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ACTIVATION OF PLASMA BY CONTACT WITH GLASS: EVIDENCE FOR A COMMON REACTION WHICH RELEASES PLASMA KININ AND INITIATES COAGULATION

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In the course of investigation of pain-inducing agents, Armstrong, Jepson, Keele & Stewart (1957) found that when blood plasma is exposed to a glass surface a pain-producing substance (PPS) is released. PPS, which is probably a polypeptide related to bradykinin (Rocha e Silva, Beraldo & Rosenfeld, 1949) and kallidin (Werle, Götze & Keppler, 1937; Werle, 1955), is also a powerful stimulant of smooth muscle. When plasma is left in contact with glass, PPS reaches a peak and then gradually disappears. When contact is interrupted the activity decays more rapidly. The substance is assayed by using its pain-producing effect on the exposed base of a blister or by its activity in stimulating plain muscle. For technical reasons the latter method has been used more extensively and has yielded results which are parallel with those obtained from pain experiments. The evidence presented in this paper is based mainly on the uterus-stimulating effect, although the crucial points have also been confirmed by pain experiments. Accordingly, it will be more appropriate to refer to the active substance as 'plasma kinin', which term has been recently proposed by Gaddum, Hilton, Keele, Lewis & Schachter (Lewis, 1958).

The term 'plasma kinin' is, therefore, used in the same sense as was 'PPS' in the paper by Armstrong *et al.* (1957). It will, however, be shown that as a result of contact with a glass surface there also appears in plasma a second active agent which stimulates both the smooth muscle and pain receptors independently of the formed plasma kinin.

It has been suggested that the reactions leading to the release of plasma kinin may be related to those involved in the initiation of clotting (Armstrong *et al.* 1957; Margolis, 1957*a*). It was shown that the latter can be attributed to a precursor, which is converted into an active substance by contact with

glass and certain other surfaces (Margolis, 1956, 1957*a*). The glass-reacting system was found in the globulin fraction and the term 'contact factor' was provisionally used to describe the clot-accelerating agent. Similar findings were described by Shafrir & De Vries (1956) who used the term 'clot-promoting activity'. Subsequent work showed that at least two stages are involved in the activation of clotting by glass (Biggs, Sharp, Margolis, Hardisty, Stewart & Davidson, 1958) and that only the first of these is directly concerned with contact. Similarly Armstrong *et al.* (1957) suggested that there may be two stages in the formation of PPS; the contact with glass may activate a proteolytic enzyme and this in turn may liberate PPS from another component of the plasma.

The object of the present paper is to produce evidence for the existence of these two stages and to show that the product of the initial contact reaction is necessary both for the liberation of plasma kinin and for activation of clotting by contact with glass and related surfaces. To avoid difficulties in terminology it is proposed to restrict the term 'contact factor' to the product of this initial reaction. 'Contact factor' is not identical with the 'clot-promoting agent', but gives rise to the latter after reacting with another coagulation factor.

Some of the material presented here was the subject of preliminary communications (Margolis, 1957*b*, *c*).

METHODS

Unless otherwise stated all operations involving blood and its components were carried out at room temperature (approx. 20° C) using siliconed or 'Polythene' apparatus. Preparations which had not been exposed to glass surface will be referred to as 'intact'.

Human venous blood was mixed with 20% trisodium citrate (0·2 ml./10 ml. blood) and freed from cells by repeated centrifuging. Ammonium sulphate fractions were dialysed during the night at 4° C against citrated saline (3·8 g trisodium citrate, 8·5 g NaCl/l.)

Samples of plasma, etc., were activated by rotation with an equal weight of acid-washed glass microspherules ('ballotini') providing a surface area of 200 cm^2/g, and carefully removed, avoiding any transfer of ballotini with the plasma. In other experiments, where maximal activation was desired and the removal of the contact surface was not essential, instead of ballotini, powdered glass or light kaolin (hydrated aluminium silicate) was added to the plasma (10 mg/ml.) and the samples, including the powder, were tested on the uterus.

Clot-promoting activity was measured by adding standard volumes of the test plasma to intact plasma, recalcifying the mixtures with $CaCl_2$-platelet reagent and recording the coagulation time (see Margolis, 1957*a*). The correcting effect of various preparations of plasma was assessed by a modification of the 'kaolin clotting time' technique: 0·2 ml. amounts of the test plasma were mixed with 0·05 ml. of the added plasma preparations and 0·05 ml. of kaolin suspension (40 mg/ml.) After incubation for 2 min at 37° C, 0·1 ml. of a suitable dilution of lysed platelets and 0·1 ml. of 0·05 M-$CaCl_2$ were added in that order (see Margolis, 1958*a*).

Plasma kinin was assayed by applying the test substance (usually 0·1 ml.) to an isolated rat uterus suspended in a physiological salt solution in a 5 ml. organ bath set at 30° C. The length of the applications is indicated by the two marks under each tracing. The organ bath was washed out three times after each application while the drum was left running. The latent period of response was the main index of activity, the magnitude of the contraction, although usually related to the

atent period, being of secondary importance for reasons discussed below. Application of glass-activated plasma to a freshly prepared uterus was often followed by a prolonged rhythmic activity. When necessary this was countered by shortening the period of application to 30 sec and/or by several preliminary applications of highly activated plasma. In later experiments it was found that a single preliminary application of soya bean trypsin inhibitor (SBTI) (0·5–1 mg/ml. bath fluid) for 1 min completely and irreversibly eliminated the rhythmic after-activity (see text).

For *pain-producing activity* the preparations were assayed on an exposed cantharidin blister base (for details of these bio-assays see Armstrong *et al.* 1957).

The indirect method for the assay of *contact factor* will be described in the text.

RESULTS

Two stages in activation of plasma kinin

When plasma was exposed to glass ballotini as described, maximum plasma kinin activity was reached in about 2 min. Thereafter the kinin decreased and it disappeared within 10–15 min. When a sample of this kinin-free plasma was removed and immediately added to intact plasma new kinin was released (Fig. 1). Evidently as a result of contact, something has appeared in the plasma, which can liberate kinin after an interaction with another plasma component.

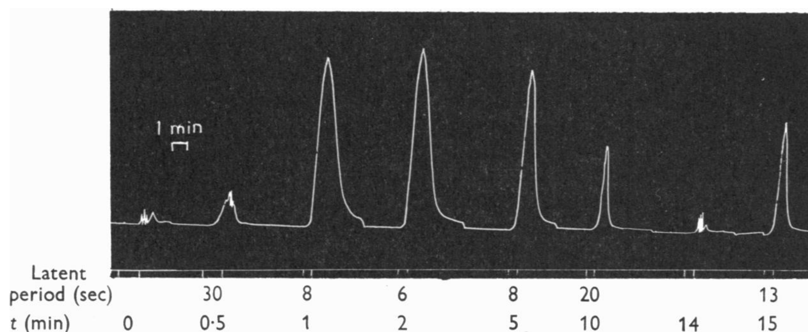

Fig. 1. Direct and indirect formation of plasma kinin. Rat uterus. 0·5 ml. amounts of plasma were rotated with equal weights of ballotini for increasing periods (t). Up to 14 min 0·1 ml. samples were applied directly to rat uterus in a 5 ml. organ bath. 0·1 ml. of the sample activated for 15 min was mixed with 0·2 ml. of intact plasma and 2 min later 0·1 ml. of this mixture was applied to the uterus (see text).

