates in the Steady State 12
Transient Inflow and Outflow Rates 14
Internal Steady-State Concentrations 17
Internal Transient Concentrations 18
Network-Structure Determination in Many-Component Systems 21
Network Determination in Living Systems 22
The Photosynthetic Network in Green Plants 22
Behavior of the Photosynthetic Network 28

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ABSTRACT

A simple chemical system in a fixed environment tends to reach a state of chemical balance in which rates of reactions and concentrations of components in the system do not change with time. A sudden change in the environment destroys this balance, resulting in transient changes in the rates of reactions and concentrations of components within the system. Such transients are gradually damped out and the system tends to a new stationary state. The responses of mathematical models of a number of simple chemical systems to environmental changes, and the lifelike appearance of these responses, are examined. Experimental methods of measuring such behavior in chemical systems and the use of this information in determining the network of chemical transformations within the system are investigated. The application of these general methods to determination of the network of chemical reactions in a living plant cell is reviewed. The close relationship between the observed behavior of the living photosynthesizing plant cell to sudden changes in environment and the behavior to be expected on the basis of the chemical network within the cell is demonstrated.

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INTRODUCTION

Very simple chemical systems behave as if they were alive, and the more complex they become the more lifelike their behavior. It would be interesting to know whether very simple living things behave as if they were chemical systems. Behavior is the response of a system to a change in its environment, so we are asking whether the complex network of chemical transformations in the living organism when thrown into a state of temporary imbalance by a change in environment will respond or behave as we would expect on the basis of what we know about chemical systems.

We shall examine in this paper some of the lifelike behavior patterns of simple chemical systems, the kinds of experiments that provide information about the network of chemical transformations in more complex chemical systems, how these methods have been applied in the study of photosynthetic algae, and how the network in these algae responds to its environment.

EQUILIBRIUM

Let us consider a system consisting of a hollow, water-filled sphere, constructed of a rigid water-permeable membrane, which is suspended in an environment of water. Molecules of a chemical A dissolved in the environment collide with the membrane and--provided their molecular dimensions are sufficiently small--they either pass through the pores of the membrane into the system or are reflected back from the solid material of the membrane into the medium. The number of molecules colliding with the membrane in a given time interval is proportional to the number of these molecules in a given volume of the medium, i.e. the external concentration of A, called (A_x). If the ratio of the penetrations to total collisions is designated as k, then the rate at which A

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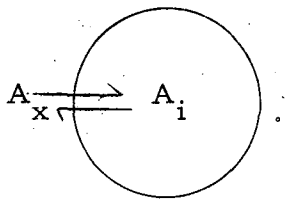
enters the system is just $k(A_x)$, and the amount of A entering the system in a given time period of duration t is $tk(A_x)$. At very long times the amount of A in the system becomes very large according to this equation:



However, if the molecules of A can pass through the membrane into the system they can also pass outward by the same pathways. The ratio of penetrations to total collisions differs for the inflow and outflow processes if the external and internal media differ markedly, but for the purposes of the discussion we shall assume them to be equal so that the flow outward is $k(A_i)$. The net rate of flow inward is the inflow rate minus the outflow rate and in the language of calculus is given the symbol $d(A_i)/dt$, so that

$$d(A_i)/dt = k(A_x) - k(A_i). \qquad (1)$$

Starting with the system and the environment containing no A, let us examine the behavior of the system when sufficient A is introduced into the medium to increase its concentration of A to unity. Assuming arbitrarily that $k = 1$, we can integrate the differential Eq. (1) to find the value of (A_i) at any time t. The calculated curve of (A_i) versus t appears in Fig. 1.

The value of (A_i) does not continue to increase linearly with time but levels off at the value of (A_x). Under these conditions the inflow and outflow proceed at the same rate so that there is no further change in (A_i) with time. The system has responded to the sudden influx in A by increasing the rate at which it excretes A back into the medium. This general type of behavioral response of chemical systems in which the system resists change is referred to as the operation of LeChatelier's Principle. The system behaves, in the sense that it responds to its environment, only when it is in a state of imbalance. Its behavior tends to bring it into a state of dynamic equilibrium with the environment in which there is no further change in either the system or the environment with time. The system remains in that state until the environment itself changes.

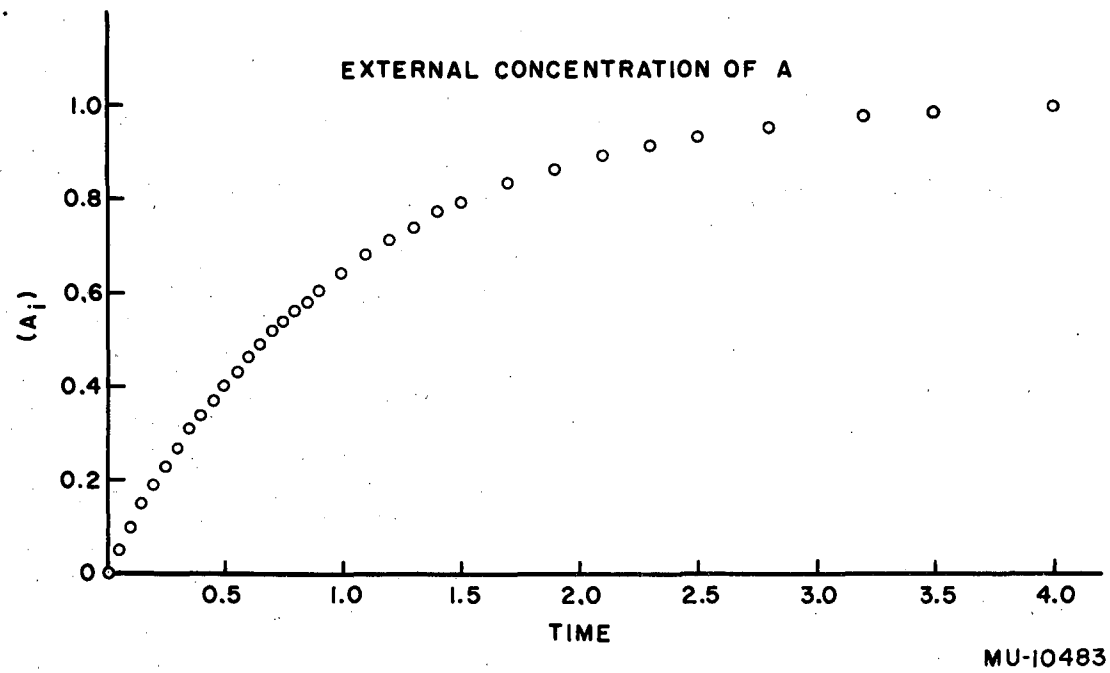
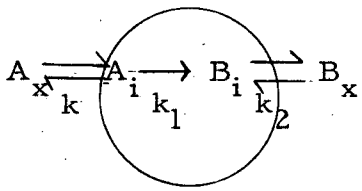


Fig. 1 The response of a one-component system to a sudden increase in the external concentration of that component.

