

## Studies on the Coagulation of Chicken Blood

### VIII. Use of Alumina, Ferric Oxide, Bismuth Sulfide, Magnesium Borate, and Zinc Tungstate for Differentiation and Assay of Strontium Carbonate Non-adsorbable Labile Factors

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Partial adsorption of the labile factor activity of strontium carbonate preadsorbed oxalated chicken plasma was obtained by use of different adsorbents. Six different categories of such adsorbents were recognized, permitting selective adsorption of six different labile coagulation factors. Provisional names for these factors are suggested:  $\alpha$  (alpha)-factor, selectively adsorbed by Mg-borate;  $\theta$  (theta)-factor, selectively adsorbed by Zn-tungstate;  $\xi$  (xi)-factor, selectively adsorbed by Fe-oxide;  $\omega$  (omega)-factor, selectively adsorbed by Bi-sulfide;  $\eta$  (eta)-factor, selectively adsorbed by hydrochloric acid treated Merck 1095 Al-oxide; and  $\zeta$  (zeta)-factor, selectively adsorbed by Hopkin & Williams 1284 Al-oxide.

These six factors are different from the eight labile factors described in preceding papers. With a few exceptions the specific activity of each of these fourteen factors is influenced in a characteristic manner by the concentrations of the other thirteen. The  $\xi$ -,  $\omega$ -,  $\eta$ -, and  $\zeta$ -factors are common components of the labile factor activities measured with thromboplastin and Russell's viper venom, with or without added cephalin, whereas the  $\alpha$ - and  $\theta$ -factors are thromboplastin specific labile coagulation factors.

In two preceding papers<sup>1,2</sup> the effects of crystalline adsorbents on the labile factor activity of strontium carbonate preadsorbed oxalated chicken plasma were investigated. Only a part of the total activity was adsorbed. These observations indicated that the labile factor activity was of complex nature, and suggested the use of such adsorbents for differentiation of the activity and characterization of the individual components. The results obtained with seventy-seven adsorbents were explained as selective adsorption of eight different labile factors (measured by thromboplastin), by eight different categories of adsorbents. Of these factors only two were parts of the labile factor activity measured by RVV \*\*-cephalin, and only one was active

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\*\* Russell's viper venom.

with RVV alone. The greater part of the labile factor activity measured by the two latter agents, was thus due to labile factors not adsorbed by any of the adsorbents tested. A few preliminary experiments also showed that the combined activities of the eight factors described did not account for more than 40–50 % of the total activity measured by thromboplastin.

A search for compounds with selective adsorption capacities for factors not adsorbed by the adsorbents described in preceding papers<sup>1,2</sup>, led to a reexamination of the adsorption properties of a sample of alumina which previously had been tested only briefly<sup>3</sup>.

It will be shown in this paper that (1) the effect of this alumina is due to adsorption of six discrete labile factors which differ from those already described<sup>1,2</sup>, (2) the six factors can be adsorbed separately by other adsorbents, including special brands of alumina, and (3) four of these six factors are common components of the labile factor activities measured by thromboplastin, RVV-cephalin and RVV, whereas the remaining two factors are inactive in the two latter systems and must be regarded as thromboplastin specific labile coagulation factors.

#### MATERIALS AND METHODS

Except for the adsorbents the methods and materials used in this study were as described previously<sup>1,2,4,5</sup>.

#### A d s o r b e n t s

The following adsorbents were prepared in this laboratory from high purity chemicals by adaption of methods described in Gmelin-Kraut's Handbuch der Anorganischen Chemie (*cf.* preceding papers<sup>1,2</sup>).

*Borate* of Mg was prepared from the oxide as described for Co-borate<sup>1</sup>.

*Tungstates* of Mg and Zn were prepared from the chlorides as described for Ca-tungstate<sup>1</sup>.

*Aluminates* of Mg and Zn were prepared from the sulfate and the chloride, respectively, as described for Co-aluminate<sup>1</sup>.

*Silicates* of Mg and Zn were prepared from the sulfate and the chloride, respectively, as described for Co-silicate<sup>1</sup>.

*Oxides* of Fe and Mn were prepared from the carbonate and the di-oxide, respectively, as described for Ce-oxide<sup>2</sup>.

*Sulfides* of Bi and Hg were prepared by precipitation with hydrogen sulfide gas, Bi-sulfide from the nitrate (dissolved in dilute hydrochloric acid) and Hg-sulfide from the acetate (dissolved in dilute acetic acid). The precipitates were thoroughly washed and dried, then heated for 3–4 days at 280°C (Bi) or 240°C (Hg) in sealed evacuated ampullae.

*Ba-metaborate* was kindly supplied by the Department of Inorganic Chemistry of this Institute.

The following brands of *alumina* were obtained commercially:

Al-oxide I: Alumina, Type H, Grade 100–200, for Chromatographic Analysis, Peter Spence & Sons.

Al-oxide II: Aluminium oxide activated, for adsorption purposes, and for chromatographic analysis, 1284, Hopkin & Williams.

Al-oxide III: Aluminium oxydatum anhydricum puriss., 1095, Merck (non-uniform particle size).

Al-oxide IV: Aluminium oxydatum anhydricum puriss., 1095, Merck (uniform particle size).

Al-oxide VI: Aluminium oxide, suitable for analysis, A 17/18/66, May & Baker.

Al-oxide VII: Aluminiumoxyd "Woelm", sauer (anionotrop), Akt.-Stufe I, zur Chromatographie.

Al-oxide VIII: Aluminiumoxyd "Woelm", alkalifrei (annähernd neutral), Akt.-Stufe I, zur Chromatographie.

Attempts were made to modify the adsorption properties of the Al-oxides I, III and IV, by stirring 50 g of the oxide with 500 ml of dilute acids at different temperatures. The details of this procedure for the modifications used in the present study were as follows:

b<sub>1</sub>: treatment with 1 M hydrochloric acid at room temperature for 48 h.

b<sub>2</sub>: treatment with 1 M hydrochloric acid on a steam bath for 24 h.

b<sub>6</sub>: treatment with 6 M hydrochloric acid on a steam bath for 3 h.

d<sub>0</sub>: a suspension of the Al-oxide in water was heated on a steam bath for 24 h and pH maintained at pH 3–3.5 by addition of nitric acid.

d<sub>2</sub>: treatment with 1 M nitric acid on a steam bath for 24 h.

d<sub>3</sub>: treatment with 4 M nitric acid at room temperature for 48 h.

