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INITIATION OF BLOOD COAGULATION BY GLASS AND RELATED SURFACES

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In spite of the rapid advances in our knowledge of the physiology of blood coagulation, the reason why blood remains fluid in the body and yet clots rapidly *in vitro* remains obscure. It is taken for granted that the initial stimulus is the exposure to a 'foreign surface', but what this 'foreigness' is and how it acts are still matters of controversy.

The subject has been reviewed amongst others by Pickering (1928), Barker & Margulies (1949), Macfarlane (1948, 1956), and Biggs & Macfarlane (1953). In contrast to the earlier emphasis on the blood platelets, recent evidence favours the view that the initial contact reaction takes place in the plasma.

Lozner, Taylor & MacDonald (1942) showed that glass contact shortens the clotting time of platelet-poor plasma and concluded that this reaction is associated with the plasma euglobulin fraction. Tocantins (1945) and his co-workers (Tocantins, Carroll & Holburn, 1951) made an extensive study of the effect of different surfaces on the clotting of plasma and decided that the basis of the reaction was the adsorption of a lipid inhibitor of clotting ('anticephalin'). Conley and collaborators (Conley, Hartmann & Morse, 1949; Hartmann, Conley & Lalley, 1949; Ratnoff & Conley, 1951; Hartmann & Conley, 1952; Dick, Jackson & Conley, 1954) studied the effect of glass contact on human and canine platelet-free plasma and its euglobulin fraction. These workers considered antihaemophilic globulin (AHG) to be the component sensitive to contact and showed that surface is a quantitative factor in prothrombin utilization. Quick, Hussey & Epstein (1953) made similar observations regarding prothrombin consumption, but attributed their findings to the auto-catalytic effect of surface-adsorbed thrombin. Fiala (1951) and Fiala & Roth (1953) isolated a thermolabile coagulation inhibitor from horse plasma and presented indirect evidence that contact activation is due to the adsorption of

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this substance, which, however, bears no relation to Tocantins's anticephalin. Biggs, Douglas & Macfarlane (1953) investigated thrombin generation in normal and pathological plasma under various conditions of glass contact and concluded that glass affects both the Christmas factor and the platelets. Ferguson (1953) showed that canine plasma, rendered incoagulable by prolonged centrifugation, was still able to respond to contact with glass. This was demonstrated by comparing the clotting times of intact and contacted samples after addition of platelets or 'cephalin'. Fantl & Nelson (1953) have shown activation by glass of dog lymph, which is completely devoid of platelets. Rapaport, Aas & Owren (1955) evaluated the effect of glass powder on Christmas factor and proconvertin and found a considerable increase in the activity of these factors. These authors disagree with the view that the removal of an inhibitor is responsible for this reaction.

The present account, which follows an earlier communication (Margolis, 1956), is an attempt to unravel the changes in the clotting ability of plasma, as a result of exposure to surfaces such as glass, kaolin and various forms of silica, which are known to activate coagulation.

METHODS

Except when deliberately exposed to surface action all blood derivatives were handled in glassware coated with silicone (MS 1107). Venous blood was collected into 19% trisodium citrate (0·2 ml./10 ml.). Platelet-rich plasma was obtained by low-speed centrifugation. Platelet-free plasma was prepared by centrifuging at 12,000 *g* for 5–10 min; the supernatant was decanted into fresh tubes and the process repeated once, or, in later experiments, twice. Direct counts on undiluted samples showed consistently less than 10 platelets/mm^3 in the plasma spun three times. Protein fractions were precipitated with saturated $(NH_4)_2SO_4$ and dialysed against citrated 0·9% NaCl solution for 18 hr at 4° C. Euglobulin was prepared by dilution and acidification with CO_2. Coagulation times were estimated at 37° C by recalcifying 0·2 ml. samples with 0·05 ml. $CaCl_2$ (M/10). Plasma which had not been in contact with glass or similar surfaces will be referred to as 'intact plasma' after Tocantins (1945).

Direct contact clotting times

A sample of plasma was recalcified in the presence of a measured amount of glass or quartz powder, kaolin or other substances. When it was desirable to achieve the shortest possible coagulation time, the plasma was incubated with the powder before adding $CaCl_2$; in other experiments it was preferable to add the powder at the last moment, e.g. by mixing it with the $CaCl_2$ solution. The optimal conditions vary according to the purpose of the particular experiment, and will be specified in the text. This procedure can be used to compare the activity of various materials, provided their specific surface areas are known.

