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THE MODE OF ACTION OF HAGEMAN FACTOR IN THE RELEASE OF PLASMA KININ

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A pain-producing and smooth-muscle-contracting substance, probably a polypeptide, is released from blood plasma by contact with glass and some other surfaces (Armstrong, Jepson, Keele & Stewart, 1957). In later work (Margolis, 1958*a*) well defined stages in the mechanism for the release of this plasma kinin have been described, and also the fact that kinin is not liberated from plasma which is congenitally deficient in Hageman factor (Ratnoff & Colopy, 1955). Hageman factor (HF) is not merely a hypothetical component whose presence is postulated to explain the steps of kinin formation, but is independently defined as the factor missing in a congenital coagulation abnormality known as the Hageman trait. Further, it is known that HF forms the connecting link between the changes involved in kinin release and those leading to blood coagulation and the formation of a permeability factor (Margolis, 1957, 1958*a*, 1959; Biggs, Sharp, Margolis, Hardisty, Stewart & Davidson, 1958; Hardisty & Margolis, 1959).

According to a concept formulated in a preliminary account (Margolis, 1958*b*), HF normally exists in the plasma in a biologically inactive state, that is, as a precursor. When plasma is brought into contact with glass and some other foreign surfaces (e.g. quartz, kaolin) HF is rapidly adsorbed by the surface, where it acquires enzymic activity and initiates the further stages of these reactions without itself returning into solution or being consumed in the process. In kinin release two such further stages have been recognized; they can be accounted for by the presence of at least two components which react successively and which have been provisionally designated components A and B. Because of the similarities in their functions and in some physical properties HF and component A were at first thought to be identical. Further work, however, has shown that this identification can no longer be maintained. Thus we can now distinguish three separate stages in the release of plasma kinin by contact with glass. The postulated order of these reactions is represented in Fig. 1. Activated

component A and plasma kinin are rapidly inactivated in plasma, but are relatively stable when partially purified. Components A and B are exhausted in the course of activation, component B being much more labile than A in this respect.

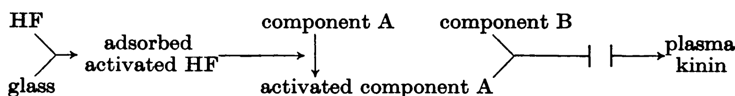

Fig. 1. Suggested mode of action of Hageman factor (HF); for details see text.

Unlike HF, which is defined by independent criteria, the other components are at present recognized only as functional entities. Something is known about their physical properties, such as heat stability, adsorbability and presence in crude protein fractions (Margolis, 1958*a*), but this information is still fragmentary and insufficient to characterize these factors as distinct substances. Future work may well reveal that each is really a complex system in itself.

The main object of this paper is to present evidence on the role of HF in the release of plasma kinin. The observations involving other components will be considered only in so far as they are necessary for the understanding of this initial stage of the process.

In the previous papers the ability of glass-activated preparations to release kinin, when added to intact plasma, was interpreted as being due to the formation of a 'contact factor' (equivalent to activated component A; see Fig. 1). This term will be avoided here because: (*a*) contact factor was at the time erroneously thought to be a direct activation product of HF, and (*b*) the term has since been justifiably used by other authors as a synonym of HF (Caen & Bernard, 1958; Soulier, Wartelle & Menache, 1958).

METHODS

Unless otherwise stated, all operations were performed in siliconed glassware. 'Intact' citrated human plasma and plasma derivatives were prepared by methods discussed in detail elsewhere (Margolis, 1957, 1958*a*).

HF-deficient (HF-dep) plasma was obtained for most of the experiments from a single patient with Hageman trait (cf. Biggs *et al.* 1958); identical results were obtained with fresh and lyophilized material. These were further confirmed on plasma from three other patients.

(1) *Plasma depleted of component B* (*B-dep plasma*) was prepared as follows. Intact plasma was rotated for 2 min with glass ballotini (microspherules 0·1 mm in diameter) 1 g/ml., i.e. about 200 cm^2 surface/ml. of plasma. It was then removed from the beads and immediately mixed with an equal volume of intact plasma. The mixture was incubated in siliconed containers for 18–24 hr at 4° C.

As has been shown before (Margolis, 1958*a*), there is an excess of component A relative to component B in normal plasma. The amount of activated component A present in the glass-contacted sample is thus sufficient to 'consume' not only its own component B but also that of the intact plasma with which it has been mixed.

(2) *Plasma depleted of components A and B* (*A+B-dep plasma*). Intact plasma was rotated with 1 g of ballotini/ml. for 60 min and then removed and kept in siliconed containers as above. It was used on the next day or kept frozen at −20° C for up to 1 week.

