

## Studies on the Coagulation of Chicken Blood

### VI. Use of Cobalt Carbonate, Barium Phosphate, Lead Carbonate and Lead Phosphate for Differentiation and Assay of Strontium Carbonate Non-adsorbable Labile Factors

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The multiple nature of the labile factor activity of strontium carbonate adsorbed oxalated chicken plasma was revealed by adsorption studies. The results obtained with fifty-two selected crystalline adsorbents are explained as adsorption of four different labile coagulation factors by four different groups of adsorbents, represented by the title compounds. Provisional names for these factors are suggested:  $\lambda$  (lambda)-factor is selectively adsorbed by cobalt carbonate,  $\pi$  (pi)-factor by lead carbonate,  $\beta$  (beta)-factor by lead phosphate, and  $\gamma$  (gamma)-factor by barium phosphate. The activity of  $\lambda$ -factor is independent of the concentrations of the other three factors. The activities of  $\beta$ - and  $\pi$ -factors are also mutually independent, whereas  $\gamma$ -factor acts as a synergist both for  $\pi$ -factor and for  $\beta$ -factor. When Russell's viper venom (RVV), with or without cephalin, is substituted for thromboplastin in the assay system,  $\gamma$ - and  $\pi$ -factors are inactive.  $\beta$ -Factor is active only if RVV is supplemented with cephalin.  $\lambda$ -Factor is part of the labile factor activity measured by all three assay accelerators. Determination of the minimum amount of the adsorbents required for maximal adsorption of labile factor activity is suggested as a possibility for quantitative determination of the adsorbable factors.

It was shown in a previous paper<sup>1</sup> that the reduced labile factor activity of strontium (or barium) carbonate adsorbed oxalated chicken plasma was due to adsorption of two labile coagulation factors. The two factors could be adsorbed separately by other adsorbents:  $\psi$ -factor by calcium carbonate, calcium oxalate or calcium molybdate and  $\sigma$ -factor by barium oxalate, barium chromate or strontium oxalate.

A number of other insoluble inorganic compounds have been tested for adsorption of the strontium carbonate non-adsorbable labile factor activity.

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In most cases only a part of the total activity could be adsorbed by each of these adsorbents. These results indicated that the labile factor activity of strontium carbonate adsorbed oxalated chicken plasma is of a complex nature and may be regarded as the combined activities of a number of separate labile coagulation factors differing characteristically in adsorbability. Our previous experiences with compounds showing partial adsorption of the strontium carbonate non-adsorbable labile factor activity, were presented in a short communication<sup>2</sup>. The present study deals with fifty-two selected adsorbents by means of which four different strontium carbonate non-adsorbable labile coagulation factors can be adsorbed separately.

#### MATERIALS AND METHODS

In addition to methods and materials described previously<sup>1,3</sup> the following materials were used in the present study.

*Strontium carbonate plasma.* Oxalated chicken plasma was adsorbed by strontium carbonate (20–25 mg/ml of a commercial preparation \*) to remove the  $\kappa$ -,  $\delta$ -,  $\varphi$ -,  $\psi$ - and  $\sigma$ -factors completely.

*Adsorbents.* The compounds listed below were used as plasma adsorbents in the present study.

Carbonates of Co and Pb, oxides of W, Sb and Bi, Sn mono- and dioxide, sulfates of Pb and Ba, ferri-ferrocyanide, Pb-metaborate, and phosphates of Ba, Sr, Mg, and Co—all were kindly supplied by the Department of Inorganic Chemistry of The Polytechnic Institute. Other adsorbents were prepared in this laboratory, according to procedures described in Gmelin-Kraut's *Handbuch der Anorganischen Chemie*. These adsorbents were prepared from high purity chemicals by (1) precipitation of aqueous solutions, (2) thermal decomposition of unstable compounds, or (3) reactions between solids in the molten state.

*Oxalates* of Co and Pb were prepared from hot (90°C) aqueous solutions of soluble salts by precipitation with excess of a hot solution of ammonium oxalate and oxalic acid in equimolar amounts.

*Fluorides* of Mg and Sr were prepared by melting a mixture containing 5 parts of magnesium chloride or 6 parts of strontium chloride mixed with 4 parts of sodium fluoride and 4 parts of sodium chloride.

*Borates* of Co and Mn were prepared from the oxide and the carbonate, respectively, by melting with an equimolar mixture of potassium bifluoride and boric acid anhydride.