Indirect contact activation method

Platelet-free plasma was gently rotated in a tube containing a weighed amount of small glass beads ('ballotini', diam. 0·12 mm). Unless otherwise stated, the standard activation 'dose' was 100%, w/v, beads, applied for 20 min at room temperature. On removal from the turn-table the beads settled within a few seconds and the supernatant sample was tested without delay. A number of siliconed tubes each containing 0·2 ml. of intact plasma were prepared beforehand; 0·1 ml. of the contacted sample was transferred into the first of these tubes. 0·1 ml. of this

mixture was then transferred into the second tube and so on. These serial dilutions were then recalcified at 37° C and the clotting times recorded. The diluent plasma will be referred to as 'indicator plasma' since it does not directly participate in the contact reaction. When the clotting times were plotted against the dilution on a log/log scale, a linear relation was obtained. Under standard conditions this relation can be used to convert clotting times into arbitrary units of activity, the undiluted sample representing 100 units, 1/3 dilution—33 units and so on. It is appreciated that because of the complexity of the system such an activity curve can act only as a rough guide. As will be shown below, in order to ensure reproducible results, it is necessary to add platelet material to the indicator plasma. Best results are obtained by adding to the $CaCl_2$ solution an aqueous 'extract' of washed platelets, lysed by freezing and thawing, and buffered at pH 7·3 with glyoxaline in order to prevent flocculation. In some earlier experiments platelet-rich plasma was added directly to the indicator plasma (e.g. in Table 4), and the resulting clotting times were somewhat longer than those in which lysed platelets were used.

RESULTS

The observations presented below are based on numerous experiments of which representative samples are given in the figures and tables.

Direct contact clotting time

Intact, platelet-free plasma prepared as described above is practically incoagulable in siliconed tubes. In the presence of an optimal amount of activating material (e.g. 20 mg kaolin/ml. plasma), the usual clotting time is 3–8 min, but occasionally much longer. The addition of 500 platelets/mm^3 eliminates these variations and, under specified contact conditions, almost identical clotting times are obtained in different normal samples. In exceptionally 'clean' specimens of plasma even 100 platelets/mm^3 will appreciably shorten the clotting time. It is therefore reasonable to assume that the unpredictable behaviour of 'platelet-free' plasma is due to the presence of traces of platelet material which is not sedimentable at 12 000 *g* and is undetectable microscopically.

At a given platelet concentration the clotting time decreases with the increase of contact area, and here again a linear relation on a log/log scale is maintained within a wide range of values (Fig. 1).

A similar set of curves is obtained if the contact area is kept constant and the platelet content is varied. It may, therefore, appear that contact and platelets are, to a certain extent, interchangeable clotting factors. When quantitative aspects are considered, however, it becomes obvious that two distinct reactions are involved. This is shown in Fig. 2 in which the simultaneous effect of the variation of platelets and contact on clotting time is schematically represented in three dimensions. It will be seen, for example, that the system is exceedingly sensitive to small increases in platelet concentration even when the contact effect is maximal.

While it is clear that platelets and contact do not replace one another, it may still be asked whether the contact reaction is the result of the activation

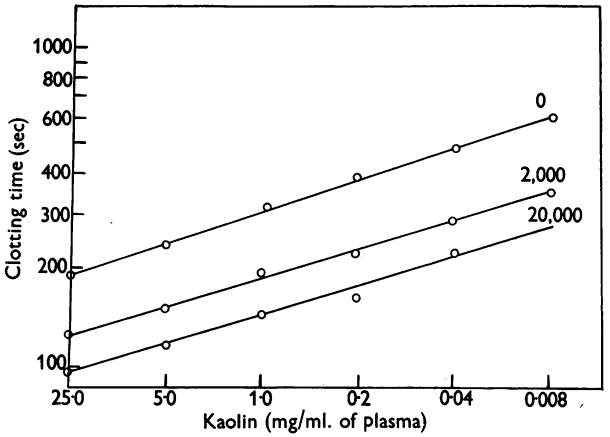

Fig. 1. The effect of contact on clotting time: 0·05 ml. of serial fivefold dilutions of a suspension of kaolin in 0·1 M-$CaCl_2$ were added to 0·2 ml. of citrated plasma in siliconed tubes at 37° C. The figures above the curves indicate the number of platelets per cubic millimetre.