After glass treatment some specimens were heated to 56 or 61° C for 20 min. The precipitated fibrinogen was discarded after centrifuging. *It should be stressed that A + B-dep plasma still contains most of the original HF*, whose complete removal requires the use of much larger surface areas (about 5000 cm^2/ml.), as is shown in Fig. 4.

(3) *Alkaline eluate from glass.* Glass ballotini which were bathed for 2–5 min in plasma or derivatives (1 g ballotini/ml. plasma) were washed with 10 changes of 0·9 % NaCl. They were next suspended in a volume of saline $\frac{1}{4}$ that of the original plasma, and sufficient 0·1 N-NaOH was added to raise the pH to 10–11. The beads were then agitated for 2–3 min and the supernatant was transferred into siliconed tubes. After addition of a little 5 % $NaHCO_3$ (0·02 ml./ml. of eluate) the eluate was brought back to neutrality with 0·1 N-HCl. Eluates from kaolin (50 mg light kaolin/ml. of plasma) were prepared as above but with the difference that the supernatant was repeatedly centrifuged before neutralizing, in order to remove the last traces of kaolin. When unheated plasma was used for 'coating' kaolin, a fine precipitate formed in the eluate below pH 8·0. This was spun down and discarded. Plasma heated to 56° C yielded eluate which remained clear in neutral solution.

(4) *Indirect test for activation of plasma by glass and biological assays of the formed plasma kinin on rat uterus.* The test has been called indirect (Margolis, 1958*a*) because it is not the amount of kinin released within the test sample itself which is measured (as in the direct method), but the ability of the test sample to liberate the kinin from intact 'substrate' plasma. The test is thus carried out in two stages.

Samples of plasma, etc., were rotated in glass tubes with 1 g of glass ballotini/ml. for 2–3 min. As soon as the beads had settled (within 10–15 sec) 0·1 ml. of the supernatant was added to 0·2 ml. of intact substrate plasma in siliconed tubes. After 2 min incubation, 0·1 ml. of the mixture was applied to one horn of a rat uterus suspended in oxygenated Ringer's solution (Armstrong *et al.* 1957) in a 5 ml. siliconed organ bath kept at 30° C. The uteruses were from virgin albino rats (180–200 g) injected with 50 μg of stilboesterol 24–48 hr previously. The latent period of contraction was measured with a stopwatch. The recording lever was counterbalanced with a cylindrical float suspended in a beaker of water; thus the weight lifted was proportional to the height of the contraction. This arrangement gave more reproducible records than with a fixed counterbalance.

With the exception of the experiments in Fig. 3 the test samples were usually diluted with 2 parts of 0·9 % NaCl immediately before activation. This was introduced for convenience and economy only. Qualitatively identical results were obtained with undiluted samples (cf. Fig. 3). In either case the intact substrate plasma was always used undiluted.

The results illustrated below are representative of a large number of experiments. In most figures the individual panels in the records (e.g. *a*, *b*, *c*) show tests carried out on different days; the contractions are therefore strictly comparable only within each panel.

RESULTS

The dosage of the test samples was such as to give a strong contraction with fully activated plasma, after a latent period of 5–10 sec, and no response within 1 min with an equal dose of intact plasma. Figure 2 illustrates that with indirectly activated samples a full-scale response of the uterus can usually be said to represent activity at least 50 (never less than 20) times greater than in samples eliciting no response. The present conclusions are based on the difference between these two extremes and no further quantitative treatment was attempted.

