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Studies on the Coagulation of Chicken Blood

XI. Determination of the φ_1- and φ_2-Factor Concentrations in Plasma, and Detection of Unidentified Dietary Factors Essential for Maximum Level of φ_2-Factor

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A method for quantitative determination of the φ_1-factor concentration of oxalated chicken plasma is presented. The method is analogous to the calcium carbonate "adsorption titration" method [1] for determination of the φ_2-factor concentration.

Nutritional studies show that the φ_1-factor concentration depends on vitamin K. No unidentified dietary requirements were discovered for this factor. φ_2-Factor concentration depends on two unidentified dietary factors in addition to vitamin K. Preliminary work on the extraction and purification of these factors is presented.

Single and double dose coumachlor administration cause depression of the φ_1- and φ_2-factor concentrations. Minimum values were observed 3—4 days after ingestion of the last dose, with full recovery after an additional 5—6 days. No change in activity or concentration of the two factors could be observed during storage of normal, oxalated plasma at 6—7°C for 12 days. Attempts were made to relate the properties of φ_1-factor with those of prothrombin, and the properties of φ_2-factor with those described for mammalian Stuart factor. Despite qualitative similarities, quantitative differences indicate that the φ_1- and φ_2-factors may prove to be coagulation factors which have not been described in mammalian plasmas.

In a preceding paper [1] it was shown in adsorption experiments that the φ-factor activity [2] of oxalated chicken plasma measured with chicken brain thromboplastin (φ_{tpl}-factor activity) was of complex nature and due to the combined activities of five discernible factors. The φ-factor activity measured with Russell's viper venom (RVV)-cephalin as assay accelerator (φ_{Vc}-factor activity) was due to the combined activities of two of these factors only. The terms φ_1- and φ_2-factor were suggested for the two factors which are active

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both in the thromboplastin and in the RVV-cephalin assay systems. These two factors were adsorbed competitively from oxalated plasma by strontium carbonate and some other adsorbents, but independently by barium carbonate, barium oxalate and barium chromate [1]. Only φ_2-factor could be adsorbed by calcium carbonate [1,3]. A method for quantitative determination of the φ_2-factor concentration in plasma was suggested by the observations that the minimum concentration of calcium carbonate necessary for complete adsorption of φ_2-factor activity was proportional to the plasma activity of this factor [1].

It will be shown in the present paper that the minimum concentration of strontium carbonate required for complete adsorption of the φ_{Vc}-factor activity of *calcium carbonate preadsorbed plasma* (φ_1-factor activity) is proportional to this activity and, therefore, represents an absolute value for the φ_1-factor concentration in the plasma.

The two adsorption methods have been applied to study the influence of coumachlor administration, and of vitamin K-deficiency and other dietary changes, on the plasma concentrations of the φ_1- and φ_2-factors. As will be shown, maximum level of φ_2-factor depends on the dietary supply of two unidentified dietary factors in addition to vitamin K.

MATERIALS AND METHODS

The materials and methods used in this study were as described in detail previously [1-4]. The φ_{Vc}-factor activity of untreated and adsorbed plasma samples was determined with substrates of vitamin K-deficient and coumachlor plasma in proportion 1:1, and RVV-cephalin as assay accelerator [2].

The chickens were raised on a commercial diet for at least 3 weeks [4], then given experimental diets (Table 1) for at least two weeks before the first blood sample was taken. The effects of variations in the composition of the experimental diets on the plasma φ_1- and φ_2-factor concentrations were followed in *individual chickens*, as described previously [4] in an analogous study on the dietary dependence of $\varkappa$-factor. Food ingredients found to be essential for maximal φ_2-factor concentration were subjected to extraction and fractionation procedures and the fractions tested for activity as described in the analogous $\varkappa$-factor study [4].

EXPERIMENTS AND RESULTS

Method for determination of the plasma concentration of φ_1-factor

Determination of the plasma concentration of φ_2-factor by adsorption "titration" of oxalated plasma with calcium carbonate was described previously [1]. An analogous method for quantitative determination of the φ_1-factor concentration was suggested by the observations [1] that the φ_1- and φ_2-factors are adsorbed competitively by strontium carbonate, proportionally to the concentration of adsorbent, and independently of the simultaneous adsorption of three other (thromboplastin specific) φ-factors.