STEADY STATE

Let us extend our system to include a second freely diffusible chemical B, formed by a very slow rearrangement of the atoms in the molecule A, and a catalyst whose sole function is to accelerate the conversion of A to B. The rate at which A is converted into B in the system depends on the nature of the catalyst, the temperature, the composition of the solvent, and other things; but with a sufficient amount of catalyst twice as many molecules of A will produce twice as many molecules of B in a given time, so that the rate of formation of B from A is proportional to (A_i) or equal to $k_1(A_i)$:



The net rate of formation of A_i with time is the inflow rate $k(A_x)$, minus the outflow rate $k(A_i)$, minus the rate of conversion of A to B, $k_1(A_i)$; i.e.,

$$d(A_i)/dt = k(A_x) - k(A_i) - k_1(A_i). \qquad (2)$$

Correspondingly, the net rate of formation of B_i is just its rate of formation from A_i, $k_1(A_i)$, plus the rate of inflow, $k_2(B_x)$, minus the rate of outflow, $k_2(B_i)$; i.e.,

$$d(B_i)/dt = k_1(A_i) + k_2(B_x) - k_2(B_i). \qquad (3)$$

How does this system initially containing neither A nor B behave when the concentrations of A and B in the environment are suddenly raised to unit concentration? Choosing arbitrary initial conditions of $(A_i) = (B_i) = 0$ and $(A_x) = (B_x) = k = k_1 = k_2 = 1$, we can integrate the differential equations (2) and (3) to find (A_i) and (B_i) as functions of time. As shown in Fig. 2, (A_i) and (B_i) rise to stationary levels that differ from their external concentrations. In this state the net rate of inflow of A is $k(A_x) - k(A_i) = 0.5$, and the net rate of outflow of B is $k_2(B_i) - k_2(B_x) = 0.5$, but the changes in concentration of A and B are negligible, so that there is no change in the rate of conversion of A to B with time. The condition in which a system is maintained in a nonequilibrium state maintained by changes in the environment is called the steady state.

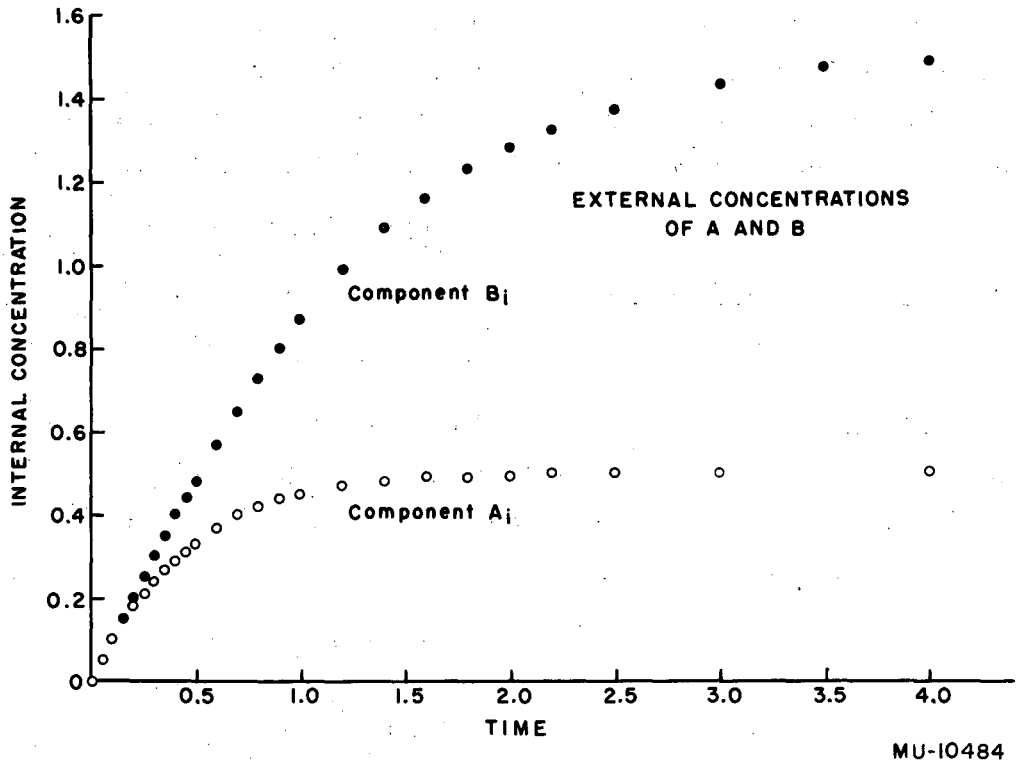


Fig. 2. Response of a two-component system to a sudden increase in the external concentrations of the components.

The process that maintains the system in an "alive" state in the sense of a nonequilibrium state is the conversion of A to B within the system. There are at least four conditions which the reaction must satisfy if the system is to exploit the environment in this way: the chemical natures of A and B must be such as to permit the conversion to proceed spontaneously; the rate at which this conversion proceeds must be slow in the environment so that A will be present in the environment for the system to utilize; the reaction may be accelerated by suitable catalysts; the system must be open to its environment so that it can absorb A from it and excrete B into it. This behavior corresponds to that of living systems, which are essentially complex systems of catalysts that maintain themselves in nonequilibrium states by the directed, stepwise utilization of the natural tendency of foodstuffs to react with the oxygen of the air.

In both equilibrium and steady states, the concentrations of components in the system do not change with time, i.e.,

$$d(A_i)/dt - d(B_i)/dt = 0.$$

Substituting these conditions into the differential equation (1), we find

$$k(A_x) = k(A_i), \text{ and therefore } (A_x) = (A_i),$$

so that, as we saw in Fig. 1, in the equilibrium case the composition of the final state is completely determined by the composition of the environment. Substituting the equilibrium or steady-state condition into the Eq. (2) and (3), we find

$$k(A_x) = k(A_i) + k_1(A_i) \quad \text{or} \quad (A_i) = (k/(k + k_1))\,(A_x),$$

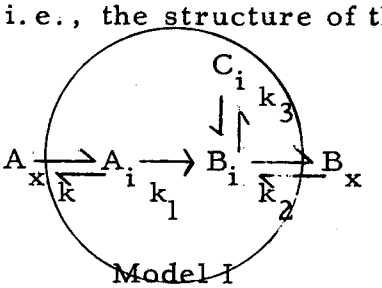
$$k_2(B_i) = k_1(A_i) + k_2(B_x) \quad \text{or} \quad (B_i) = (B_x) + \frac{k_1 k(A_x)}{k_2(k + k_1)}.$$

In the steady state, therefore, the internal composition is in part determined by the external conditions (A_x) and (B_x), but also by the internal parameter k_1, the rate constant for the conversion of A to B. Since the numerical value of this constant depends on the amount and kind of catalyst in the system, the system--by controlling the supply of catalyst--has an element of control over its internal steady state. This corresponds to the behavior of living systems, which regulate their milieu by regulating the supply of protein catalysts.