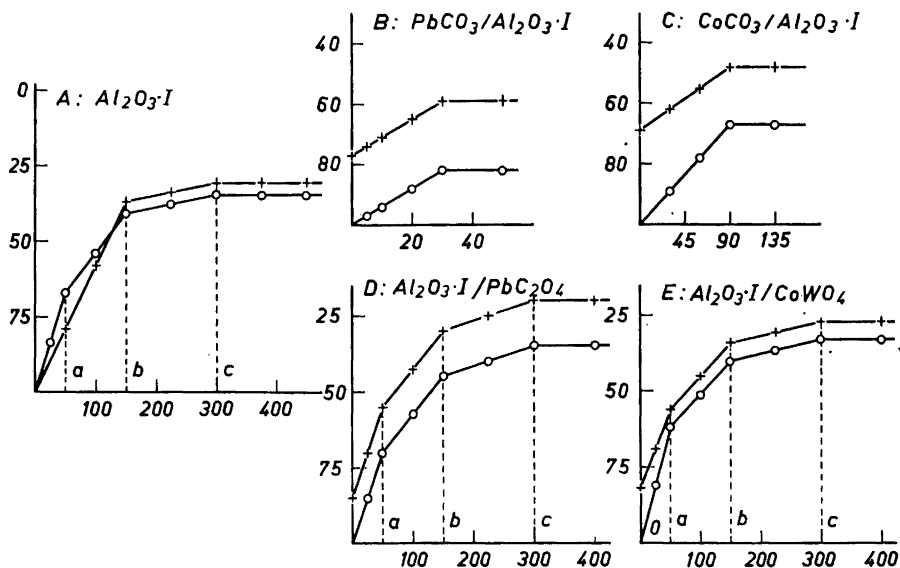
All the above modifications of Al-oxide I were prepared. Of the Al-oxides III and IV the modifications b<sub>2</sub> and b<sub>6</sub> were prepared as for I.

Before use all adsorbents, including the various preparations of Al-oxide, were washed with hot distilled water, treated with 96 % ethanol and dried, as described previously<sup>2</sup>.

## EXPERIMENTS AND RESULTS

*Adsorption of strontium carbonate non-adsorbable labile factors.* The adsorbents were tested for adsorption of the labile factor activity of fresh, strontium carbonate preadsorbed, oxalated chicken plasma (strontium carbonate plasma) as described in preceding papers<sup>1,2,4</sup>. No inhibitory effects were detected in the adsorbed plasmas, and pH was not changed after adsorption, except with Ba-metaborate and some brands of Al-oxide which increased pH to 7.5–7.7. These pH changes did not interfere with the determination of labile factor activity, but made the activity of the plasmas less stable. However, with most adsorbents the non-adsorbed activity was stable for one hour or more when stored at 0°C. Most re-adsorption experiments were done with such plasmas. When less stable, adsorbed plasmas were used for re-adsorption experiments, the results were corrected for possible minor changes in activity occurring in control samples during the re-adsorption operations.

*Adsorption by Al-oxide I.* The labile factor activity of plasma aliquots was determined after adsorption by graded amounts of Al-oxide I. Fig. 1A shows a characteristic experiment where the labile factor activity was measured with thromboplastin as well as with RVV-cephalin<sup>4,5</sup>. Al-oxide I caused only partial adsorption of the activity and could therefore be regarded as a selective adsorbent. However, the curves for adsorption of the activity were more complex than those given by the selective adsorbents tested in previous papers<sup>1,2</sup>. Thus, the curve obtained with thromboplastin appeared to be composed of three rectilinear sections. The curve obtained with RVV-cephalin had only two such sections, the end points of which corresponded to the end points of sections two and three of the thromboplastin curve. In agreement with the interpretation of the two-, three- and four-sectioned curves obtained for adsorption of  $\phi$ -factor activity by a variety of adsorbents<sup>6</sup>, the present curves were interpreted as independent adsorption of three different (groups of) labile factors, one of which must be specific for the thromboplastin assay system. The three-sectioned adsorption curve is thus regarded as the result of a superposition of three single-sectioned rectilinear adsorption curves, each



*Figs. 1-10.* All figures: Ordinates: labile factor activity in % of strontium carbonate plasma. Abscissæ: adsorbent in mg/ml.

*Fig. 1.* Effects of adsorption by Al-oxide I.

A: labile factor activity adsorption by Al-oxide I, measured by thromboplastin (O) and by RVV-cephalin (+)

B—E: Untreated strontium carbonate plasma : O  
preadsorbed » » » +

B: Pb-carbonate adsorption, preadsorption by Al-oxide I (60 mg/ml)

C: Co-carbonate adsorption, preadsorption by Al-oxide I (50 mg/ml)

D: Al-oxide I adsorption, preadsorption by Pb-oxalate (20 mg/ml)

E: Al-oxide I adsorption, preadsorption by Co-tungstate (60 mg/ml)

representing adsorption of one (group of) labile factor(s), the adsorption of which is complete at *a*, *b* and *c*, respectively.

The new possibilities for differentiation of the labile factor activity of strontium carbonate plasma offered by Al-oxide I, were investigated. The relation between the factors adsorbed by this agent and those adsorbed by the selective adsorbents described previously<sup>1,2</sup>, was studied in a series of matching experiments. Adsorption of labile factor activity by lead carbonate (adsorbing  $\pi$ -factor) before and after partial adsorption by Al-oxide I, is shown in Fig. 1B, and adsorption by Al-oxide I before and after preadsorption of the  $\pi$ -factor (by lead oxalate), in Fig. 1D. In both experiments the curves were parallel indicating that the factors adsorbed by Al-oxide I are different from  $\pi$ -factor and their activities independent of the concentration of the latter (*cf.* preceding papers<sup>1,2</sup>). Parallel curves were also obtained in the corresponding matching experiments with Al-oxide I and selective adsorbents for the  $\varrho$ -,  $\nu$ -,  $\mu$ -,  $\gamma$ -,  $\beta$ - and  $\varepsilon$ -factor activities<sup>1,2</sup>. The factors adsorbed by Al-oxide I were thus different from each of these seven factors and the activities of the

Table 1. Effect of adsorbents on the labile factor activity of strontium carbonate plasma.

Plasma no.	Adsorbent	mg/ml	Labile factor activity of adsorbed plasma in % of strontium carbonate plasma	Labile factor(s) adsorbed *
3592	Ba-metaborate	40	73	α
		70	73	
615	Mg-borate	20	85	
		50	85	
3550	Mg-tungstate	30	90	
		100	90	
5220	Mg-aluminate	30	89	
		50	89	
5220	Mg-silicate	30	89	
		50	89	
3551	Zn-tungstate	10	88	θ
		40	88	
4097	Zn-silicate	10	85	
		20	85	
5223	Zn-aluminate	1	88	ε
		10	88	
5622	Fe-sesquioxide	20	83	
		50	83	
5887	Mn-oxide (-ous, -ic)	20	86	ω
		40	86	
5906	Al-oxide VI	50	82	
		100	82	
5611	Bi-sulfide	50	80	ω
		80	80	
5874	Hg-sulfide (ic)	10	91	
		40	91	
5623	Al-oxide Id <sub>3</sub>	120	78	ς
		150	78	
4653	Al-oxide II	80	60	
		120	60	
5877	Al-oxide VIII	200	64	η
		300	64	
3859	Al-oxide IIIb <sub>2</sub>	80	78	
		180	78	
8703	Al-oxide IIIb <sub>6</sub>	100	74	α + θ
		200	74	
3859	Al-oxide IVb <sub>2</sub>	80	78	
		180	78	
3861	Al-oxide IVb <sub>6</sub>	100	72	α + θ
		250	72	
5568	Al-oxide Ib <sub>1</sub>	100	81	
		250	81	
5570	Al-oxide Ib <sub>2</sub>	100	79	α + θ
		250	79	
3858	Al-oxide b <sub>6</sub>	100	89	
		250	89	
5621	Al-oxide Id <sub>0</sub>	150	68	ξ + ω
		300	68	
5573	Al-oxide Id <sub>2</sub>	100	80	
		150	80	
5875	Al-oxide VII	100	84	ξ + ω
		200	84	