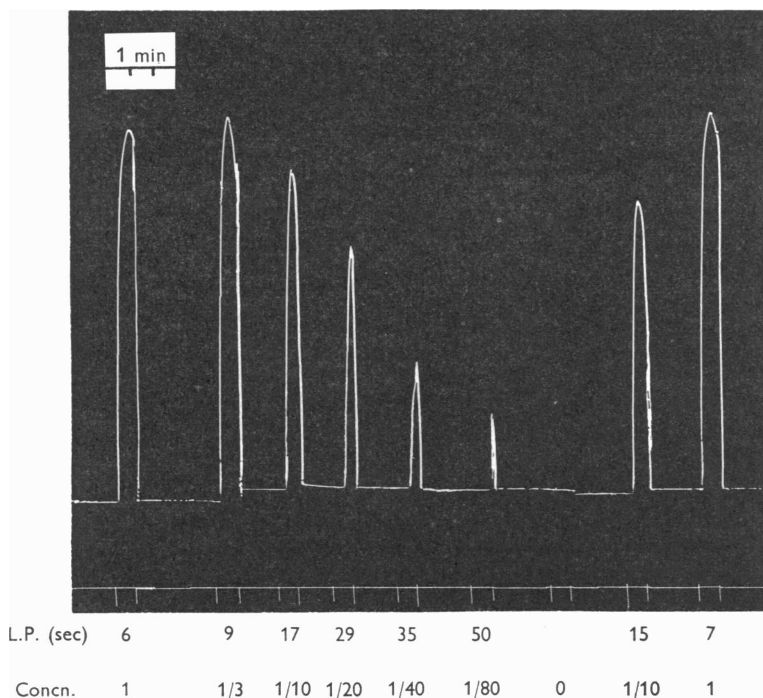

Fig. 2. Rat uterus contractions, as in all subsequent figures. Dose–response pattern of the kinin released from intact plasma by the addition of varying dilutions of glass-activated test plasma (see Methods 4). Component B-depleted plasma, diluted with 2 parts of 0·9 % NaCl, was rotated for 3 min with 1 g of glass ballotini/ml. The supernatant was diluted with 0·9 % NaCl; 0·1 ml. of this final dilution (1 to 1/80 as shown below tracing) was then added to 0·2 ml. of intact substrate plasma in siliconed tubes, incubated 2 min, and 0·1 ml. of this mixture was applied to the uterus for 1 min. L.P. = latent period of each contraction.

The interdependence of HF and Component A

Although neither HF-deficient (HF-dep) nor A + B-deficient (A + B-dep) plasma can be activated separately by contact with glass, a mixture of the two (Fig. 3, tests 5 and 7) behaves like normal plasma (test 4). HF and component A cannot therefore be identical but must be complementary to each other. As is shown in Fig. 3 the mutual supplementation takes place only when the two plasmas are mixed first and then treated with glass (tests 5 and 7) but not when they are exposed to glass surfaces separately and mixed immediately thereafter (test 6).

Evidently glass, HF and component A form the minimal reactive system in the first stage of the kinin-releasing mechanism. The reason for this will become apparent when the findings shown in Fig. 4 have been considered.

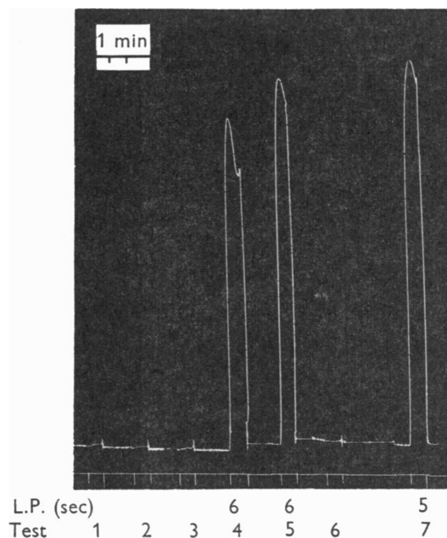

Fig. 3. Ability of HF and component A to supplement each other in kinin formation. Undiluted plasma test samples, enumerated below, were rotated for 2 min with 1 g of ballotini/ml. 0·1 ml. of supernatant was then incubated with 0·2 ml. of intact normal plasma in siliconed tubes for 2 min and 0·1 ml. of this mixture was tested on the rat uterus.

Test samples: 1, HF-dep (Hf-deficient) plasma. 2, A + B-dep (component A and B-depleted) plasma. 3, Control mixture of equal volumes of HF-dep and A + B-dep plasma, incubated without glass ballotini. 4, Normal plasma. 5 and 7, Mixture as in test 3 but rotated with ballotini. 6, HF-dep and A + B-dep plasma rotated with ballotini separately and mixed after removal from glass.

The fixation of HF to the glass surface

Glass previously 'coated' with normal plasma, or derivatives containing HF, fully activates HF-dep plasma. This property persists after repeated washing of the glass with water or neutral saline solution and, after one coating, the same glass can be used for the activation of several successive samples of HF-dep plasma without any apparent loss of activity. In Fig. 4*a* (tests 3–5) this phenomenon is illustrated by using A + B-dep plasma, which contains HF, as the coating reagent. Figure 4*b* shows that glass coated with preparations deficient in HF, such as natural HF-dep plasma or plasma deprived of HF by heating above 60° C (test 4) or by adsorption with more than 20 mg kaolin/ml. (tests 2 and 3), does not activate HF-dep plasma. There is also no evidence of activation when the order of the procedure in Fig. 4*a* is reversed, that is when the glass beads are pre-treated with HF-dep plasma and then rotated with A + B-dep plasma (Fig. 4*c*, test 4).

Together with the data in Fig. 3, these results suggest the following

mechanism: (*a*) HF is activated by adsorption to the glass surface and remains active only in the adsorbed state; and (*b*) component A is activated only after the adsorption of HF on to the glass. It should now be clear why the mixture of HF-dep and A + B-dep plasmas contacted in separate tubes (Fig. 3, test 6) was inactive. If all the activated HF in the A + B-dep plasma is discarded with the beads it obviously cannot react with the component A in the HF-dep plasma in the other tube.