These findings are consistent with the hypothesis of Armstrong *et al.* (1957). The active substance which is still present after the kinin has disappeared from contacted plasma could be a proteolytic enzyme, and the component supplied in the intact plasma could be a specific substrate from which kinin is released. Since there is still no direct evidence regarding the nature of the reaction between these two, no assumptions will be made as to which is an enzyme and which the substrate. In general terms it can be stated that an inactive precursor ('component A') is converted by contact with glass into an active

agent, which will be referred to as the 'contact factor'. The other substance(s) with which the contact factor reacts will be referred to as 'component B'.

To study the properties of contact factor without interference from formed plasma kinin, plasma must be first depleted of component B. This is done by a preliminary brief exposure to glass.

The minimal amount of glass contact, sufficient to deplete plasma of component B, was determined by repeated trials. B-depleted plasma could be prepared consistently by rotating plasma for 5 min with 1/7 of the usual quantity of ballotini (i.e. about 30 cm^2/ml.) and leaving the decanted plasma in siliconed tubes for 2–4 hr at room temperature. Such plasma did not release

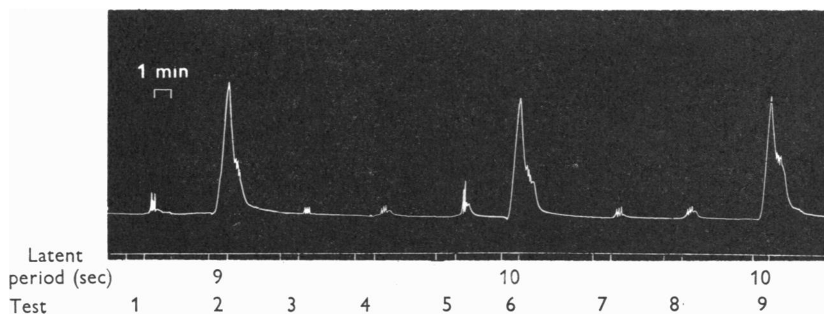

Fig. 2. Plasma depleted of component B as a source of contact factor. Responses of isolated rat uterus to 0·1 ml. doses. Soya bean trypsin inhibitor (1 mg/ml. of bath fluid) was applied to the uterus for 1 min before commencing the tests (see Methods). 1, Intact plasma. 2, Sample (1) treated for 5 min with ballotini (30 cm^2/ml.). 3, Duplicate sample (2) transferred into a siliconed tube and left for 2 hr at room temperature (= B-depleted plasma). 4, Sample (3) treated for 2 min with ballotini (200 cm^2/ml.). 5, Sample (4) mixed with twice its volume of intact plasma and tested after 10 sec. 6, Duplicate sample (5) tested 2 min after mixing (= standard procedure for indirect test). 7, Sample (3) mixed with twice its volume of intact plasma and tested after 2 min. 8, Indirect test as in (6) but 1 mg/ml. of SBTI added just before mixing the two components. 9, Indirect test as in (6) repeated.

any kinin when re-exposed to glass but could still be a satisfactory source of the contact factor. The stages of preparation of plasma depleted of component B are shown in Fig. 2. Records 5 and 6 show the development of kinin in a mixture of 1 part of glass-contacted, B-depleted plasma with 2 parts of intact plasma, and represent the second stage in the formation of kinin. It is obvious that this reaction is not instantaneous, as there was no detectable kinin activity in the mixture within 10 sec of bringing the two components together. By repeated estimations of the kinin level in such a mixture the activity was found to reach its peak at about 2 min and to decline rapidly after that time.

It is known that soya bean trypsin inhibitor (SBTI) prevents the activation of plasma by contact (Armstrong *et al.* 1957), but does not affect the response to kinin once it has been formed. The tracing No. 8 in Fig. 2 shows that when SBTI was added in the second stage of the reaction no kinin was produced.

It appears, therefore, that SBTI either inactivates the contact factor or in some manner prevents its reaction with the other component(s) of the system.

The time course of the second stage can be followed in more detail by extracting successive samples with boiling saline. The reaction is thus immediately arrested, the formed kinin is recovered in a protein-free solution (Armstrong *et al.* 1957) and the extracts, being relatively stable, can be assayed at leisure. The time relations established by this method (Fig. 3) agree well with the results of direct tests on the reacting plasma mixtures.

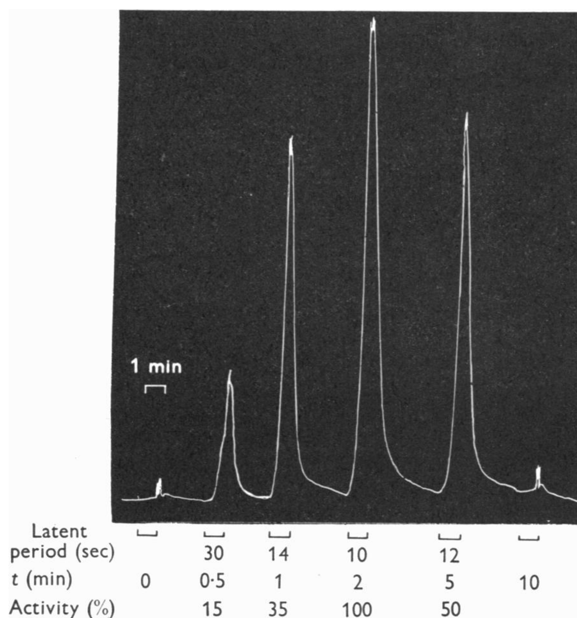

Fig. 3. Time relations of the second stage in the activation of plasma kinin. Rat uterus. Plasma depleted of component B (see Fig. 2) was activated for 2 min with ballotini and then mixed with twice its volume of intact plasma. 1 ml. samples of this mixture were removed at intervals (t) and immediately added to 2 ml. of boiling 0·9 % NaCl solution. After centrifuging to remove the coagulated protein, 0·2 ml. amounts of these extracts were tested on the uterus. The relative percentages of activity were calculated by reference to the responses to dilutions of the 2 min sample.

Formation of plasma kinin by the two-stage method was also demonstrated by testing the saline extracts of the reacting mixture on the pain area. Here also little activity was present in the initial sample as compared with the extract obtained after the two components were allowed to react for 2 min.

On the basis of these results the procedure used in the tracing No. 6 of Fig. 2 was adopted as the standard method for the indirect assay of the contact factor: one volume of the variously treated preparations was mixed with 2 vol. of intact plasma and the released kinin was assayed on the uterus after 2 min.

'Consumption' of component B

The latent amount of contact factor in plasma must be well in excess of that necessary to react with all the available component B, otherwise the two-stage experiments would not have been possible. This is shown more directly in Fig. 4. Varying amounts of glass-activated plasma were added to intact plasma. After 3 hr the mixtures were treated with ballotini and tested for kinin activity.

