remaining ten fish corresponded to the incidence and severity found in first spawners taken at the same sites. Thus, four fish taken on the high seas had spawned previously; the coronary arteries of three were normal, and the fourth had early intimal changes. Three fish taken from Puget Sound had spawned previously and were en route to their second spawning; typical lesions were present in two, and one was normal. Four fish taken from tidewater were identified as repeat spawners; two of these had typical intimal degenerative changes, and two were normal. Four fish taken from freshwater streams were repeat spawners; all were taken near spawning areas, and all had abnormal coronary arteries. The fish trapped on the spawning grounds included one repeat spawner with abnormal coronary arteries. Among these second-time spawners with abnormal coronary vessels, none had evidence of a cumulative process; that is, their lesions were indistinguishable from lesions in first-time spawners taken at the same location.

Thus, although the incidence of vascular degeneration approached 100 percent in fish at the time of spawning, some fish which were known to have spawned 1 to 2 years previously were found to be normal; and the degenerative process in second-time spawners resembled that of first-time spawners taken at the same stage of migration. These findings imply that the degenerative process regressed after spawning and that such reversal is a naturally occurring phenomenon.

We are not aware that reversal of coronary lesions is a normal event in the life cycle of any animal. Regression of human atherosclerotic lesions may occur in starvation, wasting disease, and malnutrition (*4*). Lesions induced by atherogenic diets may also regress after cessation of the diet or manipulation of hormone levels (*5*). These fish probably starve during the freshwater phase of their migration, when the lesions are in the stage of rapid development. The role of hormones in the degenerative process was suggested by Robertson *et al.* who showed that the quantity of 17 hydroxycorticosteroids was markedly increased during the spawning migration (*3*).

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Selective Phagocytosis: A New Concept in Protein Catabolism

Abstract. *The clearance of different metabolic products derived from two plasma proteins, prothrombin and fibrinogen, was studied with the aid of the isolated, perfused rat liver. Active thrombin and fibrin were rapidly cleared by the Kupffer cells. Inactive thrombin and a partially degraded fibrin molecule were also cleared but at much slower rates. This difference in clearance rates suggests the presence of a high degree of selectivity in the clearance of altered plasma proteins.*

Using the isolated, perfused rat liver for the study of phagocytosis (*1*), we have demonstrated that colloidal gold (Au^{198}), intact and disintegrated labeled rat platelets (*2*), and aggregated bovine serum albumin are cleared from heparinized rat blood at the same rate as from an artificial perfusate containing polyvinylpyrrolidone (PVP) but no heparin (*3*) (Fig. 1). Since PVP does not affect clearance by the reticuloendothelial system in the rat (*4*), one can study the clearance of materials known to be altered by blood from a perfusate containing PVP. Using the isolated, perfused rat liver technique, we have studied the fate of the metabolic products derived from two plasma proteins, fibrinogen and prothrombin.

The preparation of rat fibrinogen-Se^{75} (*5*) and of fibrinogen's metabolic products, fibrin and a product of plasmin-induced fibrin degradation (FDP) (Fig. 2B), has been described, as have techniques of liver perfusion, perfusate sampling, and the counting of the radioactivity of the perfusate, bile, and liver samples (*6*). Samples of albumin (*7*) and thrombin (*8*) were labeled with I^{131} by the method of McFarlane (*9*), to the extent that the iodination did not exceed the mean of ½ atom of I^{131} per protein molecule. The free I^{131} was removed by overnight dialysis of the protein solution in versene-treated dialysis bags against a continuous change of distilled water at 0°C (*10*). At the end of the dialysis, the supernatant fraction, after precipitation of the protein with trichloroacetic acid, contained less than 0.7 percent of the total radioactivity. Unlabeled thrombin was prepared from a commercially available bovine thrombin preparation (Parke, Davis Co.) by chromatography on an Amberlite XE64 column (*11*). To assess the effect of blockade of the reticuloendothelial system on protein clearance, we injected rats intravenously with colloidal carbon (*12*) (8 and 16 mg per 100 g of body weight) 2 hours before removal of the liver (see *13*).

Livers were initially perfused with 1 to 4 mg of bovine serum albumin-I^{131} added to the blood and the PVP perfusates. Radioactivity of these perfusates declined slightly during the first few minutes of the perfusion. This decline did not exceed 8 percent of the total activity. Subsequently there was no appreciable change in the perfusates' activities during a 2-hour perfusion. Hence the amount of bovine serum albumin-I^{131} promptly cleared is very small. This finding suggests that the labeling procedure causes little denaturation, for if denaturation were a significant problem clearance of radioactivity would probably be greater (*14*). This finding also confirms previous observations (*15*) that normal catabolism of plasma proteins by the liver takes place at a very slow rate.

