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Studies on the Coagulation of Chicken Blood

IV. Adsorption of φ-Factor Activity

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Detailed studies on the adsorption of φ-factor activity from oxalated plasma by a series of adsorbents have revealed that this activity is due to the combined activities of at least five different coagulation factors. Three of the factors were inactive when Russell's viper venom-cephalin was substituted for thromboplastin in the assay system. The experiments indicated that the minimum amount of adsorbent necessary for complete adsorption of a (group of) φ-factor(s) from oxalated plasma, may be regarded as an absolute measure of the concentration(s) of the factor(s). It is suggested that the differentiation of the φ-factors obtained by the adsorption technique depends on the presence in the surface of the adsorbents of special adsorption sites, differing in number and specificity with the various adsorbents, each causing adsorption of one or a few φ-factors only.

In a preceding paper [1] it was shown that the differences in effect between κ-, δ- and φ-factors [2] were associated with differences in the adsorbabilities of the factors by a series of adsorbents. Furthermore, it was found that calcium carbonate, which adsorbs all three types of factors, could adsorb only a part of the φ-factor activity as measured with thromboplastin and with Russell's viper venom (RVV)-cephalin. The calcium carbonate adsorbable φ-factor was considered to be a distinct coagulation factor, different from the calcium carbonate non-adsorbable φ-factors, and was designated φ_2-factor.

In the present study the adsorption of φ-factor activity by calcium carbonate and by other φ-factor adsorbing compounds was examined in greater detail to learn if a further differentiation of the φ-factor activity could be accomplished.

Only thromboplastin and RVV-cephalin were used as assay accelerators in this investigation, leaving similar studies with the cephalin assay system [2] to be carried out later.

The materials and methods used in this work have been described in previous communications [1,2].

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EXPERIMENTS AND RESULTS

The adsorption of φ-factor activity from oxalated plasma by the adsorbents of group 1 (*cf.* preceding paper) was studied in detail by determination of the φ-factor activity [2] of plasma aliquots adsorbed with graded amounts of adsorbent. The φ-factor activities determined with thromboplastin (φ_{tpl}-factor activity) and with RVV-cephalin (φ_{Vc}-factor activity) were estimated simultaneously in the adsorbed plasma aliquots.

Adsorption of φ_2-factor by calcium carbonate

A typical experiment with calcium carbonate as adsorbent is shown in Fig. 1. The adsorption of φ-factor activity is proportional to the amount of adsorbent added, until a point is reached where the curve breaks and becomes horizontal. As indicated earlier [1], this shows that only a part of the φ_{tpl}- and the φ_{Vc}-factor activities can be adsorbed by calcium carbonate. The adsorbed activity must consequently be due to a coagulation factor which may be differentiated from all other φ-factors by its adsorbability on calcium carbonate. The designation φ_2-factor was chosen for this coagulation entity.

The designation φ_1-factor has now been chosen for the non-adsorbable (by calcium carbonate) φ-factor, measured both with thromboplastin and with RVV-cephalin, and the terms φ_3-, φ_4-, *etc.* are reserved for the as yet unknown number of φ-factors, which also are non-adsorbable (by calcium carbonate), but specific for the thromboplastin assay system.

Series of comparative measurements of the φ_{tpl}- and φ_{Vc}-factor activities of different plasmas have been carried out. The experiments have shown that plasmas having identical φ_{Vc}-factor activity may have different φ_{tpl}-factor activity. These observations are further evidence for the assumption that the thromboplastin assay system registers variations in the activities of some

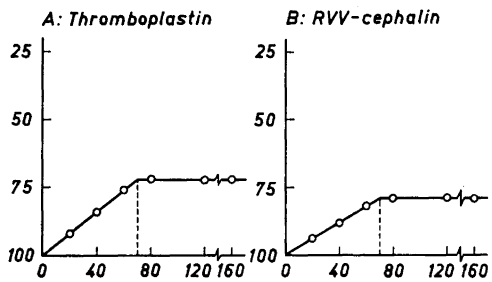

Fig. 1. Adsorption of φ-factor by calcium carbonate. Ordinates: φ-factor activity in % of original plasma. Abscissæ: Calcium carbonate in mg per ml plasma.