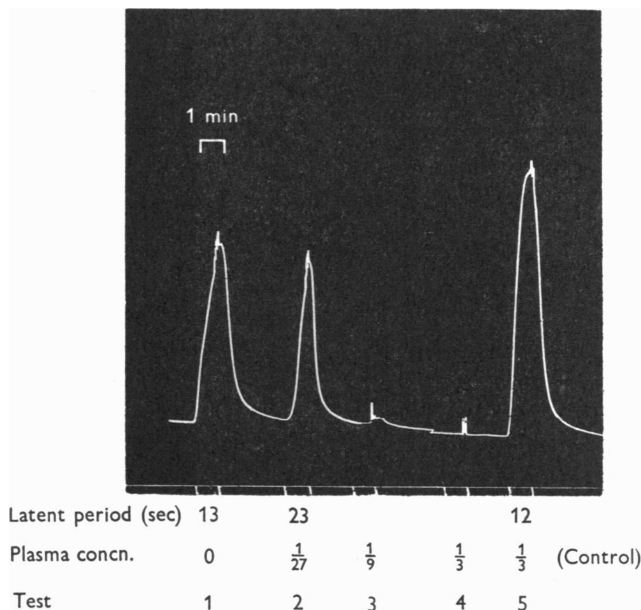

Fig. 4. 'Consumption' of component B by reaction with contact factor. Rat uterus. Plasma was activated with ballotini for 2 min and serially diluted in intact plasma (1–4); plasma concn., concentration of activated plasma in mixture. After 3 hr in siliconed containers the mixtures were again rotated for 2 min with ballotini, and 0·1 ml. amounts were tested on the uterus. A control sample (5) was also tested in which the activated and the intact plasmas were left for 3 hr in separate tubes and mixed immediately before the second activation.

The results indicate that the contact factor in a glass-treated sample can consume' all the component B in about ten times its volume of intact plasma. In fact probably much less than 10 % of the available contact factor is required, since the latter being itself unstable (see below) the amount present at any one time in glass-contacted samples represents only a fraction of the potential amount in the plasma.

Decay of contact factor

By subjecting plasma depleted of component B to various treatments some properties of contact factor were studied. When activated with the standard amount of ballotini (200 cm^2/ml.) the peak of contact factor activity was

reached in 2–3 min. If at any stage the glass was removed the contact factor rapidly decayed (Fig. 5). A rough quantitative estimate of the rate of decay can be gained from a dose–response curve which showed that more than 90% of the activity was lost in 5 min at room temperature. At lower temperatures

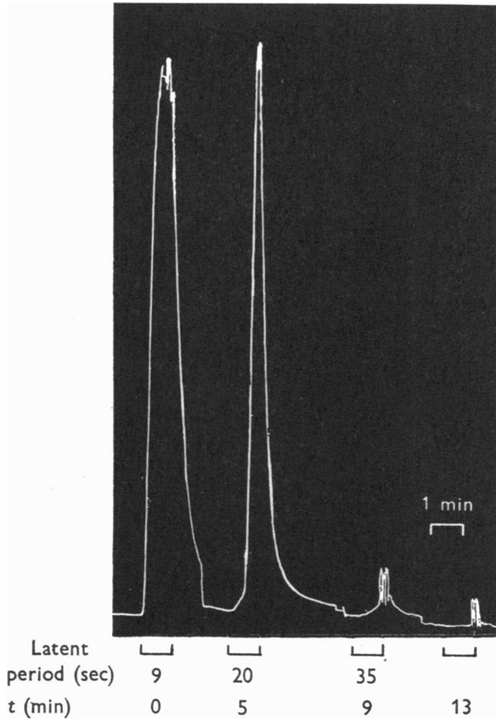

Fig. 5. Decay of contact factor. Rat uterus. Component B-depleted plasma was activated for 2 min with ballotini and transferred into siliconed tubes. At various times, *t*, after removal from glass 1 vol. was added to 2 vol. of intact plasma. After a further 2 min 0·1 ml. of this mixture was tested on the uterus (indirect test).

the decay is slower but even at 0° C it is not completely arrested. This instability of the contact factor (as well as that of kinin) must be taken into account in any attempt at a quantitative evaluation of the results; the actual yield of these substances must represent only a fraction of the potential yield.

Exhaustion of the precursor of contact factor (component A)

When contact with ballotini was continued, after 2–3 min the contact factor activity began to fall and was almost completely exhausted in 30–60 min (Fig. 6). The change was irreversible and contact factor could not be revived with fresh beads. Presumably all the available precursor of contact factor

(component A) had been exhausted. This process is quite distinct from exhaustion of component B and requires more time to complete, as is implied in the results shown in Fig. 1.

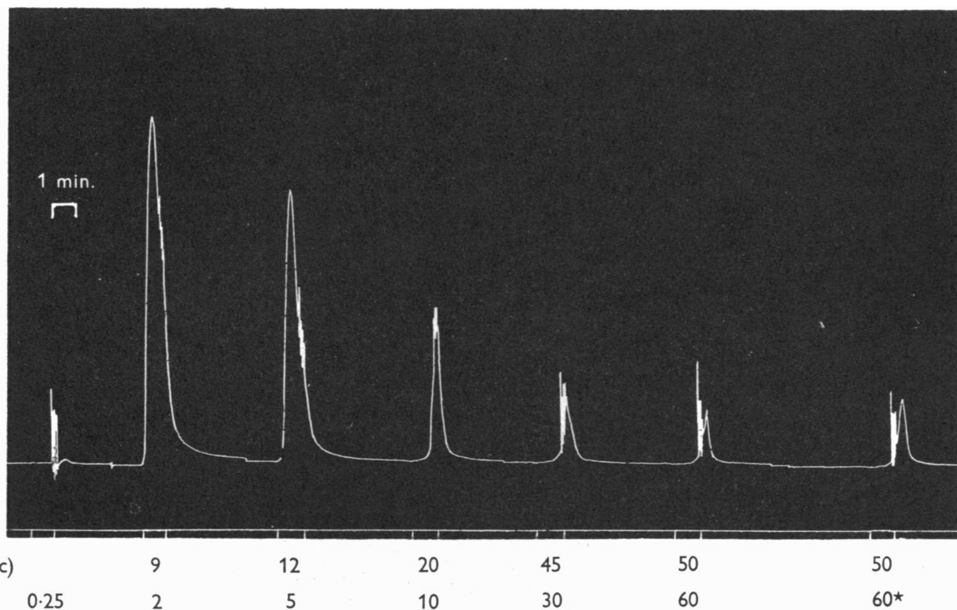

Fig. 6. Exhaustion of the precursor of contact factor. Rat uterus. 0·5 ml. amounts of B-depleted plasma were rotated with ballotini for various times, *t*, and tested on the uterus by the indirect method as in Fig. 5. * Plasma removed at 58 min and rotated for a further 2 min with fresh ballotini; indirect test as with the other samples.

Effect of heat and adsorbents

The formation of contact factor was not affected by pre-heating the plasma to 56–58° C for 20 min. In fact the activity was increased, as the decay was slower in heated than in unheated plasma. Contact factor could not be formed from plasma heated at or above 60° C for 15 min. It may be significant that this sudden change is associated with gross changes in the electrophoretic pattern of plasma heated to 60° C (Fig. 7). Treatment of plasma with 20 mg/ml. of $Al(OH)_3$ or $BaSO_4$ did not significantly influence either activation or decay. Treatment with 10–50 mg/ml. of kaolin removed the ability to form contact factor, but this is presumably only another example of 'exhaustion', since kaolin itself is even more potent than glass in activating the contact factor. A similar effect, and for the same reason, was obtained by using powdered glass or quartz.