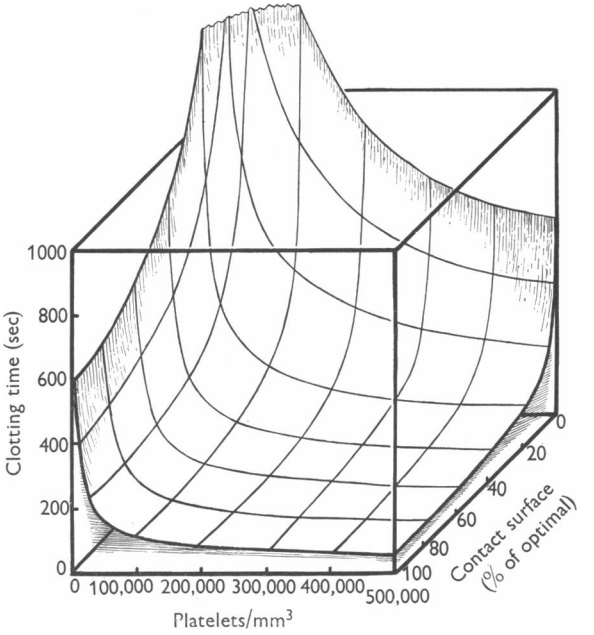

Fig. 2. Three-dimensional representation of the effect of simultaneous variation of contact area and platelet content on the clotting time of recalcified plasma. For greater clarity the concave surface has been drawn as an opaque structure and the origin is therefore concealed behind the diagram.

of platelets or of their breakdown products even when these are present in traces. To answer this question it is necessary to separate the contact stage from other stages of coagulation. This was accomplished by the indirect method.

Indirect contact activation test

Fig. 3 shows the results of an experiment in which platelet-free plasma with an exceptionally long clotting time was activated with ballotini and recalcified after serial dilution in equal portions of an intact sample of the same plasma. Lysed platelets were added with the $CaCl_2$ according to the method already described. It is apparent that the originally long clotting time of the contacted sample was not due to its failure to respond to the glass surface but to a defect in the indicator system which was corrected by addition of platelets.

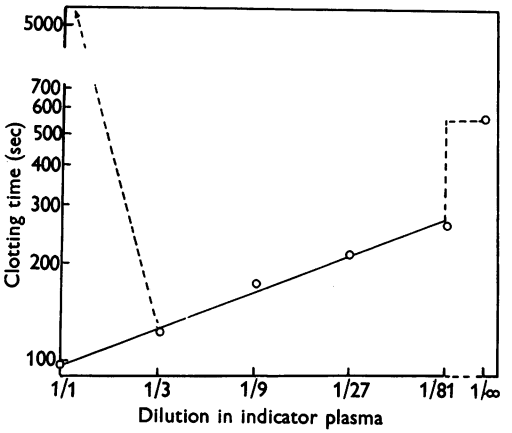

Fig. 3. Indirect contact activation of glass-incoagulable platelet-free plasma (see Methods). Lysed platelets were added to the system in $CaCl_2$. The oblique interrupted line refers to the undiluted ('1/1') sample recalcified without the platelet extract.

Since at no stage did the added platelet extract come in contact with the glass beads, the presence of platelets does not appear to be necessary for contact activation. It is not often that such a 'clean' specimen of platelet-free plasma can be obtained, but in an average experiment the clotting time of contacted samples without added platelets is still sufficiently long to illustrate the point (Fig. 4).

So far the findings support the contention that the unpredictable behaviour of different samples of normal, platelet-free plasma is due to the presence of variable traces of platelet materials, and has nothing to do with their ability to react to glass contact. What remains to be established is whether contact has any effect on platelets as such. No conclusions can be drawn from observations on washed platelets, since these are grossly damaged. In order to test the reaction of platelets in their natural plasma environment, the following

procedure was adopted. Platelet-rich and platelet-free plasma were first exposed to glass contact under identical conditions. Each was then mixed in siliconed tubes with an equal volume of intact plasma as follows:

(1) Platelet-rich intact, with platelet-free activated plasma;
(2) Platelet-rich activated, with platelet-free intact plasma.

