

## Studies on the Coagulation of Chicken Blood

### VII. Use of Nickel Oxalate, Bismuth Oxalate, Cadmium Phosphate and Bismuth Phosphate for Differentiation and Assay of Strontium Carbonate Non-adsorbable Labile Factors

ØYVIND SØRBYE\* and INGER KRUSE

*Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen, Denmark*

Twenty-five crystalline adsorbents were tested for adsorption of labile factor activity from strontium carbonate preadsorbed oxalated chicken plasma and shown to adsorb only a part of the total activity. The adsorbents belong to four different groups, represented by the title compounds, adsorbing four different labile factors for which provisional names are suggested:  $\epsilon$ (epsilon)-factor is selectively adsorbed by nickel oxalate,  $\mu$ (mu)-factor by bismuth oxalate,  $\nu$ (nu)-factor by cadmium phosphate, and  $\rho$ (rho)-factor by bismuth phosphate. These factors are different from the  $\lambda$ -,  $\pi$ -,  $\gamma$ - and  $\beta$ -factors selectively adsorbed by adsorbents tested in a previous study. The activity of each of the eight factors depends on the concentration of only one or two of the other seven. None of the factors appear to be functionally identical.

The  $\epsilon$ -,  $\mu$ -,  $\nu$ -, and  $\rho$ -factors do not contribute to the labile factor activity measured when thromboplastin is replaced by Russell's viper venom, with or without added cephalin. They are regarded as thromboplastin specific labile coagulation factors.

In a previous study on the adsorption of the labile factor activity of strontium carbonate (preadsorbed oxalated chicken) plasma<sup>1</sup>, the results obtained with fifty-two selective adsorbents were explained as adsorption of four different labile coagulation factors:  $\lambda$ -factor, selectively adsorbed by cobalt carbonate and six other cobalt compounds,  $\beta$ -factor, selectively adsorbed by lead phosphate and two other insoluble lead salts,  $\pi$ -factor, selectively adsorbed by lead carbonate and six other adsorbents, and  $\gamma$ -factor, selectively adsorbed by a group of thirty-five different adsorbents, represented by barium phosphate.

\* Fellow of the Royal Norwegian Council for Scientific and Industrial Research.

The present paper deals with the partial adsorption of labile factor activity from strontium carbonate plasma obtained by use of other groups of adsorbents. It will be shown that the observed effects can be ascribed to selective adsorption of four different labile coagulation factors, which differ from the four labile factors described previously<sup>1</sup>.

### MATERIALS AND METHODS

Except for the adsorbents the methods and materials used in the present study were as described previously<sup>1-3</sup>.

The *adsorbents* were prepared in this laboratory from high purity chemicals by adaptation of procedures described in Gmelin-Kraut's *Handbuch der anorganischen Chemie* (cf. preceding paper<sup>1</sup>), with the exception of Cd-sulfide\*, red lead oxide (minium)\* and oxides of Ti and Zr\*\*.

*Oxalate* of Ni was prepared from the chloride or sulfate as described for Co-oxalate<sup>1</sup>. Bi-oxalate was prepared from a bismuth-mannitol solution (8 g of Bi-nitrate ground with 3 g of mannitol, diluted with 200–300 ml of water) by precipitation at 50–60°C with oxalic acid in excess.

*Borate* of Ni was prepared from the oxide as described for Co-borate<sup>1</sup>. Bi-borate was prepared from a bismuth-mannitol solution by precipitation at 50–60°C with a slight excess of Na-tetraborate.

*Oxides* of Ce, Th, and Cr were prepared by melting of Na-chloride mixed with Ce-oxalate, Th-nitrate and K-bichromate, respectively.

*Phosphate* of Ni was prepared from a solution of the sulfate, by precipitation with sec. Na-phosphate. The precipitate was crystalline after 2–3 months at room temperature. Bi-phosphate was prepared from the nitrate dissolved in a small volume of nitric acid (7 N) by addition of sec. Na-phosphate, followed by dilution of the hot solution with ten volumes of distilled water. Cd-phosphate was prepared from a hot solution of the sulfate by precipitation with sec. Na-phosphate. A mixed phosphate ( $\text{Cd}_2(\text{PO}_4)_2 \cdot 2\text{CdHPO}_4$ ) is formed.

*Pyrophosphates* of Ni and Cd were prepared from the sulfates by precipitation with Na-pyrophosphate. The precipitates were dissolved in  $\text{SO}_2$ -water and crystallized by heating of the solutions on a steam bath.

*Phosphate* of Cr was prepared from the oxide by melting with Na-metaphosphate. Whether ortho- or meta-phosphate was formed was not investigated.

*Arsenates* of Cd and Bi were prepared from the sulfate and the nitrate, respectively, with sec. Na-arsenate, in analogy with the procedures for the phosphates.

Ni-arsenate was prepared from the chloride by precipitation with tert.  $\text{NH}_4$ -arsenate. The product was isolated after four months at room temperature.