\* as determined by matching experiments (pp. 2031-2033.).

former independent of the concentrations of the latter. In contrast, adsorption of  $\lambda$ -factor (by Co-carbonate) before and after partial adsorption by Al-oxide I, gave converging curves (Fig. 1C). Adsorption by Al-oxide I before and after preadsorption of  $\lambda$ -factor (by Co-tungstate) gave the curves shown in Fig. 1E, of which sections one (0– $a$ ) are converging whereas the following sections ( $a$ – $b$  and  $b$ – $c$ ) are parallel. The factors adsorbed by Al-oxide I are therefore also different from  $\lambda$ -factor, and their activities independent of the concentration of the latter, except for a factor completely adsorbed by Al-oxide I at  $a$ . This factor appears to act as a synergist for  $\lambda$ -factor, and *vice versa*.

Thus, the three (groups of) labile factors adsorbed more or less simultaneously by Al-oxide I, appeared to be different from the factors adsorbed by the selective adsorbents tested previously.

*Adsorption by more selective adsorbents.* For the purpose of separate adsorption of the factors adsorbed by Al-oxide I, a number of other adsorbents were tested. A summary of the results of a preliminary testing of these adsorbents is presented in Table 1. The selective properties of the adsorbents was demonstrated by the partial adsorption obtained.

More detailed studies revealed that with most adsorbents labile factor activity was adsorbed proportionally to the amounts of adsorbent added, up to a certain concentration of adsorbent beyond which no further adsorption of activity was obtained. As in previous studies with selective adsorbents<sup>1,2,4</sup>, the labile factor activity of plasmas adsorbed once by adequate amounts of an adsorbent, could not be reduced further by renewed treatment with the same adsorbent. Adsorption curves obtained with five different adsorbents are shown in Figs. 2 (A–E). Similar curves were obtained with all the other adsor-

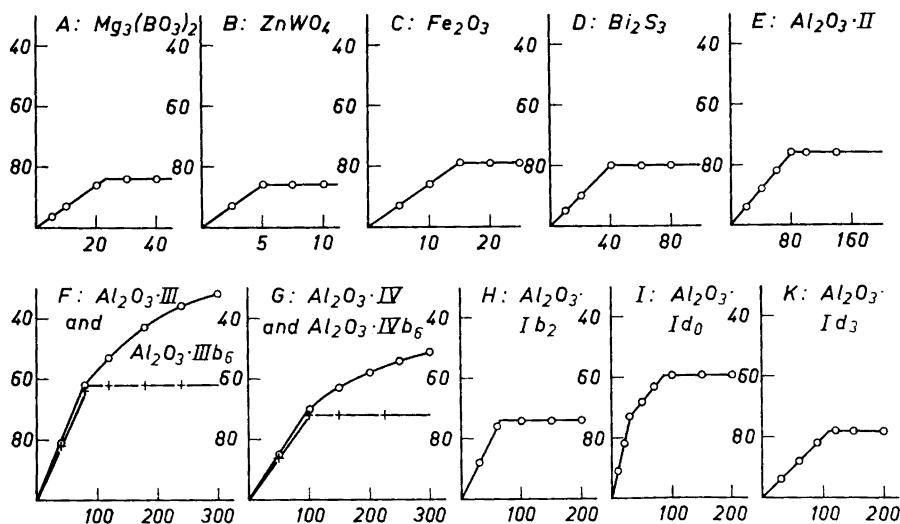


Fig. 2. Adsorption of labile factor activity by A: Mg-borate, B: Zn-tungstate, C: Fe-oxide, D: Bi-sulfide, E: Al-oxide II, F: Al-oxides III (O) and IIIb<sub>6</sub> (+), G: Al-oxide IV (O) and IVb<sub>6</sub> (+), H: Al-oxide Ib<sub>2</sub>, I: Al-oxide Id<sub>0</sub>, and K: Al-oxide Id<sub>3</sub>.

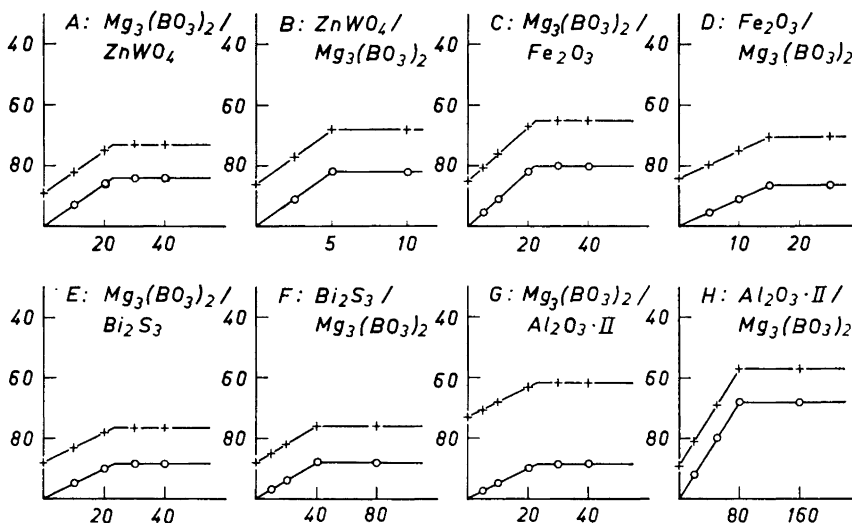


Fig. 3. Adsorption of labile factor activity from untreated (O) and preadsorbed (+) strontium carbonate plasmas.

- A: Mg-borate adsorption, preadsorption by Zn-tungstate (20 mg/ml)  
 B: Zn-tungstate adsorption, preadsorption by Mg-borate (30 mg/ml)  
 C: Mg-borate adsorption, preadsorption by Fe-oxide (20 mg/ml)  
 D: Fe-oxide adsorption, preadsorption by Mg-borate (25 mg/ml)  
 E: Mg-borate adsorption, preadsorption by Bi-sulfide (30 mg/ml)  
 F: Bi-sulfide adsorption, preadsorption by Mg-borate (25 mg/ml)  
 G: Mg-borate adsorption, preadsorption by Al-oxide II (60 mg/ml)  
 H: Al-oxide II adsorption, preadsorption by Mg-borate (25 mg/ml)

bents tested, except the Al-oxides III and IV, and the  $d_0$  and  $d_2$  modifications of Al-oxide I. Hydrochloric acid treatment of the Al-oxides III and IV changed these preparations into selective adsorbents (Figs. 2F and 2G). Acid treatment of Al-oxide I also resulted in preparations with adsorption properties markedly different from the properties of the original material. Single-sectioned rectilinear adsorption curves were obtained with the  $b_1$ ,  $b_2$  (Fig. 2H),  $b_6$  and  $d_3$  (Fig. 2K) modifications of Al-oxide I, whereas the  $d_0$  and  $d_2$  modifications of this Al-oxide gave adsorption curves composed of two rectilinear sections as shown in Fig. 2I.