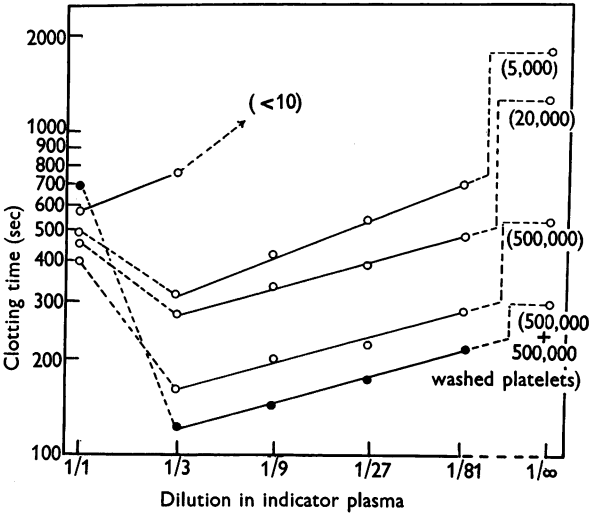

Fig. 4. Indirect contact activation test with a varying number of whole platelets in the indicator plasma (figures in brackets indicate the number of platelets/mm³ plasma). Platelet-rich plasma was added to the indicator plasma and was therefore not present in the undiluted test samples('1/1'). In the lowest curve a concentrate of washed platelets was also added (●).

This arrangement ensured that both the mixtures (1) and (2) contained an equal proportion of contacted plasma, but only those platelets contained in (2) were exposed to glass (Table 1). The clotting times were found to be dependent on the mechanical conditions of activation. In those samples which were left standing undisturbed in contact with glass beads or rods the clotting times of (1) and (2) were approximately the same. If, on the other hand, the plasma was rotated during exposure, (2) clotted well in advance of (1). In all the agitated specimens gross fragmentation of the contacted platelets was seen under the microscope. These observations suggested that the increase of platelet activity could be due simply to their mechanical disruption. It follows that it should not be observed if the platelets are disrupted before activation. The experiment was therefore repeated, the plasma samples having been first subjected to ultrasonic vibration (1 min at 35 kc/s), which treatment resulted in a total dissolution of platelets. The results of this experiment show that contact has no effect on lysed platelets. Similar findings were recorded when freezing and thawing was used as the method of disrupting

the platelets. These procedures did not destroy the activity of the platelets; on the contrary, there was a great increase of activity evident both in recalcified clotting times and in the Russell's viper venom clotting times, the latter falling from 13–15 sec in untreated plasma to 3–4 sec in the plasma containing disrupted platelets.

TABLE 1. The effect of glass contact on platelets

Platelet-rich plasma	Activated	Intact	Intact	Effect of
Platelet-free plasma	Intact	Activated	Intact	contact on platelets
Methods of exposure to glass contact				
Glass rods without agitation	215	215	600	–
Ballotini with occasional shaking	165	220	540	+
Ballotini with continuous agitation				
(*a*) Whole plasma	110	150	480	+
(*b*) Ultrasonated plasma	100	90	300	–

Recalcified clotting times (sec) of mixtures of equal volumes of platelet-rich and platelet-free plasma subjected to various contact procedures (see text).

TABLE 2. The effect of platelets on activation and decay

Platelets/mm³*		Incubation in siliconed tubes at 37° C after	Dilutions in indicator plasma					
In activated plasma	In indicator plasma	activation (min)	1/1†	1/3	1/9	1/27	1/81	1/∞
0	0	(*a*) 0	250	360	710	1500	—	2500
		(*b*) 20	720	840	1410	—	—	
0	5000	(*a*) 0	250	175	230	275	300	460
		(*b*) 20	870	330	360	—	—	
5000	5000	(*a*) 0	150	182	215	260	310	520
		(*b*) 20	225	280	370	—	—	

Recalcified clotting times (sec) of dilutions of glass-activated samples in intact indicator plasma: 0·6 ml. of plasma activated 20 min by rotation with 0·6 g of glass ballotini was recalcified in serial dilutions with intact indicator plasma (*a*) immediately, and (*b*) after incubation.

