MAXIMA IN RATE-CONCENTRATION CURVES AND THEIR RELATION TO THE STRUCTURAL ASPECTS OF CELLULAR METABOLISM*

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INTRODUCTION

In the course of an investigation into hydranth regeneration of the coelenterate *Tubularia*, it was noted (Moog and Spiegelman, 1942; Spiegelman and Moog, 1944) that some inhibitory reagents could, at certain concentrations, stimulate regenerative activity. A more systematic study was made of this phenomenon with two inhibitors, ethylurethane and phenylurethane. The relative simplicity of the biological system employed permitted quantitative measurements of regeneration rates. The results, which will be reported here, indicate that the stimulation is reproducible and is confined to a relatively small concentration range for a particular reagent.

This peculiar effect of inhibitors is not unique to regeneration but on the contrary is quite widespread in its occurrence. During the investigations of the pharmacological activity of various drugs it was often noticed that many of them were diphasic in their effect on physiological processes, stimulating them at one concentration and inhibiting them at another. This often made its appearance in terms of maxima in the rate-concentration plots. Starting at 100 per cent of normal for zero concentration, the curve would rise for very low concentrations of the reagent above this level, and then start to fall to values below the normal for higher concentrations of the drug. So widespread was this phenomenon that it led to the postulation of the so called "Arndt-Schulz law," which states that drugs which inhibit at high concentrations stimulate at low concentrations.

The same phenomenon was observed in the study of enzyme systems using more specific inhibitors. In the early quantitative studies of the effect of cyanide on oxygen consumption it was noted that low concentrations stimulated respiration (Meier, 1927; Resnitschenko, 1927; Hyman, 1919; Kisch, 1933). Later investigations using both cyanide and carbon monoxide con-

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firmed these observations (Orström, 1935; Lindahl, 1939; Borei, 1939; Commoner, 1939; Stannard, 1940). The existence of a maximum in the rate-concentration curve was noted by Krahle and Clowes (1935) in the case of the effect of dichlorophenol on the fermentation rate of yeast. The data of Commoner and Thimann (1941) on the effect of auxin and iodoacetic acid on the respiration and growth of the *Avena* coleoptile show similar phenomena.

Although comparatively rare, the phenomenon has also been observed in experiments with isolated enzyme preparations. Glick and King (1932) and Sobotka and Glick (1934) reported that octyl alcohol increased the rate of tributyrin hydrolysis by liver esterase at one concentration and inhibited it at a higher concentration. Michaelis and Stern (1931) noted the same thing in their study of the influence of iron salts on kathepsin.

The tendency to overlook this effect frequently produces needless controversy; experiments over one small range of concentrations by one author appear to contradict the results of another obtained over a different concentration range. Where these opposite effects have not been completely ignored, the disposition has been to explain their existence by assuming that the substance in question exerts its effects *via* different mechanisms at high and low concentrations. Thus Winzler (1944), in commenting on the stimulatory effect of certain concentrations of cyanide and carbon monoxide on respiration, states "... it is well to keep in mind that they (the respiratory poisons) may have other unrelated effects." It is undoubtedly true that the specificity of most reagents and in particular of even the so called "specific inhibitors" may now be seriously questioned. And, it is not unlikely that the same substance may exert its effect *via* different components of *e.g.* the respiratory mechanism. Nevertheless, the necessity for postulating entirely unrelated mechanisms for the opposing effects at different concentrations has clearly not been established. This could only be done by demonstrating that the acceptable kinetic schemes of metabolic activity do not contain extrema, and in particular maxima, in the substrate rate-concentration curves derivable from them. Thus far no such analyses have been offered.

In view of this situation and of the wide generality of the phenomenon, it seemed worthwhile to investigate the possibility that relatively simple physico-chemical mechanisms may be responsible for the rate-concentration maxima. For purposes of comparison, certain mechanisms are included which might seem to provide adequate conditions for the existence of the phenomenon, but which on closer analysis fail to do so.

It is important to emphasize that it is the purpose of the present paper, not to assert the relevance of a unique mechanism, but merely to present some which do parallel the phenomenon. Their exhibition then, opens up the possibility of explaining the phenomenon without invoking unknown and unrelated effects.

EXPERIMENTAL

A. Materials and Methods.—

Regeneration rate of hydranths was measured by the ratio L/t , a method first proposed by Barth (1938). In this, L is the length of the regenerating primordium and t is the time in hours from the removal of the old hydranth to the appearance of a constriction between the primordium of the new hydranth and the rest of the stem.