Contact factor in protein fractions

Protein fractions were precipitated by $(NH_4)_2SO_4$ from intact plasma and dialysed throughout the night (see Methods). Under these conditions kinin was not detected in any combination of the fractions exposed to a glass surface and the formation of contact factor could therefore be studied without the need for preliminary depletion of component B. It is not clear which part of the procedure destroyed the ability to form plasma kinin, but since the original plasma, dialysed in parallel with the fractions, could still release kinin,

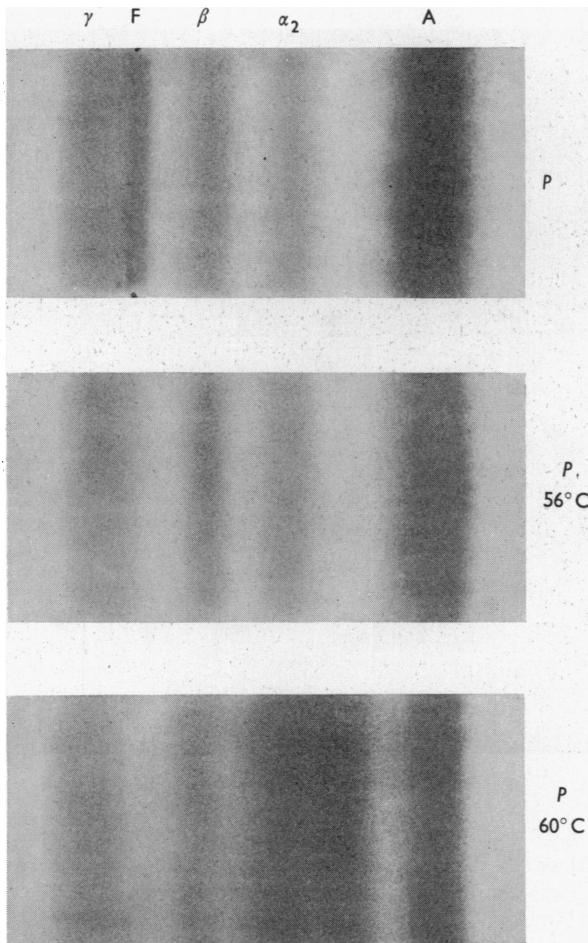

Fig. 7. Electrophoretic pattern of citrated plasma (*P*) heated for 20 min at different temperatures. Note disappearance of fibrinogen band (F) in sample heated at 56° C and the gross changes in the alpha globulin band at 60° C.

this change could not be the result of dialysis or ageing alone. The best separation was obtained by fractionation of plasma pre-heated to 56° C for 20 min. The fraction, precipitated between 25 and 45% saturation with $(NH_4)_2SO_4$, when exposed to glass developed contact factor activity comparable with that of whole plasma (Fig. 8). Relatively little activity was found in the remaining fractions. When unheated plasma was used as a starting point, the fractions precipitated below 25% saturation also contained considerable contact factor activity. As was shown in Fig. 5, contact factor rapidly decays in plasma. The decay is slower in pre-heated plasma. In the activated $(NH_4)_2SO_4$ fraction it was relatively stable (Fig. 9*b*) but when plasma was added to such fractions

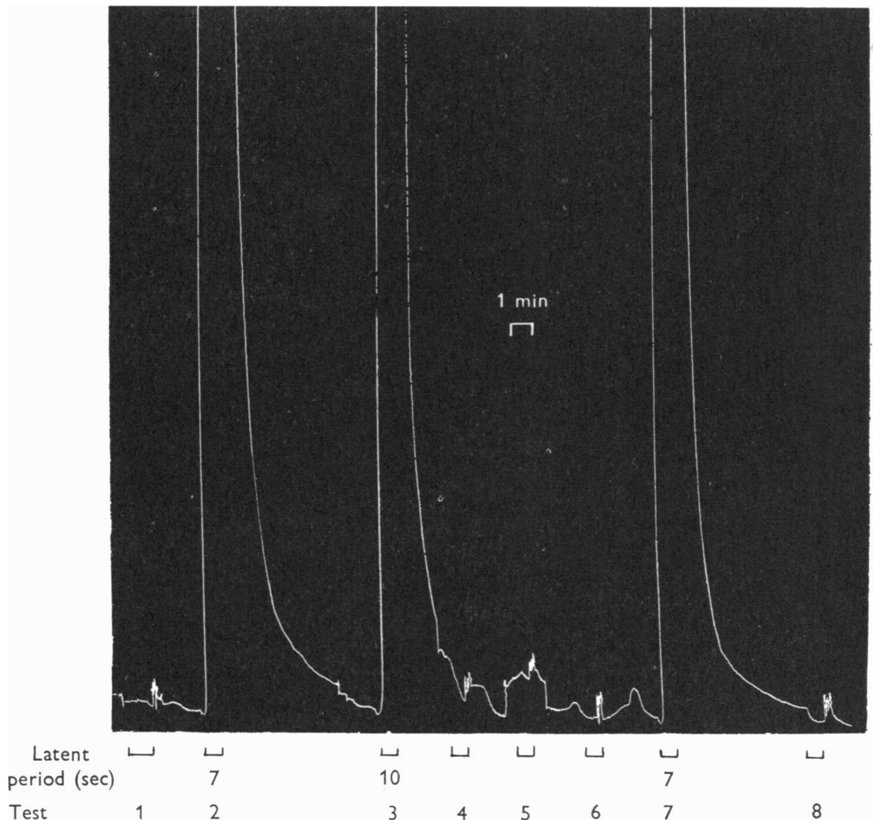

Fig. 8. Contact factor in a globulin fraction of plasma. All samples were activated and tested as described in legends to preceding figures. Fresh and dialysed plasmas are included for comparison. Direct tests: 0·1 ml. applied to the rat uterus. 1, Fresh plasma, intact. 2, Sample (1) activated by shaking with ballotini for 2 min. 3, Plasma heated at 56° C for 20 min and dialysed during the night, activated. 4, Sample (3) intact. 5, 25–45% saturated $(NH_4)_2SO_4$ fraction of (4), intact. 6, Sample (5) treated with ballotini for 2 min (note absence of response). Indirect tests: 7, Sample (6) added to intact plasma and tested after 2 min. 8, Sample (5) added to intact plasma and tested after 2 min.

the rate of decay again increased (Fig. 9*a*). These facts can be interpreted by postulating the presence in plasma of an inactivator which is relatively heat-labile and is not present in the same protein fractions as the activating system. The mechanism of inactivation of the contact factor is not the same as that of plasma kinin. Decay of kinin is inhibited by disodium edetate (EDTA) (Armstrong *et al.* 1955), which has no effect on the decay of the contact factor.