It seemed possible, therefore, that the minimum concentration of strontium carbonate required for complete adsorption of the φ_{Vc}-factor activity of *calcium carbonate preadsorbed oxalated plasma* might be proportional to the plasma activity of φ_1-factor and thus represent an absolute value for the concentration of this factor. This possibility was examined in a number of adsorp-

Table 1. Experimental diets. Composition in g per kg.

Ingredients	S-02.2A	S-07.2A	S-07.3A	S-08.2A	S-16.3	S-21.2A	S-29.2A	S-37.3	S-38.2	S-38.2A	S-39.3	S-43.2A	S-44.2A	S-45.2A	S-46.3A	969 D	969 E
Casein, Vitamin Test[a]	70	—	—	—	180	—	—	—	—	—	—	—	—	—	—	—	—
Casein, Stege[b]	—	100	100	—	—	150	170	—	—	—	—	80	80	80	80	—	—
Gelatine	30	—	—	—	70	80	—	—	—	—	—	—	—	—	—	—	—
Pancreas Powder[c]	—	100	100	170	—	80	80	220	200	200	—	170	170	170	170	—	250
Ethanol-reextracted pancreas powder[d]	—	—	—	—	—	—	—	—	—	—	200	—	—	—	—	250	—
Ground corn	200	200	200	200	—	—	—	—	—	—	—	250	250	250	—	—	—
» barley	200	200	200	200	—	—	250	—	—	—	—	180	—	—	—	—	—
» oats	100	100	100	100	—	—	100	—	—	—	—	—	—	100	—	—	—
» wheat bran	80	80	80	80	60	—	80	—	60	60	60	—	80	—	100	—	—
Ether-reextracted wheat bran[d]	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	50
Dried alfalfa	60	60	60	60	—	60	60	—	—	60	—	60	60	60	60	—	—
Refined peanut oil	50	50	—	50	—	50	50	—	50	50	—	50	50	50	—	—	—
» corn oil	—	—	50	—	50	—	—	50	—	—	50	—	—	—	50	—	40
Salt mixture[c]	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Vitamin mixture[e]	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5[f]
Cystine	—	—	—	—	5	—	—	—	—	—	—	—	—	—	—	—	—
Sucrose	155	55	55	85	580	525	155	675	635	575	635	155	255	235	485	695	605
Vitamins A and D[c]																	

a) from Genatosan Ltd., Loughborough, England. *b)* from Dansk Mejeri Industri & Export Kompagni, Stege, Denmark. *c)* as described by Dam & Søndergaard [5]. *d)* Extraction for 20—24 h in Soxhlet extractor, reextraction with fresh solvent. *e)* containing (in mg): choline chloride, 1800; inositol, 850; *p*-aminobenzoic acid, 240; thiamine hydrochloride, 6; riboflavin, 8; nicotinic acid, 100; calcium pantothenate, 24; pyridoxine, 7; biotin, 0.2; folic acid, 4; DL-α-tocopheryl acetate (Ephynal, Roche), 100; dicalcium 2-methyl-1,4-naphthohydroquinone biphosphate (Synkavit, Roche), 10; mixed with sugar to make 5 g. *f)* Synkavit omitted from this vitamin mixture.

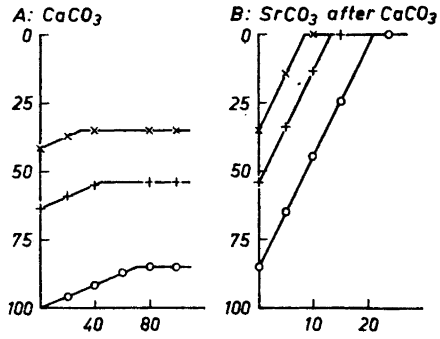


Fig. 1.