Addition of 3.5 to 4 mg of solubilized (*6*) rat fibrin labeled with Se^{75} to whole blood perfusate resulted in its

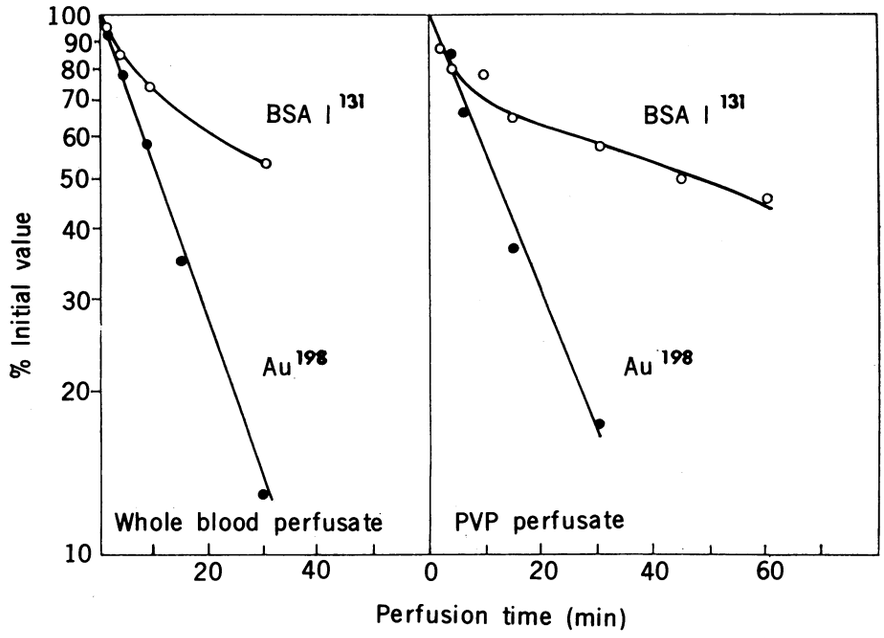

Fig. 1. The clearance of colloidal gold (Au^{198}) and aggregated bovine serum albumin-I^{131} (*BSA*) from heparinized blood occurs at the same rate as from an artificial perfusate containing PVP but no heparin.

rapid clearance. The curves for the disappearance of radioactivity from the perfusate fit a series of semilog functions, but generally they have two components. The first, a fast component, usually follows a single exponential function during the first 6 to 15 minutes, whereas the second, or slower component, can be readily separated from the first by subtraction of the slow component (the asymptotic level) from the complex general curve (Fig. 2A). The slope of the corrected first component (straight-line semilog plot) is the disappearance-rate constant K or the fraction of the material removed by the liver per minute (*16*).

$$K = (\log C_1 - \log C_2)/(T_2 - T_1)$$

or

$$0.693/T\tfrac{1}{2} \quad (13, 16)$$

Clearance curves for rat fibrin revealed a first component $T\tfrac{1}{2}$ of 8 to 10 minutes; hence, for the fibrin, K equals 6.9×10^{-2} to 8.7×10^{-2} (*17*). The loss of radioactivity from the perfusate closely paralleled the increase in activity noted in the liver. Microautoradiographs of washed liver tissue taken after the perfusion suggest a gross localization of radioactivity on or in the Kupffer cells (Fig. 2C). In contrast, the clearance of rat FDP labeled with Se^{75}, after its addition (3 to 5 mg) to the perfusate, occurs at a much slower rate. The $T\tfrac{1}{2}$ of the first component of the clearance curve is approximately 1.5 to 3 hours (Fig. 2A), hence K equals 3.8×10^{-3} to 7.7×10^{-3}. Thus, although the two metabolic products of rat fibrinogen are cleared by the rat liver, their clearance rates are different. Fibrin is removed 15 to 16 times faster than FDP.