The solutions used were made up fresh each week in filtered sea water, and when necessary were adjusted to pH 8.2 with HCl. Young, unbranched stems, uniform in translucence, length, and diameter were selected from colonies freshly gathered from the waters of Vineyard Sound or Cape Cod Bay during the months of July and August. Stem segments 6 mm. in length were cut from regions about 5 mm. proximal to the hydranth. Groups of 25 stem segments were kept in 100 ml. of the appropriate solutions in partly filled, tightly stoppered flasks which were shaken at intervals to redistribute the oxygen. Solutions were changed daily, but the stems were kept in the flasks until they reconstituted or were finally transferred to fresh sea water, after from 4 to 5 days. They were counted as totally inhibited, with regeneration rate zero, if after being transferred they developed hydranths.

B. Results.—Table I summarizes the data obtained with phenylurethane. To arrive at a clearer picture of the phenomenon, the data of Experiment 1 are plotted (Fig. 1) in terms of per cent of normal against the logarithms of the molar concentrations multiplied by 10^6 . It is seen from the table that a maximum occurs in the neighborhood of 1×10^{-8} molar. It was generally noted that although the height of the maximum with any given compound might vary considerably, the concentration range in which it occurred was relatively restricted and reproducible.

In an effort to further test the reproducibility of the phenomenon, similar experiments were performed with ethylurethane, a narcotic closely related to phenylurethane. Since its general mode of activity would presumably not be very different from that of phenylurethane, it was to be expected that ethylurethane would also yield maxima in the rate-concentration curve. Table II summarizes the results obtained over a concentration range comparable to that covered in the phenylurethane experiments. Here again maxima are exhibited and the concentration range in which they occur is relatively narrow. It is of some interest to note that the maxima occur at about the same molar concentration for both narcotics.

The data supply further evidence to the already impressive collection for the reality of the phenomenon. Its reproducibility in these measurements of a complex biological process encouraged the attempt at a search for possible mechanisms. While the data presented here supplied the stimulus for the theoretical investigations of the next section, the analysis is more general in scope. It has as its major purpose the discovery of relatively simple physico-chemical systems which would exhibit rate-concentration maxima. In the

process, it will be seen that structural restrictions are one of the peculiarities possessed by those systems which do exhibit these effects.

TABLE I
The Effect of Various Concentrations of Phenylurethane on Regeneration Rates

Experiment No.	Concentration	No. of stems	L/t	Per cent of Control
1	<i>mols/liter</i>			
	Control	21	42.9	100
	1×10^{-7}	22	42.0	98
	1×10^{-6}	24	42.9	100
	5×10^{-6}	19	42.0	98
	1×10^{-5}	17	44.2	103
	1×10^{-4}	20	53.0	120
	1×10^{-3}	21	57.4	134
	1×10^{-2}	18	48.0	112
	2.5×10^{-2}	20	41.5	97
	5×10^{-2}	22	33.4	78
	8×10^{-2}	20	23.2	54
2	Control	24	31.6	100
	1×10^{-7}	22	31.0	98
	1×10^{-6}	23	32.0	101
	1×10^{-5}	22	32.4	102
	5×10^{-5}	20	37.0	117
	1×10^{-4}	21	40.7	129
	5×10^{-4}	19	45.9	145
	1×10^{-3}	17	48.5	153
	5×10^{-3}	19	42.1	130
	1×10^{-2}	18	34.2	108
	5×10^{-2}	14	22.1	70
	8×10^{-2}	14	10.7	34

THEORY

1. Negative Cases

Case A.—The substance whose concentration effect is to be studied (hereafter referred to as the critical substance) either can be used as a substrate or reacts to yield a substrate for the reaction whose rate is being examined. At the same time the critical substance forms a compound with the enzyme governing the principal reaction, thus inactivating it. Intuitively it might seem likely that increasing the critical substance would first accelerate the reaction by furnishing additional substrate, and then, as it inactivates more and more enzyme, eventually choke off the reaction at the higher concentrations.

We adopt the following notation for concentrations: c for the critical substance, s for the substrate which it produces, e the free enzyme, a the activated complex of enzyme and substrate, p the product, and a' the inactive complex of enzyme and critical substance, e_0 being the total amount of enzyme present. The chemical equations then are:

FIG. 1

In the above equations the K 's denote velocity constants of the corresponding reactions.

We now have the steady state¹ equations, which state for the various substances that the net reaction rate producing them equals the net reaction rate consuming them (if no diffusion out of the system takes place; when such dif-

¹ It is to be noted that throughout this paper transient (time-dependent) phenomena, in which a reaction begins at a finite level and eventually drops to zero, are not being considered. Stationary states only are compared, in each of which the concentration of the critical substance is different, but is and remains constant within any given state. It is very easy for confusion to arise between the two; and there are many phenomena, such as certain chains of radioactive transformations, which show maxima with respect to variations in time, but none in their steady state values.