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

Bi-tungstate was prepared from the oxide by melting with Na-chloride and Na-tungstate in excess.

*Molybdates* of Ni and Cd were prepared from the chlorides as described for Ba-molybdate<sup>1</sup>.

All adsorbents were thoroughly washed and dried as described<sup>1</sup> and finally obtained as crystalline powders.

### EXPERIMENTS AND RESULTS

*Adsorption of strontium carbonate non-adsorbable labile factors.* The adsorbents were tested for adsorption of the labile factor activity of strontium car-

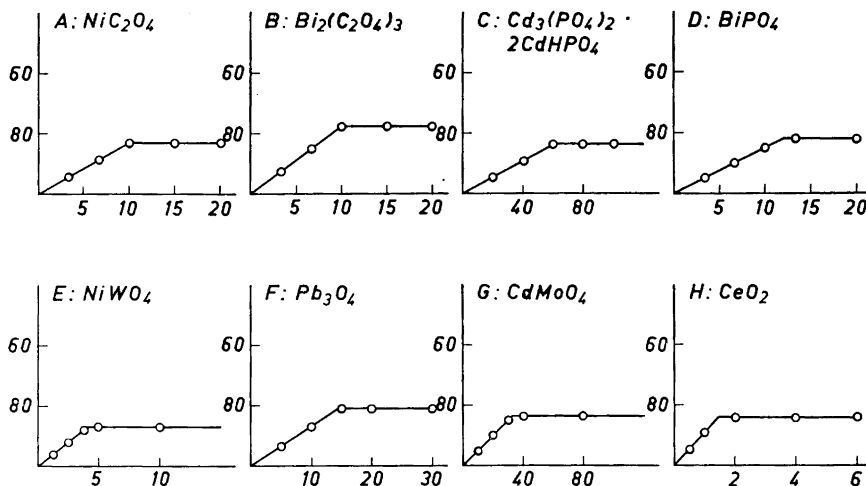
\* kindly supplied by the Department of Inorganic Chemistry, Polytechnic Institute, Copenhagen.

\*\* obtained from H. Struer's chemiske Laboratorium, Copenhagen.

Table 1. Adsorption of labile factor activity from strontium carbonate plasma.

Plasma No.	Adsorbent	mg/ml	Activity in % of strontium carbonate plasma	Labile factor adsorbed *
3585	nickel oxalate	50	82	ε
		100	82	
2436	nickel borate	20	83	
		40	83	
3532	nickel orthophosphate	50	80	
		80	80	
3557	nickel pyrophosphate	20	93	
		80	93	
3554	nickel tungstate	10	85	
		40	85	
3554	nickel molybdate	10	85	
		40	85	
5045	nickel arsenate	10	82	
		20	82	
340	bismuth oxalate	70	91	
		120	91	
621	bismuth borate	50	72	
		150	72	
460	red lead oxide (minium)	30	90	
		50	90	
3080	chromous oxide	50	86	
		80	86	
4419	titanic oxide	40	88	
		80	88	
5227	zirconium oxide	30	92	ν
		50	92	
5227	thorium oxide	30	92	
		50	92	
5575	ceric oxide	10	87	
		20	87	
340	cadmium phosphate	70	85	
		120	85	
3545	cadmium arsenate	60	85	
		100	85	
3545	cadmium pyrophosphate	20	85	ϑ
		60	85	
3551	cadmium molybdate	50	85	
		80	85	
3505	cadmium tungstate	20	93	
		50	93	
5073	chromous phosphate	10	84	
		50	84	
212	bismuth phosphate	20	92	
		100	92	
3543	bismuth arsenate	20	86	ϑ
		40	86	
5604	bismuth tungstate	30	87	
		50	87	
463	cadmium sulfide	10	89	
		30	89	

\* as determined by matching experiments (p. 1665—1669).



Figs. 1. Adsorption of labile factor activity by selective adsorbents. Ordinates: labile factor activity in % of strontium carbonate plasma. Abscissæ: adsorbent in mg/ml. A: nickel oxalate; B: bismuth oxalate; C: cadmium phosphate; D: bismuth phosphate; E: nickel tungstate; F: red lead oxide; G: cadmium molybdate; H: ceric oxide.