* Added as platelet-rich plasma which had been frozen and thawed twice to disrupt the platelets.

† In this table and in Tables 3 and 4 '1/1' signifies undiluted test sample.

Decay of activity

When contact activation is interrupted and the plasma left standing, its coagulation time rapidly increases. As tested by the indirect activation method this decay of activity occurs in all dilutions. This probably means that it is the product of the contact reaction which decays, since other limiting factors are supplied by the diluent plasma. The reaction is not significantly affected by the presence of platelet material, although, naturally, the range of clotting times is shifted (Table 2).

The rapid rate of decay could explain why not only the initial rate of activation but also the ultimate level of activity is a function of the contact area. This is shown in Fig. 5 in which the clotting times are converted into units of relative activity by reference to a curve constructed by plotting the clotting times of an activated sample against its dilution in the indicator plasma (cf. Fig. 3).

The maximal level at which the activity is sustained, presumably represents an equilibrium between activation and decay. This level can be altered in either direction by adding or removing glass surface.

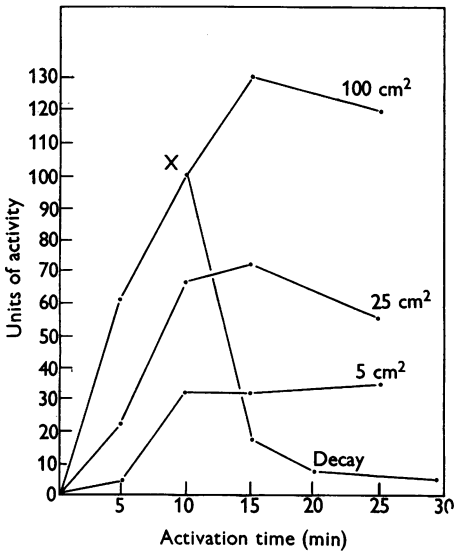

Fig. 5

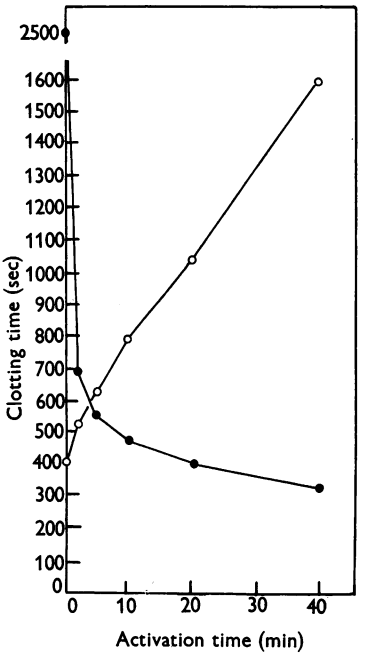

Fig. 6

Fig. 5. The rate of contact activation and decay; platelet-free plasma was rotated with various amounts of ballotini (the figures represent glass surface area per ml. of plasma). At the point marked ' × ' a volume of plasma was removed from one of the tubes and incubated in a siliconed tube at 37° C. Samples of 0·1 ml. taken at intervals were diluted with 0·2 ml. of intact indicator plasma containing 10,000 platelets/mm³ and recalcified in siliconed tubes. The clotting times were converted into relative units of activity by reference to a dilution curve constructed at the time of the experiment (see Methods).

Fig. 6. Activation and exhaustion by contact: 4 ml. of plasma were rotated with 1 g of ballotini. At intervals samples were removed and treated as follows: (*a*) immediately recalcified in siliconed tubes (●); (*b*) recalcified in the presence of 0·05 g of quartz powder after 2 hr incubation at 37° C in siliconed tubes (○).

Exhaustion of activity

Plasma which is subjected to the activation-decay sequence a number of times shows a progressively weaker response to contact and eventually will not clot at all. With platelet-free plasma this effect is very striking (Fig. 6). In contrast to the decay phenomenon, exhaustion can not be explained simply by the disappearance of the 'contact factor'. If instead of using direct contact clotting times (as in Fig. 6) the successive samples are tested by the indirect contact activation method, their clotting times increase more gradu-

ally, and the rate of exhaustion, therefore, appears slower. This means that what has been consumed can be partially replaced by the intact indicator plasma, showing that components other than the 'contact factor' have been exhausted as well.