*Oxides* of Co, Ni and Cd were prepared by thermal decomposition of the carbonates or oxalates at 500–700°C in air. A commercial preparation of Be-oxide was made crystalline by heating with boric acid anhydride at 1200°C.

*Sulfite* of Ca was prepared by SO<sub>2</sub>-gas saturation of an aqueous suspension of calcium carbonate, followed by filtration and heating of the filtrate on a steam bath.

*Sulfides* of As and Sb were prepared from dilute hydrochloric acid solutions of the oxides by precipitation with hydrogen sulfide gas or sodium thiosulfate solution. After washing and drying the products were heated in sealed ampullae at 200°C for at least 48 h.

*Phosphates* of Be, Zr, Th, Fe, Ce and Al \*\* were prepared from the oxides (Be, Zr), oxalate (Th), carbonate (Fe) and sulfates (Ce, Al), respectively, by melting with 5 parts of sodium metaphosphate at 1000°C.

The phosphate of Mn was prepared by heating of a 10 % phosphoric acid solution saturated with manganese carbonate. The phosphate of Pb was prepared from a lead acetate solution by precipitation with sec. sodium phosphate. A crystalline product was obtained by heating of the washed amorphous precipitate suspended in 8–10 % acetic acid, to 90–100°C.

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\*\* Whether ortho- or meta-phosphates were formed by this procedure was not investigated.

*Basic lead chromate* was prepared from a suspension of yellow lead chromate in slightly more than equimolar amounts of sodium hydroxide (as a 5 % (w/v) solution). The suspension was boiled until the colour had changed to bright brick red.

*Silicates* of Be, Mn and Co were prepared from the sulfates (Be, Mn) and the oxide (Co), respectively, by melting with sodium metasilicate and sodium sulfate or boric acid anhydride.

*Aluminates* of Co and Be were prepared by melting sodium chloride (sulfate) and sodium aluminate with metal chloride (sulfate) in excess. After washing and drying the insoluble product was remelted with 5 parts of boric acid anhydride.

*Tungstates* of Ca, Ba, Mn, Fe, Pb, and Co were prepared by melting one part of sodium tungstate with two parts of sodium chloride (sulfate) and a 2–3 fold excess of metal chloride (sulfate). Tungstate of Ce was prepared similarly from cerous sulfate with sodium tungstate in excess.

*Molybdates* of Ba, Pb, and Co were prepared by melting one part of sodium molybdate with two parts of sodium chloride and a 2–3 fold excess of metal chloride.

All preparations were thoroughly washed with distilled water, treated with 96 % ethanol and dried at 80°C overnight before use. The melts were treated with water to remove soluble material and pulverized before the final washing. The adsorbents were obtained as crystalline or micro-crystalline powders. For comparison, amorphous modifications of a few of the adsorbents were also prepared.

## EXPERIMENTS AND RESULTS

*Adsorption of strontium carbonate non-adsorbable labile factors.* Inorganic insoluble compounds were tested for adsorption of the strontium carbonate non-adsorbable labile factor activity as described previously<sup>1</sup>, using strontium carbonate preadsorbed oxalated chicken plasma instead of untreated plasma. The amounts of strontium carbonate used for preadsorption were more than adequate for complete adsorption of the  $\kappa$ -,  $\delta$ -,  $\varphi$ -,  $\psi$ -, and  $\sigma$ -factors. No inhibitory effects could be detected in plasmas treated with these adsorbents, and plasma pH was not changed significantly<sup>1</sup>.

The results of a preliminary testing of the compounds described in this paper, are summarized in Table 1. The adsorbents listed caused only partial adsorption of the labile factor activity of strontium carbonate plasmas and could thus be classified as selective adsorbents<sup>1</sup>.

A detailed study of the effect of the adsorbents revealed that labile factor activity was adsorbed proportionally to the amounts of adsorbent added until, at a certain concentration of adsorbent, no more labile factor activity could be adsorbed by increasing the amounts of adsorbent. Renewed treatment with the same adsorbent caused no further reduction of the labile factor activity. Typical adsorption curves obtained with 8 different adsorbents are shown in Figs. 1 (A–H). All the other adsorbents of Table 1 gave similar adsorption curves.

As pointed out previously<sup>1</sup>, partial adsorbability of the labile factor activity by an adsorbent indicates that the adsorbable and the non-adsorbable activities are not due to the same labile coagulation factors. The labile factor activity of strontium carbonate preadsorbed plasmas may be regarded, therefore, as the combined activity of distinct labile coagulation factors with characteristic differences in adsorbability.