The experiments with kaolin (tests 1, 2, 3 in Fig. 4*b*) show that in order to remove all the HF from normal plasma, a surface area of about 5000 cm^2/ml. is required, the specific surface area of light kaolin being of the order of 10 m^2/g.

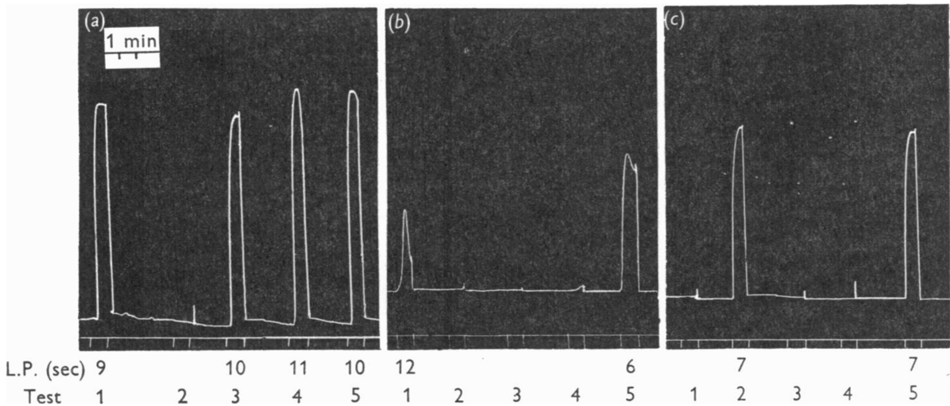

Fig. 4. Fixation of HF on to glass surface. Glass ballotini were coated as indicated below and then washed with 10 changes of 0·9 % NaCl (see Methods).

(*a*) *Test samples*: HF-dep plasma. *Treatment of ballotini* (washed with further 10 changes of saline before tests 3–5): 1, A + B-dep plasma, the source of HF. 2, 0·9 % NaCl. 3, Ballotini already used in test 1. 4, Ballotini from test 3. 5, Ballotini from test 4.

(*b*) *Test samples*: HF-dep plasma. *Ballotini* coated with: 1–3 Normal plasma adsorbed with kaolin for 8 min: (1, 20 mg/ml. 2, 50 mg/ml. 3, 100 mg/ml). 4, Normal plasma heated to 61° C for 20 min. 5, Normal plasma.

(*c*) *Test samples*: 1, 2, 5, HF-dep plasma. 3, 4, A + B-dep plasma. *Treatment of ballotini*: 1, 0·9 % NaCl. 2, A + B-dep plasma. 3, 0·9 % NaCl. 4, HF-dep plasma. 5, From test 2.

The failure of glass to remove components A and B from HF-dep plasma

The evidence for the reaction sequence postulated above was strengthened by experiments in which the conditions were so arranged that HF-dep plasma was found to be more effective than normal plasma in these activation tests. These experiments were based on the observation that

in the process of activation components A and B are consumed (Margolis, 1958*a*). Since in the absence of HF none of the later stages can take place (cf. Fig. 1), we can anticipate that after treatment with glass these components would not disappear from HF-dep as from normal plasma.

This hypothesis was subjected to the tests shown in Fig. 5 in which HF-dep plasma was treated with glass, which would normally have exhausted components A and B, and could then still be used as a source of these components in the test system. The results of Fig. 5*a* show that HF-dep plasma is not depleted of component B by a direct treatment with ballotini (see tests 1 and 2) which is more than sufficient to remove this component from normal plasma (test 3). Figure 5*b* shows that component B

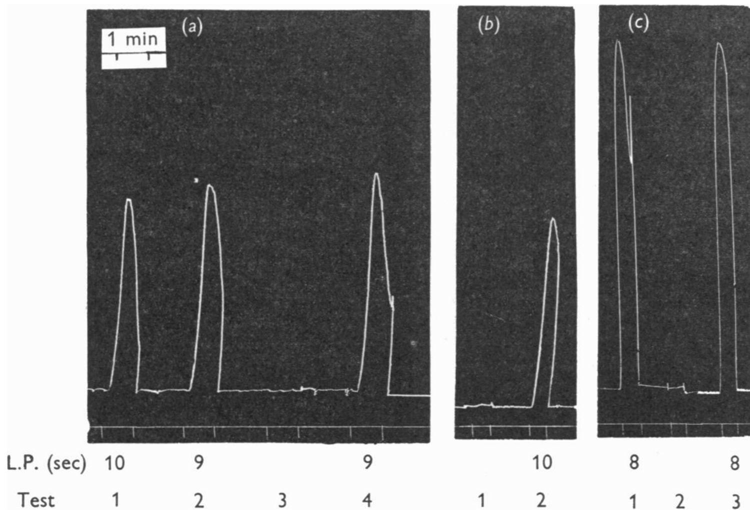

Fig. 5. Persistence of components A and B in HF-dep plasma after treatment with glass ballotini. In (*a*) and (*b*) tests for depletion of component B, in (*c*) test for depletion of component A; see text.