Activation of plasma derivatives and fractions by contact

In an attempt to localize the site of contact activity in terms of known coagulation factors, the following substances were investigated

(1) $Al(OH)_3$-adsorbed plasma;
(2) Plasma heated at 56–61° C;
(3) Plasma heated at 56–61° C and adsorbed with $Al(OH)_3$;
(4) $(NH_4)_2SO_4$ fractions of (1)–(3);
(5) Euglobulin from (1)–(4).

All these derivatives are incoagulable on recalcification and the effect of the contact can only be measured by the indirect method. In the first dilution (1/3), which contains only 66% of whole plasma, the clotting time is usually somewhat longer than with whole plasma, but in higher dilutions (1/9, etc.)

TABLE 3. Glass activation of plasma derivatives

	Dilutions in intact indicator plasma			
	Activated sample			Intact control sample
Substances tested	1/1*	1/3	1/9	1/3
(1) Whole plasma	175	250	380	880
(2) Plasma heated 15 min at 60° C†	∞	1000	930	900
(3) Plasma heated 15 min at 56° C†	∞	285	325	720
(4) As (3) but adsorbed with $Al(OH)_3$ gel	∞	300	345	720
(5) 25–45% saturated $(NH_4)SO_4$ fraction of (4)	∞	240	290	600

Indirect contact activation tests (see text): recalcified clotting times (sec) of dilutions of glass-activated and control samples in intact indicator plasma. Lysed platelets (1000/mm³ final concentration) were added in $CaCl_2$.

* I.e. undiluted.

† Denatured fibrinogen precipitate removed by high speed centrifugation.

the test conditions are comparable. The results of activation tests on (2), (3) and (4) are shown in Table 3. Apart from heating above 60° C the procedures listed do not destroy the ability of these preparations to react to glass contact. Furthermore, the uncontacted samples being practically inactive, activation is specific to contact and not incidental to the method of preparation of the fractions. The euglobulin preparations showed approximately the same activity as the fractions from which they were derived, but differed in one important respect: the intact samples were already active to begin with, and could not be further activated by glass contact. Continued rotation with glass beads resulted only in their denaturation as evidenced by the appearance

TABLE 4. Contact activation of pathological plasma

(Clotting time in seconds.)

	Dilution in normal indicator plasma								
	Patients' plasma				Normal plasma				
	Activated			Intact control	Activated			Intact control	
Clotting defect	1/1*	1/3	1/9	1/3	1/1	1/3	1/9	1/3	Platelets/mm³ in indicator system
Haemophilia									
(1)	3,600 +	225	260	900	180	255	295	915	Not counted
(2)	1,500	290	355	1,000	300	290	355	780	10,000†
(3)	5,000 +	395	530	1,300	340	380	465	1,100	6,000†
Christmas disease									
(1) Mild case (difficult veni-puncture)	455	190	320	620	250	420	680	4,400	Not counted
(2) Severe case	1,200	185	275	765	135	185	270	750	5,000‡
(3) Severe case	72,000 +	390	690	10,000 +	310	600	960	10,000 +	<10
Dindivan plasma									
(1)	285	225	280	—	160	245	295	1,260	Not counted
(2)	355	240	305	—	195	255	325	1,260	Not counted
(3)	250	225	315	1,300	175	260	360	1,800	500‡
(4)	310	250	350	1,600	175	260	360	1,800	500‡
Congenital factor V deficiency	560	200	250	540	130	175	230	570	3,000‡
Congenital factor V deficiency	1,800	400	500	3,000	310	405	510	2,200	<10

Indirect contact activation tests (cf. text and Table 3), performed in parallel on patients' and normal platelet-free plasma: the method of activation with glass ballotini was identical in all cases. The number of added platelets and the methods of addition varied in different experiments and account for the differences in the clotting times of the normal controls.

* I.e. undiluted.

† Added as platelet-rich normal plasma to the indicator plasma (hence none in the '1/1' tube).

‡ H_2O-lysed platelets added with $CaCl_2$ to all tubes.

of strands of insoluble material as well as by the gradual loss of activity. It is possible that these observations represent the phenomenon of dilution activation (Tocantins *et al.* 1951), but in the absence of further evidence the euglobulin fractions cannot be included in the same category as the other plasma derivatives studied.