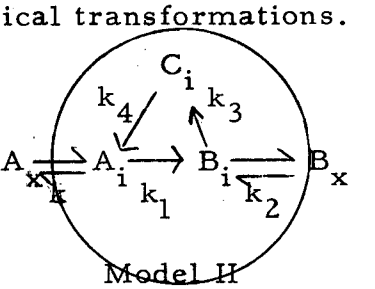
Another self-determining feature of the steady state is seen in the fact that the initial concentrations do not enter into the expressions for the final states. This means that the same balanced state is reached whatever the initial state of the system. This is shown graphically in Fig. 3, in which (A_i) is initially 2.0 rather than 0 units as in the previous example, while all the other parameters are held the same. The initial conditions influence only the transient concentration changes in reaching the steady state.

NETWORK STRUCTURE

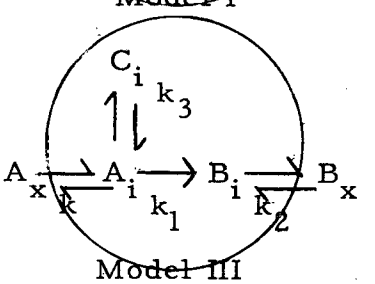
Let us include in our system a third component, C, which cannot permeate the membrane for reasons of its size, shape, or electrical charge. A number of different networks can be constructed from three components which differ as to the precursors and products of each component. Let us examine the kinds of experiments that might be carried out to determine the precursor-product relations for each component, i.e., the structure of the network of chemical transformations.



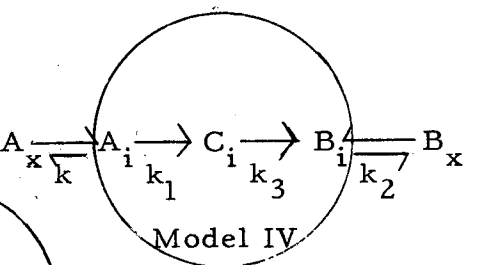
Model I



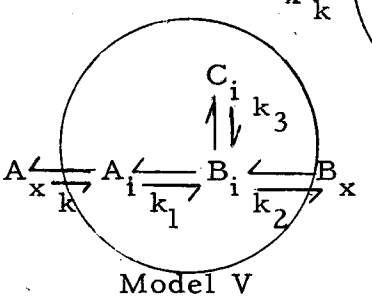
Model II



Model III



Model IV



Model V

We shall discuss only those experiments which observe and measure the intact system, and shall only mention in passing two powerful methods that provide much information about network structure through observation

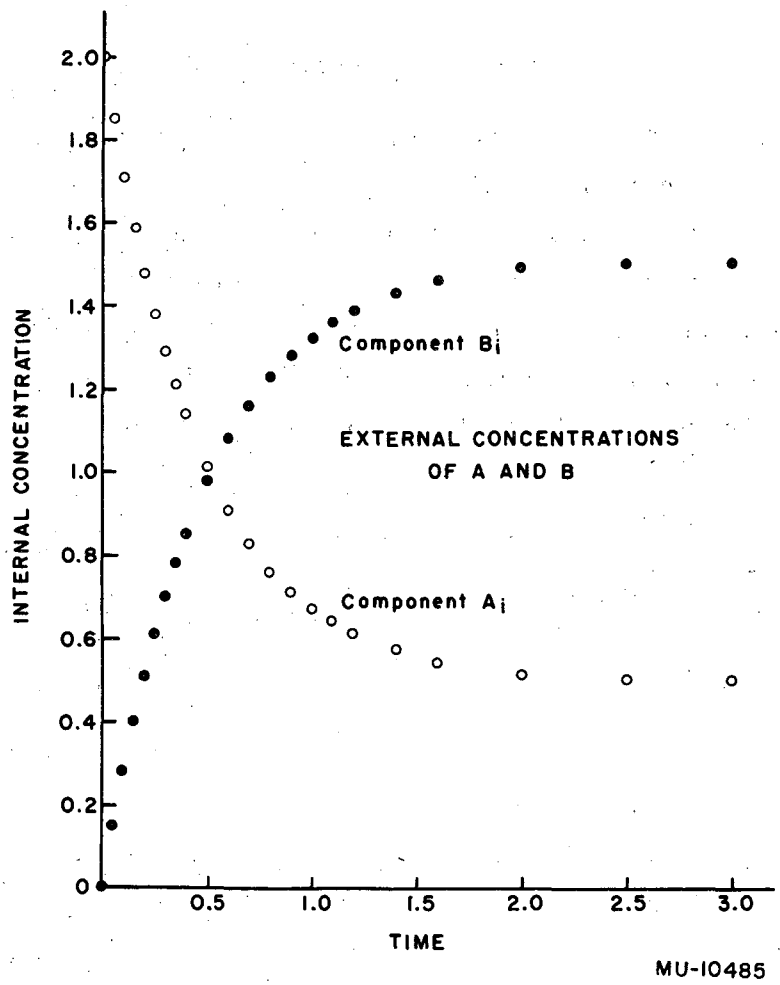


Fig. 3. Response of a two-component system to a sudden shift in the external concentrations of the components.

of parts of the fragmented system. One such method is to look at the components from the point of view of their chemical structure and make predictions as to which component will transform into which other component. Another method is based on the fact that there is a catalyst for nearly every reaction proceeding in a living system. These protein catalysts, or enzymes, may be isolated from the organism individually so that the specific reaction which each catalyzes can be studied in detail. If an enzyme is found that catalyzes the conversion of A to B, another that catalyzes the conversion of B to C, then it is assumed that the network element $A \longrightarrow B \longrightarrow C$ is operative in the organism. The current biochemical literature is crowded with papers reporting the isolation of new enzymes that catalyze new reactions, and such work has contributed mightily to our understanding of the networks in living systems.

Measurements on the intact system may be roughly divided into four classes, as indicated in Table I. In general, information about the network

Table I

Classes of measurements on intact systems

Measurements	Steady State	Transient
External	Inflow and outflow rates	The rate of change of inflow and outflow rates from one steady state to another.
Internal	Concentrations of the components.	The rate of change of concentrations of components from one steady state to another.

can be obtained only by observing the behavior of the system, i.e., the response of the system to a change in the environment. When the system is in a state of imbalance the pathway by which it returns to a steady state is a very sensitive indicator of its network.