(*a*) B-dep plasma was activated with ballotini as before and added to: 1, HF-dep plasma. 2, HF-dep plasma rotated 5 min with 1 g ballotini/ml. and then incubated at 4° C in siliconed tubes for 18 hr; this would completely remove component B from a normal plasma which then fails to release kinin when used as substrate, as is shown in 3, Normal plasma treated as in 2. 4, Normal intact plasma.

(*b*) B-depleted activated 'test' plasma as in (*a*). Substrate 1, Normal plasma activated 2 min with 1 g ballotini/ml. and added to an equal vol. of HF-dep plasma; mixture incubated at 4° C for 18 hr in siliconed tubes (see Methods: 'B-dep plasma'). 2, As in 1 but the plasmas were incubated 2 hr in separate tubes before mixing.

(*c*) Normal plasma substrate. Ballotini coated with A+B-dep plasma (source of HF). Test plasmas: in 1, normal; in 2, A+B-dep (obtained from normal plasma by rotation for 1½ hr with equal wt. of ballotini and incubation at 4° C for 18 hr); in 3, HF-dep treated as in 2.

can be removed from HF-dep plasma (test 1) by the addition of glass-treated normal plasma which contains an excess of activated component A (see Methods). Since the latter decays on incubation, component B was found to persist in a control sample (test 2) in which the two plasmas were mixed as in test 1 but after a preliminary 2 hr incubation in separate siliconed tubes.

Figure 5*c* shows that HF-dep plasma can still serve as an efficient source of component A after prolonged rotation with glass ballotini (test 3). In these experiments HF had to be supplied to complete the reacting system (cf. Fig. 1). This was done by coating the beads used in the final tests (not those employed for the prolonged rotation) with HF-containing A + B-dep plasma (cf. Fig. 4*a*). Without this step, component A in HF-dep plasma could not be activated and would therefore remain undetectable. Normal plasma treated and tested in the same manner was quite inactive (Fig. 5, test 2).

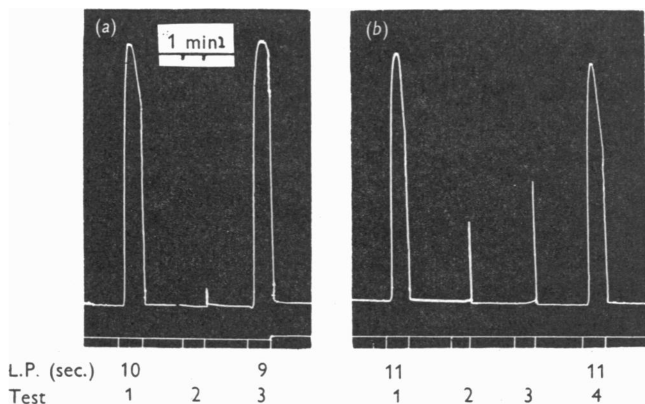

Fig. 6. Elution of HF from glass at alkaline pH. Explanation in text. *Test samples*: test 3 in *a*, normal plasma; remaining tests in *a* and *b*, HF-dep plasma. *Ballotini* were coated with the preparations indicated below and washed 10 times with 0·9 % NaCl solution before each test. In *a*, tests 2 and 3, the 4th–7th washings were at pH 10·0; in the remaining tests the pH of the saline was 7·0 throughout. *Coating of ballotini*: *a*, in all tests AB-dep plasma. *b*, Neutralized alkaline eluate (see Methods 3) from glass treated with: 1, normal plasma, 2, HF-dep plasma; 3, normal plasma heated to 60° C for 20 min; 4, A + B-dep plasma (which contains HF).

Elution of the adsorbed HF from glass

When glass, coated with preparations containing HF, is washed at pH 10–11 it loses its ability to activate HF-dep plasma (Fig. 6*a*, test 2), although it will still activate normal plasma (Fig. 6*a*, test 3). This is presumably due to the removal of the adsorbed HF at alkaline pH (cf. Bangham, Pethica & Seaman, 1958). The HF activity in the eluate can be

recovered by re-adsorption on to fresh glass beads after neutralizing the solution with dilute HCl (Fig. 6*b*, tests 1, 4).

Eluates prepared from beads coated with preparations lacking in HF are inactive (Fig. 6*b*, tests 2, 3), which shows that the effect is not an artifact incidental to the elution procedures but is specifically dependent on the presence of HF.