The adsorption properties of the adsorbents of Table 1 were compared in matching experiments. Representative experiments with eight adsorbents are shown in Figs. 3–6 (A–H). In agreement with our interpretations of similar previous experiments (p. 2028 and Refs.<sup>1,2,4</sup>) parallel curves (Figs. 3–5 (A–H) and 6 (A–D)) were interpreted as the results of matching experiments with compounds adsorbing different labile factors, the activities of which are mutually independent. Converging curves (Figs. 6E and 6F) were interpreted as the results of experiments with compounds adsorbing different labile factors, the specific activity of each of which is increased in the presence of the other (synergism). Two adsorbents giving the same maximal reduction of labile

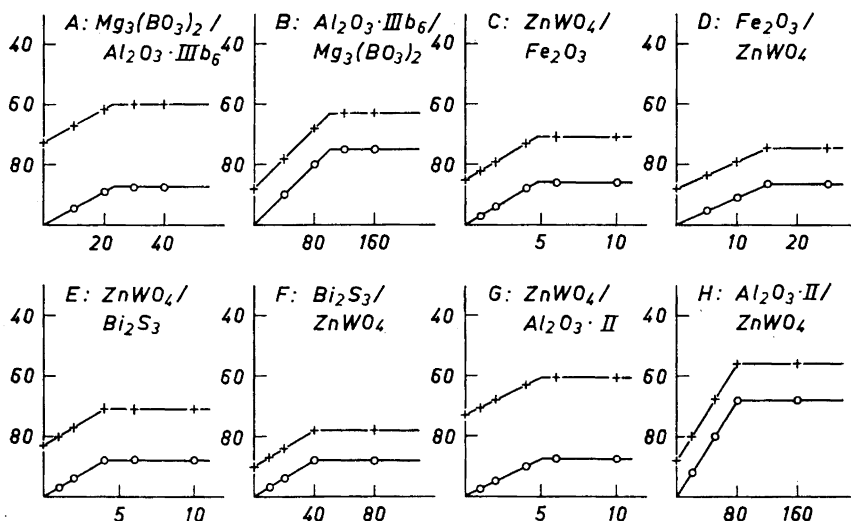


Fig. 4. Adsorption of labile factor activity from untreated (O) and preadsorbed (+) strontium carbonate plasmas.

- A: Mg-borate adsorption, preadsorption by Al-oxide IIIb<sub>6</sub> (80 mg/ml).  
 B: Al-oxide IIIb<sub>6</sub> adsorption, preadsorption by Mg-borate (30 mg/ml).  
 C: Zn-tungstate adsorption, preadsorption by Fe-oxide (20 mg/ml).  
 D: Fe-oxide adsorption, preadsorption by Zn-tungstate (7.5 mg/ml).  
 E: Zn-tungstate adsorption, preadsorption by Bi-sulfide (30 mg/ml).  
 F: Bi-sulfide adsorption, preadsorption by Zn-tungstate (7.5 mg/ml).  
 G: Zn-tungstate adsorption, preadsorption by Al-oxide II (60 mg/ml).  
 H: Al-oxide II adsorption, preadsorption by Zn-tungstate (7.5 mg/ml).

factor activity, were considered to adsorb the same factor when readsorption by one of the adsorbents had no effect on the activity of plasma adsorbed by adequate amounts of the other (Figs. 6G and 6H).

It could thus be concluded that the labile factor adsorbed by Bi-sulfide was identical with the factor adsorbed by Al-oxide Id<sub>3</sub>, and that the activity adsorbed by Ba-metaborate was due to a factor also adsorbable by Mg-borate. On the other hand, the labile factor activities adsorbed by Mg-borate (or Ba-metaborate), Zn-tungstate, Fe-oxide, Bi-sulfide (or Al-oxide Id<sub>3</sub>), Al-oxide II and Al-oxide IIIb<sub>6</sub> were due to different labile factors. The activities of these factors proved to be mutually independent (parallel curves), the only exception being the factors adsorbed by the Al-oxides II and IIIb<sub>6</sub> which had synergistic effects (converging curves). For these factors we have adopted the following terms:  $\alpha$  (alpha)-factor, selectively adsorbed by Mg-borate (or Ba-metaborate);  $\vartheta$  (theta)-factor, selectively adsorbed by Zn-tungstate;  $\xi$  (xi)-factor, selectively adsorbed by Fe-oxide;  $\omega$  (omega)-factor, selectively adsorbed by Bi-sulfide (or Al-oxide Id<sub>3</sub>);  $\zeta$  (zeta)-factor, selectively adsorbed by Al-oxide II and  $\eta$  (eta)-factor, selectively adsorbed by Al-oxide IIIb<sub>6</sub>.

In similar experiments, the effect of the other adsorbents were compared to that of the adsorbents mentioned above. Figs. 7A and 7B show that the



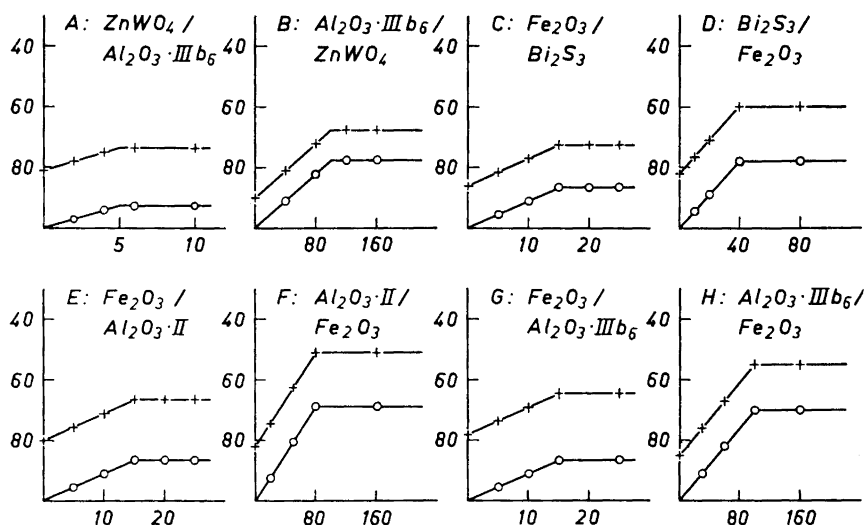


Fig. 5. Adsorption of labile factor activity from untreated (O) and preadsorbed (+) strontium carbonate plasmas.