Contact activation of pathological plasma

Glass activation reaction has been tested in cases of haemophilia, Christmas disease, congenital factor V deficiency and in patients treated with dindivan. The results in Table 4 show that indirect contact activation tests gave normal values in these cases. It may, therefore, be inferred that the factors in which these plasmas are deficient are not directly concerned in the initial contact reaction. Shafrir & De-Vries (1956) recently reported similar observations on heated haemophilic plasma.

TABLE 5. Evidence of a plasma antagonist to 'contact factor'

25–45% saturated $(NH_4)_2SO_4$ fraction of heated, adsorbed plasma	Dilutions in intact indicator plasma		
	Activated sample		Intact control
	1/3	1/9	1/3
(1) Tested immediately (see Table 3, (5))	240	290	600
(2*a*) Tested after 25 min at 37° C	300	380	600
(2*b*) As (2*a*) but incubated in 1/3 dilution in indicator plasma	530	780	600

Indirect contact activation test on a plasma fraction (cf. Table 3): The samples 2*a* and 2*b* and the indicator plasma were incubated in siliconed tubes in parallel, but in the case of 2*a* the dilutions were made at the end of incubation whilst in the case of 2*b* the first dilution (1/3) was made at the commencement of incubation.

Evidence for the presence of an antagonist

The decay phenomenon observed in whole plasma becomes less with progressive purification of plasma fractions. Thus, the activated $(NH_4)_2SO_4$ fraction of heated, adsorbed plasma is relatively stable when incubated alone. On the other hand, when it is mixed with whole plasma, the decay reaction reasserts itself (Table 5). These findings are most easily explained by assuming that an antagonist present in plasma is removed or destroyed by fractionation.

DISCUSSION

The results of the present inquiry confirm the conclusions of previous investigators that citrated plasma develops coagulant properties on exposure to glass and related materials, and that this reaction can occur in the absence of platelets or added calcium. Its independence of calcium made it possible to study the changes in two stages, that is, by the indirect activation method, in which the contact reaction is separated from those phases of clotting which require calcium for their completion. Further, this reaction was made the limiting factor by dilution of the activated samples in normal, intact plasma;

and this, in turn, enabled the investigation of incomplete clotting systems. As the first step it was necessary to determine the part played by the platelets in the contact reaction. It is not possible to guarantee the removal of the last traces of platelet fragments by the usual separation procedures, and the term 'platelet-free plasma' has, therefore, only a relative meaning. Minute quantities of unsedimentable platelet debris are sufficient to make all the difference when the object is to obtain plasma which is completely incoagulable on contact. Traces of platelet-like activity may be derived also from sources other than platelets, for example from chylomicrons (Poole, 1955). It was possible to show that whilst platelets must be present in the later stages of coagulation, which form the indicator system in the indirect activation experiments, they are not necessary for the contact reaction. Contact of platelets with glass may, under suitable mechanical conditions, result in their fragmentation and, in this way, increase their coagulant properties; but this is quite distinct from the activation of plasma. Fragmentation may be effected by other means, such as ultrasonic vibration or freezing and thawing and, in each case, the release of platelet contents produces a similar increase in the sensitivity of the indicator system.

On the basis of experiments on plasma and plasma fractions, the following conclusions were reached:

(1) An active substance appears in plasma as a result of exposure to glass contact.
(2) This reaction proceeds normally in haemophilic, Christmas disease, factor V deficient and 'dindivan' plasma.
(3) The phenomenon can be attributed to a precursor substance in the plasma, which substance survives heating to 57° C for 15 min, but is completely inactivated by heating to 60° C, is not adsorbed by $Al(OH)_3$, and is maximally precipitated from either whole or heated and adsorbed plasma between 25 and 45% saturation with ammonium sulphate.
(4) The activated substance is unstable and rapidly disappears when plasma is removed from contact with glass. This implies that the activation reaction is not autocatalytic.
(5) The decay of activity appears to be due to an antagonist present in plasma. This can be removed by fractionation procedures.
(6) The decay is not reversible, as continued or repeated activation leads to the eventual exhaustion of activity. Unlike the decay, exhaustion is not confined to the 'contact factor' alone, but affects progressively other components of the clotting system, and is, in a way, analogous to the slow ageing of plasma kept in glass vessels.