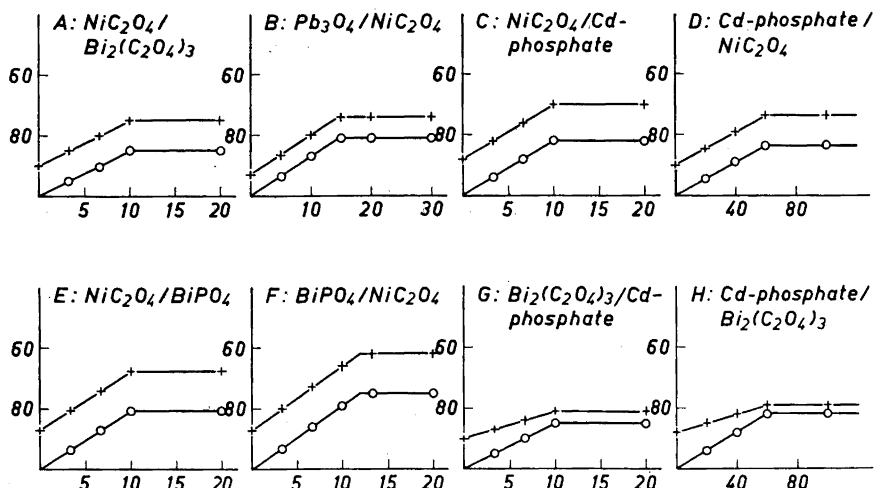
bonate (preadsorbed oxalated chicken) plasma as described in preceding papers<sup>1,3</sup>. No inhibitory effects were detected in the adsorbed plasmas and pH was not significantly altered after adsorption<sup>3</sup>.

A summary of the results of a preliminary testing of the adsorbents described in this paper is presented in Table 1. The adsorbents caused only partial adsorption of the labile factor activity of strontium carbonate plasmas and could thus be classified as selective adsorbents.

A detailed study of the effects of the adsorbents showed that 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. The labile factor activity of plasmas adsorbed once with adequate amounts of a selective adsorbent, could not be reduced further by renewed treatment with the same adsorbent. Adsorption curves obtained with eight different adsorbents are shown in Figs. 1 (A–H). The other adsorbents of Table 1 gave similar adsorption curves. These results were analogous with those obtained by use of the selective adsorbents tested previously<sup>1,3</sup>. The present adsorbents thus offered new possibilities for differentiation of the labile factor activity of strontium carbonate plasma.

*Matching experiments.* The effects of the various adsorbents of Table 1 were compared in a series of matching experiments, carried out as described previously<sup>1,3</sup>. Representative results of these experiments are presented in Figs. 2 and 3 (A–H).

The labile factor activity adsorbed by nickel oxalate was not reduced after preadsorption of strontium carbonate plasma by bismuth oxalate (Fig. 2 A), and the minimum amount of nickel oxalate required for maximal adsorp-



*Figs. 2.* Comparison of adsorbents I. Ordinates: labile factor activity in % of strontium carbonate plasma. Abscissæ: adsorbent in mg/ml.

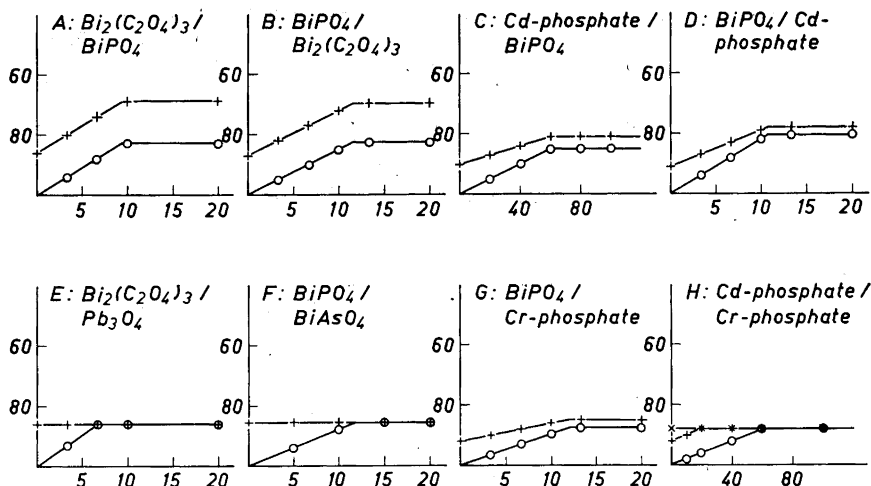
O: untreated strontium carbonate plasma. +: preadsorbed strontium carbonate plasma. A: nickel oxalate adsorption of untreated and bismuth oxalate (10 mg/ml) preadsorbed plasma. B: red lead oxide adsorption of untreated and nickel oxalate (10 mg/ml) preadsorbed plasma. C: nickel oxalate adsorption of untreated and cadmium phosphate (60 mg/ml) preadsorbed plasma. D: cadmium phosphate adsorption of untreated and nickel oxalate (10 mg/ml) preadsorbed plasma. E: nickel oxalate adsorption of untreated and bismuth phosphate (12 mg/ml) preadsorbed plasma. F: bismuth phosphate adsorption of untreated and nickel oxalate (10 mg/ml) preadsorbed plasma. G: bismuth oxalate adsorption of untreated and cadmium phosphate (50 mg/ml) preadsorbed plasma. H: cadmium phosphate adsorption of untreated and bismuth oxalate (10 mg/ml) preadsorbed plasma.

tion of activity remained unchanged after preadsorption by bismuth oxalate. The curves for adsorption of labile factor activity by red lead oxide from untreated and from nickel oxalate preadsorbed strontium carbonate plasmas were also parallel (Fig. 2 B). At the time of the latter experiment red lead oxide was used as adsorbent for the factor which is adsorbed by bismuth oxalate (*cf.* Fig. 3 E).