Other activation products on the glass surface

So long as the material on the glass surface is derived from plasma previously exhausted of components A and B, the effect of such glass can be attributed to the presence of HF alone. When, however, the beads are coated with normal plasma still containing component A, they acquire additional properties. Thus, apart from activating HF-dep plasma they will also activate A + B-dep plasma. The activation of A + B-dep plasma is not nearly as complete as that of HF-dep plasma, but it cannot be dismissed as a trace effect because here also successive samples of the A + B-dep plasma can be activated with the same coated beads. Since A + B-dep plasma is not grossly deficient in HF, the correction in this case cannot be explained only by the presence of HF on the beads. It must, therefore, be due to the adsorption of further activation products on the surface. Even so, this is still dependent on the presence of HF because, as is shown in Fig. 4*c*, test 4, glass treated with HF-dep plasma does not activate A + B-dep plasma. The above observations are relevant to the present problem, inasmuch as these secondary adsorbed agents may be present as active impurities and may give rise to difficulties in experiments in which elution is used for the purification of HF (see below).

Properties of the eluted material

The results illustrated in Fig. 6 have shown that the eluted material is active when reapplied to fresh glass. It is of theoretical interest to establish whether it also remains active in solution. In attempting to answer this question it soon became apparent that a negative result would be much more significant than a positive result. For if a given preparation is inactive, we may conclude that it contains no activated HF, but the converse statement need not be true because activity in solution may conceivably be due to further products of the reaction. The observations below must therefore be interpreted accordingly.

Eluates prepared from glass treated with A + B-dep plasma which contains HF but is devoid of the remaining known components of the system were inactive in siliconed tubes (Fig. 7*a*, test 1). Further, when such an eluate, glass and HF-dep plasma were brought together in various combinations (Fig. 7*a*, tests 2–7) activation could only be detected when

the eluate came in contact with glass at the same time as the HF-dep plasma (tests 2, 7) or before the addition of the latter (test 5). These observations suggest that HF eluted from the glass surface does not remain active in solution but returns to its precursor form, or at least that it does so as soon as it is added to plasma (normal or HF-dep).

On the other hand eluates prepared from glass treated with B-dep or with whole normal plasma (which contain respectively component A and components A and B in addition to HF) released the kinin when added in siliconed tubes to intact substrate plasma even in the absence of glass beads (Fig. 7*b*, test 2). It seems reasonable to suppose that this activity

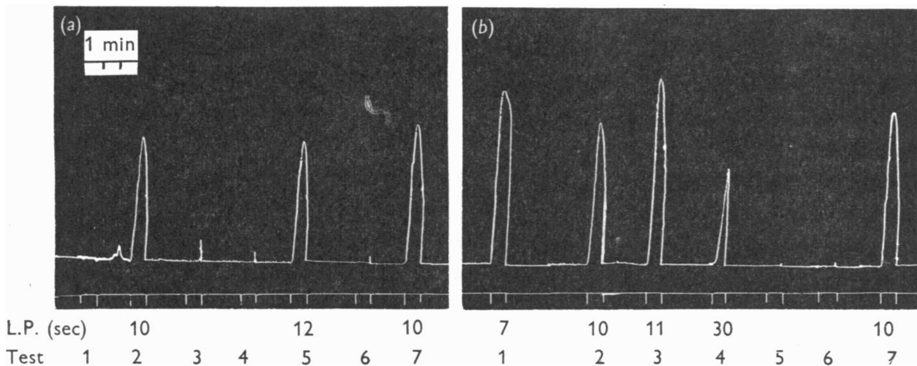

Fig. 7. Properties of eluted HF (see Text). Alkaline eluates were prepared from ballotini coated either with A + B-dep (in *a*) or with B-dep plasma (in *b*). The test samples were mixtures of equal volumes of 0·9 % NaCl, eluate and HF-dep plasma in various combinations. They were either rotated with glass ballotini (G) or incubated in siliconed tubes (S) for 3 min and then added to intact substrate plasma for tests on uterus.

(*a*) *A + B-dep eluate*. Test samples: 1, Eluate (S). 2, Saline, eluate, HF-dep plasma (G). 3, Eluate, HF-dep plasma (S). 4, Saline, eluate (G). 5, Saline, HF-dep plasma (G); ballotini from test 4. 6, Saline, HF-dep plasma (G). 7, Eluate, HF-dep plasma (G).