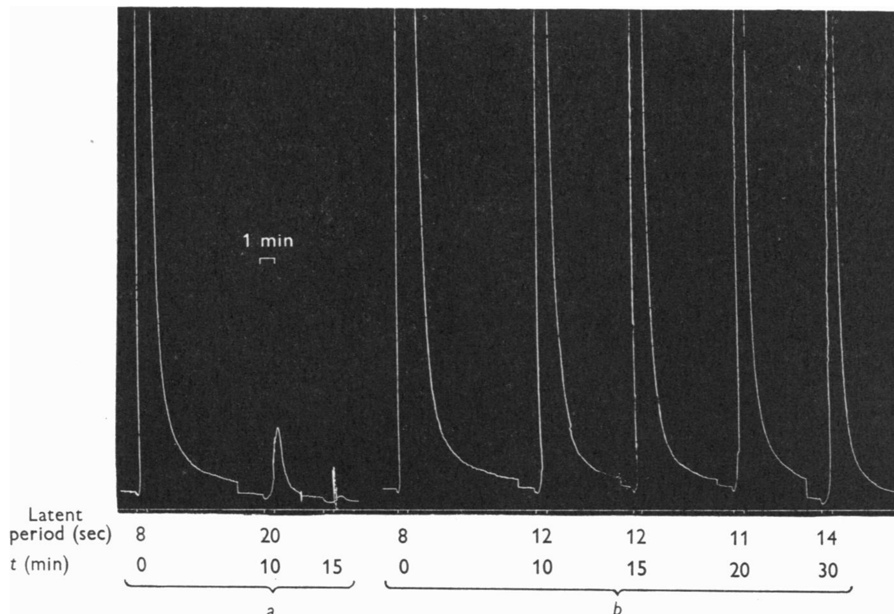

Fig. 9. Inactivation of contact factor. Rat uterus. (*a*) Equal volumes of the globulin fraction (cf. Fig. 8) and of B-depleted plasma were separately activated for 2 min with ballotini and then mixed; at intervals (*t*) samples of the mixtures were added to twice their volumes of intact plasma and 0·1 ml. amounts were applied to the uterus after 2 min incubation. (*b*) The samples were activated and tested as in (*a*) but the fraction and the B-depleted plasma were kept separately and mixed only immediately before each addition to the intact plasma.

Contact factor in pathological plasma

Because of the suspected relation with blood clotting, formation of plasma kinin was studied both by the direct and by the two-stage methods in the plasma of patients with various coagulation deficiencies. The reaction was found to be normal in cases of haemophilia, Christmas disease, deficiency of plasma thromboplastin antecedent (P.T.A.) as well as in factor VII deficiency and thrombocytopenia and in patients under phenindione treatment (J. W. Stewart, personal communication). Since Biggs *et al.* (1958) have shown that plasma from a case of 'Hageman trait' (cf. Ratnoff & Colopy, 1955) did not develop any clot-promoting activity after exposure to glass, it was of particular

interest to test such plasma for the production of kinin. Fig. 10 shows that there was no release of plasma kinin when this plasma was treated with ballotini or kaolin. A two-stage analysis indicated that this was due to the inability to form contact factor and not to the lack of component B, and when a normal source of contact factor was added to the patient's intact plasma, kinin was formed in normal amounts. As a corollary to this and in contrast to normal plasma, it was found that 'Hageman' plasma was not depleted of component B by treatment with ballotini.

Further, glass-treated 'Hageman' plasma also failed to produce pain when tested on an exposed blister base (D. Armstrong, personal communication).

It is therefore reasonable to conclude that in 'Hageman trait' component A, that is, the precursor of the contact factor, is absent.

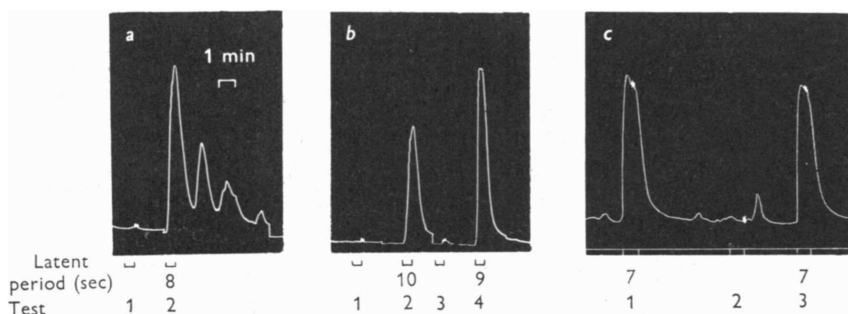

Fig. 10. Absence of glass activation in the plasma from a patient with 'Hageman trait'. (*a*) *Direct tests* (0·1 ml. applied to the rat uterus). 1, Patient's plasma rotated for 2 min with ballotini. 2, Normal plasma treated as in (1). (*b*) *Indirect tests*: 0·1 ml. of glass-treated (*G*) samples added to 0·2 ml. of intact plasma; 0·1 ml. of the mixture tested after 2 min incubation. 1, Patient's plasma (*G*) + normal, intact. 2, B-depleted, normal plasma (*G*) + patient's, intact. 3, B-depleted, normal (*G*); control, tested directly as in (*a*). 4, B-depleted, normal (*G*) + normal, intact. (*c*) Plasma activated with kaolin (10 mg/ml.) for 1 min, 0·1 ml. tested directly (see Methods). 1, Normal. 2, Patient's. 3, Normal. The tests under (*c*) were performed with a different sample from the same patient 7 months after those in (*a*) and (*b*). 1 mg/ml. of SBTI was applied to the uterus before commencing this experiment (see Methods).

Relation between the initiation of clotting and the formation of plasma kinin

The possibility that plasma kinin itself contributes to the clot-promoting activity of glass was tested by adding to plasma potent protein-free extracts of the kinin and recalcifying the mixtures under standard conditions (see Methods). The results were entirely negative, there being no difference between the clotting times with added kinin and with saline control. On the other hand, the properties of the contact factor with respect to plasma kinin formation resemble those described in connexion with blood coagulation (Margolis, 1957*a*).

What is more important is that 'Hageman' factor is an essential component for both sets of reactions.

It has been shown that after activation by the glass surface 'Hageman' factor interacts with P.T.A. to give rise to clot-promoting activity (Biggs *et al.* 1958). The sequence of these reactions is thus parallel to that described above in relation to components A and B. The analogy may be carried still further. Just as component B is 'consumed' by interaction with the contact factor leaving behind an excess of available component A, so P.T.A. is lost long before 'Hageman' factor, and as a result of a similar interaction. Table 1 shows the corrective effect of added plasma on the clotting time of plasma

TABLE 1. Exhaustion of P.T.A. by treatment of plasma with glass

Test plasma (0·2 ml.)	Added plasma (0·05 ml.)							
	P.T.A.-deficient		'Hageman' trait			Normal		
	0′ *G*	5′ *G**	0′ *G*	5′ *G*	10′ *G*	0′ *G*	5′ *G*	10′ *G*
P.T.A.-deficient	205	—	140	141	143	132	163	184
P.T.A.-deficient (5′ *G*)*	—	300	166	165	165	135	193	228
'Hageman' trait	—	—	800	—	—	105	115	115
Normal	90	—	93	—	—	90	—	—

Kaolin clotting times in seconds (see Methods) of plasma mixtures. The samples marked 5′ *G* and 10′ *G* were rotated for 5 or 10 min with equal weight of glass ballotini, transferred into siliconed tubes and left for 2 hr at room temperature. The samples marked 0′ *G* were not exposed to glass. *P.T.A.-deficient sample was treated with glass to accentuate the deficiency. Note (1) rapid exhaustion of P.T.A. but not of 'Hageman' factor by contact with glass; (2) better correction of P.T.A. deficiency by glass-treated plasma from 'Hageman' trait than by glass-treated normal plasma.

deficient in these respective factors. It will be seen that, after 10 min treatment with ballotini, normal plasma has lost most of its ability to correct P.T.A. deficiency, but little if any of its ability to correct the 'Hageman' defect. Moreover, when 'Hageman' plasma was treated in the same way, its corrective effect on P.T.A.-deficient plasma remained unchanged, which implies that 'consumption' of P.T.A. does not take place in the absence of 'Hageman' factor.