*Matching experiments.* The effects of different adsorbents were compared in a series of matching experiments, carried out as described previously<sup>1</sup>

Table I. Adsorption of labile factor activity from strontium carbonate plasma.

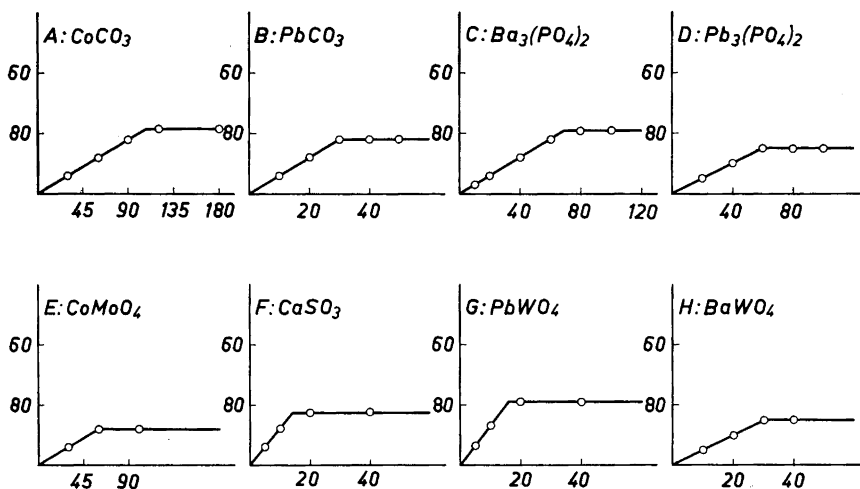
Plasma No.	Adsorbent	mg/ml	Activity in % of strontium carbonate plasma	Labile factor ad-sorbed *	Plasma No.	Adsorbent	mg/ml	Activity in % of strontium carbonate plasma	Labile factor ad-sorbed *
2031	lead carbonate	40	70	π	5351	cobalt aluminate	40	85	λ
3162 a	tungsten trioxide	80	70		5371	barium phosphate	70	85	
	100	89	100		78				
3361	cadmium oxide	20	89		3166	strontium phosphate	10	78	
3375	lead sulfate	100	69		3162 b	magnesium phosphate	60	94	
	20	89	100		79				
3600	lead metaborate	50	89		3163	cobalt phosphate	100	79	
3570	lead oxalate	100	84		1137	manganous phosphate	50	86	
	20	84	100		90				
3523	lead molybdate	100	86		5060 a	cerous phosphate	100	90	
321	lead phosphate	20	84		5060 b	beryllium phosphate	40	84	
	100	76	100		60				
3505	lead tungstate	200	76		5073	ferric phosphate	40	86	
3530	basic lead chromate	50	81		5060 b	aluminium phosphate	60	88	
	20	81	100		88				
1695	cobalt carbonate	80	87	5329 a	thorium phosphate	90	86		
3585	cobalt oxalate	120	60	5329 b	zirconium phosphate	20	86		
	150	60	70	20					
1113	cobalt borate	120	82	3352	antimony trioxide	40	89		
	20	82	50	89					
3554	cobalt molybdate	50	86	3589	bismuth oxide	20	86		
3561	cobalt tungstate	60	86	3361	stannous oxide	100	86		
	120	86	60	69					
3696	cobalt silicate	120	90	3361	stannic oxide	50	85		
	50	78	100	85					
		100	78				85		

\* Determined by matching experiments (pp. 1473-1477).

Table 1 (cont.)

Plasma No.	Adsorbent	mg/ml	Activity in % of strontium carbonate plasma	Labile factor ad-sorbed *	Plasma No.	Adsorbent	mg/ml	Activity in % of strontium carbonate plasma	Labile factor ad-sorbed *
3162 a	cobaltous oxide	20	78	}	3530	calcium tungstate	20	89	}
1979	nickel sesquioxide	100	78		3503	barium tungstate	80	89	
5219	beryllium oxide	20	88		5633	cerous tungstate	20	92	
5569	beryllium oxide	40	79		5889	manganous tungstate	80	89	
5569	arsenic trisulfide	40	79		5890	ferrous tungstate	70	89	
5569	arsenic trisulfide	10	90		5209	beryllium silicate	40	86	
5890	antimony trisulfide	30	90		5889	manganous silicate	40	86	
5890	antimony trisulfide	30	83		3583	barium molybdate	60	86	
3568	calcium sulfite	50	88		5896	manganous borate	20	85	
3568	calcium sulfite	40	88		5209	beryllium aluminate	100	85	
3561	barium sulfate	80	88				40	86	
3561	barium sulfate	50	85				40	86	
3596	ferri-ferro cyanide	100	85				60	86	
457	magnesium fluoride	20	77				20	85	
457	magnesium fluoride	100	77				100	85	
1110	strontium fluoride	100	75				40	86	
1110	strontium fluoride	200	75				60	86	
1110	strontium fluoride	50	92			40	86		
1110	strontium fluoride	100	92			60	86		