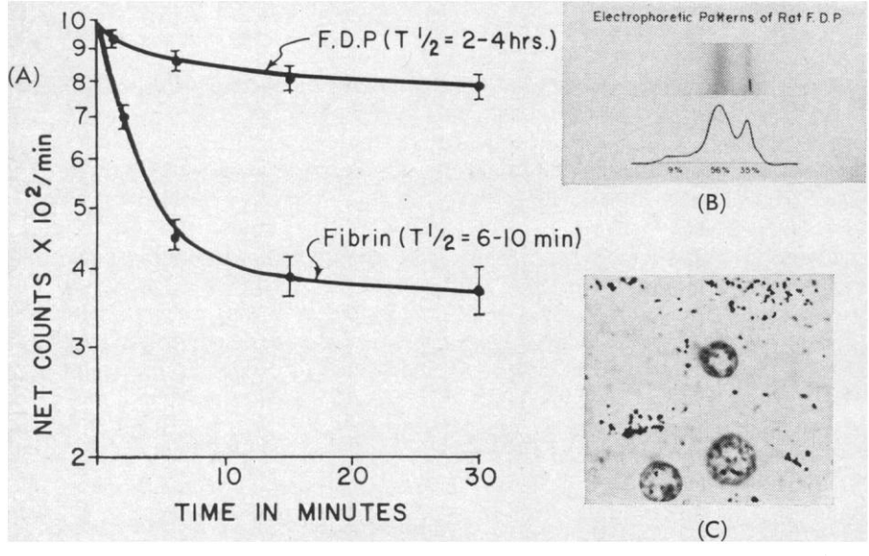

Fig. 2. Uptake of rat fibrin-Se^{75} and FDP by an isolated perfused rat liver. (A) Clearance curves for FDP and fibrin; (B) electrophoretic pattern of the FDP preparation; (C) automicroradiograph of liver after perfusion with a solution containing fibrin-Se^{75}.

The intravascular formation of fibrin in both man and the experimental animal is invariably associated with activation of a blood-borne proteolytic mechanism that breaks down fibrin (*18*). Quantitative assessment of the capabilities and limitations of this proteolytic system are currently not available. The results of our study suggest, however, that, in instances in which the proteolytic mechanism fails to complete this function, phagocytosis serves as a backup mechanism that rapidly eliminates unchanged fibrin. This process proceeds at a rate which exceeds that of an enzyme reaction in preference to the clearance of partially degraded fibrin. This finding uncovers an aspect concerning the selectivity of phagocytosis, the mechanism of which is presently unknown.

The intravascular formation of fibrin requires the generation of thrombin. Thrombin, in addition to forming fibrin, has been implicated in activation of plasminogen (*19*), an indication that the interaction of fibrinogen and thrombin in vivo and the formation of fibrin and FDP occur simultaneously and hence, are difficult to study separately. Part of the thrombin is absorbed onto fibrin (*20*); part is inactivated by the antithrombin of plasma and serum (*21*).

With these considerations in mind, we studied the uptake of thrombin labeled with I^{131} by the isolated, perfused rat liver. Labeled bovine thrombin (1000 to 2000 units) and sufficient unlabeled bovine thrombin to obtain a final amount of 3 to 4 mg were added to a perfusate containing PVP. After perfusion began, there was a rapid clearance of the label. Curves with an early rapid-phase $T\tfrac{1}{2}$ of 1.5

to 2.5 minutes were obtained (Fig. 3A), hence K equaled 0.28 to 0.46. The loss of thrombin-bound radioactivity from the perfusate closely paralleled the increase in activity noted in the liver. The isotope appeared in the bile. In addition, there was a definite increase in the fraction of the perfusate soluble in trichloroacetic acid. This increase was linear. Thus the liver can eliminate bovine thrombin (*22*) at a rapid rate (three times that for rat fibrin). After carbon blockade of the liver with 8 mg of carbon per 100 g of body weight, thrombin clearance from a PVP perfusate is only slightly reduced. However, the reduction in clearance when 16 mg of carbon is used is marked, resulting in a $T½$ of 12 to 15 minutes. This finding indicates that the thrombin is cleared by the Kupffer cells (see *13*).

In contrast to the findings obtained with a PVP perfusate, thrombin-I^{131} was cleared from heparinized rat blood as a perfusate at a much slower rate and to a much lesser extent. After the addition of thrombin-I^{131} to rat blood, the early rapid-phase $T½$ value was 25 to 30 minutes.

Since a variety of materials are cleared at the same rate from perfused PVP as from blood, we have to assume that the difference in clearance rates observed in these two experiments resulted from an alteration of the thrombin by the blood. Since blood contains an antithrombin system that slowly inhibits the thrombin, and since the activity of this system is greatly enhanced by heparin (*23*), which is used in our system to prevent the clotting of blood, the differences between the results obtained with blood and those obtained with a PVP perfusate may be due to alteration of thrombin by antithrombin. This change of the thrombin molecule may affect its subsequent clearance by the isolated, perfused rat liver.