Thus there is a qualitative correspondence between the reaction sequences of clotting and of kinin formation in both their first and second stages. In the latter, however, the two sets of reactions have already diverged and the components involved are no longer identical. Apart from the fact that kinin formation takes place normally in P.T.A. deficiency, this is also illustrated by the different time courses in the development of clot-promoting activity, on the one hand, and of plasma kinin on the other. As is shown in Fig. 11 'Hageman' plasma is not significantly activated by contact with glass. P.T.A.-deficient plasma responds normally within the first 1–1½ min, but then stops short, whereas the activity of normal plasma continues to increase for another 5–10 min. The initial acceleration of clotting time may thus be attributed

mainly to the activation of 'Hageman' factor and it corresponds in time with the formation of contact factor (cf. Fig. 6). In the second phase, P.T.A. becomes the limiting factor and this part of the reaction is slower than the parallel stage in the formation of plasma kinin (cf. Fig. 1).

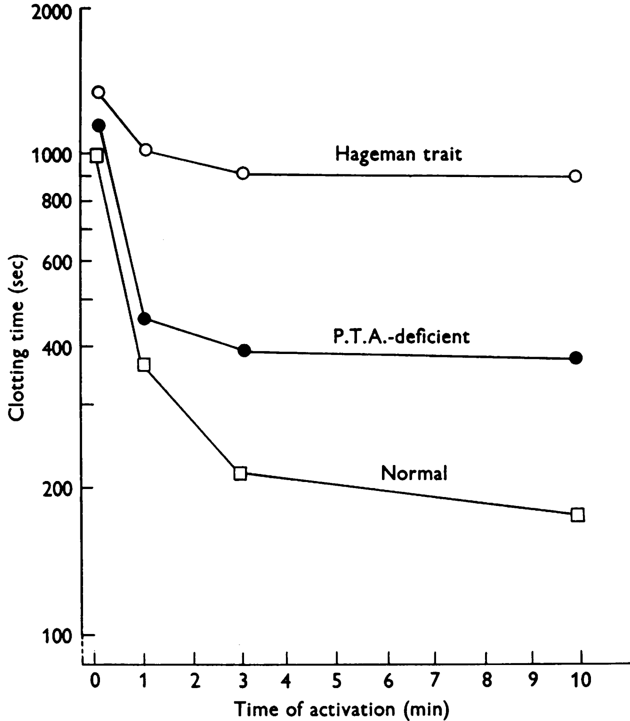

Fig. 11. Clot-promoting activity of 'Hageman trait', P.T.A. deficient and normal plasmas. 1 ml. of each plasma was rotated with ballotini. At intervals 0·1 ml. amounts were removed, mixed with 0·2 ml. of intact normal plasma and recalcified with 0·75 ml. of 0·1 M-$CaCl_2$ containing lysed platelets (see Methods). Ordinate, clotting time, log. scale.

Other effects of contact factor

'Direct' uterus-stimulating activity. In addition to prompt contraction due to plasma kinin, another, delayed type of response was also observed. This response merged with the kinin contraction when activated whole plasma was used, but it could be isolated by using B-depleted plasma.

The active principle differed from kinin in the following features: (1) It could not be recovered in protein-free extracts. (2) For a comparable latent period the contraction was much higher than with kinin and it was followed by 'spontaneous' rhythmic activity after washing. (3) The responses progressively decreased with repeated applications (Fig. 12). (4) They were irreversibly suppressed by a single application of SBTI to the uterus bath (Fig. 13).

Uterus preparations varied in their sensitivity to this type of stimulation. The effect occurred consistently with plasma activated by powdered glass, quartz or kaolin (cf. Fig. 12), which act in the same way as glass ballotini but are more potent because of the greater surface which they present to plasma. It was not always detectable with the usual doses (0·1 ml.) of ballotini activated plasma. When the effect was marked (as in Figs. 10*a*, 13), and when it interfered with two-stage tests for contact factor, the uterus could be desensitized with SBTI (see Methods).

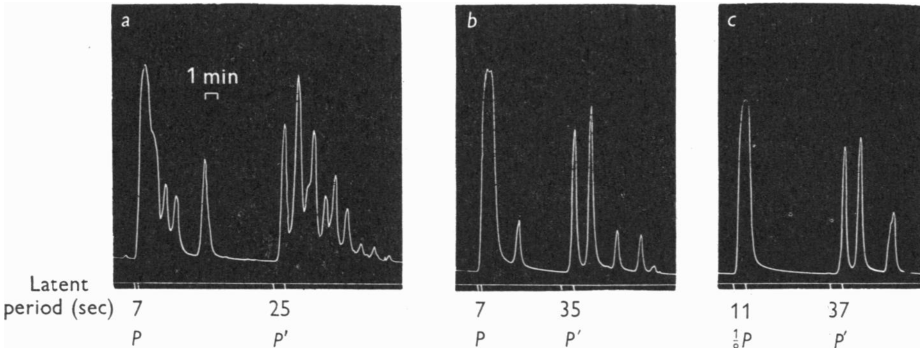

Fig. 12. 'Direct' response of the rat uterus to contact factor. Samples of plasma (*P*) and B-depleted plasma (*P'*) were activated for 1 min with 10 mg/ml. of kaolin. 0·1 ml. of each was alternately applied to the uterus for 20 sec and 60 sec respectively. *a*, Second application o *P* and *P'*. *b*, Fifth application. *c*, Sixth application: ⅛ dose of *P* applied for 60 sec. Note the different patterns of response to *P* and *P'* and the decreasing effect of *P'* after repeated applications.

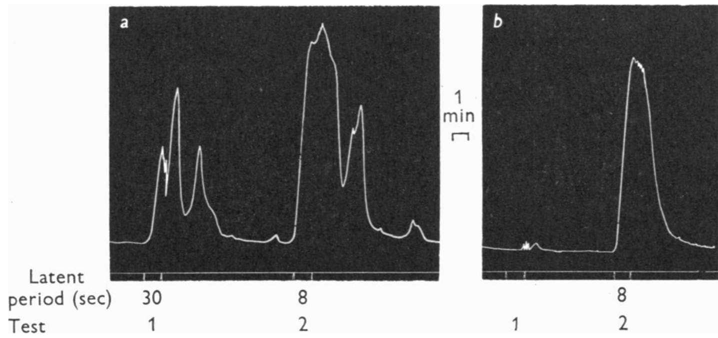

Fig. 13. Suppression by SBTI of the 'direct' uterus response. All tests samples were activated with 1 g/ml. of ballotini. Responses of rat uterus to 0·1 ml. doses. 1, B-depleted plasma; 2, whole plasma. Test (*a*), before SBTI; test (*b*), 20 min after a single application of SBTI (1 mg/ml. bath fluid) for 1 min. Note the composite nature of the contraction with whole plasma (2) and the suppression of the delayed phase by SBTI. In B-depleted plasma (1) the delayed phase was isolated and the whole of the response was suppressed by SBTI.

