Protective Effect of Fibrinogen on Accelerator-Globulin. (17917)

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Data has been obtained which indicates that the presence of fibrinogen may play an important rôle, not only in protecting accelerator-globulin (Ac-globulin) against various chemical and physical agents, but also, in inhibiting Ac-globulin activity in the conversion phase of the normal clotting process. In a previous study(1), oxalated bovine plasma was subjected to the action of chemical and physical agents which involved denaturation, adsorption, and precipitation phenomena. It was observed repeatedly that the amount of Ac-globulin and prothrombin removed or inactivated, in large part, depended upon whether or not the plasma had been defibrinated initially. Almost invariably, agents which effected a reduction of Ac-globulin activity and prothrombin concentration in undefibrinated plasma, effected a decidedly greater reduction in defibrinated plasma. Also, when precipitated fibrinogen was removed from plasma which had been frozen and subsequently thawed, Ac-globulin activity was significantly greater than that in fresh unfrozen plasma. Preliminary quantitative data suggest that there is probably an inverse relationship, within limits, between Ac-globulin activity and the amount of fibrinogen in plasma.

Methods. The source of prothrombin and an outline of the method used in the quantitative assay of Ac-globulin have been described elsewhere(1). Prothrombin concentrations were determined by the modified 2 stage technic(2,3). The thromboplastin and fibrinogen preparations were of the same type as those used previously(1). Parke-Davis topical thrombin was used exclusively. Beef, dog, and rat blood was collected in 1.85% potassium oxalate; human blood was collected in 2.85% sodium citrate; 7 parts of blood to 1 part of anticoagulant were used.

Observations. An example of the protective action of fibrinogen on Ac-globulin and prothrombin, and one which may be taken as representative of an adsorption phenomenon, is the treatment of plasma with kaolin. (Table I) When 5 g of kaolin was added to 100 ml of non-defibrinated bovine plasma, only 30% of the prothrombin and Ac-globulin was adsorbed. In contrast, when 100 ml of plasma was defibrinated with thrombin, and then treated with 5 g of kaolin (NF Powder), approximately 40-50% of the prothrombin and Ac-globulin was adsorbed. It required 17 g of kaolin per 100 ml of non-defibrinated plasma to adsorb 40-50% of the prothrombin and Ac-globulin. The removal or inactivation of Ac-globulin and prothrombin effected by the addition of protein precipitants of the heavy metal group is another instance in which the protective action of fibrinogen on Ac-globulin and prothrombin was demonstrated. In one experiment, when native bovine plasma was treated with an equal volume of a 1.5% $HgCl_2$ solution, centrifuged, and the mercuric ions removed with coagulated egg albumin, the Ac-globulin activity of the supernatant was approximately 10%, and the prothrombin concentration 35% of that in the native plasma. However, when defibrinated plasma was subjected to similar treatment, no Ac-globulin activity or prothrombin could be detected. Furthermore, the presence in the plasma of precipitated fibrinogen or of clumps of formed fibrin, which may result from freezing and subsequent thawing of the plasma, interferes with the removal or inactivation of Ac-globulin and prothrombin by $HgCl_2$ solution. When the precipitated fibrinogen and/or fibrin are removed from the plasma, how-

1. Carter, J. R., and Warner, E. D., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 30.

2. Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, v114, 667.

3. Ware, A. G., and Seegers, W. H., *Am. J. Clin. Path.*, 1949, v19, 471.

TABLE I.
Effect of Kaolin on Prothrombin Concentration and Ac-Globulin Activity.

Physical state of plasma	% prothrombin conc.	% area	% Ac-G activity
1. Fresh native beef plasma	100	100	100
2. (1) treated with kaolin	69	92	70
3. (1) defibrinated with thrombin and treated with kaolin	58	61	51

The kaolin conc. was 5 g per 100 ml of plasma. Initially kaolin was mixed with distilled water, the mixture centrifuged, the supernatant decanted, and the tube inverted and allowed to drain. Beef plasma was added to kaolin, mixed, swirled for 5 min., centrifuged, and the clear supernatant plasma removed. For each Ac-globulin determination, .03 ml of plasma was used.

TABLE II.
Effect of Freezing on Ac-Globulin Activity.

Physical state of plasma	ml of plasma	% area	% Ac-G activity
1. Fresh pooled human plasma, 28°C	.01	100	100
1a. (1) stored at −40°C, 5 hr, thawed at 28°C; precipitated fibrinogen removed	.01	177	334
2. Fresh beef plasma, 28° C	.0009	100	100
2a. (2) same as (1a), stored 3 days	.0009	136	175
3. Fresh rat plasma, 28°C	.1	100	100
3a. (3), 28°C, spontaneous precipitation of fibrinogen; precipitate removed	.1	187	353
4. Fresh dog plasma, 28°C	.02	100	100
4a. (4) same as (1a), but stored 4 hr	.02	133	220
4b. Fibrinogen redissolved in dog plasma, 5°C	.02	107	125
5. Fresh dog plasma, 28°C	.002	100	100
5a. (5) same as (1a), but stored 24 hr; no precipitation of fibrinogen	.002	80	60
6. Fresh beef plasma, 28°C	.009	100	100
6a. (6) defibrinated with thrombin, 28°C	.009	141	182
6b. (6a) same as (5a), stored 3 hr	.009	144	185