To test the validity of this hypothesis we incubated 1000 to 1500 units of thrombin-I^{131} with 1 and 2 ml of rat serum at 37°C for 2 hours. In a parallel experiment similar amounts of thrombin-I^{131} were incubated for 2 hours with saline (1 to 2 ml) at 37°C. At the end of this period, thrombin clotting activity, as determined by established techniques (*24*), was unaltered in the mixture of thrombin and saline. However, at the end of 2 hours there remained less than 5 units of thrombin clotting activity per milliliter of the

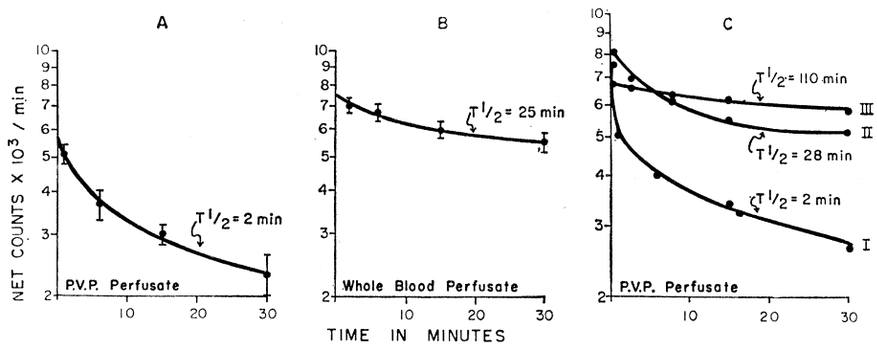

Fig. 3. Uptake by the isolated, perfused rat livers of bovine thrombin-I^{131} from a PVP perfusate (A) occurs at a rate which is much faster than that from perfused whole blood (B). Thrombin-I^{131} incubated with saline for 2 hours at 37°C is cleared from a PVP perfusate more rapidly (C, curve I) than thrombin incubated for 2 hours with rat serum at 37°C (C, curves II and III).

mixture of serum and thrombin. This finding suggests that the serum had inactivated the thrombin. The clearance of this inactive labeled thrombin preparation from an artificial perfusate was slow (Fig. 2C); $T½$ for the early rapid-phase clearance for thrombin incubated with serum ranged from 25 to 110 minutes (K = 0.026 to 0.0058), a rate similar to that for clearance of thrombin from heparinized blood. Thus the liver clears products derived from prothrombin, namely active and inactive thrombin, but inactive thrombin is cleared much more slowly. The similarity between the changes in the elimination ratios during the conversion of rat fibrin to FDP and during the changes of active to inactive bovine thrombin is striking. Active thrombin is a noxious agent and is slowly neutralized by antithrombin. The thrombin which escapes this reaction is rapidly eliminated by phagocytosis. Hence the reticuloendothelial system, in eliminating active thrombin, serves as a backup mechanism for the antithrombin system. The clearance of the innocuous inactive thrombin hardly interferes with that of active thrombin because the latter proceeds 12 to 55 times faster than the former.

Since clearance in vivo probably greatly exceeds those we observed, the significance of this mechanism is increased. The similarity in the clearance mechanism of the products of fibrinogen and prothrombin metabolism suggests that certain active enzymes derived from an inactive plasma protein precursor, as well as certain products of enzyme activity derived from a plasma protein substrate, are eliminated by the reticuloendothelial system.

The lung probably functions in the clearance of fibrin (*25*) and an inactive (diisopropyl phosphoryl-P^{32})–thrombin preparation (*26*) from blood after their intravenous injection into rabbits. The results of our study suggest that the liver, which constitutes a filter for arterial and portal-vein blood, has two functions in the metabolism of these proteins: the production of fibrinogen and prothrombin and the clearance of products derived from them.