The only other step in purification so far taken has been the precipitation of euglobulin from the active fractions, but it is still to be proven that its clot-

accelerating action bears any relation to the contact phenomenon. The distinction is more than one of academic interest, as the recently described 'Hageman' clotting defect (Ratnoff & Colopy, 1955) has been identified in a euglobulin preparation very similar to the one used in the present investigation.

Because of the complex nature of the reactions involved, objections may be raised against drawing quantitative conclusions from changes in clotting times by converting these into arbitrary units of activity. It is for this reason that throughout this investigation, with the exception of Fig. 5, such quantitative deductions were avoided. On the other hand, it may be noted that in Fig. 5, where such interpretation was attempted, the resulting initial rates of activation proved to be roughly proportional to the contact surface areas as would be expected on theoretical grounds.

The contact reaction is an important variable in tests for clotting efficiency. Thus the recalcified clotting times at 37° C can vary from under 1 min to over 24 hr, according to the amounts of contact and of platelet material present (Fig. 2). In routine tests these extremes are not realized, but the wide normal range and the lack of agreement between laboratories bear witness to the need for a more rational control of these conditions. The present data suggest that recalcification with a near-maximal surface exposure and a low but controlled platelet concentration provides a very sensitive test system. The former can be provided by the addition of kaolin or kieselguhr (10–50 mg/ml.), the latter by adding lysed platelets (1000–10 000/mm^3) to high-spun plasma.

Certain implications of a more general nature should also be considered. It is obvious that haemostasis is not normally initiated by glass or other forms of silica, but by contact of blood with injured tissue. In both cases, coagulation is dependent on the formation of blood thromboplastin. Although the initial stimuli appear to be so widely different, there are some indications that injured tissue supplies an equivalent of the already activated plasma 'contact factor', and this may be the connecting link between haemostasis and *in vitro* coagulation.

The emphasis in literature on glass as a clot-promoting surface presumably arose from its common use as a container. What may be more important is that other, naturally-occurring, forms of silica are equally active in this respect, whilst most other compounds are relatively inert. It is interesting to note that silica is a potent agent in producing other forms of injury manifestations, for example coagulative necrosis, proliferative foreign body reaction (Kettle, 1935; Miller & Sayers, 1934), toxicity towards leucocytes (Marks, Mason & Nagelschmidt, 1956) and 'pain producing substance' (Armstrong, Jepson, Keele & Stewart, 1955; Jepson, Armstrong, Keele & Stewart, 1956). These considerations lend support to the view that blood coagulation is an evolutionary adaptation of a more general irritability reaction as suggested by

Biggs & Macfarlane (1953) and Robb-Smith (1955). If, as seems possible, various injury reactions have their initial stages in common the relatively simple coagulation techniques could be used as an indicator to study certain aspects of such reactions.

Further investigation of the physical properties and biological activity of 'foreign surface' is in progress. For the present, such terms as 'activation', 'decay', 'exhaustion', 'contact factor' and 'antagonist' are used in a purely descriptive sense and are not necessarily intended to imply any specific chemical substances or reactions.

SUMMARY

1. Recalcified clotting time of citrated plasma is quantitatively dependent on the area and duration of contact with active surfaces. Contact activation of coagulation has also been demonstrated in partial clotting systems by the use of an indirect procedure in which the contact reaction was made the limiting factor.

2. Evidence is presented that the phenomenon is due to the activation of a plasma component which is stable at 57° C but not at 60° C, is not adsorbed by $Al(OH)_3$ and is maximally precipitated between 25 and 45% saturation with $(NH_4)_2SO_4$.

3. Contact activation proceeds normally in haemophilic, Christmas disease, factor V deficient and 'dindivan' plasma.

4. The induced activity declines rapidly after removal of the contact material, probably due to the presence of a plasma antagonist which can be eliminated in fractionation procedures.

5. Continued or repeated activation results in a gradual exhaustion of plasma, and this may be a factor in the ageing process on storage in glass containers.

6. It is suggested that surface activation may be an important aspect in a wider injury reaction of which blood coagulation is only one facet.

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