ever, the same concentration of $HgCl_2$ solution effects complete removal or inactivation of both Ac-globulin and prothrombin. If fibrinogen does manifest some type of protective action toward Ac-globulin, as our evidence would suggest, it may, in an analogous manner, serve to inhibit Ac-globulin activity in the clotting process. This would provide a probable explanation for the observation that Ac-globulin activity is decidedly greater in plasma that has been frozen than in plasma that has not. The data in Table II, substantiate this observation in 4 species of animals. It should be noted that increased Ac-globulin activity occurs only when fibrinogen is precipitated out of the solution, either by freezing and subsequent thawing (1, 1a, 2, 2a, 4, 4a), or by spontaneous precipitation (3, 3a). When, in spite of freezing and thawing, no fibrinogen is precipitated, there is some decrease of Ac-globulin activity (5, 5a). When the fibrinogen is redissolved in the same plasma from which it was precipitated, Ac-globulin activity is reduced almost to that of fresh unfrozen plasma(4). We have been unable, however, to reduce the Ac-globulin activity of frozen plasma to that of unfrozen plasma by the addition of Armour bovine fibrinogen. There is no significant difference in Ac-globulin activity in frozen and unfrozen defibrinated plasma, but there is a decided difference in activity in defibrinated (with thrombin) and non-defibrinated plasma (6, 6a, 6b). There is only a slight difference in Ac-globulin activity in plasma defibrinated with thrombin (6a) and that defibrinated by freezing (2a), the latter, moreover, not being free entirely from fibrinogen. Prothrombin concentrations of the species tested are not altered detectably by the initial freezing and thawing of plasma.

Discussion. These studies serve to intro-

4. Quick, A. J., and Stefanini, M., *J. Lab. Clin. Med.*, 1948, v33, 819.

duce the concept that fibrinogen apparently acts as a buffer or protector substance to Ac-globulin and to prothrombin. More significantly, they call attention to a probable rôle of fibrinogen in the conversion phase of the normal clotting process. Although the prothrombin concentration is not altered detectably by the removal of fibrinogen, Ac-globulin activity is. This qualitative difference, therefore, focuses attention on the Ac-globulin-fibrinogen relationship particularly. It is conceivable that Ac-globulin "molecules" are enveloped by, or form a complex with fibrinogen molecules, in which state the former may be considered to be inactive. With the liberation of small amounts of thrombin, or by adding small amounts of thrombin to plasma, the fibrinogen is converted to fibrin, thereby releasing "active" Ac-globulin (serum type) "molecules." Such an hypothesis is consistent with many observed, but hitherto unexplained phenomena in blood clotting, and can serve as a starting point for sound speculation and experiment. Recently, for example, Quick and Stefanini(4) have shown that the effect of rabbit labile factor on stored human plasma is increased over that on fresh plasma. They have called attention to the similarity between this observation and that of Ware, Murphy, and Seegers(5) regarding the conversion of an inactive plasma Ac-globulin to an active serum Ac-globulin. The release of fibrinogen from an Ac-globulin-fibrinogen complex incident to the storage of plasma could account for the increase in the sensitivity of the stored human plasma to labile factor. Similarly, the influence of varying amounts of thrombin on Ac-globulin activity in oxalated bovine plasma(6) could be explained by the removal of fibrinogen. The conversion of an inert pro-enzyme plasma Ac-globulin to an active serum-type Ac-globulin may be mediated by thrombin, by freezing and thawing, or, in all probability, by any chemical or physical agent which effectively removes fibrinogen without concomitant removal of Ac-globulin. Quantitative studies of this problem are in progress. Further, it is tentatively postulated that with additional study of the fibrinogen-Ac-globulin relationship, the differences and incongruities between the various accelerator factors described may, in part at least, be reconciled.

The increase of Ac-globulin activity subsequent to the removal of fibrinogen is of considerable practical importance in the routine assay of plasma Ac-globulin. From the above discussion, it would seem evident that the Ac-globulin activity in plasma is a function of several variables, of which fibrinogen, and agents which alter it, is of appreciable significance. As a corollary, it would seem equally evident that Ac-globulin as a quantity or entity can be measured accurately only in serum or in completely defibrinated plasma.

Summary. Evidence is presented which indicates that fibrinogen serves as a buffer or protector substance to Ac-globulin. The removal of fibrinogen from plasma by the addition of thrombin or by freezing and subsequent thawing enhances Ac-globulin activity. The data provide a probable explanation for the conversion of an inactive pro-enzyme accelerator in plasma to an active accelerator in defibrinated plasma and in serum.

5. Ware, A. G., Murphy, R. C., and Seegers, W. H., *Science*, 1947, v106, 618.

6. Ware, A. G., and Seegers, W. H., *Am. J. Physiol.*, 1948, v152, 567.

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