Visual Excitation and Blood Clotting

Abstract. *Both processes involve extremely large amplification between the stimulus and the response. In vision it had been suggested that this might be achieved by a chain of successive proenzyme-enzyme activations. Such a chain has now been found to underlie the mechanism of blood clotting. Methods are suggested for pursuing this comparison further.*

In the human eye a dark-adapted rod can be stimulated by a single photon. The absorption process presumably involves a single molecule of the visual pigment, rhodopsin (*1, 2*). A major problem in vision is to try to understand how one molecule of visual pigment can trigger so large an effect. Clearly a large amplification, by a factor of thousands or millions of times, must intervene between the photochemical event and the first perceptible electrical response (*2*).

A similar problem exists in the photographic plate, where the absorption of one or a few photons makes a whole grain developable (*3*). Here the mechanism is catalysis: one photon reduces a silver ion to an atom of metallic silver, which during development acts as a catalytic center for the reduction of the whole grain.

A comparable answer was suggested some time ago for visual excitation (*4, 5*). It was proposed that rhodopsin may be a proenzyme or zymogen, whose catalytic center is covered by the chromophore, 11-*cis*-retinaldehyde, or by some aspect of folding of the protein, opsin. Light, in isomerizing the chromophore from the 11-*cis* to the all-*trans* configuration, or through the subsequent configurational changes in opsin (*2, 4,* 5), exposes the catalytic center, activating the enzyme. One molecule of enzyme might turn over a large number of molecules of substrate, thus constituting one stage of amplification. If the substrate were also a proenzyme, and if the product of the catalysis were a second active enzyme, that would be a second stage of amplification. One could imagine a train of proenzymes, each activating the next in line, all triggered by the action of one photon on a molecule of rhodopsin. Such a train would make of the rod a biochemical photomultiplier, capable of yielding a large biochemical product very rapidly in return for a minimal initial input (Fig. 1).

This notion has lately received encouragement from an unexpected source. It has long seemed baffling to outsiders, as also to workers in the field, that the process of blood clotting is so complicated. An extraordinarily large number of factors seem to take part in this process, and the problem of terminology has grown so formidable that some time ago an International Committee on Blood Clotting Factors was convened to straighten it out.

A recent proposal promises to clarify this situation on a very simple basis (*6*). The clotting factors in human blood seem to form a chain of proenzymes, each on activation activating the next in line. The initial factor (factor XII, the Hageman factor), activated by surface contact, activates the next presumed proenzyme, factor XI, which in turn activates the Christmas factor (factor IX), and so on down the chain until prothrombin, activated to thrombin, splits a peptide fragment from fibrinogen, converting it to fibrin, the substance of the clot (Fig. 2) (*7*). Macfarlane speaks of this arrangement as a "cascade of proenzyme-enzyme tranformations" and recognizes its role as a "biochemical amplifier in which enzymes are analogous to photomultiplier or transistor stages" (*6*).

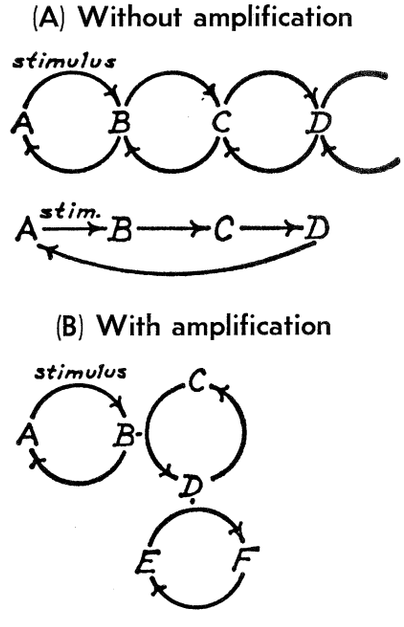

Fig. 1. Hypothetical arrangements of types of reaction chain that may be concerned in excitation processes. The schemes shown in (*A*) regenerate, but do not provide amplification; the transformation caused by the stimulus yields less than a mole-equivalent of product. In (*B*) the action of the stimulus on A produces a catalyst, B, which promotes a second reaction; and D, the product of that second reaction, in turn catalyzes the third reaction. In this case the transformation of A can result in formation of many mole-equivalents of D, and this in turn as many more mole-equivalents of F. Such a system might involve almost any degree of amplification, depending only on the turnover constants of B and D as catalysts and the duration of the reaction (from Wald, *4*).