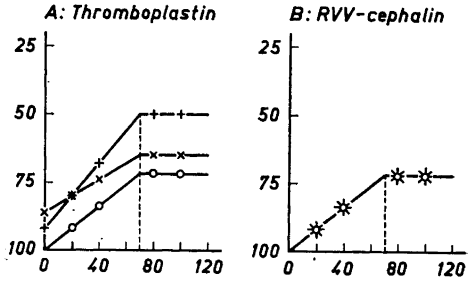

Fig. 2. Adsorption of φ-factor by calcium carbonate.
O: Plasma No. 1217.
+: » » 1219.
×: » » 1218.
Ordinates: φ-factor activity in % of plasma No. 1217.
Abscissæ: Calcium carbonate in mg per ml plasma.

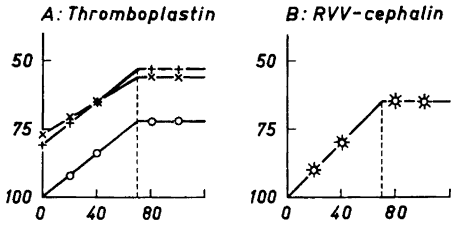

Fig. 3. Adsorption of φ-factor by calcium carbonate.
O: Plasma No. 1722.
+: » » 1721.
×: » » 1724.
Ordinates: φ-factor activity in % of plasma No. 1722.
Abscissæ: Calcium carbonate in mg per ml plasma.

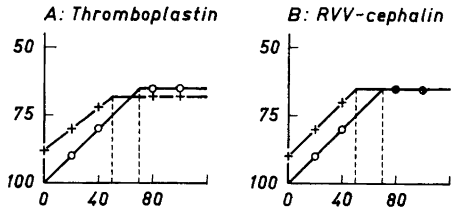

Fig. 4. Adsorption of φ-factor by calcium carbonate.
O: Plasma No. 1221.
+: » » 1220.
Ordinates: φ-factor activity in % of plasma No. 1221.
Abscissæ: Calcium carbonate in mg per ml plasma.

φ-factors which do not influence the results of the RVV-cephalin assay system [2]. Such plasmas were used in the experiments of Figs. 2 and 3. In both these cases the curves for adsorption of the φ_{Vc}-factor activity of three different plasmas were identical. The relation between adsorbed φ_{tpl}-factor activity and amount of adsorbent was, however, not always identical for different plasmas in the same experiment. The three curves of Fig. 2A were not parallel, and the adsorption curve for plasma No. 1 724 (Fig. 3A) differed in slope from the curves for the two other plasmas (Nos. 1 721 and 1 722). However, the minimum amount of adsorbent necessary for maximal adsorption of φ_{tpl}- and φ_{Vc}-factor activities was the same for all plasmas in these experiments, *viz.* 70 mg per ml of plasma. Differences in φ_2-factor activity, as measured with thromboplastin, may thus be found in plasmas having identical φ_2-factor activity as measured with RVV-cephalin. These differences were ascribed to differences in the activity of one or more hypothetical thromboplastin specific factors, acting as synergists for φ_2-factor.

Adsorption experiments were also carried out with plasmas showing different φ_{Vc}-factor activity (Figs. 4 and 5). In each experiment the curves for adsorption of the φ_{Vc}-factor activity of two different plasmas had the same slope. Their φ_{tpl}-factor activity adsorption curves were, however, not parallel. This fact again indicated differences in the activity of one or more thromboplastin specific factors acting as synergists for φ_2-factor. However, for each plasma the two assay systems gave the same value for the minimum amount of adsorbent necessary for maximal adsorption of φ-factor activity, *viz.* 70 mg/ml for plasmas Nos. 1 221 and 1 725, 50 mg/ml for plasma No. 1 220 and 40 mg/ml for plasma No. 1 723.

From Figs. 4 and 5 it was apparent that the observed differences in φ_{Vc}-factor activity of the plasmas were due to differences in the activity of φ_2-factor alone, since the maximally adsorbed plasma had identical φ_{Vc}-factor activity. The φ_2-factor activity of plasmas may consequently be expected to vary independently of the activity of φ_1-factor. The differences in the activi-

ty of φ_2-factor in these plasmas are reflected in proportional differences in the minimum amount of adsorbent necessary for maximal adsorption of φ-factor activity. The observed differences in φ_{tpl}-factor activity of the plasmas were not due to differences in the activity of φ_2-factor only. The activities of the maximally adsorbed plasmas were not identical, indicating additional differences in the activity of thromboplastin specific factors.

A comparison of the experiments in Figs. 1—5 shows that whereas the slope of the curves for adsorption of φ_{Vc}-activity was the same for all plasmas used in the same experiment, the adsorption curves of different experiments varied slightly in slope. This could be expected since a different set of substrate plasmas was used in each experiment. It must be ascribed to small differences in the relative sensitivity of the substrate mixtures to φ_1- and φ_2-factors.