(*b*) *B-dep eluate*. Test samples: 1, B-dep plasma (G). 2, Eluate (S). 3, Saline, eluate, HF-dep plasma (G). 4, Saline, eluate, HF-dep plasma (S). 5, Saline, eluate (G). 6, Saline, HF-dep plasma (G). 7, Saline, HF-dep plasma (G); ballotini from test 5.

is not due to HF being active in solution but to the presence in the eluate of a further activation product or products (e.g. activated component A), which, unlike HF, does not revert to the precursor form after removal from the glass surface. Otherwise the results in this panel are similar to those in Fig. 7*a*, except that when the mixture of the eluate, saline and HF-dep plasma was incubated without contact with glass some residual activity could still be detected (test 4).

More concentrated preparations of the eluted proteins than the one

used in Fig. 7*a* were obtained by using kaolin as the adsorbent. These were, however, already active before the exposure to glass. It is not yet clear whether this discrepancy between the eluates from glass and those from kaolin is due to quantitative factors, or is of a more fundamental nature. Theoretical considerations favour the latter alternative (see Discussion).

Failure of glass pre-treated with HF-dep plasma to adsorb HF from normal plasma

Glass which had been treated with HF-dep plasma loses much of its ability to accelerate coagulation of normal plasma (Hardisty & Margolis, 1959). We interpreted this as being due to inert substances which combine with glass and block its capacity to adsorb HF. The same phenomenon can be demonstrated in the activation of the kinin-releasing mechanism. Thus, there was no detectable activation in a mixture of normal and HF-dep plasma when the HF-dep plasma was added to the glass beads first and

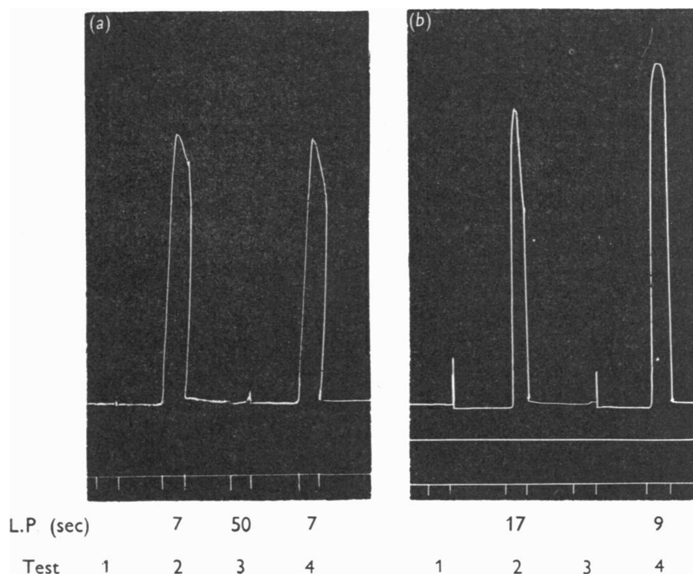

Fig. 8. Suppression by HF-dep plasma (*a*) and by rat kidney homogenate (*b*) of glass activation of normal plasma. See text.

(*a*) To 0·4 g of ballotini were added 0·2 ml. of normal diluted plasma (1/10 in 0·9 % NaCl) and 0·2 ml. of undiluted HF-dep plasma. The tubes were rotated for 3 min and the supernatant tested. *Test samples*: 1, HF-dep plasma alone. 2, Normal plasma added to ballotini 30 sec before HF-dep plasma. 3, HF-dep added to ballotini 30 sec before normal plasma. 4, Normal and HF-dep plasma mixed in a siliconed tube and added to ballotini together.

(*b*) Indirect activation test on normal plasma using ballotini treated with homogenized rat kidney (see Text) and then washed 10 times with neutral 0·9 % NaCl. *Concentration of homogenate*: 1, Undiluted. 2, 1/10. 3, 1/2. 4, 0 (control).

was then followed by normal plasma (Fig. 8*a*, test 3). On the other hand, full responses were obtained when the order of addition was reversed (test 2), or when the two plasmas were first mixed in a siliconed tube and then exposed to glass (test 4). This deleterious effect of HF-dep plasma could not be removed by washing the beads with saline, which is a clear indication that the surface had become blocked. There is, however, nothing in these results to point to a specific inhibitor in HF-dep plasma. Similar observations were made with normal plasma in which HF was destroyed by heating to 61° C, as well as with some tissue extracts when a number of tissues were tested for the presence of HF. In Fig. 8*b* the material used was rat kidney, previously perfused with saline to remove all traces of blood, since rat plasma contains HF. Instead of HF, a powerful blocking effect was discovered.

The most obvious interpretation of the above results is that there are in plasma and tissues substances which compete with HF for the adsorptive sites on the glass surface.

DISCUSSION

HF is actually deposited on the glass surface

In the preceding description the relevant adsorbed material was referred to as HF. However, in the absence of a positive chemical identification, it must be shown that the substance in question: (*a*), can replace HF in HF-dep plasma; (*b*), cannot be obtained from HF-dep plasma and (*c*), corrects only HF deficiency.