- A: Zn-tungstate adsorption, preadsorption by Al-oxide IIIb<sub>6</sub> (80 mg/ml).  
 B: Al-oxide IIIb<sub>6</sub> adsorption, preadsorption by Zn-tungstate (10 mg/ml).  
 C: Fe-oxide adsorption, preadsorption by Bi-sulfide (30 mg/ml).  
 D: Bi-sulfide adsorption, preadsorption by Fe-oxide (20 mg/ml).  
 E: Fe-oxide adsorption, preadsorption by Al-oxide II (60 mg/ml).  
 F: Al-oxide II adsorption, preadsorption by Fe-oxide (20 mg/ml).  
 G: Fe-oxide adsorption, preadsorption by Al-oxide IIIb<sub>6</sub> (80 mg/ml).  
 H: Al-oxide IIIb<sub>6</sub> adsorption, preadsorption by Fe-oxide (20 mg/ml).

factor completely adsorbed by Al-oxide Id<sub>0</sub> at *a*, was identical with  $\omega$ -factor, whereas the factor completely adsorbed at *b* was identical with  $\xi$ -factor. The effect of Al-oxide Id<sub>0</sub> is thus explained as independent adsorption of the  $\omega$ - and  $\xi$ -factors. Figs. 7C and 7D (simultaneous experiments with the same plasma) demonstrate that the activity adsorbed by Al-oxide Ib<sub>2</sub> was equal to the combined activities of the factors adsorbed by Mg-borate and Zn-tungstate, and that the same adsorbents had no effect on the labile factor activity of Al-oxide Ib<sub>2</sub> preadsorbed plasma. It was concluded that the effect of Al-oxide Ib<sub>2</sub> was due to adsorption of the  $\alpha$ - and  $\vartheta$ -factors. In similar experiments (Figs. 7E and 7F) it was shown that Al-oxide VII adsorbed the  $\omega$ - and  $\xi$ -factors simultaneously. Al-oxide VIII adsorbed a factor acting as a synergist for  $\eta$ -factor (Fig. 7G). This factor proved to be  $\zeta$ -factor (Fig. 7H). By the same technique the labile factors adsorbed by the remaining adsorbents of Table 1 were identified. The final results of the matching experiments are shown in the last column of this table.

As in previous experiments with selective adsorbents<sup>1,2,4</sup>, the relative activity of an adsorbable factor varied in different experiments with the same adsorbents or with compounds shown to adsorb the same labile factor. Differences in the sensitivity of the substrate plasmas to adsorbable and non-adsor-

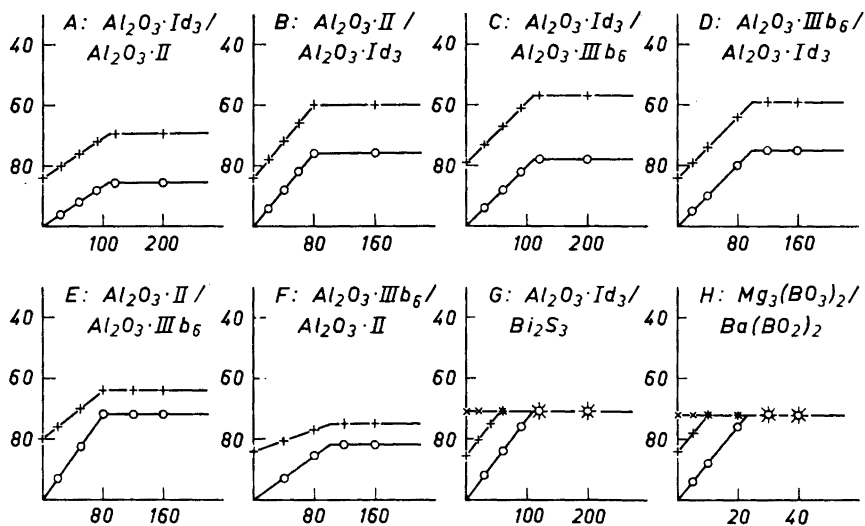


Fig. 6. Adsorption of labile factor activity from untreated (O) and preadsorbed (+ and  $\times$ ) strontium carbonate plasmas.

- A: Al-oxide  $\text{Id}_3$  adsorption, preadsorption by Al-oxide II (60 mg/ml).  
 B: Al-oxide II adsorption, preadsorption by Al-oxide  $\text{Id}_3$  (70 mg/ml).  
 C: Al-oxide  $\text{Id}_3$  adsorption, preadsorption by Al-oxide  $\text{IIIb}_6$  (70 mg/ml).  
 D: Al-oxide  $\text{IIIb}_6$  adsorption, preadsorption by Al-oxide  $\text{Id}_3$  (80 mg/ml).  
 E: Al-oxide II adsorption, preadsorption by Al-oxide  $\text{IIIb}_6$  (50 mg/ml).  
 F: Al-oxide  $\text{IIIb}_6$  adsorption, preadsorption by Al-oxide II (60 mg/ml).  
 G: Al-oxide  $\text{Id}_3$  adsorption, preadsorptions by Bi-sulfide (20 mg/ml: +, and 50 mg/ml:  $\times$ ).  
 H: Mg-borate adsorption, preadsorptions by Ba-metaborate (20 mg/ml: +, and 40 mg/ml:  $\times$ ).

bed factors, and possible differences in the relative concentrations of these factors in the fresh plasmas employed, were regarded as the causes of such variations.

*Identification of the factors adsorbed by Al-oxide I.* Adsorption by the selective adsorbents described above was compared with adsorption by Al-oxide I for an identification of the factors adsorbed by the latter. Figs. 8A and 8B show that the  $\alpha$ - and  $\vartheta$ -factors were no longer present in plasmas preadsorbed by 55 mg/ml of Al-oxide I. The reverse experiments: adsorption by Al-oxide I of labile factor activity from untreated plasmas and plasmas preadsorbed by Mg-borate or Zn-tungstate, are seen in Figs. 8D and 8E. The amounts of Al-oxide I required for complete adsorption of the first of the three (groups of) factors, discernible by this adsorbent, were reduced after preadsorption by Mg-borate and Zn-tungstate (from  $a$  to  $a'$ , a reduction of about 50% in each case). The amounts of Al-oxide I required for complete adsorption of the two other (groups of) factors were not affected by these preadsorptions.