INFLOW AND OUTFLOW RATES IN THE STEADY STATE

Let us consider a real system of three components which we think may have one of the five networks modeled above. Can we distinguish them by measuring the steady-state rate at which A flows into the system and B flows out of it? We can best answer this question by calculating the

steady-state flow rate of A (which is equal to the flow rate of B in the steady state) for the five model systems, using the methods illustrated for the two-component system. The five models fall into two groups, (II and V, and I, III, and I_v), which differ in their response to varying (B_x) (Table II). Measurement of the dependence of steady-state flow rates of the real system on (B_x) will thus discriminate between the two groups. However, since this method does not determine the numerical values of the rate constants it cannot discriminate between models within the same group.

Further information may be gained by altering one or more rate constants by the addition of chemicals which either stimulate or inhibit the catalytic activity of a given enzyme. If, for example, a chemical is

Table II

Steady-State Flow Rates

Model	Inflow Rate of A
I	$\left(k - \frac{k^2}{k + k_1}\right) (A_x)$
II	$\left(k - \frac{k^2}{k + k_1 - \frac{k_1 k_3}{k_2 + k_3}}\right)(A_x) - \left(\frac{kk_2k_2}{(k_2+k_3)(k+k_1 - \frac{k_1k_3}{k_2+k_3})}\right)(B_x)$
III	$\left(k - \frac{k^2}{k + k_1}\right) (A_x)$
IV	$\left(k - \frac{k^2}{k + k_1}\right) (A_x)$
V	$\left(k - \frac{k_1k^2+k_2k^2}{k_1k+k_1k_2+kk_2}\right) (A_x) - \left(\frac{kk_1k_2}{k_1k + k_1k_2 + kk_2}\right) (B_x)$

added to the system that is known to completely inhibit the reaction $B \rightarrow C$ the effect is comparable to reducing k_3 to zero in Models I, II, and V. As can be seen in Table II, k_3 appears only in the steady-state flow rate of Model II, and in such a form that when $k_3 = 0$ the flow rate no longer depends upon (B_x). This method would distinguish between the two models

whose flow rates depend upon (B_x). Inhibitors of other reactions may be used in the same way to eliminate possibilities, but the method in general is limited to discrimination solely on the basis of the form of the dependence of flow rate upon experimental variables.

TRANSIENT INFLOW AND OUTFLOW RATES

The limitation of the method just described results from the fact that both the rate parameters and the structure of the network affect the observed quantity. The time course of the flow rate as it changes from one steady state to another, i.e., as it "behaves", is less dependent on rate parameters and more so on net structure.

A transient flow process frequently followed in biochemical systems is the excretion of radioactive atoms injected into the system in the form of one of the components. In such experiments it is customary to assume that a molecule containing a radioactive atom behaves just like a normal one until the radioactive atom explodes. This is not strictly true, since the difference in weight between the radioactive and normal atoms affects somewhat the rate at which the molecule reacts, but in what follows we shall neglect such effects altogether.

The rate at which radioactive atoms in a molecule A are transferred to molecule B is simply the rate at which all the molecules of A are transformed into molecules of B times the fraction of the molecules of A that contain radioactive atoms. This fraction we will designate as X_a and although, strictly speaking, it is the mole-fraction activity we will refer to it as the specific activity.

The differential equations for the transfer of components within the system can therefore be transformed into the differential equations for radioactivity transfer by multiplying each term for the rate of reaction of a compound in a particular reaction by the specific activity of that component. The set of equations for Model III would be, for example,

$$d(A_i)X_a/dt = k(A_x)X_{ax} + k_3(C_i)X_c - k(A_i)X_a - k_3(A_i)X_a - k_1(A_i)X_a,$$

$$d(B_i)X_b/dt = k_1(A_i)X_a + k_2(B_x)X_{bx} - k_2(B_i)X_b,$$

$$d(C_i)X_c/dt = k_3(A_i)X_a - k_3(C_i)X_c.$$

These equations are difficult to integrate in the general case because they involve terms such as $k(A_i)X_a$ in which both (A_i) and X_a vary with

time and in different ways. If these differential equations cannot be solved then we cannot compare the behavior of the real system and model systems. This situation is avoided in practice by maintaining the real system in a state that corresponds to a condition of the model system that is more easily treated mathematically. Most commonly the concentrations of the components are held constant while the specific activities undergo transient changes.

What kinds of information can be obtained about our three-component system if isotopically labeled component C is injected into the system, in such a small amount as not to disturb the concentration steady state, and the outflow of radioactive component B is measured as a function of time? To answer this question we shall again examine the differences in the behavior of the model systems under consideration. In general, the further the component is from the outlet in the network the longer the time required to excrete radioactivity from that component. We will therefore investigate the two models that differ most in this respect, Models III and IV. The outflow was calculated by assigning values of unity to (A_x) and (B_x) and all the rate constants, solving the steady-state equations for the concentrations of the components, and inserting these concentrations into the differential equations for radioactive flow, with the assumptions that initially all specific activities are zero except for C, which is unity, and that the environment is much larger than the system. The amount of B excreted into the medium calculated by numerical integration appears in Fig. 4.

There are two principal differences between the behavior of Model III and that of Model IV: Model III excretes radioactivity more slowly than Model IV, and the total amount at very long times is only 50% as great. The low total excretion reflects the excretion of radioactivity as A_x in Model III, a process impossible in Model IV in which there is no flow from C to A. Measurement of the final yield thus would experimentally distinguish between Models II, III, and V, which excrete activity as A_x, and Models I and IV, in which all of the activity is excreted as B_x. This type of measurement is therefore useful in determining whether a network segment has multiple outflow pathways.