The delayed response could also be induced by activated globulin fraction containing contact factor, but not by plasma pre-heated to 60°C or by 'Hageman' plasma, neither of which can form contact factor. Hence, the activity appears to be dependent on the formation of contact factor.

This action of contact factor has been referred to as 'direct' by analogy to trypsin, which can stimulate the uterus either directly, or indirectly by releasing bradykinin from plasma globulins (Rocha e Silva *et al.* 1949).

Direct pain-producing activity. In agreement with the above findings, plasma depleted of component B and re-activated with glass induced pain in the blister base. Here also the activity was not recoverable in protein-free solutions and the effect was prevented by a previous application of SBTI to the base of the blister (Fig. 14). In these properties the active agent differed from plasma

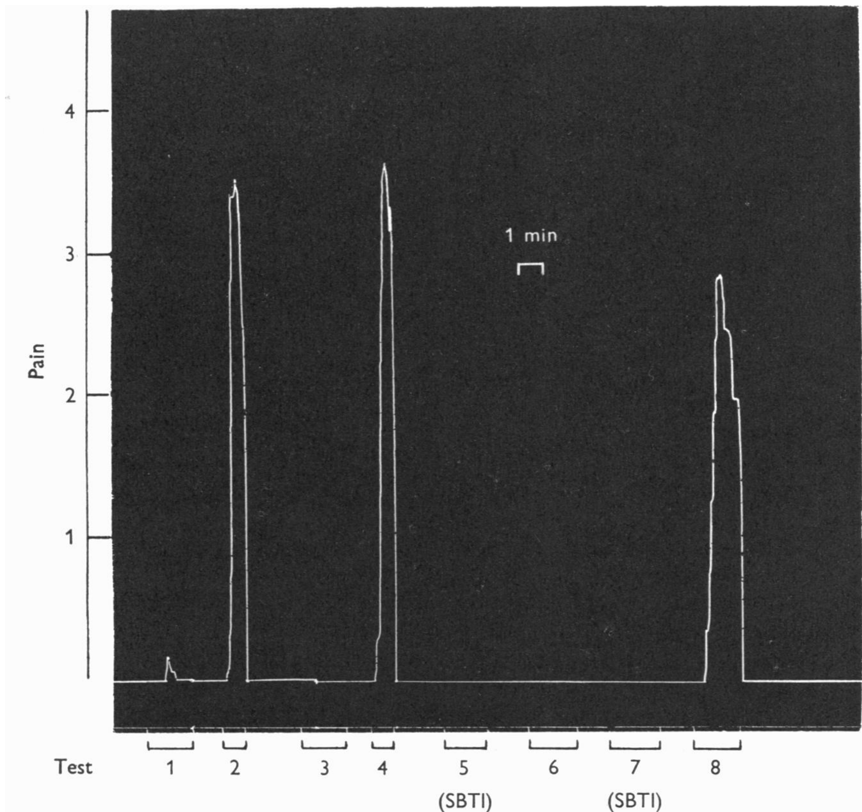

Fig. 14. 'Direct' pain response to contact factor and its inhibition by SBTI. 1, B-depleted plasma. 3, Intact plasma. 2 and 6, B-depleted plasma treated for 2 min with ballotini (=contact factor). 4 and 8, Plasma activated for 2 min with ballotini (=plasma kinin + contact factor). 5 and 7, SBTI (1 mg/ml.) applied to the area for 2 min and then washed off. Note the suppression by SBTI of response to contact factor (6) but not to kinin (8).

kinin. In pain experiments, as with the uterus, no response could be elicited with 'Hageman' plasma (D. Armstrong, personal communication). Thus it is evident that glass-activated plasma can stimulate both the uterus and the pain receptors in two different ways. The responses are normally superimposed but, under suitable conditions, they can be dissociated.

DISCUSSION

The investigation of the mechanism whereby kinin is released from plasma by contact with glass was guided by the results of a similar study on the initiation of blood coagulation (Margolis, 1957*a*). Most of the findings relating to clotting applied also to plasma kinin. Some apparent discrepancies led to a further investigation of the coagulation mechanism and helped to clarify the parts played by 'Hageman' factor and plasma thromboplastin antecedent (P.T.A.). The development in guinea-pig plasma of a factor increasing capillary permeability also appears to be related to the above phenomena (Margolis, 1958*b*).

There seems little doubt that glass contact initiates blood coagulation and the release of the plasma kinin via a common reaction. It is also likely that the permeability effect is an expression of the same initial change, although more work is needed to prove this, and in particular an investigation of this phenomenon in man is required. The essential change underlying these reactions is an alteration of a specific plasma component by contact with certain, as yet incompletely specified, types of foreign surface to form what has been termed the 'contact factor'.

The physical characteristics of active surfaces are being studied and the results will be reported separately. It is sufficient to say here that among the materials tested there is a close correlation between their clot-promoting and plasma kinin-releasing activity.

The first step of activation having been completed, contact factor in turn initiates diverging chains of reactions, the products of which are recognized by their different biological effects. A suggested sequence of events is outlined in Fig. 15. Unlike the subsequent stages of coagulation, activation of component A (i.e. 'Hageman' factor) to contact factor and the interaction of the latter with component B and with P.T.A. are not arrested by citrate or sodium edetate and are therefore independent of calcium ions.

Quantitative evaluation of the changes described is complicated by the fact that their products are rapidly destroyed by inactivators in plasma and the level of each is a resultant of a dynamic equilibrium between formation and decay. In the process of activation the precursor substances (i.e. components A and B and P.T.A.) are used up. The finding that component B and P.T.A. are exhausted long before the precursor of contact factor afforded a simple way of isolating the contact stage from the other stages of the reaction.

Component A, which has been identified with the 'Hageman' factor, is defined by some of its physical properties, by its function in relation to clotting and plasma kinin formation, and by its absence in a specific hereditary trait. P.T.A. is defined in a similar way except that all the evidence is based on its clot-promoting effect, since this factor does not participate in the formation of plasma kinin. There is as yet no known naturally occurring condition in which component B is missing; its definition is therefore limited to fewer criteria.

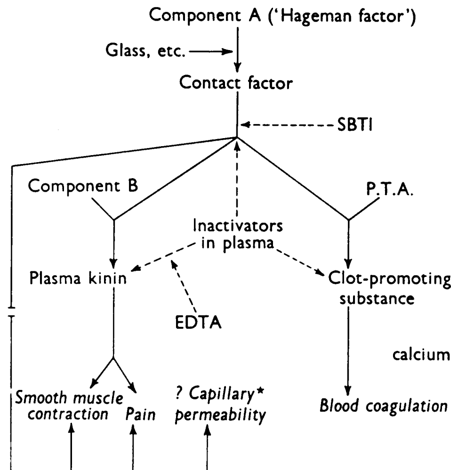

Fig. 15. Relations between various biological effects of the plasma foreign surface reaction. Solid lines indicate activation, interrupted lines indicate inhibition. *Guinea-pig plasma (Margolis, 1958*b*).

It is recognized as a constituent of plasma which is necessary for the formation of kinin by contact factor and which is consumed in this reaction. The present data do not indicate the nature of the immediate substrate from which plasma kinin is split off, nor the role of component B in relation to this substrate. Thus component B could equally well be the immediate precursor of kinin or a co-factor in its production. For similar reasons it is not clear whether the 'direct' uterus and pain responses to preparations containing contact factor and free from kinin are to be regarded as due to kinin formation *in situ* or to some other action. Whatever the case may be, it should be emphasized that in plasma activated by glass there appear two distinct muscle-contracting and pain-producing substances: (1) plasma kinin, and (2) contact factor, which differs from the former in that it is antagonized by soya bean trypsin inhibitor and is not extractable in protein-free solutions.