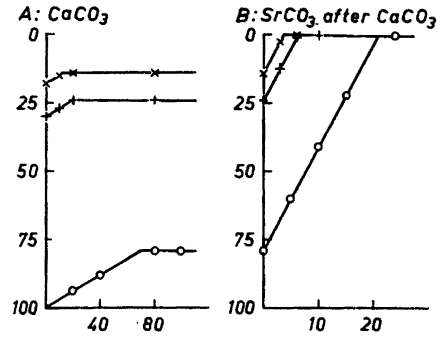


Fig. 2.

Figs. 1—3. Adsorption of φ_{Vc}-factor activity from oxalated plasmas. A: calcium carbonate adsorption of untreated plasma (φ_2-factor adsorption); B: strontium carbonate adsorption of calcium carbonate (80 mg/ml) preadsorbed plasmas (φ_1-factor adsorption). Ordinates: φ_{Vc}-factor activity in % of untreated control plasma. Abscissae: adsorbent in mg/ml.

Figs. 1 and 2. × and +: coumachlor plasmas. O: control plasma (diet No. S-07.3A).

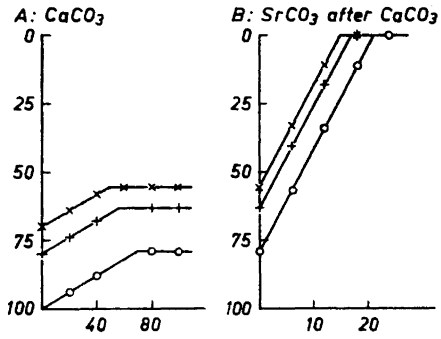


Fig. 3. × and +: plasmas from slightly vitamin K-deficient chickens (diet No. 969 E). O: control plasma (diet No. 969 E supplemented with Synkavit (10 mg/kg)).

tion experiments, using plasmas from coumachlor treated chickens and plasmas from chickens with a slight deficiency in vitamin K (diet No. 969 E). Representative experiments are presented in Figs. 1—3. In each of these experiments the three φ_{Vc}-factor adsorption curves obtained by strontium carbonate adsorption of calcium carbonate preadsorbed plasmas (Figs. 1 B, 2 B and 3 B) were parallel. Variations in φ_1-factor activity are thus associated with proportional variations in the minimum concentration of strontium carbonate required for complete adsorption of the activity. Analogous results were obtained for adsorption of the φ_2-factor activity of the corresponding untreated plasmas by calcium carbonate (Figs. 1A, 2A and 3A) in confirmation of the results of previous experiments [1].

A comparison of the experiments in Figs. 1—3 shows that the slopes of the φ_1- and φ_2-factor adsorption curves may vary from one experiment to another. As pointed out previously [1], this may be ascribed to differences in the relative sensitivity to the φ_1- and φ_2-factors of the substrate mixtures used in different experiments. The minimum concentration of strontium carbonate required for complete adsorption of the φ_{Vc}-factor activity of calcium carbonate preadsorbed oxalated plasma is independent of such differences and may thus be regarded as an absolute measure of the concentration of φ_1-factor in the plasma. It appears that a value of 21 (mg of our preparation of strontium carbonate per ml of calcium carbonate preadsorbed plasma) represents the value for maximum concentration of φ_1-factor in chicken plasma.

Dependence of φ_2-factor concentration on unidentified dietary factors

In a number of nutritional experiments (*cf.* Ref.[1]) it was found that certain diets were inadequate for maintenance of the φ_2-factor concentration at the maximum value, whereas other diets, Table 1, Nos. S-02.2A, S-07.2A and S-08.2A, were capable of maintaining the φ_2-factor concentration at the maximum level throughout the experimental periods.

Dietary ingredients essential for maximum φ_2-factor concentration were identified by feeding of diets lacking one or more of the ingredients of the adequate diets. In Table 2 is shown that exclusion of corn (diet No. S-29.2A) resulted in reduced levels of φ_2-factor, whereas exclusion of oats and wheat bran (diet No. S-43.2A), of barley and oats (diet No. S-44.2A), or of barley and

Table 2. φ_2-Factor concentration in relation to composition of diet.