It is thus evident that an estimation of the *concentration* of φ_2-factor in a plasma by determination of its *relative* φ-factor activity ($\varphi_2/\varphi_{total}$) will give different results depending on: (1) whether thromboplastin or RVV-cephalin is used as assay accelerator, (2) the relative sensitivity of the substrate to φ_2-factor and to the calcium carbonate non-adsorbable factor(s), (3) the concentration of φ_1-factor, and (4) the concentrations of thromboplastin specific factors, one or more of which may act as synergists for φ_2-factor. However, the minimum amount of calcium carbonate necessary for maximal adsorption of φ-factor activity appears to be intimately related to the *concentration* of φ_2-factor. It is independent of the nature of the assay accelerator, independent of small differences in the sensitivity of the substrate, and independent of the concentrations of thromboplastin specific factors. It is probably also independent of the concentration of φ_1-factor.

Therefore, the minimum amount of calcium carbonate necessary for maximal adsorption of φ-factor activity from oxalated plasma is regarded as a reliable

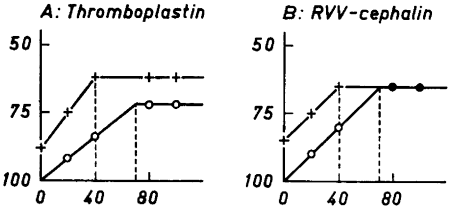

Fig. 5. Adsorption of φ-factor activity by calcium carbonate.
O: Plasma No. 1725.
+: » » 1723.
Ordinates: φ-factor activity in % of plasma No. 1725.
Abscissæ: Calcium carbonate in mg per ml plasma.

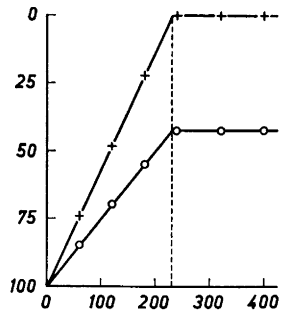

Fig. 6. Adsorption of φ-factor activity by magnesium arsenate.
O: Thromboplastin assay.
+: RVV-cephalin assay.
Ordinate: φ-factor activity in % of original plasma.
Abscissa: Magnesium arsenate in mg per ml plasma.

measure of the concentration of φ_2-factor in the plasma. It appears that a value of 70 (mg calcium carbonate per ml of oxalated plasma) represents the maximum concentration of φ_2-factor in chicken plasma. The experimental background for the lower values occasionally observed will be dealt with in a coming publication.

Adsorption of φ-factor activity by other adsorbents

Less extensive studies on the adsorption of φ-factor activity were carried out with other φ-factor adsorbing compounds (*cf.* preceding article).

Curves for adsorption of φ-factor activity from oxalated plasma by magnesium arsenate are shown in Fig. 6. The φ_{Vc}-factors could be adsorbed completely, whereas only partial adsorption of the φ_{tpl}-factor activity was accomplished with this compound. The experiment indicated that φ_1- and φ_2-factors, and possibly a thromboplastin specific φ-factor were adsorbed simultaneously and proportionally to the amount of adsorbent added to the plasma. Other thromboplastin specific φ-factors appeared to be non-adsorbable by magnesium arsenate.

Fig. 7 shows a typical set of curves for adsorption of φ-factor activity from oxalated plasma by lead sulfide. As with magnesium arsenate, the adsorption of φ_{Vc}-factor activity was proportional to the amount of adsorbent added. The curve indicated that the φ_1- and φ_2-factors could be adsorbed completely and were adsorbed simultaneously. The φ_{tpl}-factor activity was also completely adsorbable by lead sulfide, but the curve for adsorption of this activity was more complex and appeared to be composed of two rectilinear sections. The end point of the curve for adsorption of φ_{Vc}-factor activity coincided with the end point of the second section of the curve for adsorption of φ_{tpl}-factor activity (at B). This point thus represents complete adsorption of a group

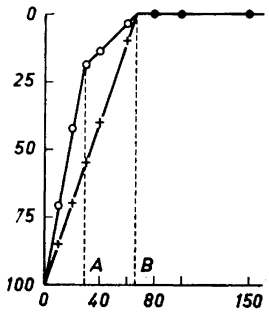

Fig. 7. Adsorption of φ-factor activity by lead sulfide.
O: Thromboplastin assay.
+: RVV-cephalin assay.
Ordinate: φ-factor activity in % of original plasma.
Abscissa: Lead sulfide in mg per ml plasma.