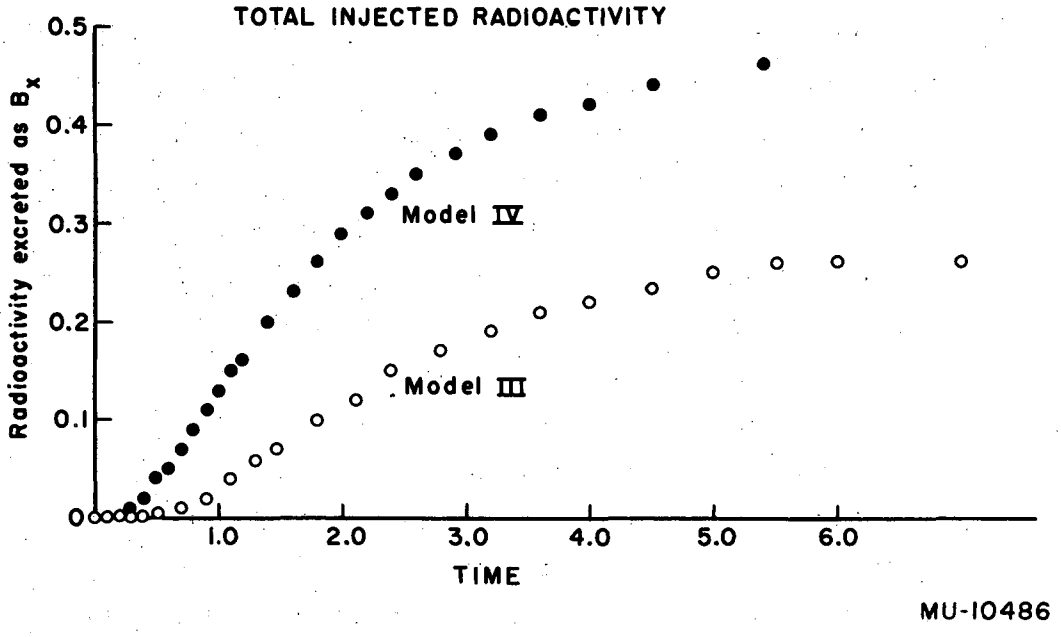


Fig. 4. Excretion of radioactivity injected as labeled component C.

Unfortunately the curves for all the models have similar shapes, consisting of an initial rise in activity--which is approximately proportional to the square of the time--a straight portion, and an asymptotic approach to the final value. Models I and IV, which have the same final activity, therefore differ only in the fine details, such as how rapidly the initial rise and approach to the final value occur. These details are functions of the as yet unknown flow parameters as well as of the network structure, so that such experiments cannot distinguish between models with only a single network outlet.

INTERNAL STEADY-STATE CONCENTRATIONS

Difficulties were encountered in determining network structure from measurements made in the environment, because the observed behavior was a function of both network structure and flow parameters. On the other hand the flow parameters themselves could not be ascertained from such measurements even if the net were known, because the observed quantities are involved functions of the flow parameters and are fewer in number than the parameters to be determined.

The internal concentrations in the steady state, however, depend in a less complex manner upon the flow parameters, as can be seen in the example of the two-component system studied above. If the network is known, a single determination of the component concentrations provides enough information to determine all the flow parameters. We shall discuss later just how such measurements may be made experimentally.

In general, the response of a system to change is more sensitive to structure than to flow; thus we would expect to find out something about the network of our real system by measuring the change in steady-state concentrations when a change in the environment occurs. We shall again examine the behavior of our model systems to gain ideas as to the information value of such experiments. Let us consider a steady state in which all the rate constants as well as (A_x) and (B_x) are unity, which is changed to a new steady state by the addition of an inhibitor that reduces the rate constant of the conversion of A to B to one-half of its previous value. The steady-state concentrations for these two conditions appear in Table III.

Table III

Steady-state concentrations of components of model systems

Model	(A_i)		(B_i)		(C_i)	
	$k_1=1$	$k_1=1/2$	$k_1=1$	$k_1=1/2$	$k_1=1$	$k_1=1/2$
I	0.50	0.67	1.50	1.33	1.50	1.33
II	1.00	1.20	1.00	0.80	1.00	0.80
III	0.50	0.67	1.50	1.33	0.50	0.67
IV	0.50	0.67	1.50	1.33	0.50	0.33
V	1.00	1.00	1.00	1.00	1.00	1.00

In drawing conclusions from Table III we must recall that the numerical values of the concentrations depend on unknown flow parameters in the real system, so that our analysis must depend only on the changes in concentrations between two steady states. The most important characteristic of the data in Table III is that the concentration of every precursor of the inhibited reaction increases and that of every product of the inhibited reaction decreases after the reaction is inhibited. This type of measurement appears therefore to provide much information, since--in principle at least--we can try different inhibitors and determine the precursor and product side of each reaction.

In Model II and Model V component C is both a precursor and a product of the inhibited reaction. In general, cyclic network segments such as these tend to resist external changes more effectively than linear segments because the steady-state shifts described tend to cancel out in components that are both precursors and products of the disturbed reaction. As we shall see later on, these cyclic subnetworks are responsible for the ability of living systems to maintain a constant internal environment.

INTERNAL TRANSIENT CONCENTRATIONS

We have seen that external and steady-state measurements often cannot separate structure and flow factors of the net. We might

expect, on the other hand, that internal transient measurements would permit the separation of these two aspects of the net.

In the section on external transients the time course of radioactivity excretion was calculated. Let us examine the form of the differential equations for radioactive flow in Model III and Model IV for this process at times very shortly after the injection of radioactive C. At these short times only C has an appreciable specific activity, so that the sets of differential equations for the models reduce to

Model III:

$$d(A_i)X_a/dt = k_3(C_i)X_c, \quad d(B_i)X_b/dt = 0, \quad -d(C_i)X_c/dt = k_3(C_i)X_c;$$

Model IV:

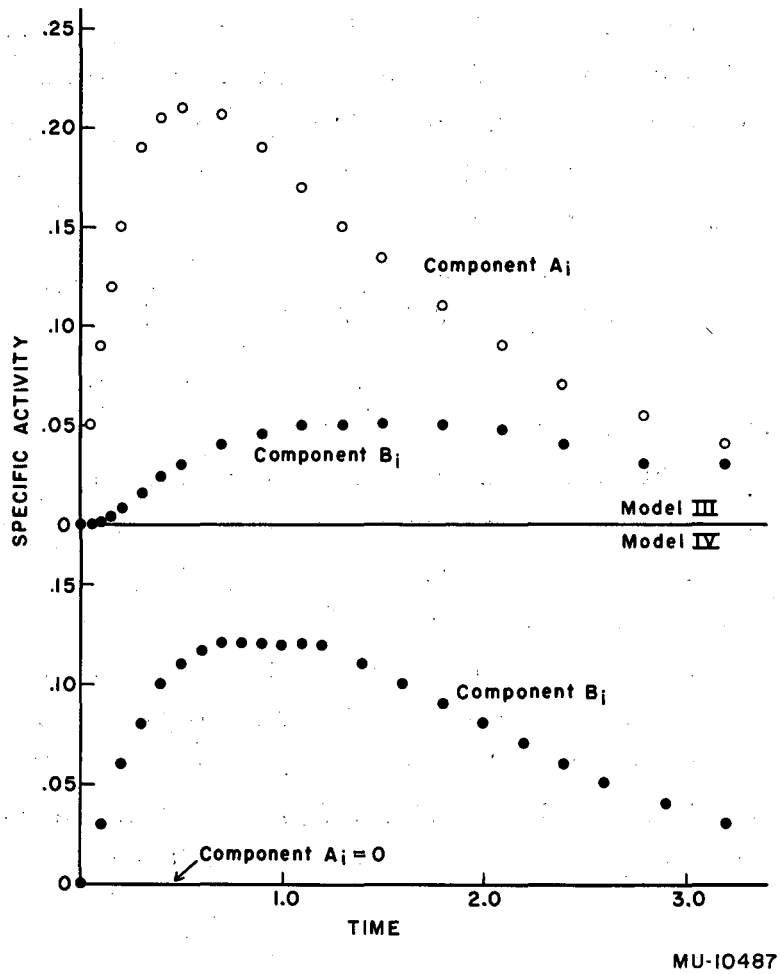
$$d(A_i)X_a/dt = 0, \quad d(B_i)X_b/dt = k_3(C_i)X_c, \quad d(C_i)X_c/dt = -k_3(C_i)X_c.$$

These equations suggest that the first product of C in the network can be determined by measuring the rate of increase in activity of the various components shortly after the injection of radioactivity. The first product has the only activity curve that increases linearly with time from zero time; none of the products of C--which are in turn also products of this first product of C--increase in activity until later times. The complete activity-time curves for Model III and Model IV appear in Fig. 5.