Chicken No.	Diet *	φ_2-Factor concentration in % of maximal							
		plasma sample No.**							
		1	2	3	4	5	6	7	8
799	S-02.2A	100	100	100	100	—	—	—	—
794	S-07.2A	100	100	100	—	—	—	—	—
1221	S-08.2A	100	100	100	100	100	—	—	—
624	S-21.2A	61	43	44	—	—	—	—	—
1220	S-29.2A	89	71	71	—	—	—	—	—
1721	S-43.2A	100	100	100	100	100	—	—	—
1722	S-44.2A	100	100	100	100	100	100	—	—
1723	S-45.2A	100	100	100	100	100	—	—	—
1725	S-46.3A	100	100	100	100	100	—	—	—
1993	S-16.3	100	100	100	100	100	100	100	100
2840	S-39.3	100	100	100	100	100	100	100	100
2863	S-37.3	49	43	—	—	—	—	—	—
2546	S-38.2	67	57	—	—	—	—	—	—
4707	S-38.2A	80	74	—	—	—	—	—	—
2841	969 D	69	54	49	47	47	51	49	49

* *cf.* Table 1.
** the plasma samples were taken at intervals of 6—8 days.

wheat bran (diet No. S-45.2A) had no such effects. Corn could be omitted from a complete diet provided corn oil was supplied (diet No. S-46.3A). Alfalfa was not an essential ingredient (diet No. S-16.3), but omission of wheat bran in the absence of barley and oats (diet No. S-37.3), as well as substitution of peanut oil for corn oil (diet No. S-38.2) resulted in reduced levels of φ_2-factor. Casein and pancreas powder, both were adequate as protein sources. The fact that ethanol-reextracted pancreas powder (diet No. S-39.3) also was an adequate source of protein indicated that pancreas does not provide easily extractable material essential for maximum φ_2-factor level. The supply of vitamins was above usual requirements in these diets, and corn oil in combination with barley, oats or wheat bran thus appeared to supply unidentified dietary factors essential for maximum φ_2-factor concentration in chicken plasma. The active principles in these materials were extracted and fractionated according to the general procedures described in detail previously [4]:

(1) *Concentration of the active factor in corn (Ma) oil.* In Table 3 are summarized the results of testing the fractions obtained from corn oil. After saponification and isolation of the unsaponifiables, the bulk of sterols were separated from a hot, saturated solution in methanol by cooling. The filtrate was subjected to partition between light petroleum and 90 % methanol. Both the epiphasic (Ma-11) and the hypophasic (Ma-12) fractions were active. The sterol fraction (Ma-13) and the fatty acids (Ma-14) were found to be inactive. The two active fractions (Ma-11 and Ma-12) were combined and further purification of this fraction (Ma-10) was attempted by chromatography.

Following application of Ma-10 to a column of calcium carbonate and development with light petroleum, the ethyl ether eluate (Ma-103) and the material eluted with 20 % methanol in ethyl ether (Ma-104) were inactive. The material which passed through the calcium carbonate column with light petroleum, was applied to a column of alumina. The column was developed with light petroleum (eluate Ma-100), followed by 30 % benzene in light petroleum (eluate Ma-101), dry ethyl ether (eluate Ma-102) and finally 20 % methanol in ethyl ether (eluate Ma-102a). The eluate Ma-102 was the only active fraction.

Table 3. Effect of corn oil fractions fed as supplements to diet No. S-38.2 or S-38.2A.

Supplements	Daily amounts, in equivalents of unfractionated corn oil	φ_2-Factor concentration in % of maximal	
		Before feeding of supplement	After feeding of supplement
Ma-11	2 g	*71*	*100*
Ma-12	2 g	*57*	*100*
Ma-13	4 g	57	57
Ma-14	2 g	67	57
Ma-100 + Ma-101	2 g	50	53
Ma-102	2 g	*44*	*100*
Ma-102a	2 g	53	44
Ma-103 + Ma-104	2 g	57	50

Table 4. Effect of fractions of barley (By), oats (Ha) and wheat bran (Hk) fed as supplements to diet No. S-37.3.