Parallel adsorption curves were also obtained for the adsorbent pairs: nickel oxalate and cadmium phosphate (Figs. 2 C and 2 D), and for nickel oxalate and bismuth phosphate (Figs. 2 E and 2 F).

As in the experiments reported previously<sup>1,3</sup>, parallel adsorption curves were interpreted as the results of matching experiments with compounds adsorbing two different labile factors, the activities of which are mutually independent. The factor adsorbed by nickel oxalate is thus different from the factors adsorbed by bismuth oxalate (or red lead oxide), cadmium phosphate and bismuth phosphate.

Matching experiments with bismuth oxalate and cadmium phosphate are shown in Fig. 2 G and 2 H. The minimum amounts of each of these two adsorbents, required for maximal adsorption of activity were not changed after



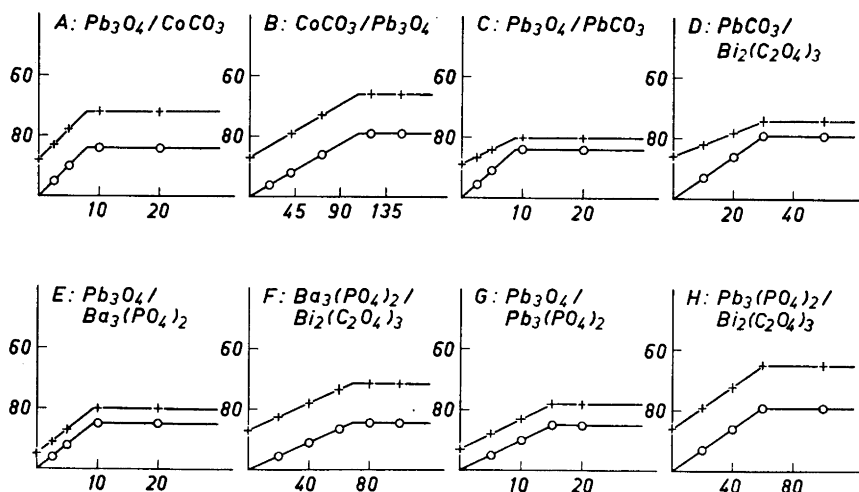
Figs. 3. Comparison of adsorbents II. Ordinates: labile factor activity in % of strontium carbonate plasma. Abscissæ: adsorbent in mg/ml.

O: untreated strontium carbonate plasma. + and ×: preadsorbed strontium carbonate plasma. A: bismuth oxalate adsorption of untreated and bismuth phosphate (10 mg/ml) preadsorbed plasma. B: bismuth phosphate adsorption of untreated and bismuth oxalate (10 mg/ml) preadsorbed plasma. C: cadmium phosphate adsorption of untreated and bismuth phosphate (10 mg/ml) preadsorbed plasma. D: bismuth phosphate adsorption of untreated and cadmium phosphate (50 mg/ml) preadsorbed plasma. E: bismuth oxalate adsorption of untreated and red lead oxide (15 mg/ml) preadsorbed plasma. F: bismuth phosphate adsorption of untreated and bismuth arsenate (20 mg/ml) preadsorbed plasma. G: bismuth phosphate adsorption of untreated and chromous phosphate (40 mg/ml) preadsorbed plasma. H: cadmium phosphate adsorption of untreated plasma and of plasmas preadsorbed by 40 mg/ml (+) and by 60 mg/ml (×) of chromous phosphate.

preadsorption by the other adsorbent, but the adsorbed activities were reduced. Therefore, in agreement with our previous interpretation of converging adsorption curves<sup>1</sup>, bismuth phosphate and cadmium phosphate are selective adsorbents for two different labile factors, the activity of each of which is increased in the presence of the other. A synergistic relationship thus exists between these factors.

The activities adsorbed by bismuth oxalate and bismuth phosphate were due to two different labile factors with independent activities (Figs. 3 A and 3 B), whereas the two different factors adsorbed by cadmium phosphate and bismuth phosphate (Figs. 3 C and 3 D), had synergistic effects.