An allied problem which still awaits investigation is the relation between the phenomena described in this paper and the activation of plasma by dilution, trypsin, saliva, urine and some snake venoms (Rocha e Silva *et al.* 1949; Tocantins, Carroll & Holburn, 1951; Miles & Wilhelm, 1955; Hilton & Lewis,

1955; Werle, 1955; Schachter, 1956; Holdstock, Mathias & Schachter, 1957; Stewart & Bliss, 1957). The observation that plasmin is a potent releaser of plasma kinin (Lewis, 1958) deserves special attention, since plasmin has also been shown to activate coagulation (Travis & Ferguson, 1951). It has also been shown that 'Hageman' factor is precipitated in the same protein fraction as plasmin (Ratnoff & Colopy, 1955). However, clot lysis in the presence of 'Streptokinase' has been found to be quite normal in 'Hageman' plasma (unpublished observations). 'Hageman' factor cannot therefore be identified with plasminogen or its 'proactivator'.

At present little is known about the physicochemical nature of contact activation, but the phenomenon seems to be specific enough to merit a functional definition. The proposed concept of the 'plasma foreign surface reaction' may be appropriate in that it directs attention to the possible physiological significance of these events. Thus it may be significant that there appears to be some correlation between the ability of a number of substances to activate contact factor in plasma and their activity in producing inflammatory tissue reactions *in vivo*. At one extreme there are such highly active surfaces as glass, quartz and kaolin which are known to provoke intense tissue reactions (Miller & Sayers, 1934; Kettle, 1935), at the other 'Polythene', $BaSO_4$, $Al(OH)_3$ and carbon which are relatively inert both in plasma and in tissues.

The most obvious objection against drawing such direct inferences is that the key component of the contact reaction is missing in persons with the 'Hageman trait' and that in spite of the deficiency the few cases of this trait described in literature (Ratnoff & Colopy, 1955; Frick & Hagen, 1956; Ramot, Singer, Heller & Zimmerman, 1956; Jim & Goldfein, 1957; Biggs *et al.* 1958) do not appear to have any haemostatic defects or other obvious physical disabilities. As has been remarked elsewhere (Margolis, 1957*a*; Biggs *et al.* 1958), haemostasis *in vivo* is not normally initiated by contact with glass or similar materials and an alternative mechanism must exist in the body. P.T.A. deficiency, however, is accompanied by a tendency to bleeding. We may therefore postulate that injured tissue releases substances which by-pass or substitute for contact factor and interact with P.T.A. to initiate normal coagulation in the body.

It is more difficult to evaluate the significance of the 'Hageman' defect in relation to plasma kinin and (possibly) permeability factor since the physiological and pathological role of these substances is still a matter for speculation. Plasma kinins, formed in different ways, produce vasodilatation and pain, which together with coagulation and increased permeability are amongst the cardinal features of inflammation (Menkin, 1956). How far all these changes depend on the reactions demonstrated in this paper cannot yet be stated but it may be said that cell-free plasma can be activated outside the body so that it could evoke most, if not all, of the features of inflammation.

Another aspect of the question is to what extent the phenomena detected in the plasma reflect what are essentially intracellular mechanisms. It is conceivable that the inflammatory responses *in vivo* are brought about chiefly by the changes in the cytoplasm of the cells but that, in the course of evolution, some of these mechanisms may have been transferred also to the body fluids (Robb-Smith, 1955; Biggs & Macfarlane, 1957). It is known, for instance, that cytoplasmic processes involving a sol-gel transformation, such as amoeboid movement and mitosis, are in many ways akin to blood coagulation (Monné, 1948; Heilbrunn, 1952). Thus it is possible that the plasma reactions, whilst of relatively subsidiary physiological importance, may serve as a model of intracellular reactions. If this view is correct, then the absence of the plasma contact-reaction in 'Hageman trait' could indicate an intracellular defect. It would be interesting to learn whether these patients show normal inflammatory responses to injurious agents, and especially to such materials as glass, quartz and kaolin. On the other hand, in view of the absence of clinical abnormality in this condition, it is probable that the defect is confined to plasma only. The precursor of contact factor or its equivalent might still be present within the cells so that cellular mechanisms of inflammation could occur normally. The 'Hageman trait' could then be considered to be merely an interesting genetic character, of no more importance to the affected individuals than is the inheritance of secretor or non-secretor state of the blood group substances.

Whilst the above speculations are still several steps removed from experimental facts, they suggest an approach to the study of inflammatory reactions to foreign surfaces and perhaps also to other forms of injury, and offer a working hypothesis as a guide for such an investigation.

SUMMARY

1. The mechanism of release of plasma kinin (originally termed 'pain-producing substance') by contact with glass was investigated. The reaction was resolved into two stages: in the first, a precursor in plasma (component A) is converted into an active agent, termed 'contact factor', in the second stage contact factor interacts with another substance (component B) to release plasma kinin.

2. The properties of contact factor were studied by an indirect method in which plasma was depleted of component B by brief exposure to glass. Contact factor was then allowed to react with the component B of intact plasma. The activity was assayed on the isolated rat uterus and also, for pain production, on an exposed blister base.

3. The formation of contact factor was not affected by prior treatment of plasma with $BaSO_4$ or $Al(OH)_3$ or by pre-heating at 56° C, but was prevented

by heating to 60° C for 15 min. Contact factor was also formed from a globulin fraction precipitated between 25 and 45% saturation with $(NH_4)_2SO_4$.

4. Contact factor is rapidly destroyed by an inactivator in plasma. The inactivator is largely removed by fractionation.

5. Prolonged treatment with large amounts of glass eventually exhausts plasma of component A.

6. Plasma from a case of 'Hageman trait' did not form contact factor when treated with glass or kaolin. Plasma from subjects of other coagulation deficiencies behaved normally in this respect.

7. Contact factor forms a connecting link between the initiation of clotting and the release of plasma kinin. Clot-promoting activity is produced by interaction of contact factor with plasma thromboplastin antecedent (P.T.A.). Plasma kinin is released by interaction of contact factor with component B, which is independent of P.T.A. (Fig. 15).

8. In addition to indirect excitation of plain muscle and pain receptors through the release of plasma kinin, contact factor acts independently on these receptors. Soya bean trypsin inhibitor suppresses this effect, but does not inhibit the action of plasma kinin.

9. Apart from glass, some other surfaces are potent activators of plasma. There may be a correlation between this action and the ability to provoke intense inflammatory reactions.

10. Plasma 'foreign surface reaction' may serve as a model for certain aspects of inflammatory reactions.

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Note added in proof. An intradermal test with 0·05 ml. of kaolin suspension (5 mg/ml.) was performed on a patient with Hageman trait. There was no trace of early whealing which in normal controls persisted for up to 2 hr. The onset of local erythema was delayed and the reaction less marked than in normal subjects. Thus Hageman factor may be a necessary component of an early 'humoral' phase of foreign surface response which may accelerate and intensify a later cellular reaction.

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