Supplement	Daily amounts, in equivalents of unfractionated material	φ_2-Factor concentration in % of maximal	
		before feeding of supplement	after feeding of supplement
Hk-1	10 g	39	34
Hk-3	5 % of diet	34	49
Hk-211 + Hk-221	4 g	44	43
Hk-212 + Hk-222	4 g	57	54
Hk-213	4 g	43	49
Hk-223	4 g	*49*	*100*
Hk-2231	4 g	*61*	*100*
Hk-2232	4 g	49	43
Hk-2233	4 g	43	46
Hk-2234	4 g	46	43
Ha-213	6 g	43	41
Ha-2231	6 g	*41*	*100*
By-213	15 g	57	57
By-2231	15 g	*57*	*100*

(2) *Concentration of the active factor in wheat bran (Hk), oats (Ha) and barley (By).* In Table 4 are summarized the results of testing the fractions obtained from wheat bran, and of some of the corresponding fractions obtained from oats and barley. The active factor in wheat bran was not extracted with ether (extract Hk-1), and the residue after reextraction with ethanol (Hk-3) was almost inactive. After partition of the ethanol extracts (cold soluble and cold insoluble fractions) with ether and water, the ether layers (Hk-211 and Hk-221) did not contain the active factor. Butanol extracts (Hk-212 and Hk-222) of aqueous solutions of the lower phases were also inactive. Of the butanol extracted solutions (Hk-213 and Hk-223) only Hk-223 was active. The active factor had thus been precipitated completely by cooling of the original ethanol extract to −15°C (*cf.* Ref.[4]). Further fractionation of the active extract (fraction Hk-223) revealed that the active factor could be precipitated completely by addition of barium hydroxide solution to pH 9 (precipitate Hk-2231). Fractions obtained from the filtrate by precipitation with lead salts at pH 6 (Hk-2232) and at pH 8 (Hk-2233), and the final filtrate (Hk-2234) were all inactive.

During fractionation of the ethanol extracts of oats and barley, the active factor was concentrated in the fractions corresponding to Hk-2231 (Ha-2231 and By-2231, respectively).

Relation of φ_1-factor concentration to composition of the diet

No diets have as yet been designed which will give reduced plasma levels of φ_1-factor due to deficiency in an unidentified dietary factor. All diets which are adequate in their supply of protein and micro-nutrients have given

Table 5. φ_1 -Factor concentration in relation to composition of diet.

Chicken No.	Diet *	φ_1-Factor concentration in % of maximal							
		plasma sample No.**							
		1	2	3	4	5	6	7	8
2545	S-37.3	100	100	—	—	—	—	—	—
2546	S-38.2	100	100	—	—	—	—	—	—
2840	S-39.3	100	100	100	100	100	100	100	100
2841	969 D	100	100	100	100	100	100	100	100

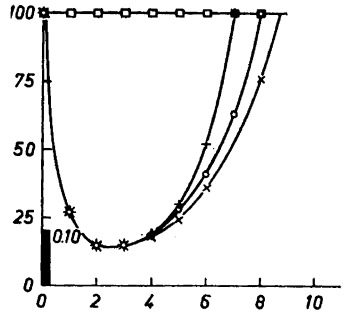
* *cf.* Table 1.
** the plasma samples were taken at intervals of 6—8 days.

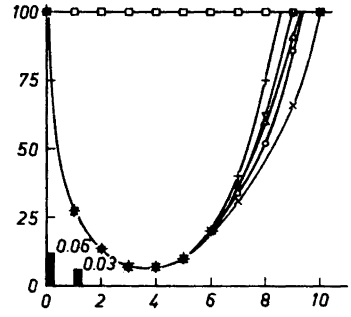
maximum value for the φ_1-factor concentration in the plasma. Thus, a diet composed of ethanol-reextracted pancreas powder, mineral salts, vitamins and sucrose (diet No. 969D) proved adequate for maintenance of maximum φ_1-factor concentration throughout the experiments (Table 5).