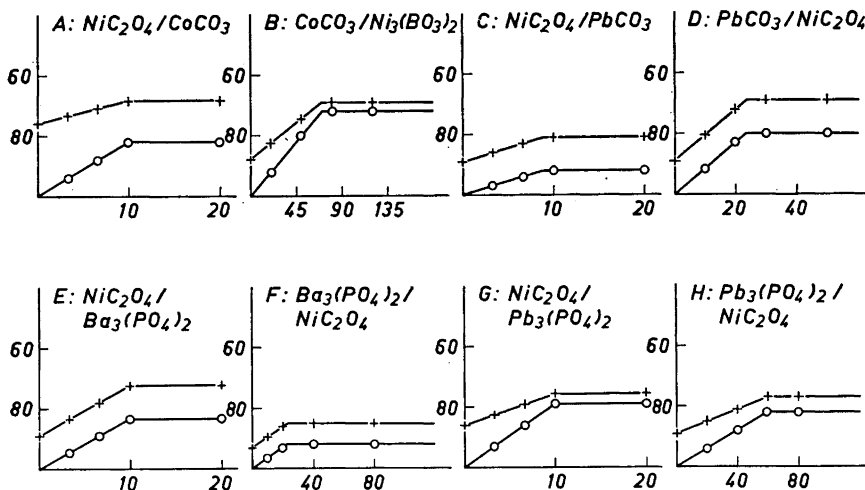
The activity adsorbed by red lead oxide was due to adsorption of the factor which could be adsorbed by bismuth oxalate. Fig. 3 E shows that adsorption by red lead oxide can reduce the labile factor activity to the same level as that obtained by use of bismuth oxalate, and that bismuth oxalate causes no further reduction of the activity of plasma preadsorbed by adequate amounts of red lead oxide. Red lead oxide was therefore, as mentioned above, occasionally used as a substitute for bismuth oxalate.



*Figs. 4.* Comparison of adsorbents III. Ordinates: labile factor activity in % of strontium carbonate plasma. Abscissæ: adsorbent in mg/ml. O: untreated strontium carbonate plasma. +: preadsorbed strontium carbonate plasma. A: red lead oxide adsorption of untreated and cobalt carbonate (100 mg/ml) preadsorbed plasma. B: cobalt carbonate adsorption of untreated and red lead oxide (20 mg/ml) preadsorbed plasma. C: red lead oxide adsorption of untreated and lead carbonate (40 mg/ml) preadsorbed plasma. D: lead carbonate adsorption of untreated and bismuth oxalate (10 mg/ml) preadsorbed plasma. E: red lead oxide adsorption of untreated and barium phosphate (60 mg/ml) preadsorbed plasma. F: barium phosphate adsorption of untreated and bismuth oxalate (10 mg/ml) preadsorbed plasma. G: red lead oxide adsorption of untreated and lead phosphate (60 mg/ml) preadsorbed plasma. H: lead phosphate adsorption of untreated and bismuth oxalate (10 mg/ml) preadsorbed plasma.

The results obtained by use of these five adsorbents can thus be explained as adsorption of four different labile coagulation factors. For these factors we have adopted the following terms:  $\epsilon$ (epsilon)-factor for the labile factor selectively adsorbed by nickel oxalate,  $\mu$ (mu)-factor for the factor selectively adsorbed by bismuth oxalate,  $\nu$ (nu)-factor for the factor selectively adsorbed by cadmium phosphate, and  $\rho$ (rho)-factor for the labile factor selectively adsorbed by bismuth phosphate. The activities of  $\epsilon$ -factor,  $\mu$ -factor and  $\rho$ -factor are apparently mutually independent as are the activities of  $\epsilon$ -factor and  $\nu$ -factor. The latter factor, however, appears to act as a synergist for  $\mu$ -factor as well as for  $\rho$ -factor.

The effects of the other adsorbents were by similar experiments compared to that of the adsorbents described. Fig. 3 F shows that bismuth arsenate adsorbed  $\rho$ -factor. Chromium phosphate adsorbed a factor acting as a synergist for  $\rho$ -factor (Fig. 3 G). This factor was shown to be  $\nu$ -factor (Fig. 3 H). By the same technique the labile factors adsorbed by the remaining adsorbents of Table 1 were identified. The final results of these matching experiments are shown in the last column of Table 1.



Figs. 5. Comparison of adsorbents IV. Ordinates: labile factor activity in % of strontium carbonate plasma. Abscissæ: adsorbent in mg/ml.

O: untreated strontium carbonate plasma. +: preadsorbed strontium carbonate plasma. A: nickel oxalate adsorption of untreated and cobalt carbonate (80 mg/ml) preadsorbed plasma. B: cobalt carbonate adsorption of untreated and nickel borate (20 mg/ml) preadsorbed plasma. C: nickel oxalate adsorption of untreated and lead carbonate (30 mg/ml) preadsorbed plasma. D: lead carbonate adsorption of untreated and nickel oxalate (20 mg/ml) preadsorbed plasma. E: nickel oxalate adsorption of untreated and barium phosphate (60 mg/ml) preadsorbed plasma. F: barium phosphate adsorption of untreated and nickel oxalate (10 mg/ml) preadsorbed plasma. G: nickel oxalate adsorption of untreated and lead phosphate (60 mg/ml) preadsorbed plasma. H: lead phosphate adsorption of untreated and nickel oxalate (10 mg/ml) preadsorbed plasma.