TABLE II
The Effect of Various Concentrations of Ethyl Urethane on Regeneration Rates

Experiment No.	Concentration	No. of stems	<i>L/t</i>	Per cent of control
1	<i>mols/liter</i>			
	Control	25	24.7	100
	1×10^{-7}	21	24.2	98
	1×10^{-6}	20	24.7	100
	1×10^{-5}	23	24.0	97
	2×10^{-5}	18	24.2	98
	5×10^{-5}	16	24.0	97
	1×10^{-4}	19	25.1	101
	4×10^{-4}	22	25.7	102
	1×10^{-3}	18	29.0	118
	5×10^{-3}	22	27.5	106
	1×10^{-2}	21	22.2	90
	5×10^{-2}	17	10.2	42
	8×10^{-2}	14	4.9	20
2	Control	22	22.5	100
	1×10^{-7}	18	22.0	98
	1×10^{-6}	21	21.7	97
	6×10^{-6}	17	22.2	99
	1×10^{-5}	19	22.0	98
	5×10^{-5}	20	22.5	100
	1×10^{-4}	23	23.0	101
	5×10^{-4}	18	21.7	97
	1×10^{-3}	17	25.2	112
	5×10^{-3}	20	22.0	98
	1×10^{-2}	21	18.3	82
	5×10^{-2}	17	8.3	37
3	Control	19	27.6	100
	1×10^{-4}	21	28.7	102
	5×10^{-4}	24	26.6	97
	1×10^{-3}	19	32.0	116
	5×10^{-3}	22	28.7	102
	1×10^{-2}	21	25.1	91
	5×10^{-2}	23	13.1	51

fusion occurs, the rate of inflow or outflow must be included in the material balance equations). These are:

$$(4) \quad K_2c - K_4's + K_1'a - K_1se = 0$$

$$(5) \quad K_4ce - K_4'a' = 0$$

$$(6) \quad K_1se - K_1'a - K_2a = 0$$

The critical substrate c is assumed to be maintained at a constant concentration in the system. Solving equations (3) to (6), we get:

$$(7) \quad a = \frac{\{(mc + n) \pm [(mc + n)^2 - 4K_2e_0K_3c]^{\frac{1}{2}}\}}{2K_2}$$

$$(8) \quad m = K_3 + \frac{(K_1' + K_2)K_3'K_4}{K_1K_4'}$$

$$(9) \quad n = K_2e_0 + \frac{(K_1' + K_2)K_3'}{K_1}$$

$$(10) \quad e = \frac{K_4'(e_0 - a)}{K_4c + K_4'}$$

$$(11) \quad s = \frac{K_3c - K_2a}{K_3'}$$

Equation (7) presents two solutions (both positive and therefore admissible). The one corresponding to the positive square root increases indefinitely with c . The solution corresponding to the negative square root approaches, as c is increased without bound, the saturation value K_3e_0/m . The conclusion that no maximum with respect to c exists can easily be confirmed by taking the derivative of (7) and setting it equal to zero; since the principal reaction in which we are interested is the production of p , whose rate is K_2a , and therefore proportional to (7). Why the second case does not result in a final zero reaction rate may be seen by examining (10) and (11) (whose product gives the rate at which the activated complex a is formed). As c is increased, e approaches zero, but s becomes infinite correspondingly fast, so that the product approaches a constant value. If an enzyme molecule is momentarily freed, it is certain to be seized upon by one of the dense horde of substrate molecules present.

This is of course the case only if the inactivation of enzyme is reversible. If it is made irreversible by setting K_4' equal to zero, it is evident from (5) that e will be zero in the *steady state* for all finite values of c and s , and so will a ; this may be confirmed by inspecting (8), which becomes infinite, so that (7) is either infinite for all values of c (which is nonsense) or, if we take the negative square root, is identically zero for all values of c .

Case B.—In this case it is assumed that the critical substance is simply a substrate for the reaction being studied. Although it does not inactivate the enzyme, it might be supposed that with very high substrate concentrations the products of the reaction would pile up sufficiently for the back reactions to lower the rate. This case illustrates the necessity for a precise differentiation between transient maxima and those occurring in a time-independent state.