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Trehalose Regulation of Glucose-6-Phosphate Hydrolysis in Blowfly Extracts

Abstract. *The presence of trehalose enhances the rate of glucose-6-phosphate hydrolysis in extracts of fat body and other tissues of adult* Phormia regina *Meigen. The activation appears to be specific, and increasing the concentration of trehalose changes both* V_{max} *and* K_m *for glucose-6-phosphate. There is, however, no easily recognizable phosphotransferase in the extract.*

α,α-Trehalose [(α-D glucosido)-α-D-glucoside] is the predominant hemolymph carbohydrate in a large number of insect species and is known to be synthesized in certain of them (*1*) through the same series of reactions that has been demonstrated in yeast (*2*):

glucose-6-phosphate + uridine diphosphate glucose → trehalose-6-phosphate + uridine diphosphate (1)

trehalose-6-phosphate → trehalose + P_i (2)

Reaction 1, catalyzed by UDP glucose: D-glucose-6-phosphate 1-glucosyltransferase (2.4.1.15), has never been completely separated from reaction 2, whereas the second enzyme, trehalose-6-phosphate phosphohydrolase (3.1.3.-12), catalyzing the hydrolytic cleavage of trehalose-6-phosphate, has been isolated and partially purified (*3*).

Since insect fat body appears to be an important site of production of α,α-trehalose, research concerned with the control of trehalose synthesis has necessarily centered around this organ. In the course of a recent investigation of this problem in the adult blowfly, *Phormia regina* Meigen, I observed that the in vitro release of trehalose by fat body was decreased in the presence of relatively small amounts of added trehalose (*4*). Murphy and Wyatt, having previously noted a similar phenomenon in Cecropia larvae, were able to demonstrate that trehalose in some way inhibited the trehalose synthetase complex (reactions 1 plus 2) prepared from fat body extracts (*5*).

There is now reason to speculate that in the blowfly, at least, one of the ways in which trehalose may exert a measure of control over trehalose synthesis is through its action on a system responsible for the hydrolysis of glucose-6-phosphate, since a preparation has been obtained from adult *Phormia* fat body and other tissues in which the rate of hydrolysis of this important substrate is specifically increased by trehalose (*6*).

This discovery provides an explanation for some heretofore poorly understood results described in another report (*3*). In those experiments crude adult blowfly tissue extracts were shown to catalyze reactions resulting in the liberation of inorganic phosphate (P_i) from adenosine triphosphate (ATP) at a rate which was greater in the presence of trehalose than glucose. Enzymes have now been found in these extracts which rationalize the trehalose effects. These include low levels of trehalose (catalyzing the reaction: trehalose → 2 glucose), hexokinase (glucose + adenosine triphosphate → glucose-6-phosphate + adenosine diphosphate), and a trehalose-activated glucose-6-phosphate hydrolyzing system (glucose-6-phosphate → glucose + P_i).

Table 1 contains the results of an experiment in which a partially purified extract was incubated with the substrates noted. The presence of a phosphatase activated by trehalose and inhibited by glucose is established in the last three lines of the table. In Table 2 it may be seen that trehalose does not change the balanced liberation of glucose and P_i, and in Table 3 the specificity of trehalose in this activation process is demonstrated. In this experiment the inhibition of P_i liberation by maltose is due to glucose contamination of the maltose preparation.

The extract, as it is assayed, has little adenosine triphosphatase activity (see Table 1), but it does hydrolyze inorganic pyrophosphate (PP_i). Recent studies on rat microsomes have uncovered what appears to be a single enzyme which is responsible for both the synthesis and hydrolysis of glucose-6-phosphate through the coordination of three activities: glucose-6-phosphatase, inorganic pyrophosphatase, and glucose-PP_i phosphotransferase (*7*). The enzyme is nonspecific, mannose acting as a phosphate acceptor from both PP_i and glucose-6-phosphate. In view of this finding, the blowfly preparation was examined for similar activity, the examination including, however, an investigation of the influence of trehalose on the rate of hydrolysis of PP_i

Table 1. Inorganic phosphate released from various substrates by an extract of adult *Phormia regina* Meigen. The reaction mixture consisted of substrate in the final concentration shown below, $MgCl_2$ ($4.5 \times 10^{-3}M$), tris HCl (*p*H 7.2, $4.5 \times 10^{-2}M$), enzyme (0.2 ml) (40 to 60 percent ethanol fraction of centrifuged homogenate), and H_2O up to 0.45 ml. The mixture was incubated for 30 minutes at 32°C and P_i was determined by an unimportant modification of the method of Fiske and Subba Row (see *8*).

Substrate	P_i released (μg)
ATP ($3.3 \times 10^{-3}M$)	0.8
Glucose ($1.8 \times 10^{-2}M$) + ATP	4.3
Trehalose ($9 \times 10^{-3}M$) + ATP	12.1
Glucose-6-phosphate ($1.3 \times 10^{-2}M$)	26.4
Trehalose + G-6-P	55.4
Glucose + G-6-P	13.0