\* Determined by matching experiments (pp. 1473-1477).



Figs. 1. Adsorption of labile factor activity.

Ordinates: labile factor activity in % of strontium carbonate plasma.  
Abscissæ: adsorbent in mg/ml.

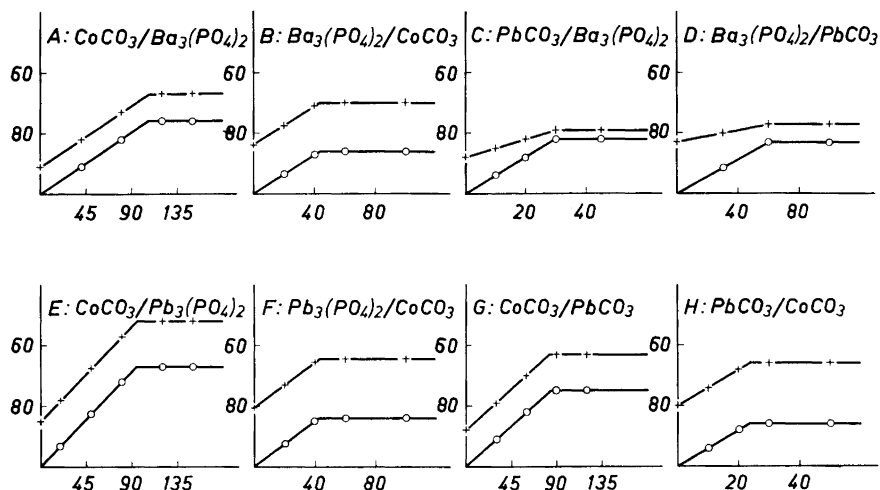
A: cobalt carbonate;  
B: lead carbonate;  
C: barium phosphate;  
D: lead phosphate;

E: cobalt molybdate;  
F: calcium sulfite;  
G: lead tungstate;  
H: barium tungstate.

with strontium carbonate plasma instead of untreated plasma. Representative results of such experiments are shown in Figs. 2—4 (A—H). The effects obtained by use of cobalt carbonate, barium phosphate, lead carbonate and lead phosphate were first compared.

The labile factor activities adsorbed by cobalt carbonate and barium phosphate were not reduced after preadsorption of strontium carbonate plasmas by barium phosphate and cobalt carbonate, respectively (Figs. 2A and 2B), and the minimum amounts of each of the two adsorbents required for maximal adsorption of activity remained unchanged after preadsorption by the other adsorbent. The labile factor activities adsorbed by these adsorbents are therefore due to two different labile coagulation factors, and the activities of the factors are mutually independent. Parallel adsorption curves are thus the results of matching experiments with compounds adsorbing different factors with additive effects.

Experiments with lead carbonate and barium phosphate gave the two sets of curves shown in Figs. 2C and 2D. The minimum amounts of each of these adsorbents necessary for maximal adsorption of activity, were not changed after preadsorption by the other adsorbent, but the adsorbable activities were reduced. The experiments indicate that the activities adsorbed by lead carbonate and barium phosphate are due to two different labile factors, the activity of each of which is increased in the presence of the other. Converging



Figs. 2. Comparison of adsorbents I.

Ordinates: labile factor activity in % of strontium carbonate plasma.

Abscissæ: adsorbent in mg/ml.

O: untreated strontium carbonate plasma.

+ : preadsorbed strontium carbonate plasma.

A: cobalt carbonate adsorption of untreated and barium phosphate (80 mg/ml) preadsorbed plasma.

B: barium phosphate adsorption of untreated and cobalt carbonate (80 mg/ml) preadsorbed plasma.

C: lead carbonate adsorption of untreated and barium phosphate (80 mg/ml) preadsorbed plasma.