Using the same symbols as previously, the equations for this system take the form:

The steady state equations are:

$$(3) \quad K_1'a - K_1ce + K_2a - K_2'pe = 0$$

$$(4) \quad K_2a - K_2'pe = h(p - p_0)$$

$$(5) \quad e_0 = e + a$$

Here h is the permeability coefficient of the product, p , multiplied by the ratio of cell surface to cell volume, and p_0 is the external concentration of product. Solving, we obtain:

$$(6) \quad p = \frac{\{[(K_1hc + r)^2 + 4K_2'h(sK_1c + t)]^{\frac{1}{2}} - (K_1hc + r)\}}{2K_2'h}$$

$$(7) \quad r = (K_1' + K_2)(K_2' + h) - K_2'(K_2e_0 + hp_0)$$

$$(8) \quad s = K_2e_0 + hp_0$$

$$(9) \quad t = hp_0(K_1 + K_2)$$

$$(10) \quad e = \frac{s - hp}{K_2 + K_2'p}$$

The rate of production of p from a is the principal reaction by (4). It is equal to $h(p - p_0)$, and so will have a maximum if p has a maximum. But it is evident from (6) that, as c is increased, p will approach a saturation value. The other solution, with a negative square root, has been discarded because it leads always to negative and therefore meaningless values of the concentration p . If one had expected that increasing c would first act to increase the formation of a , and then, by increasing p , raise the back reaction in equation (2) to the point of halting the reaction, it is evident that this will not be the case. Whatever the permeability of p , it will always diffuse out in sufficient quantities so that, if a steady state is attained at all, it will exhibit a non-zero reaction rate no matter how large c is made. However, it may be noted that transient maxima in the rates may occur during the time in which the system is approaching the steady state. It is evident from (4) that, if there is no diffusion whatever, the reaction will in the steady state be in true equilibrium with a zero reaction rate; but this will be the case for any value of c . The total amount of p present in this equilibrium state will be simply proportional to c .

Case C.—For future reference, as well as comparison with case A, it is of interest to examine the following for maxima: s , the substrate, is adsorbed on a surface, where it reacts to form a product p , which is then desorbed. The “poison” or critical substance stimulates the production of s , but also inactivates the enzyme by reversible adsorption on its surface. While this system is formally analogous to that of case A, emphasis is here placed on the kinetics at the activating surface in terms of active centers and thus permits the analysis and detection of any differences which may stem from the heterogeneity of the system. The steady state equations are:

$$\begin{aligned} (1) \quad & g' N_e = gc (N - N_s - N_o) \\ (2) \quad & VKc = as (N - N_s - N_o) - a' N_s \\ (3) \quad & LN_s = as (N - N_s - N_o) - a' N_s \\ (4) \quad & Vc^* = V_o + N_o \end{aligned}$$

N is the number of free places on the surface. g is the adsorption coefficient and g' is the desorption coefficient of c . c and s refer to concentrations of critical substance and substrate in solution, while N_o and N_s refer to the corresponding number of molecules adsorbed on the surface. c^* is the total concentration of “poison.” L is the reaction constant of s on the surface. The solution of the system is given by:

$$\begin{aligned} (5) \quad & c = \frac{\{(fc^* - m) - [(fc^* - m)^2 + 2f'c^*n]^{\frac{1}{2}}\}}{n} \\ (6) \quad & n = 2f \left(1 - \frac{K}{L}\right) \\ (7) \quad & m = gN + f' \\ (8) \quad & f = gV; \quad f' = g'V \\ (9) \quad & LN_s = VKc \end{aligned}$$

It is evident here, as in case A, that the reaction rate, which is proportional to (5) by (9), either becomes infinite with the critical concentration c^* , or attains a saturation value, in no case giving a maximum.

Case D.—Again for purposes of later comparison as well as a further investigation into the consequences of heterogeneity, consider the following case: a substrate A is adsorbed on an enzyme surface; another substance B , if it colloides with an activated A molecule on the surface, reacts with it to form a product P , which is then desorbed. Here either A or B may be considered as the experimentally varied critical substance. With the notations c_a and c_b for concentrations in solution, N' for number of A molecules adsorbed on the surface, and N_p for number of product molecules on the surface, and N for total number of places on the surface, we have the steady state equations for

the number of free places on the surface and for the number of places occupied by product molecules:

$$(1) \quad ac_a (N - N_a - N_P) - a' N_a - KN_P = 0$$

$$(2) \quad bC_b N_a - KN_P = 0$$

The solution is:

$$(3) \quad n_p = \frac{abc_a c_b N}{[K(ac_a + a') + bc_b(ac_a + K)]}$$

The rate at which product P is released into solution, which is proportional to (3), behaves the same way with respect to c_a and c_b . As either one is increased, the rate approaches a saturation value, and exhibits no maximum.