I wrote Macfarlane to ask whether one result of this arrangement might be that a single molecule of Hageman factor can activate a clot. He replied: "I am sure that only a relatively few molecules of Hageman factor can be involved in the surface activation of the next stage, but there are no quantitative data as yet. But the fact that 0.001 microgram/ml. of Russell's viper venom will clot blood in about the same time as its normal Hageman mechanism, may give some indication. Russell's viper venom activates Factor X, while a Hageman factor operates 3 stages further back, so that the clotting mechanism might be more sensitive by a factor of 100 or 1000 to the Hageman factor than to the venom."

There is a simple way to go at this problem, and it has already played a large part in analysis of quantum relations in the photographic process (*3*) and the visual process (*1*): it is based upon Poisson's so-called law of small numbers (*8*).

The Poisson relation states the probability, P_x, that an event will occur x times in a certain number of trials, when its mean occurrence is m. The relation is

$$P_x = e^{-m}m^x/x!$$

In the present context, x becomes the number of molecules of factor needed to initiate a clot, and m is the concentration of that factor. One need not know its absolute concentration; relative concentrations are quite enough. Since, however, clotting will occur if a sample contains x or more molecules of activating factor, the Poisson relation applies in its integral form,

$$\sum_{x=1,2,3}^{x=\infty} P_x = \sum_{x=1,2,3}^{x=\infty} e^{-m}m^x/x!$$

This is easily evaluated from tables of the Poisson function (*9*) by adding up, over a range of m, all the P_x's for a given value and all higher values of x. It is not a formidable undertaking, since the series converges rapidly, so that P_x for values of x much higher than the minimum being considered ceases to affect the result. The results of such a computation are shown in

Fig. 3 for the cases: 1 molecule or more required to activate a clot ($x \geq 1$); 2 molecules or more; and 3, 4, 5, 7, and 10 molecules or more.

All that is needed therefore is a preparation of the clotting factor to be tested—the Hageman factor or any other—and a test mixture containing adequate amounts of all subsequent and accessory factors needed to form a clot. The activating factor would be brought into a range of concentrations such that at the low end of the range the addition of a sample to the test mixture never yielded a clot (perhaps within a specified time) ($P_x = 0$) and at the upper end of the range it always yielded a clot ($P_x = 1$). Then intermediate concentrations would be tested over this range, each on a standard number of samples of the test mixture —say 20 to 40—to determine the fraction of samples that clot. Plotting this fraction—the frequency of clotting—against the logarithm of the relative concentration is expected to yield a curve that matches in shape one curve in a family of Poisson curves such as those shown in Fig. 3. The corresponding value of x represents the minimum number of molecules of factor required to activate a clot. It is important to note that this is the *effective* number, the number of molecules actually taking part in the reaction. The method takes no account of molecules that may be present but for some reason are not actively engaged. There is every reason to hope that here, as in vision and in the photographic process, such a trial would yield a meaningful result.

Another feature of the clotting mechanism offers a significant parallel with excitation systems. It is important not only that an excitation system respond quickly when a stimulus goes on, but that it quickly cease to respond when the stimulus goes off. It is equally important that clotting, having responded quickly to an injury, be limited in scope by cessation of the reaction soon after it has begun. This appears to be achieved by rapid inactivation of the clotting factors. The mean life of a thrombin molecule is estimated to be 24 seconds (*10*), and other factors may be comparably short-lived.

There may also be an important difference between the clotting and visual excitation mechanisms, in that the former is in solution, the latter at least in part in the solid state, the visual pigments forming highly oriented components of the membranes of rod

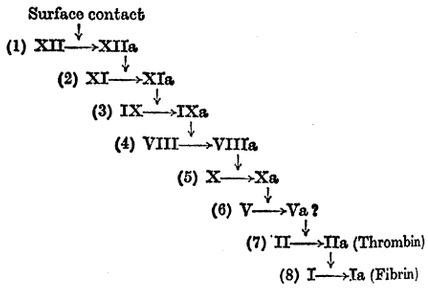

Fig. 2. Diagram of the proenzyme-enzyme cascade involved in human blood clotting. The Roman numerals stand for the various factors. Factor XII, the Hageman factor, on surface activation catalyzes the activation of factor XI, this in turn activates factor IX, and so on until prothrombin (II), activated to thrombin, catalyzes the transformation of fibrinogen (I) to fibrin, the substance of the clot (from Macfarlane, *6*).

and cone outer segments. Could an enzyme cascade operate in a system even partly in the solid state?