D: barium phosphate adsorption of untreated and lead carbonate (40 mg/ml) preadsorbed plasma.

E: cobalt carbonate adsorption of untreated and lead phosphate (70 mg/ml) preadsorbed plasma.

F: lead phosphate adsorption of untreated and cobalt carbonate (100 mg/ml) preadsorbed plasma.

G: cobalt carbonate adsorption of untreated and lead carbonate (40 mg/ml) preadsorbed plasma.

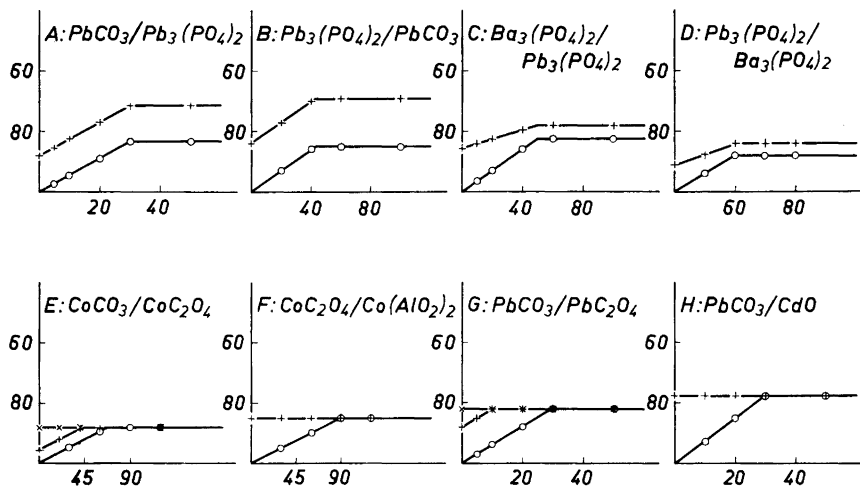
H: lead carbonate adsorption of untreated and cobalt carbonate (120 mg/ml) preadsorbed plasma.

adsorption curves are thus the results of matching experiments with compounds adsorbing two different labile factors with synergistic effects.

The activities adsorbed by cobalt carbonate and lead phosphate were due to two different labile factors with independent activities (Figs. 2E and 2F). The activities adsorbed by cobalt carbonate and lead carbonate were also different and unrelated (Figs. 2G and 2H).

The activity adsorbed by lead carbonate was different from and unrelated to the activity adsorbed by lead phosphate (Figs. 3A and 3B), whereas the activities adsorbed by barium phosphate and lead phosphate are due to two different factors with synergistic effects (Figs. 3C and 3D).

The results obtained by the use of these four adsorbents can thus be explained as adsorption of four different labile coagulation factors. For these factors we have adopted the following terms:



Figs. 3. Comparison of adsorbents II.

Ordinates: labile factor activity in % of strontium carbonate plasma.

Abscissæ: adsorbent in mg/ml.

O: untreated strontium carbonate plasma.

+ and ×: preadsorbed strontium carbonate plasma.

A: lead carbonate adsorption of untreated and lead phosphate (60 mg/ml) preadsorbed plasma.

B: lead phosphate adsorption of untreated and lead carbonate (40 mg/ml) preadsorbed plasma.

C: barium phosphate adsorption of untreated and lead phosphate (60 mg/ml) preadsorbed plasma.

D: lead phosphate adsorption of untreated and barium phosphate (60 mg/ml) preadsorbed plasma.

E: cobalt carbonate adsorption of untreated plasma and plasma preadsorbed by 20 mg/ml (+) and by 60 mg/ml (×) of cobalt oxalate.

F: cobalt oxalate adsorption of untreated and cobalt aluminate (40 mg/ml) preadsorbed plasma.

G: lead carbonate adsorption of untreated plasma and plasma preadsorbed by 10 mg/ml (+) and by 20 mg/ml (×) of lead oxalate.

H: lead carbonate adsorption of untreated and cadmium oxide (10 mg/ml) preadsorbed plasma.