Case E.—An extremely interesting system to analyze, and one of some physiological importance, is one in which the critical substance c is involved in a reaction which is coupled to the over-all one whose rate is being studied; *i.e.*, supplies an activated complex utilized for substrate transformation in the main reaction. The critical substance c diffuses in and an excited or activated complex p^* is formed (Equation 1, below). This is then deactivated by collision with either the solvent, or with the reactant or product of the main reaction (Equations 2 and 3 below). The substrate s of the main reaction is kept at a constant concentration, and goes over into a product u , which is removed at a rate proportional to the difference between the internal and external concentrations ($u - u_0$) (Equation 4). The schematic diagram for this system with the appropriate velocity constants and employing the subscript 0 for external concentrations may be written:

The steady state equations for the critical substance and the activated complex take the form:

$$(5) \quad h(c_0 - c) = K_1 c - K_1' p^* = K_2 p^*$$

where

$$(6) \quad K_2 = a + bs + gu$$

In (6) a is the deactivation coefficient for collisions of p^* with solvent molecules, whereas b and g perform the same function for collisions with s and u respectively (see (2) and (3)). The steady state equation for the substrate transformation takes the form:

$$(7) \quad k_1 s - k_1' u = m(u - u_0)$$

For purposes of calculation k_1 and k_1' may be put in the form

$$(8) \quad k_1 = a_1 + b p^*; \quad k_1' = a_1' + g p^*$$

The a_1 in the above measures the contribution to activated substrate, s^* , due to collisions with solvent and this is added to those obtained by collision p^* . Similarly a_1' measures the amount of back transformation of u into s^* through collisions with solvent in addition to that which occurs because of collisions of u with p^* . Since the over-all rate, according to equation (7), may be put in the form $m(u - u_0)$, we may solve for u as a function of c_0 and examine for maxima. Solving, we get u of the form:

$$(9) \quad u = (A c_0 + B) + [(A c_0 + B)^2 + (r c_0 + D)]^{1/2}$$

where A , B , r , and D are constants which are combinations of the other parameters.

Differentiating u with respect to c_0 in the above it is clear that no maximum exists and the curve is of the saturation type. If we suppose that c is identical with u , we obtain all the equations the same as before (with c instead of u), except the very first, which becomes:

$$(10) \quad h(u_0 - u) = K_1 u - K_1' p^* + k_1' u - k_1 s$$

Equation (7) disappears. The result, however, is the same as above, with no maximum.

It is important to realize for later reference that homogeneity has been assumed throughout the analysis of the present case. All activations and deactivations were assumed effected by collisions between molecules in a homogeneous system.

2. Positive Cases

The following cases do exhibit maxima in the rate-concentration curve. It will be noted that they are modifications of those already discussed in the previous section. The detailed comparison will be considered in the discussion.

Case A'.—Let us suppose that two substrates, A and B , either of which may be considered as the critical substance, are present in solution in concentrations c_a and c_b . A single large enzyme molecule of surface area S is present, containing N identical sites, upon which substrate is adsorbed; the number of

adsorbed molecules of each species shall be denoted by N_a and N_b . In order that A and B may react, they must be upon neighboring sites of the surface. The product molecules are desorbed at a certain rate.

Two cases are possible. The first which we treat is one in which adsorption equilibrium is rapidly attained in comparison with chemical reaction, so that we may calculate this equilibrium without taking the reaction rate into account.

If the coordination number (number of sites surrounding a given site) of the surface is z , the average number of neighboring pairs of sites on the surface is:

$$(1) \quad n_{ab} = \frac{1}{2} zN$$

The average number of neighbor sites occupied at any time by an A and a B molecule is given by this, multiplied by the probability that a neighbor pair is so occupied. This is given by:

$$(2) \quad P_{ab} = P(a1)P_{a1}(b2) + P(b1)P_{b1}(a2)$$

In this notation, $P(a1)$ is the probability of finding an A molecule on site 1 of a neighbor pair. $P_{a1}(b2)$ is the conditional probability that a B molecule is on site 2 if an A molecule is on site 1. A similar interpretation is obvious for the other two symbols. If we assume for the sake of simplicity that the presence of an A or B molecule in a given region of the surface does not influence the adsorption of others, except insofar as it occupies a site, we obtain for the probabilities:

$$(3) \quad P(a1) = \frac{N_a}{N}; \quad P(b1) = \frac{N_b}{N}$$

$$(4) \quad P_{a1}(b2) = \frac{N_b}{N-1}; \quad P_{b1}(a2) = \frac{N_a}{N-1}$$

We thus obtain:

$$(5) \quad P_{ab} = \frac{2N_a N_b}{N(N-1)}$$

Thus we have for the average number of A - B neighbor pairs on the surface:

$$(6) \quad N_{ab} = n_{ab} P_{ab} = \frac{N_a N_b z}{(N-1)}$$

The reaction rate on the surface will be denoted by:

$$(7) \quad R = KN_{ab}$$

We now write the steady state equations, which state that the rate at which A is adsorbed on the surface (proportional to the number of free places and to its concentration in solution) is equal to the rate of desorption, and similarly for B .