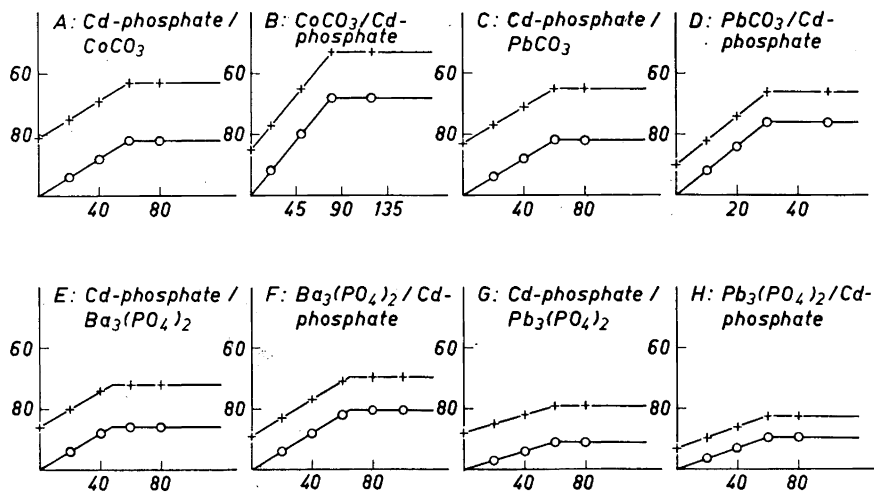
In analogy with previous observations<sup>1,3</sup>, the present experiments show that the relative activity of an adsorbable factor varies in different experiments with the same adsorbents or with compounds shown to adsorb the same factor. As already pointed out<sup>1,3</sup>, such variations may be ascribed partly to differences in the sensitivity of the substrate plasmas to adsorbable and non-adsorbable factors, partly to differences in the relative concentrations of these factors in the fresh plasmas employed.

*Further matching experiments.* The effects of the adsorbents used in this study were also compared with the effects of the adsorbents used previously for selective adsorption of the  $\lambda$ -,  $\pi$ -,  $\gamma$ - and  $\beta$ -factors<sup>1</sup>. The results of these matching experiments are presented in Figs. 4—7 (A—H).

Figs. 4 A and 4 B show that  $\mu$ -factor and  $\lambda$ -factor (selectively adsorbed by cobalt carbonate) are different labile factors. The activities of the two factors are mutually independent.

The non-identity and synergistic effects of  $\mu$ -factor and  $\pi$ -factor (selectively adsorbed by lead carbonate) are seen from Figs. 4 C and 4 D. Figs. 4 E and 4 F, and Figs. 4 G and 4 H show that  $\mu$ -factor is different from  $\gamma$ -factor (selectively adsorbed by barium phosphate) and from  $\beta$ -factor (selectively





*Figs. 6.* Comparison of adsorbents V. Ordinates: labile factor activity in % of strontium carbonate plasma. Abscissæ: adsorbent in mg/ml.

O: untreated strontium carbonate plasma. +: preadsorbed strontium carbonate plasma. A: cadmium phosphate adsorption of untreated and cobalt carbonate (80 mg/ml) preadsorbed plasma. B: cobalt carbonate adsorption of untreated and cadmium phosphate (60 mg/ml) preadsorbed plasma. C: cadmium phosphate adsorption of untreated and lead carbonate (30 mg/ml) preadsorbed plasma. D: lead carbonate adsorption of untreated and cadmium phosphate (60 mg/ml) preadsorbed plasma. E: cadmium phosphate adsorption of untreated and barium phosphate (70 mg/ml) preadsorbed plasma. F: barium phosphate adsorption of untreated and cadmium phosphate (60 mg/ml) preadsorbed plasma. G: cadmium phosphate adsorption of untreated and lead phosphate (40 mg/ml) preadsorbed plasma. H: lead phosphate adsorption of untreated and cadmium phosphate (60 mg/ml) preadsorbed plasma.

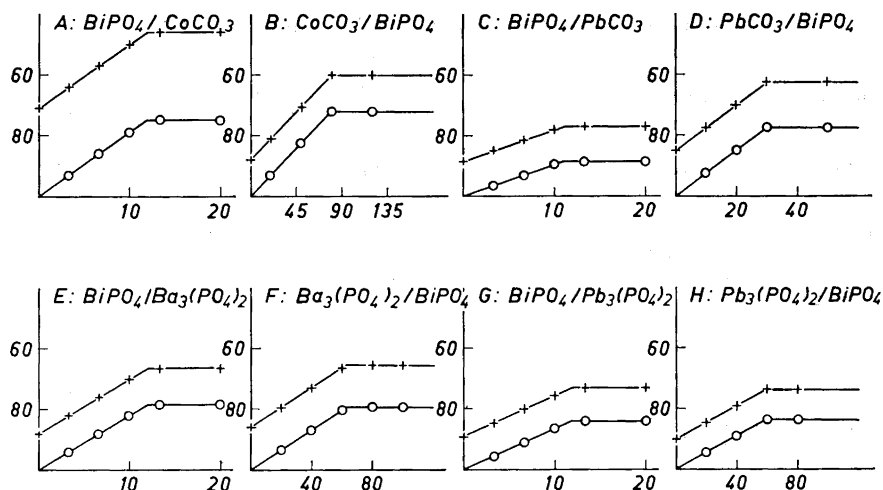
adsorbed by lead phosphate), respectively. The activity of  $\mu$ -factor is independent of the concentrations of the two latter factors, and *vice versa*.