$\lambda$ (lambda)-factor for the labile factor adsorbed by cobalt carbonate,

$\pi$ (pi)-factor for the labile factor adsorbed by lead carbonate,

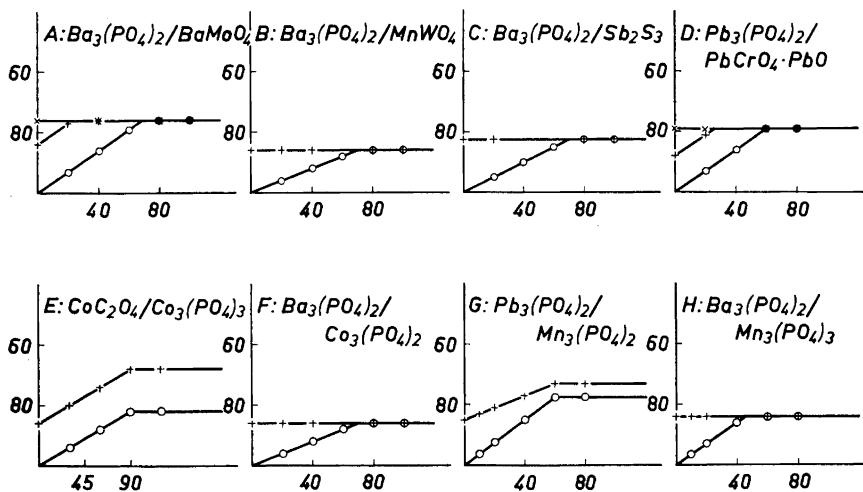
$\gamma$ (gamma)-factor for the labile factor adsorbed by barium phosphate, and

$\beta$ (beta)-factor for the labile factor adsorbed by lead phosphate.

The effects of  $\lambda$ -factor,  $\pi$ -factor and  $\beta$ -factor are apparently purely additive as are the effects of  $\lambda$ -factor and  $\gamma$ -factor. The latter factor, however, appears to act as a synergist both for  $\pi$ -factor and  $\beta$ -factor.

The effects of the other adsorbents could by similar experiments be compared to that of each of these four prototypes of adsorbents.





Figs. 4. Comparison of adsorbents III.

Ordinates: labile factor activity in % of strontium carbonate plasma.

Abcissæ: adsorbent in mg/ml.

O: untreated strontium carbonate plasma.

+ and ×: preadsorbed strontium carbonate plasma.

- A: barium phosphate adsorption of untreated plasma and plasma preadsorbed by 10 mg/ml (+) and by 20 mg/ml (×) of barium molybdate.
- B: barium phosphate adsorption of untreated and manganese tungstate (40 mg/ml) preadsorbed plasma.
- C: barium phosphate adsorption of untreated and antimony trisulfide (30 mg/ml) preadsorbed plasma.
- D: lead phosphate adsorption of untreated plasma and plasma preadsorbed by 30 mg/ml (+) and by 60 mg/ml (×) of basic lead chromate.
- E: cobalt oxalate adsorption of untreated and cobalt phosphate (30 mg/ml) preadsorbed plasma.
- F: barium phosphate adsorption of untreated and cobalt phosphate (30 mg/ml) preadsorbed plasma.
- G: lead phosphate adsorption of untreated and manganese phosphate (50 mg/ml) preadsorbed plasma.
- H: barium phosphate adsorption of untreated and manganese phosphate (50 mg/ml) preadsorbed plasma.

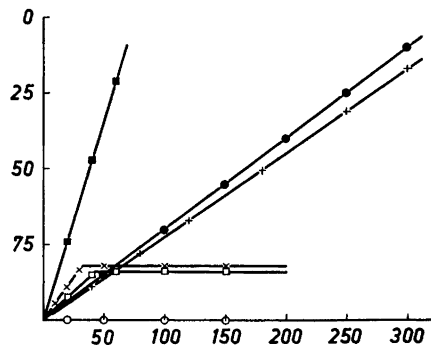
Figs. 3E and 3F show that adsorption by cobalt oxalate and cobalt aluminate can reduce the labile factor activity to the same level as obtained with cobalt carbonate and cobalt oxalate, respectively, and that cobalt carbonate and cobalt oxalate cause no further reduction of the activity of plasmas preadsorbed by adequate amounts of cobalt oxalate or cobalt aluminate, respectively. The effects of the latter two adsorbents are thus due to adsorption of  $\lambda$ -factor.

In the same way it was shown that the effects of lead oxalate or cadmium oxide were due to adsorption of  $\pi$ -factor (Figs. 3G and 3H). Barium molybdate, manganese tungstate and antimony trisulfide were all shown to adsorb  $\gamma$ -factor (Figs. 4 A—C). The factor adsorbed by basic lead chromate was identified as  $\beta$ -factor (Fig. 4D). The factor adsorbed by cobalt phosphate was unrela-

Fig. 5. Differences in effect between crystalline and amorphous adsorbents.