$$(8) \quad ac_a (N - N_a - N_b) = a' N_a$$

$$(9) \quad bc_b (N - N_a - N_b) = b' N_b$$

We solve this system, assuming c_a and c_b to be maintained constant, and get:

$$(10) \quad N_a = \frac{Nab'c_a}{a'bc_b + b'(ac_a + a')}$$

$$(11) \quad N_b = \frac{Na'bc_b}{a'bc_b + b'(ac_a + a')}$$

Combining the solutions with (7), we find for the reaction rate:

$$(12) \quad R = \frac{Mc_ac_b}{[a'bc_b + b'(ac_a + a')]^2}$$

$$(13) \quad M = \frac{Kab'a'b'zN^2}{(N-1)}$$

R is evidently the same considered as a function of either c_a or c_b . It begins at zero, rises, and drops off again to zero as the concentration increases indefinitely. This is confirmed by differentiation; choosing A as the critical substance, differentiating with respect to c_a and setting the result equal to zero, we obtain:

$$(14) \quad c_a = \frac{a'bc_b + a'b'}{ab'}$$

The larger c_b , the larger is the value of c_a for which R attains a maximum and begins to decrease. The interpretation is quite clear: for some finite value of c_b , if we begin with no A present and gradually increase its concentration, the number of A - B pairs formed on the surface will increase. Then, as c_a increases still further, A molecules will tend to occupy more and more of the surface, leaving less and less room for B molecules (as is clear from (11), since N_b tends to zero with increasing c_a). Since it is necessary for A and B molecules to become neighbors on the surface in order that the reaction may proceed, this implies that the reaction rate will drop off for lack of A - B pairs to work with, and eventually become zero.

Case B'.—In case A' we considered that the adsorption equilibrium on the enzyme surface was attained quite rapidly as compared with the reaction rate. We now modify this for cases where the rates are comparable. If a fraction m of the AB molecules produced per second is desorbed, the desorption rate is mkN_{ab} , which is calculated from equation (6) of case A . The steady state equations each assert that the rate at which substrate is adsorbed is balanced by the rate at which it is desorbed free plus the rate at which it is desorbed in the form of the AB compound:

$$(1) \quad Ac_a(N - N_a - N_b) = a'N_a + LN_aN_b$$

$$(2) \quad bc_b(N - N_a - N_b) = b'N_b + LN_aN_b$$

$$(3) \quad L = \frac{mKz}{(N-1)}$$

This system may be solved, and N_{ab} calculated. We first introduce some abbreviations:

$$\begin{aligned} (4) \quad & G = LNb' \\ (5) \quad & f = a'(bc_b + b') + LNbcb \\ (6) \quad & f' = f - 2LNBcb \end{aligned}$$

Taking A as the critical substance, and using the notation x for ac_a , we have:²

$$(7) \quad 2LN_a = \frac{\{[f + (b' - LN)x]^2 + 4Gx(a' - bc_b + x)\}^{\frac{1}{2}} - f - (b' + LN)x}{(a' - bc_b + x)}$$

$$(8) \quad 2LN_b = \frac{\{[f + (b' - LN)x]^2 + 4Gx(a' - bc_b + x)\}^{\frac{1}{2}} - f' - (b' + LN)x}{(b' + bc_b - x)}$$

The reaction rate is proportional to the product of (7) and (8). To determine the presence of a maximum, one should differentiate the product and set the result equal to zero, solving for x . The result is an equation of unwieldily high degree; it appears preferable to study the rate by semiquantitative methods. We shall do this by analyzing (7) and (8) separately, whereupon it will be possible to draw certain conclusions regarding the behavior of the product.

Considering (7) plotted against x , it appears that N_a is zero when x equals zero, and approaches the limiting value N as x becomes infinite. In this range of x , the denominator of (7) may change sign if $(a' - bc_b)$ is negative. But this does not affect the sign of N_a ; when the denominator is positive, the square root in the numerator is larger than the rest of the numerator, so that the quotient is positive. When the denominator is negative, the square root is less than the remainder of the numerator; numerator and denominator are both negative, and the fraction again positive. It remains only to determine whether (7) has a singularity for that value of x at which the denominator is equal to zero. The limit is easily found by the usual procedure of differentiating numerator and denominator separately, and turns out to have the finite value:

$$(9) \quad N_a' = \frac{Nb'(bc_b - a')}{[f + (b' - LN)(bc_b - a')]}$$

For this same value of x , N_b also has a finite and positive value, $Na'/(a' + b')$. The slope of the $N_a - x$ curve at the origin is equal to $Nb'/^2a'(b' + bc_b) + LNbcb^2$, which is positive. The slope when x becomes very large goes to zero as $1/x^2$. It is evident from this that N_a rises from zero through positive values to the asymptotic value N .

² The solutions for N_a and N_b with the minus sign of the radical yields negative values for either N_a or N_b or both and are therefore not considered.