The criteria (*a*) and (*b*) are easily satisfied. But the specificity of the activity (*c*) can only be assessed by exclusion. All that can be said at present is that it is possible to obtain preparations which correct HF deficiency but have no such effect on plasma containing HF and deficient in other hitherto recognized components of the system. Such negative evidence can, of course, never be entirely satisfactory. Additional proof, however, may be implicit in the experiments of Fig. 7.

Thus, while an already active substance (whether on glass or in solution) could be either HF or a further product of the reaction, it is probable that a preparation which is not active to begin with, but becomes so in contact with glass, must contain HF. If this reasoning is admissible, then it follows that HF must have been adsorbed to the glass from which the eluates such as those in Fig. 7*a* were obtained.

Activity of HF probably only in the adsorbed form

According to previous concepts (Margolis, 1958*a*; Soulier *et al.* 1958) the activated HF was supposed to be released back into the plasma where it could be recognized by various tests. The present observations speak

against such an interpretation. First, while A + B-dep plasma seems to contain an ample supply of HF, this plasma when shaken with glass and then immediately added to intact plasma does not release any kinin (Fig. 3), which would be expected should any activated HF have escaped from the glass surface. Secondly, there are the observations that one sample of 'coated' glass beads completely corrects the defect in successive specimens of HF-dep plasma (Fig. 4). This indicates that there is no apparent loss of the adsorbed HF from the surface and also that the amount of HF combined with the surface is adequate to account for all the detectable activity. Thus we can probably accept without further reservations that only this part of HF which is actually carried on the surface is capable of initiating further events in the plasma.

The above reasoning applies to the state of HF as it occurs in the plasma. It does not necessarily apply to HF eluted from glass by treatment with alkali. It would, however, be difficult to explain the results of Fig. 7*a* except by assuming that the surface activation of HF is, at least partly, reversible. This agrees with present concepts of protein denaturation (cf. Fraser, 1957). Activation of HF by adsorption most likely involves some unfolding of the protein molecules which results in uncovering of enzymically active groups. The degree of unfolding (i.e. of denaturation) depends on the amount of available surface. With a relatively small surface such as is provided by glass beads, this is restricted by 'overcrowding' and is evidently insufficient to produce irreversible changes in the (tertiary) protein configuration. Hence, upon elution, HF tends to resume its native precursor form. The presence of a larger surface area (e.g. kaolin) leads to a more advanced denaturation due to the disruption of stronger intramolecular bonds. The protein structure is still sufficiently specific to retain its enzymic activity but it cannot return to its native form. Thus HF eluted from kaolin remains active in solution. Finally, when the surface is so large that it is no longer a limiting factor, not only the tertiary but also the secondary convolutions of the adsorbed proteins are disrupted and all specific function is lost. Under these conditions HF activity can no longer be detected (unpublished observations).

Some inadequacies in the present scheme

The mode of action of HF in kinin release seems to be fairly intelligible and is entirely consistent with the studies of its role in coagulation (Hardisty & Margolis, 1959) and the formation of a permeability factor (Margolis, 1959). However, there still remains a residue of facts which cannot be easily fitted into the scheme outlined in Fig. 1. These refer to the nature of component A, which was originally defined as the immediate precursor of the kinin-releasing agent detected by the indirect activation

test (Margolis, 1958*a*). Since in the indirect test glass is removed from the system, the activated component A must be present in solution. Yet, as was stated above, some of the lost component-A activity in A + B-dep plasma can be partially replaced by substances other than HF, which are adsorbed to the glass particles. This makes it difficult to account for the functions of component A in terms of a single factor. It may thus be more proper to speak of a 'complex A' rather than 'component A'. The semantic difficulties could, of course, be avoided if the experiments could be described without any reference to incompletely specified factors but such an account would then be extremely cumbersome. The terms 'component A' and 'B' were therefore retained for the present and, with the reservations mentioned, they are still useful in providing a relatively simple theoretical framework on which to hang a large number of diverse observations.

SUMMARY

1. The first detectable stage in the release of plasma kinin by contact with glass is the adsorption of Hageman factor (HF) by the glass surface.

2. The adsorbed HF activates catalytically a 'component A' which then interacts with a 'component B' in the plasma.

3. At the pH of plasma the activated HF remains fixed to the surface, but it can be eluted with alkali and then reapplied to a fresh surface after neutralization. There is some evidence that the eluted HF returns to its precursor form in solution.

4. In the presence of HF a further activation product is adsorbed by the glass surface. This is distinct from HF and remains active in solution upon elution.

5. Plasma and tissues contain substances which block the capacity of glass to adsorb HF.

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