Selective Phagocytosis: A New Concept in Protein Catabolism

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Reprinted from
SCIENCE
January 5, 1968, Vol. 159, No. 3810, pages 107-110

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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 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

$$0.693/T½ \quad (13, 16)$$

Clearance curves for rat fibrin revealed a first component $T½$ 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

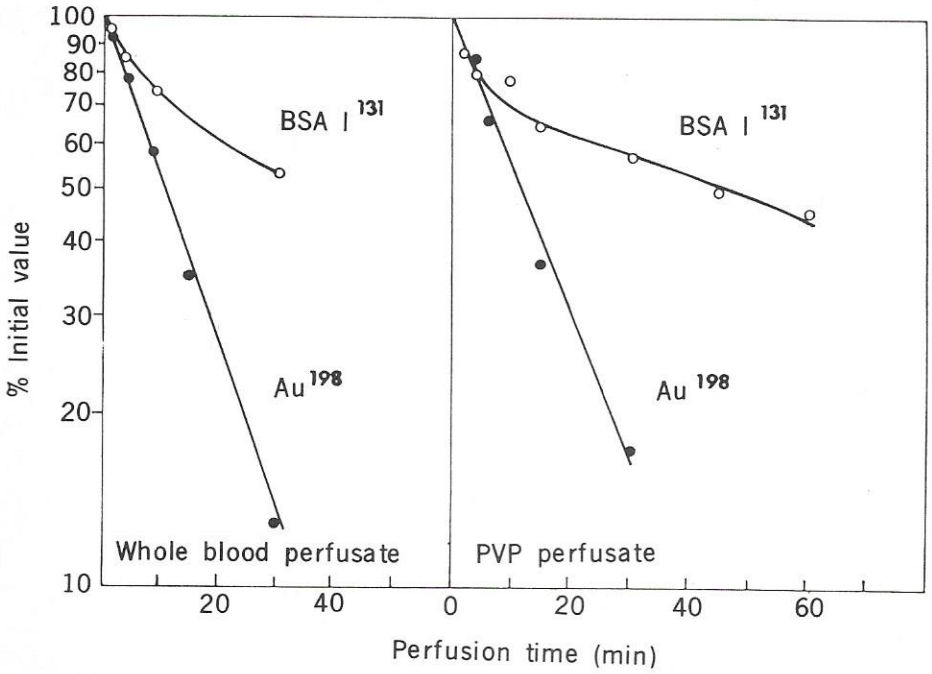

Fig. 1. The clearance of colloidal gold (Au^{198}) and aggregated bovine serum albumin-I^{131} (*BSA*) from heparinized blood occurs at the same rate as from an artificial perfusate containing PVP but no heparin.

labeled with Se^{75}, after its addition (3 to 5 mg) to the perfusate, occurs at a much slower rate. The $T½$ 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.

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.

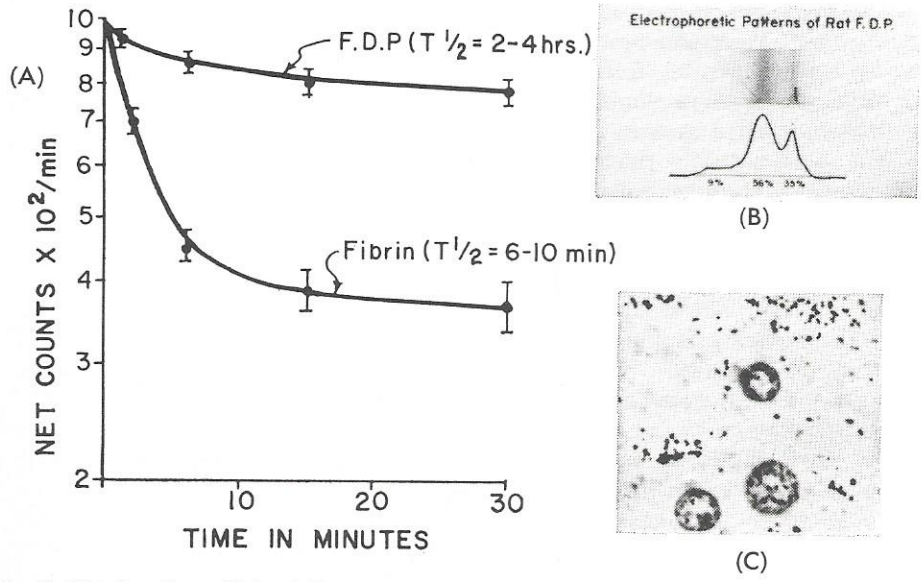

Fig. 2. Uptake of rat fibrin–Se^{75} and FDP by an isolated perfused rat liver. (A) Clearance curves for FDP and fibrin; (B) electrophoretic pattern of the FDP preparation; (C) automicroradiograph of liver after perfusion with a solution containing fibrin-Se^{75}.

The intravascular formation of fibrin 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 $T½$ 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 as-

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