The equations also suggest that the flow parameter for this first reaction of C can be determined by dividing the rate of increase of activity in A by the total activity in C (in Model III), i.e.,

$$k_3 = (A_i)dX_a/k_3(C_i)dt.$$

This method is quite general, since it completely separates the network structure from the network flow and therefore can be applied to systems with a large number of components, such as living cells. Although this method for determining initial products does not yield information about more distant transformations in the network, the fact that we can label every component with radioactive atoms permits, in principle, the determination of the initial product of every component in the network, and hence the entire network structure.



ig. 5. Activity-time curves for components following the injection of radioactive component C.

The success of this initial-change method depends on the fact that the disturbance that throws the system into imbalance is highly localized in the network, and it is only this area which responds initially. At longer times the imbalance spreads throughout the network and the entire network begins responding to bring the system back into the steady state. When the entire network responds the kinetic relationships become too involved for analysis.

There are other types of localized changes that may be applied to the system in practice, such as sudden changes in environmental supply factors, inhibitors, stimulants, etc., which would bring about transient concentration changes. The initial changes observed under these conditions permit the same kind of precursor-product analysis as the radioactivity changes described above.

NETWORK-STRUCTURE DETERMINATION IN MANY-COMPONENT SYSTEMS

As the number of components in the system increases, external and steady-state measurements become less informative. Transient internal measurements that result from localized disturbances in the network retain their information value, but many more experiments are required to determine the entire network.

Often a complex network may operate as a set of independent or quasi-independent subnets, in which the flow between the subnets is either negligible or of a known value. Such a network of subnets appears in Fig. 6, in which the flow from E to F is either much higher than from E to H or of a known value. In such systems of subnets the methods described can be applied to each subnet individually.

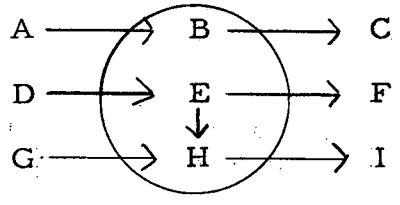


Fig. 6. A network of subnets.

NETWORK DETERMINATION IN LIVING SYSTEMS

The systems we have been discussing have been internally homogeneous, i.e., without concentration gradients within the system. Even the simplest living system is remarkably heterogeneous, especially as regards the distribution of its catalytic enzymes. The enzymes are not only not uniformly distributed in the cell but are even grouped into systems that catalyze long cyclic or chain network segments. This results in the inhomogeneous distribution of the components whose reactions they catalyze. Some components are formed and consumed without ever leaving the enzyme-system surface. The same component may be formed and consumed by two different enzyme systems, giving rise to two pools of the component which may or may never mix with each other.

We have assumed a surplus of catalysts in our model systems, but the simplest living cell may have a surplus of some and a deficiency in others, and because of its synthetic capacity constantly alters the amounts of the enzymes in an effort to maintain a constant internal milieu.

THE PHOTOSYNTHETIC NETWORK IN GREEN PLANTS

Although it was known at the beginning of the nineteenth century that green plants utilized light energy, carbon dioxide, and water to synthesize organic matter and oxygen, the network of chemical transformations involved in this complicated photosynthetic process long eluded investigators.*

Measurements of the inflow and outflow of the reactants and products established that the over-all chemical reaction was essentially

$$n\,CO_2 + n\,H_2O + \text{light} \longrightarrow (CH_2O)_n + nO_2,$$

that the reaction stored light energy as chemical energy with high efficiency, and that the oxygen evolved was produced from the water in the plant rather than from the carbon dioxide. This suggested that the over-all reaction in photosynthesis could be broken into two parts:

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* (A comprehensive review of investigations in photosynthesis is contained in Photosynthesis and Related Processes, I and II, by E. I. Rabinowitch, Interscience, N. Y., 1945 and 1951)

(I) $2n\ H_2O + \text{light} \longrightarrow 4n\ (H) + n\ O_2$,

(II) $4n\ (H) + n\ CO_2 \longrightarrow (CH_2O)_n + n\ H_2O$.

This suggestion was strengthened by the observation that other materials could be substituted for CO_2 in the second reaction. Since the two partial reactions undoubtedly are complex reactions in themselves it seemed plausible to consider the photosynthetic network as consisting of two networks, the light-reaction subnets (I), and the carbon-dioxide-reducing reaction subnet (II), which are relatively isolated from the other metabolic systems in the plant and which have only a single known link between each other, i.e., (H) transfer.

Steady-state concentration measurements of selected compounds within the plant did not elucidate the structure of the network, chiefly because of the coexistence of several independent nonphotosynthetic subnets within the plant network all of which contained the same components.

Essentially nothing was known about the chemical intermediates in photosynthesis prior to the availability of carbon-14 and paper chromatographic methods in the late 1940's. These two tools made possible internal transient measurements of the single subnet of a plant involved in the carbon-dioxide-reducing reactions. In general the kinds of experiments performed were as follows: a suspension of unicellular photosynthetic algae in water was illuminated and air bubbled through until a steady state in the algae was reached. At zero time the $C^{12}O_2$ in the air was replaced with $C^{14}O_2$ so that carbon-14 was flowing into the algae and entering their carbon-dioxide-reducing subnet. At various times after the carbon-14 was added small samples of the algal suspension were treated with hot ethanol, which immediately stopped all further chemical reactions by denaturation of the protein catalysts. The soluble components were separated first from the dead cell debris and then from each other by paper chromatography. The amount of carbon-14 in each component was then measured with a Geiger counter.