At x equals zero, N_b has the finite positive value $Nbc_b/(bc_b + b')$. For very large values of x it goes to zero as $1/x$. Investigating the zero of the denominator we find at this point the value:

$$(10) \quad N_b' = \frac{N'a'bc_b}{[(a' + b')(b' + bc_b) + LNb']}$$

This is positive. For the same value of x , N_a has the positive value $Nb'/(a' + b')$. The initial slope of the $N_b - x$ curve is given by the initial slope of the $N_a - x$ curve multiplied by the factor $-bc_b^2(b' + bc_b)^{-1} + LN(b' + bc_b)^{-2}$. The slope is therefore negative. For very large values of x , the slope goes to zero as $-1/x^2$. The N_b curve consequently begins at a finite value and drops off asymptotically to zero.

One function is finite and the other zero at the origin; their product is therefore zero at the origin. One function is zero and the other finite for infinite values of x ; their product is therefore zero for infinite values of x . They are positive and free of singularities between the extremes of the x range. Consequently, there must exist at least one maximum of their product for some positive and finite value of x . If either of the two factors is not monotonic, there may be more than one maximum.

Case C'.—It was pointed out in the analysis of case E that the assumptions which led to the steady state equations of the system were based purely on reactions in a homogeneous system. It is clearly of no little importance to examine the coupled reaction system modified to consider the effects of imposing geometrical constraints by assuming that the reactions take place on enzyme surface. Using the same reaction system we assume here that c is adsorbed on an enzyme as c_s and is activated as p^* . The latter goes into p_s , which is then desorbed. The deactivation of p^* takes place by transfer of energy to s , which is adsorbed on the enzyme surface as s_s . The transfer of energy converts s_s to s^* , which then transforms to u_s , which is then desorbed as u . If N_s is the total number of places on the enzyme and N_0 the number of free places, the assumption of time independence for adsorption and activation of c_s leads to

$$(1) \quad \alpha cN_0 = k_s c_s$$

where α is the adsorption coefficient and k_s the activation coefficient. Setting activation and deactivation rates equal yields:

$$(2) \quad k_s c_s = Lp^* + k s_s p^*$$

where L is the deactivation coefficient when the energy is not transferred to the adsorbed substrate s_s , and k is the deactivation coefficient when p^* transfers its energy to the substrate. Setting the deactivation rate of p^* equal to the rate of desorption of the deactivated product p_s gives:

$$(3) \quad Lp^* + k s_s p^* = b p_s$$

where b is a desorption coefficient. Turning now to the substrate s of the main reaction, the net rate of adsorption is set equal to the rate of substrate activation; thus

$$(4) \quad g s N_0 - g' s_* = k s_* p^*$$

where g and g' are the adsorption and desorption coefficients respectively, and k is the activation coefficient of the adsorbed substrate. Finally, setting the activation rate equal to rate of inactivation of the substrate and the desorption of the resulting product u_* , we obtain:

$$(5) \quad k s_* p^* = e s^* = d' u_*$$

The desired reaction rate R of the main reaction is given by $d' u_*$. The solutions for R as a function of c , the concentration of the critical substance, are given by;

$$(6) \quad R = -\frac{1}{2} \frac{B}{A} + \left[\left(\frac{1}{2} \frac{B}{A} \right)^2 - \left(\frac{D}{A} \right) \right]^{\frac{1}{2}}$$

where

$$(7) \quad \begin{cases} A = (ac + b')(a'c + b) \\ B = \left(\frac{k_e N_0 g s}{\alpha} \right) (a'c + b) + k_* N_* c (ac + b') + \left(g' \frac{L}{k} \right)^2 (ac + b)^2 \\ D = \left(\frac{g s}{\alpha} \right) (k_* N_*)^2 c \end{cases}$$

in which the constants a , a' , b , and b' are combinations of the other constants. It is seen that R is zero for zero and infinite values of c , and consequently an extremum of the rate concentration curve exists. Without going into the algebraic detail, it is sufficient here to note that under the restriction that R be real and positive, this extremum is a maximum if A and B are both negative or of opposite sign and is a minimum if they are both positive.