The matching experiments presented in Figs. 5 were obtained with nickel oxalate (or nickel borate) and selective adsorbents for the  $\lambda$ -,  $\pi$ -,  $\gamma$ - and  $\beta$ -factor activities. It is seen that  $\epsilon$ -factor is different from these four factors. It acts as a synergist for  $\lambda$ -factor (Figs. 5 A and 5 B) and for  $\beta$ -factor (Figs. 5 G and 5 H), whereas its activity is independent of the concentrations of  $\pi$ -factor (Figs. 5 C and 5 D) and of  $\gamma$ -factor (Figs. 5 E and 5 F).

Finally,  $\nu$ -factor and  $\rho$ -factor were both shown to be different from  $\lambda$ -factor,  $\pi$ -factor,  $\gamma$ -factor and from  $\beta$ -factor (Figs. 6 and 7 (A-H)). The  $\nu$ - and  $\rho$ -factor activities are independent of the  $\lambda$ -,  $\pi$ -,  $\gamma$ -, and  $\beta$ -factor concentrations.

*The labile factor activities measured by RVV-cephalin\* and RVV.* The effects of the adsorbents described in this paper were also studied with RVV, with or without added cephalin, replacing thromboplastin in the test system<sup>1-3</sup>.

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



Figs. 7. Comparison of adsorbents VI. Ordinates: labile factor activity in % of strontium carbonate plasma. Abscissæ: adsorbent in mg/ml.

O: untreated plasma. +: preadsorbed strontium carbonate plasma. A: bismuth phosphate adsorption of untreated and cobalt carbonate (80 mg/ml) preadsorbed plasma. B: cobalt carbonate adsorption of untreated and bismuth phosphate (12 mg/ml) preadsorbed plasma. C: bismuth phosphate adsorption of untreated and lead carbonate (30 mg/ml) preadsorbed plasma. D: lead carbonate adsorption of untreated and bismuth phosphate (12 mg/ml) preadsorbed plasma. E: bismuth phosphate adsorption of untreated and barium phosphate (70 mg/ml) preadsorbed plasma. F: barium phosphate adsorption of untreated and bismuth phosphate (10 mg/ml) preadsorbed plasma. G: bismuth phosphate adsorption of untreated and lead phosphate (60 mg/ml) preadsorbed plasma. H: lead phosphate adsorption of untreated and bismuth phosphate (12 mg/ml) preadsorbed plasma.

Representative experiments are summarized in Table 2. Adsorption of  $\varepsilon$ -factor (by nickel oxalate), of  $\mu$ -factor (by bismuth oxalate), of  $\nu$ -factor (by cadmium phosphate) or of  $\rho$ -factor (by bismuth phosphate) had no effect on the labile factor activities measured by RVV-cephalin or by RVV alone. These four factors must consequently be regarded as thromboplastin specific labile coagulation factors.

#### DISCUSSION

The present study is a continuation of previous studies<sup>1,3</sup> on the effects of crystalline adsorbents on the labile factor activity of fresh oxalated chicken plasma.

Like the adsorbents described in the preceding study<sup>1</sup>, the present adsorbents adsorb only a part of the total labile factor activity of strontium carbonate preadsorbed oxalated plasma. This is a further demonstration of the complex nature of this activity and lends additional support to the idea that the labile factor activity of fresh plasma represents the combined activities of discrete labile coagulation factors differing characteristically in adsorbability<sup>1,3</sup>. New possibilities for characterization of the individual components

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>
Nickel oxalate	$\left\{ \begin{array}{l} 3 \frac{1}{3} \\ 6 \frac{2}{3} \\ 10 \\ 20 \end{array} \right\}$	ε	95 (95.5)	100	100
			90 (91)	—	—
			85 (86.5)	—	100
			85 (86.5)	100	100
Untreated			100 <sup>a</sup> (100) <sup>b</sup>	100 <sup>a</sup>	100 <sup>b</sup>
Bismuth oxalate	$\left\{ \begin{array}{l} 3 \frac{1}{3} \\ 6 \frac{2}{3} \\ 10 \\ 20 \end{array} \right\}$	μ	95 (95.5)	100	100
			90 (91)	—	—
			85 (86.5)	—	100
			85 (86.5)	100	100
Untreated			100 <sup>c</sup> (100) <sup>d</sup>	100 <sup>c</sup>	100 <sup>d</sup>
Cadmium phosphate	$\left\{ \begin{array}{l} 20 \\ 40 \\ 60 \\ 80 \end{array} \right\}$	ν	95 (96)	100	100
			90 (92)	—	—
			85 (88)	—	100
			85 (88)	100	100
Untreated			100 <sup>c</sup> (100) <sup>d</sup>	100 <sup>c</sup>	100 <sup>d</sup>
Bismuth phosphate	$\left\{ \begin{array}{l} 3 \frac{1}{3} \\ 6 \frac{2}{3} \\ 10 \\ 13 \frac{1}{3} \\ 20 \end{array} \right\}$	ϑ	96 (96)	100	100
			92 (92)	—	—
			88 (88)	—	—
			85.5 (85.5)	100	100
			85.5 (85.5)	100	100

a: plasma No. 5044; b: plasma No. 3589; c: plasma No. 5043; d: plasma No. 3589.