Figs. 8C, 9A and 9B demonstrate that preadsorption by Al-oxide I (50, 60 and 60 mg/ml, respectively) reduced the amounts of Fe-oxide, Bi-sulfide and Al-oxide  $\text{IIIb}_6$  required for complete adsorption of the  $\xi$ -,  $\omega$ -, and  $\eta$ -factors, respectively (by 33, 40 and 40%, respectively). In the reverse experiments

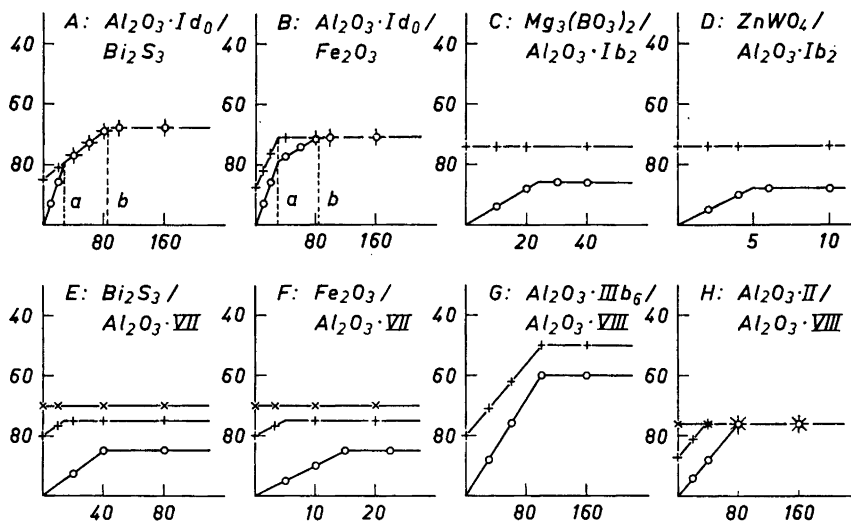


Fig. 7. Adsorption of labile factor activity from untreated (O) and preadsorbed (+ and x) strontium carbonate plasmas.

A: Al-oxide  $Id_0$  adsorption, preadsorption by Bi-sulfide (45 mg/ml).

B: Al-oxide  $Id_0$  adsorption, preadsorption by Fe-oxide (18 mg/ml).

C: Mg-borate adsorption, preadsorption by Al-oxide  $Ib_2$  (80 mg/ml).

D: Zn-tungstate adsorption, preadsorption by Al-oxide  $Ib_2$  (80 mg/ml).

E: Bi-sulfide adsorption, preadsorptions by Al-oxide VII (50 mg/ml: +, and 100 mg/ml: x).

F: Fe-oxide adsorption, preadsorptions by Al-oxide VII (50 mg/ml: +, and 100 mg/ml: x).

G: Al-oxide  $IIIb_6$  adsorption, preadsorption by Al-oxide VIII (80 mg/ml).

H: Al-oxide II adsorption, preadsorptions by Al-oxide VIII (80 mg/ml: + and 160 mg/ml: x).

(Figs. 8F, 9D and 9E), preadsorptions by the same three adsorbents reduced only the amounts of Al-oxide I required for complete adsorption of the second of the three adsorbable (groups of) factors (from  $b$  to  $b'$ , reductions of 29, 29 and 42 %, respectively).

Preadsorption by Al-oxide I (75 mg/ml) also reduced (by 25 %) the amounts of Al-oxide II required for complete adsorption of  $\zeta$ -factor (Fig. 9C), and the factor completely adsorbed by Al-oxide I at the end of the third section (at  $c$ ) of the curve for untreated plasma, was no longer present after preadsorption by Al-oxide II (Fig. 9F).

In confirmation of the above results, Fig. 10A shows that the factors completely adsorbed by Al-oxide I at the end of the first section (at  $a$ ) of the curve for untreated plasma, were absent after preadsorption of the  $\alpha$ - and  $\vartheta$ -factors by Al-oxide  $Ib_6$ . Finally, preadsorption of the  $\xi$ -,  $\omega$ -, and  $\eta$ -factors by a mixture of Al-oxide  $Id_0$  and Al-oxide  $IIIb_6$  removed the factors which were completely adsorbed by Al-oxide I at the end of the second section (at  $b$ ) of the curve for untreated plasma (Fig. 10B).

It was therefore concluded that the effect of Al-oxide I on the labile factor activity of strontium carbonate plasma is fully explained and due to adsorp-

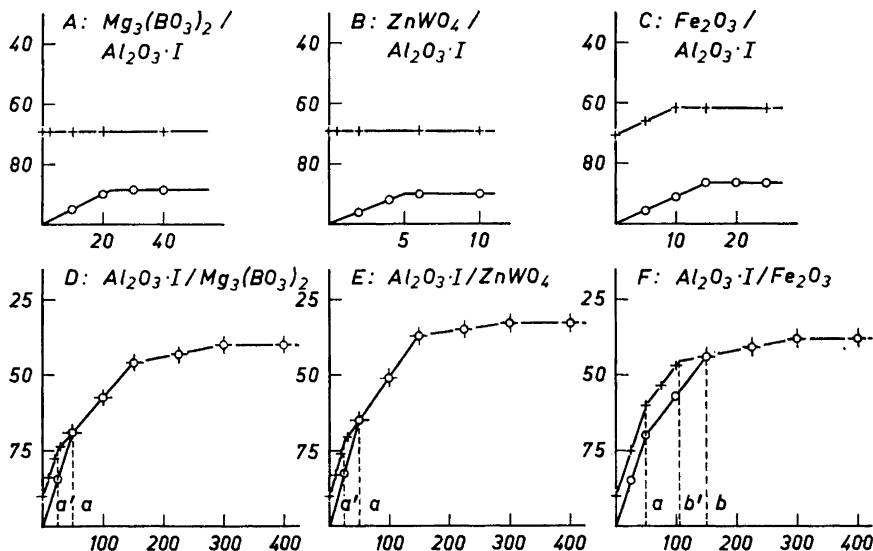


Fig. 8. Adsorption of labile factor activity from untreated (O) and preadsorbed (+) strontium carbonate plasmas.

- A: Mg-borate adsorption, preadsorption by Al-oxide I (55 mg/ml).  
 B: Zn-tungstate adsorption, preadsorption by Al-oxide I (55 mg/ml).  
 C: Fe-oxide adsorption, preadsorption by Al-oxide I (50 mg/ml).  
 D: Al-oxide I adsorption, preadsorption by Mg-borate (30 mg/ml).  
 E: Al-oxide I adsorption, preadsorption by Zn-tungstate (7.5 mg/ml).  
 F: Al-oxide I adsorption, preadsorption by Fe-oxide (18 mg/ml).

tion of the six labile factors adsorbed separately by Mg-borate, Zn-tungstate, Fe-oxide, Bi-sulfide, Al-oxide IIIb<sub>6</sub> and Al-oxide II.

*Additional matching experiments.* The non-identity of the factors adsorbed by Al-oxide I and the factors adsorbed by the selective adsorbents used in the preceding papers was disclosed by the experiments cited on p. 2028 (Figs. 1 B–E). In confirmation of these results and for an identification of the factor acting as a synergist for  $\lambda$ -factor, the selective  $\rho$ -,  $\nu$ -,  $\mu$ -,  $\pi$ -,  $\gamma$ -,  $\beta$ -,  $\varepsilon$ -, and  $\lambda$ -factor adsorbents were compared with the adsorbents for separate adsorption of the  $\alpha$ -,  $\vartheta$ -,  $\xi$ -,  $\omega$ -,  $\eta$ -, and  $\zeta$ -factors, in a series of additional matching experiments. Of the ninety-six different experiments carried out with adsorbent pairs from these two groups of adsorbents, only four are shown here because of space limitations. Parallel curves, similar to those shown in Figs. 10C and 10D, were obtained in ninety-four of these experiments. Selective adsorbents for the  $\alpha$ -factor and for the  $\lambda$ -factor activities were the only adsorbent pairs giving converging curves (Figs. 10E and 10F). These experiments permitted identification of  $\alpha$ -factor with the Al-oxide I adsorbable factor acting as a synergist for  $\lambda$ -factor.