Ordinate: labile factor activity in % of strontium carbonate plasma.

Abscissæ: adsorbent in mg/ml.



○: crystalline barium oxalate, plasma No. 3747.

●: amorphous barium oxalate, plasma No. 3510.

□: crystalline lead phosphate, plasma No. 313.

■: amorphous lead phosphate, plasma No. 222.

×: crystalline lead molybdate, plasma No. 3565.

+ : amorphous lead molybdate, plasma No. 3510.

ted to  $\lambda$ -factor (Fig. 4E) and shown to be  $\gamma$ -factor (Fig. 4F). Manganese phosphate adsorbed a factor, acting as a synergist for  $\beta$ -factor (Fig. 4G). It was identified as  $\gamma$ -factor (Fig. 4H). By the same technique the effects of all the other adsorbents could be identified as adsorption of  $\lambda$ -,  $\pi$ -,  $\gamma$ -, or of  $\beta$ -factor. The results of all these experiments are seen from the last column of Table 1.

From the recorded experiments it is apparent that the ratio of adsorbable to total activity of strontium carbonate plasmas can vary in different experiments with the same adsorbent or with compounds shown to adsorb the same factor. Such variations, previously observed for the relative activities of  $\psi$ - and  $\sigma$ -factors<sup>1</sup>, were ascribed partly to differences in the sensitivity of the substrate plasmas to adsorbable and nonadsorbed factors, and partly to possible differences between individual fresh plasmas in the relative concentrations of these factors. The present discovery of synergistic effects of certain labile factors and purely additive effects of others, shows that the influence of the concentration of non-adsorbed factors on the relative activity of an adsorbable factor is complex. The influence of *increased* concentrations of factors with additive effects is thus equivalent to *decreased* concentrations of factors with synergistic effects.

*Crystalline versus amorphous adsorbents.* It has been stated<sup>4</sup> that selective adsorption of the  $\kappa$ -,  $\delta$ - and  $\varphi$ -factors was accomplished only by crystalline adsorbents, whereas no clear-cut differentiation of these coagulation activities could be obtained with the corresponding amorphous preparations. For the adsorption of labile factor activity similar phenomena were observed as illustrated by the experiments presented in Fig. 5. The differences in effect of crystalline and amorphous preparations of (1) barium oxalate, of (2) lead phosphate, and of (3) lead molybdate on the labile factor activity of strontium carbonate plasma are quite pronounced.

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>b</sup>
Lead phosphate	$\left\{ \begin{array}{l} 20 \\ 40 \\ 60 \\ 80 \end{array} \right.$	$\beta$	93.5	94.5	100
			87	89	100
			80.5	83.5	—
			80.5	83.5	100
Untreated			100 <sup>c</sup>	100 <sup>b</sup>	100 <sup>b</sup>
Cobalt oxalate	$\left\{ \begin{array}{l} 30 \\ 60 \\ 90 \\ 120 \end{array} \right.$	$\lambda$	95	96	94.5
			90	92	89
			85	88	83.5
			85	88	83.5
Untreated			100 <sup>d,e</sup>	100 <sup>d</sup>	100 <sup>e</sup>
Lead carbonate	$\left\{ \begin{array}{l} 10 \\ 20 \\ 30 \\ 40 \end{array} \right.$	$\pi$	94	100	100
			88	—	—
			82	—	—
			82	100	100
Untreated			100 <sup>d,e</sup>	100 <sup>d</sup>	100 <sup>e</sup>
Barium phosphate	$\left\{ \begin{array}{l} 20 \\ 40 \\ 60 \\ 80 \\ 100 \end{array} \right.$	$\gamma$	95	100	100
			90	—	—
			85	—	—
			82.5	100	100
			82.5	100	100

<sup>a</sup> plasma No. 5617; <sup>b</sup> plasma No. 5045; <sup>c</sup> plasma No. 5351; <sup>d</sup> plasma No. 5051; <sup>e</sup> plasma No. 3445.

The labile factor activity measured by RVV \* cephalin or RVV. The effects of the adsorbents were also studied with RVV, with or without added cephalin, replacing thromboplastin in the test system<sup>1,3</sup>. Representative experiments are summarized in Table 2.