The two types of system may not differ as greatly in this regard as at first seems likely. The clotting reaction does not proceed entirely in solution but involves one or more surface adsorptions. Macfarlane writes in this connection: "several of the clotting reactions take place on surfaces. The Hageman factor is adsorbed to and activated by suitable surfaces, and factor XI is probably adsorbed and activated by the Hageman factor before being released into the plasma. Phospholipid is the next surface catalyst, probably affecting two stages at least, and finally fibrin itself may be involved in the adsorption of thrombin and other factors which it may activate." Nor need the visual excitation reactions proceed wholly in the solid state. The membranes containing what is probably a single layer of visual pigment are in contact with intracellular spaces in the rods and with both intra- and extracellular spaces in most types of cone, which could permit interaction with components in solution. It should be added furthermore that possibilities exist for reactions between enzymes and other proteins in the solid state. Mazia and Hayashi (*11*) have prepared insoluble monolayers of mixed pepsin and egg albumin which digest themselves when brought near the optimal *p*H, about 1.5, each pepsin molecule accounting for hydrolysis of 20 to 40 molecules of albumin. They found that such solid mixtures digest more rapidly than comparable systems in which the enzyme is in solution.

Perhaps it would be well after all these comparisons to say plainly that such an enzyme cascade, having been to a degree established in the mechanism of clotting, remains only an unsupported suggestion in visual excitation. There is as yet no evidence that rhodopsin is a proenzyme, still less that it stands at the head of such a reaction chain as I have described. Nevertheless, such a biochemical arrange-

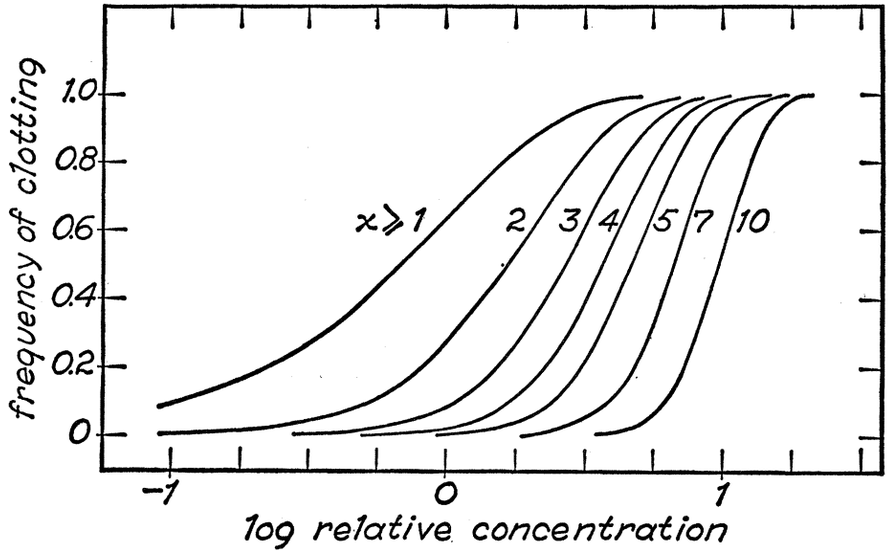

Fig. 3. Sample Poisson curves brought into form for testing the minimum number of molecules (x) of a blood-clotting factor, effective in stimulating a clot. If the frequency of clotting of a given test mixture is determined as a function of the relative concentration of the activating factor, the plot of such observations is expected to match in shape one of such a family of Poisson integrals as that shown here. In that case the corresponding value of x should represent the minimum number of molecules of factor involved in activating the clot.

ment for multistage amplification, capable of drawing a large return very rapidly from a minimal input, seems so applicable to the general problem of excitation, whether in receptors, nerve, muscle, or indeed eggs, that it would seem worthwhile to look for it in all these structures.

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Blood-Flow Relation between Hepatic Artery and Portal Vein

Abstract. *Blood flows in the hepatic artery and portal vein have been measured with a square-wave, electromagnetic flow meter. Hepatic arterial flow increased when portal venous flow was decreased, but, when hepatic arterial flow was decreased, portal venous flow also decreased. The relation between the two blood systems can be explained as the simple mechanical effect of interposing a slower-flowing stream in the path of a faster-flowing stream.*

The dual blood supply of the liver has prompted much speculation about the relations between the blood flows of the hepatic artery and the portal vein. The two principal current theories are (i) that the hepatic arterial flow and the portal venous flow do not influence each other (*1*) and (ii) that a reciprocal and compensatory relation exists between changes in flow in the two systems (*2*). The development of electromagnetic flow meters has made possible the direct measurement of blood flows in the hepatic artery and portal vein. A definite relation between the two blood supplies has been established under experimental conditions (blood vessels exposed but not cannulated).