The components that acquired carbon-14 in short-term exposures to $C^{14}O_2$ were presumed to be a part of the carbon-dioxide-reducing network. These compounds were: two compounds with three carbon atoms each, phosphoglyceric acid and triose phosphate; a four-carbon compound, malic

acid; a five-carbon compound, ribulose diphosphate; a six-carbon compound, hexose phosphate; and a seven-carbon compound, sedoheptulose phosphate. When the activity in each component was plotted against time only the activities in phosphoglyceric acid and in malic acid increased linearly with time, indicating that they are the first products formed from carbon dioxide. Subsequent experiments with inhibitors showed malic acid to be part of an independent subnet, and it is now well established that there is only one photosynthetic carbon-dioxide-reducing subnet input.

Since phosphoglyceric acid is a three-carbon compound its formation must involve the addition of a carbon dioxide molecule to a two-carbon precursor compound. Extensive experiments of the above type failed to reveal the two-carbon precursor in the network, although the fact that the two atoms in phosphoglyceric acid not formed directly from carbon dioxide became labeled rapidly with carbon-14 showed the two-carbon precursor to be cyclically produced from the phosphoglyceric acid by a small number of transformations.

When the algal cells were exposed to $C^{14}O_2$ for relatively long periods of time (5 to 10 minutes) the specific activities of all the components in the carbon-dioxide-reducing subnet became equal to that of the incoming $C^{14}O_2$, and a steady state with respect to specific activity within the net was reached. The activity in each compound was then directly proportional to the concentration of the compound. Measuring steady-state concentrations in this way failed to provide network information because of the dependence of such values on flow as well as on structure factors.

The two-carbon precursor of phosphoglyceric acid was finally found by suddenly dropping the concentration of carbon dioxide in the environment of a steady-state algal suspension. The transient concentration changes that followed are shown in Fig. 7 and are taken from the Ph. D. Thesis of Dr. Alex T. Wilson (University of California, Berkeley, 1954). As the reaction between the two-carbon compound and carbon dioxide was suddenly stopped, the concentration of the product, phosphoglyceric acid (PGA), dropped whereas that of the two-carbon compound increased. The ribulose diphosphate (RDP) was the first of many components to rise, and it is now well established that the first reaction in photosynthesis is the reaction of the five-carbon ribulose disphosphate with the one-carbon carbon dioxide to form two three-carbon phosphoglyceric acids.

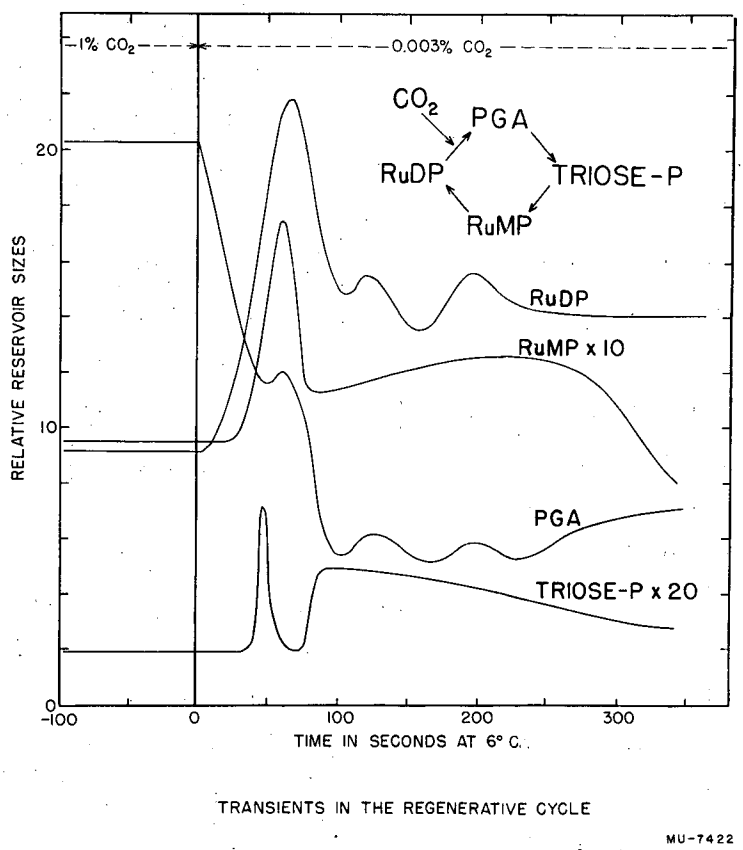

Fig. 7. Transients in the regenerative cycle.

Since the carbon-dioxide-fixing subnet depends upon an energy supply from the light-reaction subnet it became of interest to determine the cross-link between the two subnets. When an illuminated algal system in a radioactivity- and concentration-steady state was suddenly placed in the dark the flow of light-produced reducing agent, (H), was stopped. The concentration of phosphoglyceric acid suddenly rose, as is shown in Fig. 8, indicating that it is the precursor of the reduction reaction. Triose phosphates and other sugar phosphates known to be produced when phosphoglyceric acid is reduced fall rapidly in the dark, confirming the location of the reduction reaction. The data in Fig. 8 are taken from "Phosphate Transients in Scenedesmus," by D. F. Bradley (in press).

When such transients were followed for longer times and for more components it was possible to determine network segments more distantly related to the CO_2 and light inputs. We are only now developing analytical tools that permit us to draw inferences about the network from the general form of transient curves. For example, the larger the number of maxima and minima the closer the component is to the primarily affected reaction. The longer the time required for the transient of a compound to reach its first maximum or minimum the further the compound is from the primarily disturbed reaction. The smaller the first maximum or minimum the further away is the component in the net.

By use of the techniques described it has been possible to identify the elements of the carbon-dioxide-fixing subnet in photosynthesis as shown in Fig. 9.

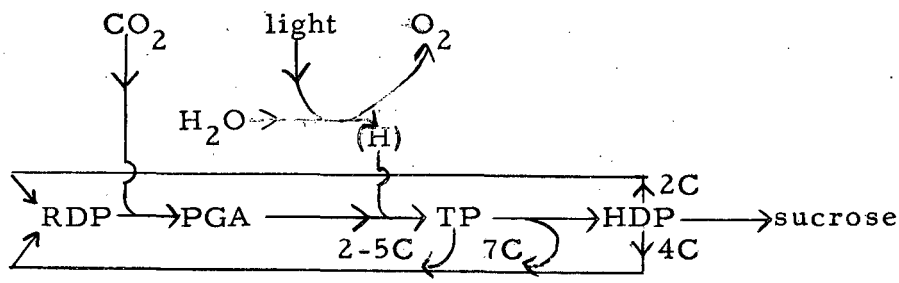

Fig. 9. Carbon-dioxide-fixing subnet.