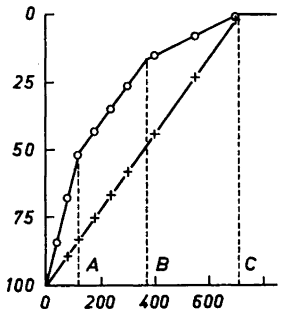

Fig. 8. Adsorption of φ-factor activity by manganese carbonate.
O: Thromboplastin assay.
+: RVV-cephalin assay.
Ordinate: φ-factor activity in % of original plasma.
Abscissa: Manganese carbonate in mg per ml plasma.

of factors which includes the φ_1- and φ_2-factors. It is not known whether or not a thromboplastin specific factor belongs to this group. The end point of the first rectilinear section of the φ_{tpl}-factor adsorption curve (at A) was considered to represent complete adsorption of φ-factors which are thromboplastin specific. It should be stressed that the change in slope occurring at A (29 mg of lead sulfide per ml of plasma), was an abrupt change similar to that occurring at B where the adsorption of the φ_{Vc}-factors was complete. The curve for adsorption of φ_{tpl}-factor activity by lead sulfide should therefore be regarded as two independent adsorption curves superimposed, representing the adsorption of two different groups of φ-factors.

Adsorption of φ-factor activity from oxalated plasma by manganese carbonate is shown in Fig. 8. The curve for adsorption of φ_{Vc}-factor activity showed that the φ_1- and φ_2-factors were completely adsorbable by manganese carbonate and were adsorbed simultaneously. The φ_{tpl}-factors could also be adsorbed completely by this adsorbent, but the curve for adsorption of the latter activity was more complex than the corresponding curve obtained with lead sulfide (Fig. 7). It appeared to be composed of *three* rectilinear sections. The end point of the third section (at C) coincided with the end point of the curve for adsorption of φ_{Vc}-factor activity. This point thus represents complete adsorption of a group of φ-factors which includes φ_1- and φ_2-factors and possibly a thromboplastin specific factor. The end point of the first and of the second rectilinear sections (at A and at B) were considered to represent complete adsorption of two different (groups of) thromboplastin specific φ-factors.

Curves for adsorption of φ_{tpl}- and φ_{Vc}-factor activities from oxalated plasma by calcium oxalate and strontium oxalate are shown in Figs. 9 and 10, respectively. The two sets of curves were very similar. The curves for adsorption of φ_{Vc}-factor activity indicated complete adsorbability and simultaneous

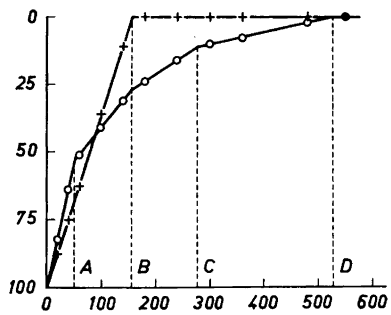

Fig. 9. Adsorption of φ-factor activity by calcium oxalate.
O: Thromboplastin assay.
+: RVV-cephalin assay.
Ordinate: φ-factor activity in % of original plasma.
Abscissa: Calcium oxalate in mg per ml plasma.

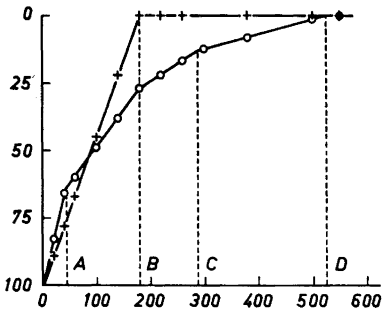

Fig. 10. Adsorption of φ-factor activity by strontium oxalate.
O: Thromboplastin assay.
+: RVV-cephalin assay.
Ordinate: φ-factor activity in % of original plasma.
Abscissa: Strontium oxalate in mg per ml plasma.

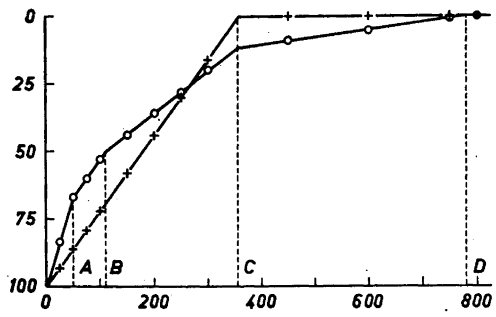

Fig. 11. Adsorption of φ-factor activity by calcium molybdate.
O: Thromboplastin assay.
+: RVV-cephalin assay.
Ordinate: φ-factor activity in % of original plasma.
Abscissa: Calcium molybdate in mg per ml plasma.