Vitamin K-deficiency, however, causes a reduction in the plasma concentrations of both φ_1- and φ_2-factor. In plasmas with thromboplastin coagulation times of more than six times the value for normal plasma, the φ_1- and φ_2-factor concentrations were usually below 15 % of maximal.

Effect of coumachlor administration on the φ_1- and φ_2-factor concentrations in plasma

Coumachlor administered to chickens fed on a diet adequate for maintenance of maximum φ_2 (and φ_1)-factor concentration (diet No. S-07.3A) had a profound effect on the concentrations of the φ_1- and φ_2-factors in the plasma. The results of two experiments are shown in Figs. 4 and 5, where coumachlor





Figs. 4 and 5. Effect of coumachlor administration on the concentrations of φ_1- and φ_2-factor.
Ordinates: φ_{Vc}-factor activity, φ_1-factor and φ_2-factor concentrations, in % of control plasma (diet No. S-07.3A).
Abscissae: time in days.
Solid bars: coumachlor dose in mg per g body weight.
□ : control plasmas.
+, ×, O, △ and ▽: plasmas from individual coumachlor chickens (diet No. S-07.3A).

was given orally as a single dose (0.10 mg per g body weight) and on two consecutive days (0.06 and 0.03 mg/g). Coumachlor caused a depression of the φ_1- and φ_2-factor concentrations. Both factors were reduced to the same extent, indicating identical turn-over rates. The minimum concentrations of the two factors was observed 2—3 days after ingestion of the last dose, with full recovery after an additional 5—6 days.

Stability of the φ_1- and φ_2-factors to storage

The design of diets adequate for maximum φ_1- and φ_2-factor concentration in plasma also permitted comparison of the φ_1- and φ_2-factor activities of fresh plasma with those of plasma stored at 6—7°C for different periods of time. No deterioration of the φ_{Vc}-factor activity of oxalated plasma was observed during storage at 6—7°C for 12 days. There were no changes in the relative activities of the two factors, and no changes in the minimum concentration of the adsorbents required for complete adsorption of the φ_1- and φ_2-factors according to the procedures described. In fact, the adsorption curves for fresh and stored plasmas were identical.

Attempts to correlate the φ_1- and φ_2-factors with established mammalian coagulation factors

It has been pointed out previously [1,2] that of the coagulation factors presently recognized in mammalian plasmas, only Stuart factor and prothrombin might possibly correspond to the φ_1- and φ_2-factors in chickens. Stuart factor and prothrombin, both are reported to be sensitive to vitamin K-deficiency and to coumarin drugs [6]. Other vitamin K dependent and coumarin drug sensitive mammalian factors (factor VII and IX [6]) do not influence the plasma coagulation rate measured with RVV-cephalin.

However, it is not possible at present to decide if Stuart factor and prothrombin (like the φ_1- and φ_2-factors) are reduced below *optimal levels* in chickens, *i.e.* become rate limiting coagulation factors, during vitamin K-deficiency and shortly after administration of high doses of dicumarol or cou-

Table 6. Coagulation time of calcium carbonate adsorbed plasma aliquots.

Calcium carbonate in mg/ml	Coagulation time in min/100			
	Plasma No./Assay accelerator			
	321/Thr.pl.	325/Thr.pl.	325/RVV-ceph.	325/RVV
0	25	28	22.4	38
2	25	—	—	—
3	26.1	—	—	—
5	26.1	30	23.3	41
20	—	30	23.3	41
60	—	30	23.3	41
100	26.1	30	23.3	41
200	—	30	23.3	41

Table 7. Coagulation time and φ-factor activity of adsorbed plasma aliquots.