Adsorption of  $\beta$ -factor (by lead phosphate) reduced the labile factor activity measured by RVV-cephalin, but not that measured by RVV alone. Maximal adsorption of the activities measured by thromboplastin and RVV-cephalin was obtained with the same concentration of lead phosphate, viz. 60 mg/ml. Adsorption of  $\lambda$ -factor (by cobalt oxalate) reduced the labile factor activities measured by all three assay accelerators, and maximal adsorption of activity was obtained with the same concentration of adsorbent, viz. 90 mg/ml. Removal of  $\pi$ -factor (by lead carbonate) or of  $\gamma$ -factor (by barium phosphate) had no effect on the labile factor activities measured by RVV-cephalin or RVV. By these experiments  $\lambda$ -factor was established as a labile factor common to all three coagulation systems.  $\beta$ -Factor is only active in coagulation systems

\* Russell's viper venom, kindly supplied by The Wellcome Research Laboratories, Langley Court, Beckenham, England.

containing added cephalin, as such or in the form of thromboplastin. The  $\pi$ - and  $\gamma$ -factors must be regarded as thromboplastin specific labile factors.

#### DISCUSSION

The present study is part of a systematic investigation on the effects of adsorbents on the labile factor activity of strontium carbonate preadsorbed oxalated chicken plasma. Partial adsorption of the activity by crystalline adsorbents proved its multiple nature and suggested the use of such adsorbents for characterization of the individual components. By matching experiments fifty-two adsorbents tested in this study were found to belong to four different categories of adsorbents, causing adsorption of four different labile coagulation factors. Differences in physico-chemical properties are thus the basis for regarding these factors as different. Differences in the functions of the factors so defined, were indicated by the observed synergistic effects of certain factors and the merely additive effects of others.  $\lambda$ -Factor is thus functionally different from  $\beta$ - and  $\pi$ -factors because it is not a synergist for  $\gamma$ -factor, and functionally different from  $\gamma$ -factor because of its non-synergistic relations to  $\pi$ - and  $\beta$ -factors.  $\beta$ -Factor and  $\pi$ -factor differ functionally from  $\gamma$ -factor by not being synergists for  $\pi$ -factor or  $\beta$ -factor, respectively.

Further evidence of the functional non-identity of the factors was obtained in experiments with RVV-cephalin or RVV replacing thromboplastin as assay accelerator. Both  $\pi$ -factor and  $\gamma$ -factor were inactive in the RVV-cephalin and RVV assay systems. The activity of  $\beta$ -factor was found to depend on the presence of added cephalin (or thromboplastin).  $\lambda$ -Factor was the only factor active in all three assay systems. These observations and the fact that the effect of each adsorbent can be duplicated by at least two others, appear to rule out the possibility that the results can be due to artifacts.

We have not discovered any simple rules as to which factor is adsorbed by what type of adsorbent.  $\lambda$ -Factor was adsorbed by cobalt compounds only, but cobalt phosphate and cobalt oxide adsorbed  $\gamma$ -factor, sharing this property with a multitude of apparently unrelated compounds.  $\beta$ -Factor was adsorbed by a few lead compounds only. All other lead compounds adsorbed  $\pi$ -factor, duplicating the effects of tungsten and cadmium oxides. The pronounced selective properties of the crystalline adsorbents contrasted sharply with the completely indiscriminate adsorption of labile coagulation factors obtained with amorphous preparations. This is compatible with our previous suggestion<sup>1,5</sup> that selective adsorption of coagulation factors depends on a rigid spatial pattern of molecules in the surface of the adsorbent which "fits" the structure of a characteristic part of the adsorbable factor, permitting adsorption of one or a few factors only.

It has been pointed out previously<sup>1</sup> that in a determination of the relative labile factor activities of different plasmas, the same sample of stored plasma and the same solution of accelerator should be employed. The present results indicate that a comparison of the *activities* adsorbed from two different plasmas by a selected adsorbent, may not permit any conclusions as to the relative *concentrations* of the adsorbable factor since possible differences in adsorbed

activity may be caused by differences in the concentrations of non-adsorbed synergists. Another possibility for quantitative determination of individual labile coagulation factors is suggested by the observed proportionality between adsorbed activity and amounts of adsorbent. The minimum concentration of adsorbent required for maximal adsorption of labile factor activity may thus be proportional to the concentration of the adsorbable factor, and represent an absolute value for the concentration of the factor. Such values will be independent of the concentration of non-adsorbed factors, but may also reflect possible variations in the concentrations of inert plasma factors if such factors are adsorbed in competition with the labile factor.

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