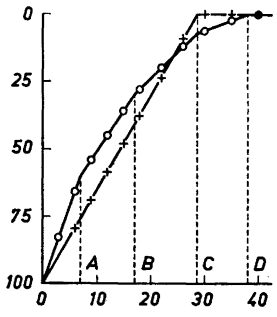

Fig. 12. Adsorption of φ-factor activity by strontium carbonate.
O: Thromboplastin assay.
+: RVV-cephalin assay.
Ordinate: φ-factor activity in % of original plasma.
Abscissa: Strontium carbonate in mg per ml plasma.

adsorption of φ_1- and φ_2-factors by these adsorbents. The curves for adsorption of φ_{tpl}-factor activity by calcium and strontium oxalates were composed of no less than four rectilinear sections. The end points of each of these four sections were throught to represent complete adsorption of four different (groups of) φ-factors. In both curves the end point of the second section (at B) coincided with the point representing complete adsorption of φ_1- and φ_2-factors. The end point of the first, third and of the fourth section (at A, C, and D) may, therefore, represent complete adsorption of three different (groups of) thromboplastin specific φ-factors.

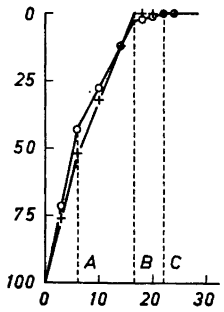

Fig. 13. Adsorption of φ-factor activity by barium carbonate.
O: Thromboplastin assay.
+: RVV-cephalin assay.
Ordinate: φ-factor activity in % of original plasma.
Abscissa: Barium carbonate in mg per ml plasma.

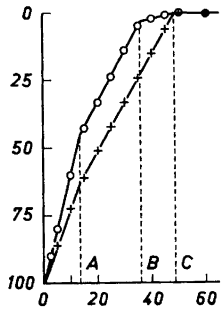

Fig. 14. Adsorption of φ-factor activity by barium oxalate.
O: Thromboplastin assay.
+: RVV-cephalin assay.
Ordinate: φ-factor activity in % of original plasma.
Abscissa: Barium oxalate in mg per ml plasma.

Adsorption of plasma by calcium molybdate and strontium carbonate gave the adsorption curves shown in Figs. 11 and 12. The curves showed a similar complexity as the curves in Figs. 9 and 10, but the adsorption of φ_1- and φ_2-factors by calcium molybdate and strontium carbonate was complete at C *i.e.* at the end point of the *third* part of the four-sectioned curves for adsorption of φ_{tpl}-factor activity.

Figs. 13, 14, and 15 show the curves obtained for adsorption of plasma by barium carbonate, barium oxalate and barium chromate, respectively. The curves for adsorption of the φ_{Vc}-factor activities were composed of two rectilinear sections and indicated that complete adsorption of one φ_{Vc}-factor was accomplished with a smaller amount of adsorbent than that required for complete adsorption of a second φ_{Vc}-factor. The curves for adsorption of the φ_{tpl}-factor activities had three rectilinear sections and indicated independent adsorption of three different (groups of) φ-factors. The end points of two of these sections coincided with the end points of the two sections of the φ_{Vc}-factor adsorption curves. The end point of the third section therefore, represents complete adsorption of a thromboplastin specific φ-factor.

Adsorption of φ-factor activity from preadsorbed plasma

In the interpretation of the curves for adsorption of φ-factor activity from oxalated plasmas, each rectilinear section was thought to represent simultaneous adsorption of one or more (groups of) φ-factors. A sudden change in the slope of the adsorption curve, *i.e.* the end of one rectilinear section and the beginning of a following, was thought to indicate that a (group of) φ-factor(s) had been completely adsorbed at this point, whereas the adsorption of other φ-factor(s) progressed, proportionally to the increase in the amount of adsorbent. This implied that the adsorption of one (group of) φ-factor(s) took place independently of the adsorption of other (groups of) φ-factors. With lead sulfide as adsorbent it was possible to distinguish between two such groups of φ-factors. A similar distinction between three different groups was possible with manganese carbonate, barium carbonate, barium oxalate or barium chromate, and four groups of φ-factors were discernible with calcium oxalate, strontium oxalate, calcium molybdate or strontium carbonate. In general, a two-, three-, or four-sectioned adsorption curve was interpreted as the results of superposition of two, three of four independent adsorption curves, respectively. Each of the independent adsorption curves could be expected to be analogous to the curves obtained with calcium carbonate, and represent the adsorption of one (group of) φ-factor(s).