Table 3. Effect of adsorption of single factors on the specific activities of the others.

Adsorption of	Effect on the specific activity of							
	ϑ	ν	μ	π	γ	β	ε	λ
ϑ		+	○	○	○	○	○	○
ν	+		+	○	○	○	○	○
μ	○	+		+	○	○	○	○
π	○	○	+		+	○	○	○
γ	○	○	○	+		+	○	○
β	○	○	○	○	+		+	○
ε	○	○	○	○	○	+		+
λ	○	○	○	○	○	○	+	

Symbols: ○ indicates no change, *i.e.* the factors have independent (additive) effects.  
+ indicates reduced specific activity, *i.e.* the factors have synergistic effects.

of this activity were offered by the twenty-five adsorbents described in the present paper. According to results of matching experiments, these adsorbents belong to four different categories of selective adsorbents, adsorbing four different labile coagulation factors. Comparison of the adsorbents described here, with representatives of the four categories of adsorbents described previously<sup>1</sup>, showed that the factors adsorbed by the former were different from those adsorbed by the latter. Eight different strontium carbonate non-adsorbable labile coagulation factors can thus be distinguished by us of the adsorbents tested until present. This distinction is based on differences in the physico-chemical properties of the individual factors, and does not *per se* permit definite conclusions concerning their possible *functional* non-identity.

However, the factors, thus defined by their adsorbabilities, do appear to be functionally different entities as judged from the observed synergistic effects of certain factors, and the purely additive effects of others. A summary of these interrelationships (Table 3) shows that none of these factors have identical functional properties. Thus,  $\rho$ -factor differs from all other factors by being a synergist for  $\nu$ -factor and for none of the other factors,  $\nu$ -factor is the only factor acting as a synergist for  $\rho$ -factor as well as for  $\mu$ -factor.  $\mu$ -Factor is distinguished by its synergistic effects on the  $\nu$ - and  $\pi$ -factor activities, and  $\pi$ -factor by its synergistic effects on the  $\mu$ - and  $\gamma$ -factor activities. The functional non-identity of the remaining factors is recognized by similar argumentation.

Further evidence for the functional non-identity of some of the factors was obtained by substituting RVV or RVV-cephalin for thromboplastin as assay accelerator. Six of the factors ( $\varepsilon$ -,  $\gamma$ -,  $\pi$ -,  $\mu$ -,  $\nu$ -, and  $\rho$ -) proved inactive in the RVV and the RVV-cephalin assay systems, and the activity of  $\beta$ -factor was shown to depend on the presence of added cephalin<sup>1</sup>.  $\lambda$ -Factor is thus the only one of these eight factors which is a common component of the labile factor activities measured by these three assay systems, and the  $\varepsilon$ -,  $\gamma$ -,  $\pi$ -,  $\mu$ -,  $\nu$ -, and  $\rho$ -factors must be regarded as thromboplastin specific labile factors. As to the relation between human factor V and chicken labile factor activity, the prolonged thromboplastin and RVV-cephalin accelerated coagulation times of congenitally factor V-deficient human plasma<sup>4</sup> indicate that factor V is a rate limiting coagulation factor in both of these two assay systems. Of the eight labile factors detected in fresh chicken plasma, only  $\lambda$ -factor and  $\beta$ -factor represent activities which may possibly correspond to human factor V activity, if the latter is to be regarded as the activity of a single coagulation factor.

A method for quantitative determination of the  $\varepsilon$ -,  $\mu$ -,  $\nu$ -, and  $\rho$ -factor concentrations in fresh plasma is suggested by the observed proportionality between adsorbed labile factor activity and concentration of adsorbent. The minimum concentration of adsorbent required for maximal adsorption of activity may be proportional to the concentration of the adsorbable factor and represent an absolute value for the concentration of this factor. As pointed out previously<sup>1,3</sup>, such values are independent of the concentrations of non-adsorbed factors. However, variations in the concentrations of possible inert plasma factors, adsorbed in competition with the adsorbable labile factor,

must also be considered as a cause of possible variations in the results obtained by this procedure.

In some experiments (unpublished data) the combined activity of the eight labile factors did not account for more than 40 % of the total labile factor activity measured with thromboplastin. Factors differing from those adsorbed by the adsorbents tested until present, are also responsible for the activities measured by RVV and RVV-cephalin, which are not due to  $\lambda$ -factor and the combined activity of the  $\lambda$ - and  $\beta$ -factors, respectively. Selective adsorption of these factors by other categories of adsorbents will be reported in a forthcoming paper.

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