Healthy mongrel dogs were anesthetized lightly with sodium pentobarbital (30 mg/kg of body weight), intubated, and placed on a respirator. The arterial blood pressure was monitored during the experiment through a needle in the femoral artery connected to a Sanborn pressure transducer, model 267B. Hepatic arterial and portal venous blood flows were measured with a square-wave electromagnetic flow meter (*3*). The portal venous flow was measured at the point just before the vein branched to enter the liver and the hepatic arterial flow, at a point distal to any gastroduodenal branches.

Attempts to decrease the portal vein flow by direct or partial occlusion were followed by a decrease in the systemic arterial pressure as blood pooled in the intestines. A significant decrease of the blood flow in the portal vein was produced, without causing a decrease in systemic blood pressure, by occluding the superior mesenteric artery. This decrease in portal venous blood flow was associated with an increase in the hepatic arterial blood flow, while release of the occlusion of the superior mesenteric artery resulted in restoration of normal flows in both the portal vein and the hepatic artery.

After these blood-flow measurements had been made, a side-to-side portacaval shunt was formed. The portal venous blood flow between the shunt and the liver was measured with the shunt first open and then closed. The hepatic arterial blood flow was also measured with the shunt open and closed. The remarkable decrease in the blood flow through the portal vein was again accompanied by an increase of the flow in the hepatic artery. Closing the shunt restored the portal flow through the liver and decreased the hepatic artery flow to its previous levels. The relation between the blood flows in the portal vein and hepatic artery is shown in Fig. 1. The reduction in portal venous blood flow is compared to the increase in hepatic arterial flow, and the amount of this increase appears to be directly related to the amount of decrease in portal venous flow.

Occlusion of the hepatic artery, which decreases the arterial flow to the liver, is associated not with an increase but with a decrease in the portal venous flow. Figure 2 shows the effect on the blood flow in the portal vein of a decrease in the hepatic arterial flow. A decrease in the arterial flow was accompanied by decreased portal venous flow.

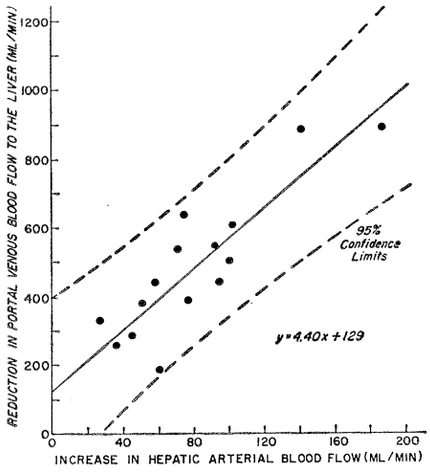

Fig. 1. The effect of reduction in portal venous blood flow to the liver on blood flow in the hepatic artery. The reduction in portal venous flow was produced by occlusion of the superior mesenteric artery or by formation of a side-to-side portacaval shunt. The increase of blood flow in the hepatic artery is directly related to the decrease of the flow in the portal vein.

Two similar experiments were performed in dogs that had been given reserpine (5 mg/kg of body weight) 24 hours before the experiment to eliminate a possible effect of epinephrine. The systemic pressure in these dogs was lower than that in the untreated dogs, and the systolic pressure stayed at about 100 mm-Hg throughout the experiment. In these animals the re-

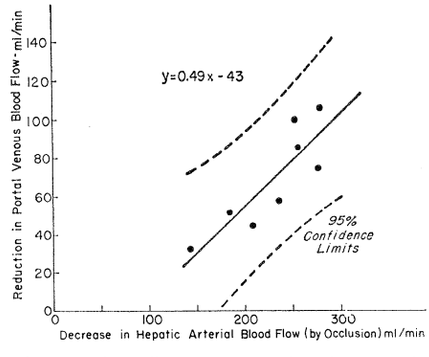

Fig. 2. The effect of reduction in hepatic arterial blood flow to the liver on blood flow in the portal vein. A decrease in hepatic arterial flow results in a decrease in portal venous flow. The amount of decrease of the blood flow in the portal vein is directly proportional to the increase of the flow in the hepatic artery.