Adsorption analysis of the φ-factor activity of a plasma with, *e.g.*, strontium carbonate would thus give four characteristic values: A, B, C, and D (*cf.* Fig. 12). Values A, B, and D could be regarded as related to the concentrations of three different (groups of) thromboplastin specific factors. Value C would then be related to the concentrations of a group of factors which includes the φ_1- and φ_2-factors. Two plasma samples, differing only in the concentrations of one of the φ-factors, could consequently be expected to have three of the values in common and differ only in the fourth.

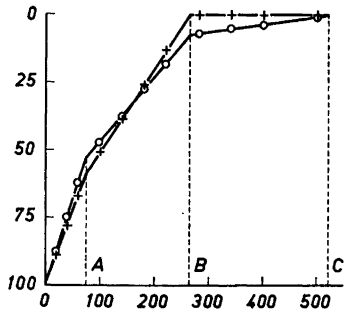

Fig. 15. Adsorption of φ-factor activity by barium chromate.
O: Thromboplastin assay.
+: RVV-cephalin assay.
Ordinate: φ-factor activity in % of original plasma.
Abscissa: Barium chromate in mg per ml plasma.

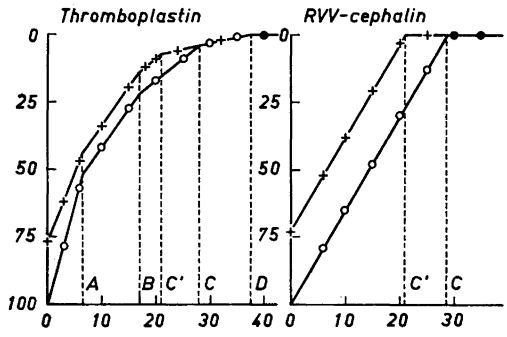

Fig. 16. Adsorption of φ-factor activity by strontium carbonate.
O: Untreated plasma.
+: Plasma preadsorbed with calcium carbonate (80 mg/ml).
Ordinates: φ-factor activity in % of original plasma.
Abscissæ: Strontium carbonate in mg per ml plasma.

The validity of these assumptions has been checked by a few experiments. Plasma pairs, differing only in the concentrations of φ_2-factor, were prepared by adsorbing one portion of a plasma with calcium carbonate to remove φ_2-factor completely. Another portion of the same plasma was left untreated. Curves for adsorption of the φ-factor activity of the untreated plasma were then compared with the curves obtained for the preadsorbed plasma. Typical experiments of this type are shown in Figs. 16—18.

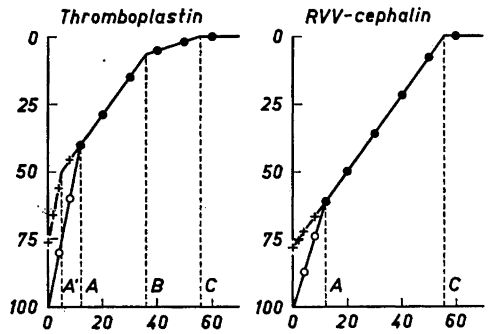

Fig. 17. Adsorption of φ-factor activity by barium oxalate.
O: Untreated plasma.
+: Plasma preadsorbed with calcium carbonate (80 mg/ml).
Ordinates: φ-factor activity in % of original plasma.
Abscissæ: Barium oxalate in mg per ml plasma.

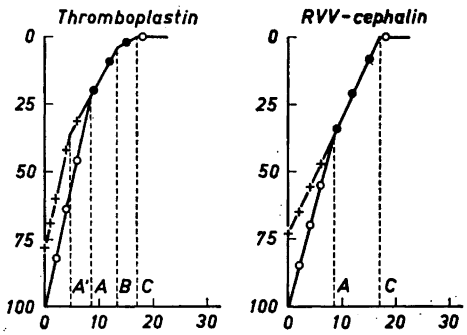

Fig. 18. Adsorption of φ-factor activity by barium carbonate.
O: Untreated plasma.
+: Plasma preadsorbed with calcium carbonate (80 mg/ml).
Ordinates: φ-factor activity in % of original plasma.
Abscissæ: Barium carbonate in mg per ml plasma.