Case D'.—Of some interest, in view of possible relations between enzyme activity and protein denaturation, is the case where the critical substance can not only inactivate the enzyme, but also may protect it from denaturation. Thus, suppose an enzyme is present in total concentration E_0 . The reaction being studied takes place at a rate R proportional to the active concentration of enzyme, E_A

$$(1) \quad R = k E_A$$

The enzyme is in equilibrium with its denatured and inactive form E_d described by

$$(2) \quad E_d = K_d E$$

where E is concentration of free enzyme. A substance S also reacts with the enzyme. If it forms a compound E_s given by

$$(3) \quad E_s = K_1 S E$$

it protects the enzyme from denaturation. But if it reacts further with E_s to form E_{ss} , where

$$(4) \quad E_{ss} = K_2 S E_s$$

it occupies an essential group in the enzyme, and renders it inactive. The total concentration of enzyme E_0 is given by:

$$(5) \quad E_0 = E + E_s + E_{ss} + E_A$$

and the active form E_A by

$$(6) \quad E_A = E + E_s$$

Solving for R as a function of S , the concentration of critical substance, and the velocity constants, we obtain,

$$(7) \quad R = \frac{k E_0 (1 + K_1 S)}{1 + K_3 + K_1 S + K_1 K_2 S^2}$$

Differentiating R with respect to S we find that R does have a maximum, which is attained when S has the value:

$$(8) \quad S_{\max.} = \frac{-1 + \left(1 + \frac{K_1 K_2}{K_3}\right)^{\frac{1}{2}}}{K_1}$$

which it is seen is greater than zero.

It will be noted that $S_{\max.}$ increases with K_3 , the denaturation constant of the enzyme. Thus, if heat denaturation is involved, one would expect a series of rate-concentration curves at different temperatures to have the location of their maxima displaced towards higher concentrations with increasing temperature.

This case is of interest as illustrating what may occur if an inhibitor acts on an enzyme both competitively and non-competitively with respect to the substrate. Even if the non-competitive poison partially inactivates the enzyme, a stimulating effect may be found. For if only a fraction f of E_s remains active, we get instead of (8):

$$(9) \quad S_{\max.} = \frac{-1 + \left[1 + \frac{f K_1}{K_3} \{1 - (1 + K_3) f\}\right]^{\frac{1}{2}}}{f K_1}$$

$S_{\max.}$ increases towards the value (8) as f approaches unity. It is equal to zero when

$$(10) \quad f = \frac{1}{1 + K_3}$$

For larger values of f , a maximum always occurs. The critical value of f is smaller the larger is K_3 ; *i.e.*, we might say that a non-competitive poison will stimulate at low concentrations if its inhibitory effect on the enzyme is less severe than the effect of denaturation.

DISCUSSION

It is clear from the discussion of both the positive and negative cases that it is intuitively difficult to decide whether a particular mechanism will yield a maximum in the rate-concentration curve. A decision requires a detailed analysis of the steady state equations in all but the most naive situations.

An interesting characteristic which seems to emerge from the positive mechanisms analyzed here is the existence of a more definite type of geometrical constraint than was evident in the corresponding negative cases. Thus, if the positive cases A' and B' are compared with the parallel negative one D , it is seen that the geometrical conditions imposed on the reactions in the former two systems are more severe than those assumed in the case of D . The distinguishing mark of A' and B' is the proviso that the various substrate molecules must occupy certain relative positions on a surface in order to react at all. Again the homogeneous coupled reaction of case E which was negative was made positive (case C') by introducing the geometrical condition of heterogeneity. It is to be by no means concluded that the geometrical conditions of structure are *necessary* for the exhibition of maxima in rate concentration curves, although they are, as we have seen, sufficient if of a relatively rigid nature. Nevertheless, in view of the present study, it is perhaps not surprising to find such phenomena relatively common in the structured systems cells are presumed to be, whereas they are relatively rare in "test-tube" experiments. In any case, it is evident that the existence of a maximum in the rate-concentration curve of a substance does not necessarily imply a dichotomy in the mechanism of its activity at different concentration.

Interpretation of the experiments reported here cannot be made directly in terms of the positive cases described since it is hardly likely that the narcotics enter directly as substrates in the synthetic reactions of regeneration. However, the existence of maxima in their rate-concentration curves would be explained if they affected the enzymes involved in the formation of the substrates used in regenerative activity. The narcotics would thus influence the concentration of the critical substance whose variation leads to maxima.

One other important point may be noted; it is evident from the analysis that a unique mechanism for these maxima does not obtain. Hence, its existence in any particular instance is not diagnostic of a particular reaction mechanism. While certain types of reactions are ruled out for any process or system which exhibits a maximum, there remain too many positive possibilities for it to be particularly useful as a tool for investigating mechanisms.

SUMMARY

1. Reproducible maxima are exhibited in the rate-concentration curves obtained by studying the effects of ethyl- and phenylurethanes on regeneration rates of hydranths in *Tubularia*.
2. The general problem of maxima in rate-concentration curves is analyzed in terms of reaction kinetics of relatively simple systems.
3. Certain systems were shown to exhibit this phenomenon. A comparison is made of these with similar ones which fail to do so.
4. The possible rôle in this phenomenon of cellular structure and its attendant geometrical constraints is discussed in terms of the above comparison.

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