*The labile factor activities measured by RVV-cephalin and RVV.* The effects of the adsorbents described in this paper were also studied in test systems

Table 2. Labile factors measured by RVV-cephalin or RVV.

Treatment of strontium carbonate plasma		Labile factor adsorbed	Labile factor activity in % of strontium carbonate plasma			
Adsorbent	mg/ml		Tpl.		RVV-ceph.	RVV
Untreated			100 <sup>a</sup>	100 <sup>b</sup>	100 <sup>a</sup>	100 <sup>b</sup>
Mg-borate	{ 10 20 30 40	}	91.5	93	100	100
			83	86	—	—
			80.5	84	—	—
			80.5	84	100	100
Untreated			100 <sup>a</sup>	100 <sup>b</sup>	100 <sup>a</sup>	100 <sup>b</sup>
Zn-tungstate	{ 2 4 6 10	}	95	94	100	100
			90	88	—	—
			87.5	85	—	—
			87.5	85	100	100
Untreated			100 <sup>c</sup>	100 <sup>c</sup>	100 <sup>c</sup>	100 <sup>c</sup>
Fe-sesquioxide	{ 5 10 15 20 30	}	94	95	95	96
			88	90	90	92
			82	85	85	88
			82	85	85	88
			82	85	85	88
Untreated			100 <sup>d</sup>	100 <sup>d</sup>	100 <sup>d</sup>	100 <sup>d</sup>
Bi-sulfide	{ 10 20 30 40 60	}	96	96.5	96.5	97
			92	93	93	94
			88	89.5	89.5	91
			84	86	86	88
			84	86	86	88
Untreated			100 <sup>e</sup>	100 <sup>e</sup>	100 <sup>e</sup>	100 <sup>e</sup>
Al-oxide II	{ 20 40 60 80 120	}	92	90	90	89.5
			84	80	80	79
			76	70	70	68.5
			68	60	60	58
			68	60	60	58
Untreated			100 <sup>f</sup>	100 <sup>f</sup>	100 <sup>f</sup>	100 <sup>f</sup>
Al-oxide IIIb <sub>6</sub>	{ 25 50 75 100 140	}	89	88.5	88.5	87.5
			78	77	77	75
			67	65.5	65.5	62.5
			56	54	54	50
			56	54	54	50

<sup>a</sup>: plasma No. 5050,  
<sup>d</sup>: plasma No. 3860,

<sup>b</sup>: plasma No. 3591,  
<sup>e</sup>: plasma No. 3856,

<sup>c</sup>: plasma No. 3862  
<sup>f</sup>: plasma No. 3857

where thromboplastin was replaced by RVV, with or without added cephalin<sup>1,2,4</sup>.

Representative experiments are summarized in Table 2. Adsorption of  $\alpha$ -factor (by Mg-borate) or  $\vartheta$ -factor (by Zn-tungstate) had no effect on the labile factor activities measured by RVV-cephalin or RVV alone. In contrast, adsorption of  $\xi$ -factor (by Fe-oxide), of  $\omega$ -factor (by Bi-sulfide), of  $\eta$ -factor (by

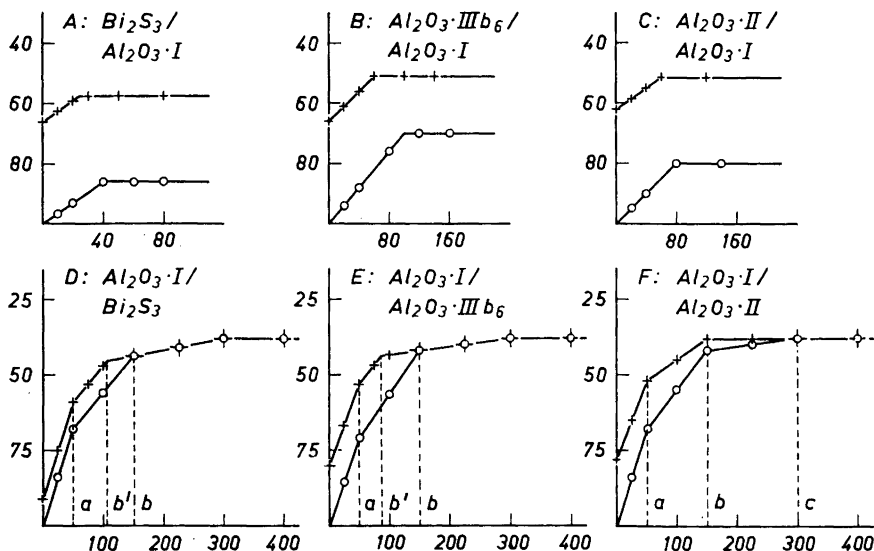


Fig. 9. Adsorption of labile factor activity from untreated (O) and preadsorbed (+) strontium carbonate plasmas.

- A: Bi-sulfide adsorption, preadsorption by Al-oxide I (60 mg/ml).  
 B: Al-oxide IIIb<sub>6</sub> adsorption, preadsorption by Al-oxide I (60 mg/ml).  
 C: Al-oxide II adsorption, preadsorption by Al-oxide I (75 mg/ml).  
 D: Al-oxide I adsorption, preadsorption by Bi-sulfide (45 mg/ml).  
 E: Al-oxide I adsorption, preadsorption by Al-oxide IIIb<sub>6</sub> (100 mg/ml).  
 F: Al-oxide I adsorption, preadsorption by Al-oxide II (90 mg/ml).

Al-oxide IIIb<sub>6</sub>), and of ζ-factor (by Al-oxide II) reduced the labile factor activity measured by all three coagulation accelerators, maximal reduction of activity being obtained at the same concentration of adsorbent. The ξ-, ω-, η-, and ζ-factors are therefore regarded as common components of the labile factor activities measured in the three different assay systems, whereas the α- and ϑ-factors must be thromboplastin specific labile coagulation factors.