The adsorption by strontium carbonate of the φ-factor activity of untreated and of calcium carbonate preadsorbed plasmas is shown in Fig. 16. It will be seen that the preadsorbed plasma requires less adsorbent (C′) for complete adsorption of the φ_{Vc}-factor activity than does the untreated plasma (C). The quantities of adsorbent causing complete adsorption of the three thromboplastin specific φ-factors (A, B, and D) in the two plasma samples are identical. This experiment thus shows that adsorption of the thromboplastin specific φ-factors occurs independently of the adsorption of φ_2-factor. The latter factor must be considered to compete with φ_1-factor for adsorption on strontium carbonate. The first section of the φ_{tpl}-factor adsorption curves for the untreated and the preadsorbed plasma did not have the same slope, but the following sections of the curves were parallel. This indicates that φ_2-factor may act as a synergist for a factor which is completely adsorbed at A. In fact, this factor may be the hypothetical thromboplastin specific factor, referred to previously (p. 905).

Figs. 17 and 18 show the curves for adsorption of φ-factor activity from untreated and from calcium carbonate preadsorbed plasmas by barium oxalate and barium carbonate, respectively. It will be seen that the curves for adsorption of φ_{Vc}-factor activity from untreated plasma had two rectilinear sections, whereas the curves obtained with preadsorbed plasma were straight lines, merging into the curve for the untreated plasma at A. The φ_{Vc}-factor which was completely adsorbed at A in the untreated plasma was thus no longer present in the preadsorbed plasma. It is concluded that this factor is φ_2-factor, and that φ_1- and φ_2-factor do not compete for adsorption on these adsorbents. The curves for adsorption of φ_{tpl}-factor activity show that the factors completely adsorbed at A in the untreated plasma are completely adsorbed at A′ in the preadsorbed plasma. The amounts of adsorbent required for complete adsorption of the two other groups of φ_{tpl}-factors, are the same for the two plasmas. The experiments show that the φ-factors which are completely adsorbed at B and at C, are adsorbed independently of the adsorption of φ_2-factor. On the other hand, the thromboplastin specific factor which is completely adsorbed at A in the untreated plasma and at A′ in the preadsorbed plasma, competes with φ_2-factor for the adsorption on barium oxalate and barium carbonate. The fact that sections one of the φ_{tpl}-factor adsorption curves for the untreated and for the preadsorbed plasmas are parallel, indicates that the factor adsorbed in competition with φ_2-factor does not act as a synergist for the latter.

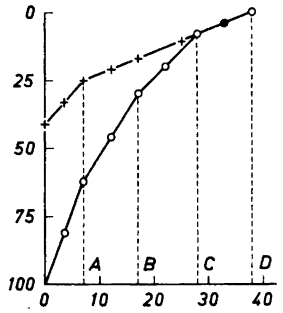

Fig. 19. Adsorption of φ-factor activity by strontium carbonate.
O: Untreated plasma.
+: Plasma preadsorbed with magnesium arsenate (240 mg/ml).
Ordinates: φ-factor activity in % of original plasma.
Abscissæ: Strontium carbonate in mg per ml plasma.

In Fig. 19 are shown the curves for adsorption by strontium carbonate of the φ_{tpl}-factor activities of untreated plasma and of plasma preadsorbed with magnesium arsenate for complete removal of the factors which are adsorbable by this compound (*cf.* Fig. 6). The untreated plasma gave the usual four-sectioned adsorption curve. The adsorption curve for the preadsorbed plasma had only two rectilinear sections. The last part of the second section merged into the fourth section of the curve for the untreated plasma. The changes in slope occurring at B and at C in the curve for the untreated plasma were thus not seen with preadsorbed plasma, whereas the end points of the two sections of the curve for the latter coincided with the end points of sections one and four of the curve for the untreated plasma. It is concluded that the factors removed by preadsorption with magnesium arsenate are the factors which were completely adsorbed at B and at C, and that the adsorption of these factors by strontium carbonate has no influence on the adsorption of the factors adsorbed completely at A and at D. Since the adsorption of φ_1- and φ_2-factors is complete at C and the adsorption of a thromboplastin specific φ-factor is complete at B, it is evident that the factors which are adsorbable by magnesium arsenate include φ_1-factor and φ_2-factor plus a thromboplastin specific φ-factor.