This represents only the bare beginning in the entire network determination of the intact living plant. There are other subnets active which have been characterized chiefly by isolation of enzymes that carry out the controlled

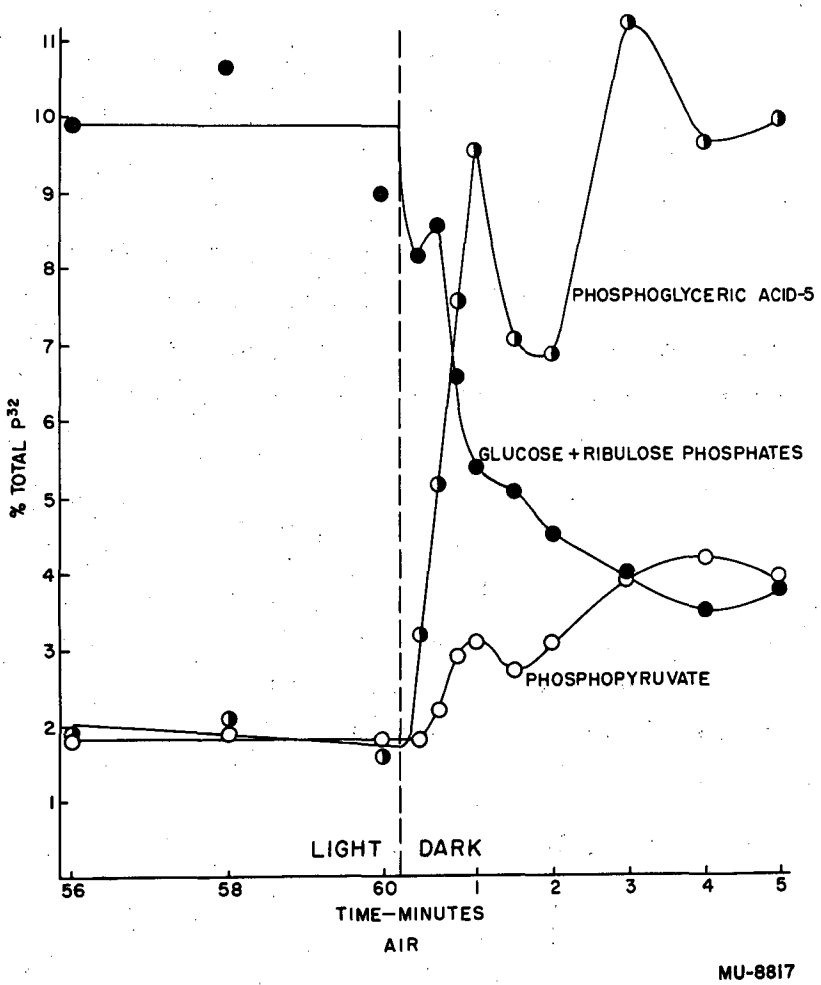

Fig. 8. Response of the photosynthetic network to sudden darkening.

combustion of these photosynthetically produced food stuffs. The group of subnets with their interflow as known today in the plant is shown in Fig. 10, which is taken from the paper by J. A. Bassham and M. Calvin, "Photosynthesis," in Currents in Biochemistry, edited by D. E. Green, Inter-Science Publishers, Inc. (in press). (University of California Radiation Laboratory Report No. UCRL-2853).

BEHAVIOR OF THE PHOTOSYNTHETIC NETWORK

How does the network of chemical transformations in a living plant cell respond to changes in its environment? At first violent transient changes occur, which are just what would be expected from the network operative. Very rapidly, however (15 seconds), the balancing mechanisms inherent in the network begin to operate; these transients reverse themselves and undergo damped oscillations until a new steady state is reached. This new steady state is much like the old one even when its sole energy source is completely stopped or its material source is reduced 300-fold. The model systems that we have studied do not posess this high degree of independence of the internal state from the environment. There were indications, however, even in the simple systems studied, that cyclic nets were particularly self-determining, and all the known nets in plants are cyclic in nature.

It thus appears that when a system is thrown into a state of imbalance by a change in its environment the system behaves in such a way as to eliminate the effect of this change and to return to a steady state that is as nearly like the former state as is possible. The degree to which the system can maintain the steady state depends both on the complexity of the system and the magnitude of the external change. When the change is so great that the network of chemical transformations cannot compensate, the system fails to return to the steady state, and death occurs.

This work was done under the auspices of the U. S. Atomic Energy Commission.

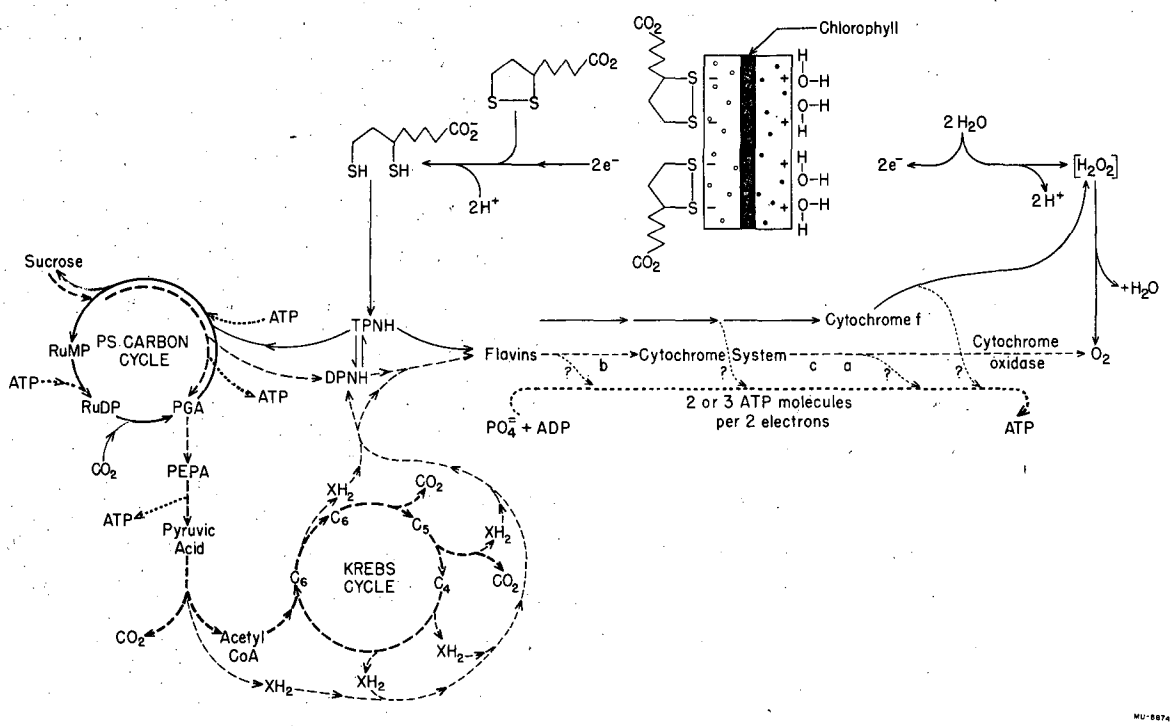

Fig. 10. Subnets and their interflow.