#### DISCUSSION

The purpose of the experiments reported here was to find adsorbents which would permit characterization, by selective adsorption, of labile coagulation factors not adsorbed by the selective adsorbents tested previously<sup>1,2</sup>. The adsorption studies presented in the first part of this work indicated that this could be attained by use of Al-oxide I. However, Al-oxide I was not ideally suited for the purpose. The three-sectioned adsorption curve obtained with this adsorbent, pointed to the presence of three different "types" of adsorption sites in Al-oxide I, each causing selective adsorption of one or a few labile factors. A more satisfactory solution was reached only after tedious studies with other adsorbents which finally led to the recognition of no less than six different categories of selective adsorbents, each adsorbing only part of the Al-oxide I adsorbable activity. By means of these selective adsorbents

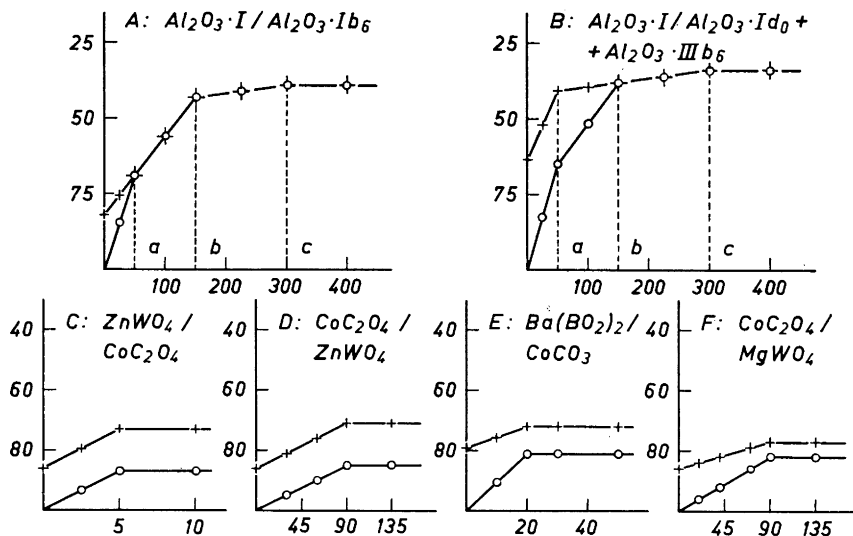


Fig. 10. Adsorption of labile factor activity from untreated (O) and preadsorbed (+) strontium carbonate plasmas.

- A: Al-oxide I adsorption, preadsorption by Al-oxide  $Ib_6$  (90 mg/ml).  
 B: Al-oxide I adsorption, preadsorption by Al-oxide  $Id_0$  (95 mg/ml) and Al-oxide  $IIIb_6$  (105 mg/ml).  
 C: Zn-tungstate adsorption, preadsorption by Co-oxalate (100 mg/ml).  
 D: Co-oxalate adsorption preadsorption by Zn-tungstate (10 mg/ml).  
 E: Ba-metaborate adsorption, preadsorption by Co-carbonate (100 mg/ml).  
 F: Co-oxalate adsorption, preadsorption by Mg-tungstate (30 mg/ml).

recognition and characterization of six different Al-oxide I adsorbable labile factors was achieved. The three-sectioned adsorption curve obtained with Al-oxide I, could then be explained as the results of: (1) competitive adsorption of the  $\alpha$ - and  $\vartheta$ -factors by one of the three different "types" of adsorption sites on Al-oxide I, (2) competitive adsorption of the  $\xi$ -,  $\omega$ -, and  $\eta$ -factors by a second "type", and (3) selective adsorption of  $\zeta$ -factor by a third "type" of adsorption sites. Moreover, the six categories of selective adsorbents permitted studies on the interrelationships between each of these six labile factors and the factors adsorbed by representatives of the eight categories of selective adsorbents described previously<sup>1,2</sup>. The conclusions reached by corresponding matching experiments with Al-oxide I, concerning the non-identity and activity interrelationships of the two groups of factors were confirmed. Furthermore, the final series of matching experiments permitted identification of  $\alpha$ -factor with the Al-oxide I adsorbable synergist for  $\lambda$ -factor.

The observed differences in the adsorption properties of different brands of alumina may possibly be due to differences in the surface structure of Al-oxide particles prepared by different procedures. The effects of acid treatment on the adsorption properties of the Al-oxides I, III and IV are less readily deciphered. Thus, nitric acid treatment ( $d_0$  and  $d_2$ ) of Al-oxide I led to preparations which gave *independent adsorption* of the  $\xi$ -, and  $\omega$ -factors,

but *no adsorption* of the  $\alpha$ -,  $\vartheta$ -,  $\eta$ -, and  $\zeta$ -factors, while *only the  $\alpha$ -, and  $\vartheta$ -factor adsorption capacity* was retained after similar treatment of Al-oxide I with hydrochloric acid. On the other hand,  *$\eta$ -factor was the only factor* adsorbed by the hydrochloric acid treated Al-oxides IIIb<sub>2(6)</sub> and IVb<sub>2(6)</sub>. No explanation of these phenomena can be suggested by the authors at present.

However, the selective adsorbents described in this paper offered an opportunity for differentiation and characterization of the individual factors which are adsorbed by Al-oxide I. This differentiation, as well as the differentiation of these six factors from the eight factors described previously<sup>1,2</sup>, is thus based on differences in the physico-chemical properties of the factors. However, important differences in the functional properties of the factors, so defined, were also apparent. The specific activities of the  $\xi$ -,  $\omega$ -,  $\eta$ -,  $\zeta$ -, and  $\vartheta$ -factors were independent of the concentrations of each of the eight factors studied previously<sup>1,2</sup>. In this respect, all members of the former group of factors differed functionally from the members of the latter (*cf.* preceding paper<sup>1</sup>).  $\alpha$ -Factor differed functionally from each member of both of these two groups of factors by acting as a synergist for  $\lambda$ -factor, but not for any of the other factors. As for the remaining five factors,  $\xi$ -factor,  $\omega$ -factor and  $\vartheta$ -factor differed from  $\eta$ -factor and from  $\zeta$ -factor by being independent of the concentrations of  $\zeta$ -factor and  $\eta$ -factor, respectively. The synergistic relationship between  $\eta$ -factor and  $\zeta$ -factor reflects the unique functional properties of each of these two factors.

Finally, differences between  $\vartheta$ -factor and the  $\xi$ - and  $\omega$ -factors were revealed by substituting RVV-cephalin for thromboplastin. Only  $\xi$ -factor and  $\omega$ -factor were active in the RVV-cephalin assay system. These factors are accordingly the only strontium carbonate non-adsorbable labile coagulation factors for which no differences in function have been detected so far.

Methods for quantitative estimation of the  $\alpha$ -,  $\vartheta$ -,  $\xi$ -,  $\omega$ -,  $\eta$ -, and  $\zeta$ -factor concentrations in fresh plasma are suggested by the observed proportionality between adsorbed activity and concentration of the selective adsorbent employed. The minimum concentration of a selective adsorbent required for maximal adsorption of activity may be proportional to the concentration of the adsorbable factor and represent an absolute value for the plasma concentration of this factor. As with similar methods, suggested for quantitative estimation of the labile factors described previously<sup>1,2,4</sup>, the values obtained will be independent of the plasma concentrations of non-adsorbed factors. However, due attention should be paid to the possibility that variations in the concentrations of inert plasma factors, adsorbed in competition with the adsorbable labile factor, may be the cause of possible variations in the results obtained by this procedure. These problems will receive further attention in future publications.

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