The proposed interpretation of the curves for adsorption of φ-factor activity from oxalated plasma is supported by these experiments. The various shapes of the adsorption curves may be explained as the results of adsorption of different coagulation entities, some of which are adsorbed competitively, whereas the adsorption of others is mutually independent. Factors which are adsorbed competitively by one adsorbent, may be adsorbed independently of each other by another adsorbent. With the adsorbents used in these studies it appears possible to distinguish between three different thromboplastin specific φ-factors (φ_{3-5}), and between two φ-factors (φ_1- and φ_2-factor) with contribute to the measured φ-factor activity both with thromboplastin and with RVV cephalin as assay accelerators. The present differentiation of the φ-factor activity of plasma may not represent the final answer as to the number of different coagulation entities measured as φ-factors in the assay systems used here.

DISCUSSION

The possibility that prothrombin and Stuart factor may be registered as φ_{vc}-factors and proconvertin as a thromboplastin specific φ-factor has been discussed earlier [2]. A final answer to these questions cannot be given at present.

The curves for adsorption of φ-factor activity from oxalated plasma give adequate support for the earlier assumptions [2] that different coagulation entities contribute to the activity measured as φ-factor activity under the conditions described. From the present results it may be concluded that at least five different coagulation factors contribute to the φ-factor activity of plasma as measured with thromboplastin. Three of the factors do not influence the coagulation rate of plasma when RVV-cephalin is used as assay accelerator instead of thromboplastin. They are therefore regarded as thromboplastin specific factors. The two remaining factors (φ_1- and φ_2-factors) are involved in

reactions, the speed of which may limit the coagulation rate of plasma, *both* when thromboplastin and when RVV-cephalin are used as accelerators.

The technique for assay of φ-factor activity [2] usually permits repeated determinations of the clotting times with no more than 0.1 sec. difference. The readings of the φ-factor activity of the adsorbed plasma samples are therefore very accurate. With this in mind it would not be permissible to draw a smooth curve between the observed values so as to fit an adsorption isotherm. Strict proportionality between adsorbed activity and amount of adsorbent is observed for each section of the curves for adsorption of the φ_{Vc}- and the φ_{tpl}-factor activities. The proportionality suggests that the minimum amount of adsorbent necessary for complete adsorption of a (group of) φ-factor(s) may be regarded as an absolute measure of the concentration of the factor(s). This was found to be the case when calcium carbonate was used as adsorbent. With this compound all observed variations in φ_2-factor concentration were associated with proportional variations in the minimum amount of adsorbent necessary for complete adsorption of the factor from oxalated plasma. With the other adsorbents the problem is more complex because of more or less simultaneous adsorption of a number of φ-factors. It is probable that the amount of adsorbent, corresponding to the end point of a rectilinear section of an adsorption curve, is related to the concentration of the factor(s) completely adsorbed at this point, but it is not yet clear if variations in these values for different plasmas can be correlated with variations in the concentrations of the various φ-factors only. Inert plasma material may be adsorbed simultaneously with the φ-factors, but should not be expected to affect the amount of adsorbent necessary for complete adsorption of a φ-factor, *unless it is adsorbed in competition with the factor*. The thromboplastin specific factors behave as inert material in the RVV-cephalin assay. The end points at A in Figs. 13—15, 17, and 18, representing complete adsorption of φ_2-factor and a thromboplastin specific factor in competition, could therefore not be expected to vary with variations in the concentration of φ_2-factor only, but also with variations in the concentration of the inert thromboplastin specific factor. These problems need further consideration. Further experiments with adsorption of preadsorbed plasmas may give additional information concerning the relation between the factors responsible for the adsorption curves obtained with the different adsorbents.

Adsorption of φ-factor by a crystalline adsorbent implies that the adsorbent has a certain spatial pattern of surface molecules which acts as an adsorption site for the factor, probably being the reverse model of a characteristic pattern of molecular groups in the adsorbable factor. Competitive adsorption of some φ-factors by one adsorbent and independent adsorption of the same factors by another adsorbent indicate that different adsorbents can distinguish between different factors. When two or more φ-factors are adsorbed competitively by an adsorbent, the adsorption sites involved are considered to be identical or to have overlapping patterns of surface molecules. In case of independent adsorption entirely different adsorption sites must be engaged in the adsorption of different factors. It would thus seem necessary to regard each adsorbent as having a certain number of adsorption sites with different and very specific adsorption properties. It is not known if the differences

between the various adsorption sites of an adsorbent may be correlated with differences in surface structure between different regions of a single crystal or between crystals of different form. In any case, adsorption analysis appears to represent a valuable technique for differentiation of chicken coagulation factors which cannot be distinguished by other means in the assay systems available at present.

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