Adsorbent in mg/ml		Coagulation time in min/100		φ-Factor activity in % of activity of untreated plasma	
		Accelerator			
		Thr.pl.	RVV-ceph.	$\varphi_{tpl.}$	φ_{Vc}
Calcium oxalate	0	23.2	17.2	100	100
	70	30	23.8	44	55
	140	48	33	19.5	10
	180	*55*	*38*	13	*0*
	240	*112*	*63*	5	*0*
	300	> 24 h	~500	1.5	0
	400	> 24 h	> 24 h	0	0
Strontium oxalate	0	23.2	16.2	100	100
	80	28	19	50	55
	160	35	24.8	28.5	10
	200	*40*	*26.9*	20	*0*
	240	*46*	*31*	14	*0*
	300	*64*	*39*	5.5	*0*
	400	750	250	2	0

machlor. This could be ascertained by use of chicken plasmas with established single deficiencies in each of the two factors, or preparations of physiologically pure factors. In the absence of such reagents, information concerning the possible equivalence of the above mammalian and chicken coagulation factors could be obtained by indirect experiments only.

One-stage coagulation time of adsorbed plasmas. The coagulation times of oxalated, normal plasma aliquots, adsorbed by increasing concentrations of calcium carbonate are given in Table 6. It is seen that a slight prolongation of the clotting time occurs with 3 mg $CaCO_3$/ml, corresponding to complete adsorption of the ψ-factor [7]. Higher concentrations of calcium carbonate — up to three times the concentration sufficient for complete adsorption of the φ_2-factor — had no further effect on the coagulation time of the plasma. The effect of φ_2-factor on the coagulation rate of plasma may, therefore, depend on the presence of ψ-factor. In any case the effect is so small that an identification of φ_2-factor with prothrombin is impossible and an identification with Stuart factor very unlikely.

Similar experiments using calcium or strontium oxalates as adsorbents (Table 7) showed that plasma samples with φ_{Vc}-factor activity reduced to zero are still able to clot, although at a reduced rate. Thus, prothrombin is still present (but probably in reduced concentrations) in the plasma samples from which the φ_1- and φ_2-factors have been completely removed by adsorption. This seems to exclude an identification of φ_1-factor with prothrombin.

Behavior of the φ_1- and φ_2-factors during coagulation of blood. The φ_{Vc}-factor activity of oxalated plasma was compared to that of the corresponding serum obtained by spontaneous coagulation of blood at 37°C. Three percent (v/v) of veronal buffer or thromboplastin solution (centrifuged extract of 100 mg

Table 8. φ_{Vc}-Factor activity of sera.

Experiment No.	Activity in % of φ_{Vc}-factor activity of corresponding oxalated plasma					
	1	2	3	4	5	6
Citrate serum φ_{Vc}-factor (A)	27	27	20	35	34	20
Oxalate serum φ_{Vc}-factor (B)	17	—	12	—	20	—
Oxalate plasma φ_2-factor (C)	28	28	21	35	35	21
(A)/(C) in %:	96	96	95	100	97	95
Citrate serum "prothrombin" in %	13	4	<3	10	10	11

acetone-dried chicken brain with 2 ml of buffer) was added to the native blood. To the serum, obtained by centrifugation after incubation at 37°C for 2 1/2—3 h, was added 1/6 vol. of oxalate or citrate anticoagulant [2]. Without addition of thromboplastin the blood clotted after 35—60 min. In Table 8 is shown that the serum prothrombin activity [2] of the citrated sera could amount to 13 % of the corresponding plasma value. The residual φ_{Vc}-factor activity of the citrated sera was practically identical to the φ_2-factor activity of the corresponding plasmas. In the oxalated sera the residual φ_{Vc}-factor activity was reduced by about 40 %, presumably due to adsorption by the precipitated calcium oxalate.

The φ_{Vc}-factor activity of the oxalated sera could be completely adsorbed by calcium carbonate. The adsorption curves obtained were parallel to those of the corresponding oxalated plasmas (Fig. 6). It is very unlikely that the calcium oxalate precipitated in the oxalated sera should have caused a *preferen-*

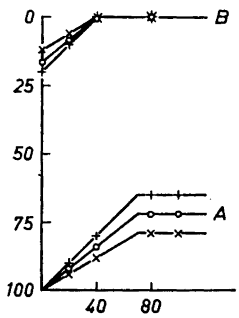


Fig. 6. Adsorption of φ_{Vc}-factor activity by calcium carbonate.
Ordinate: φ_{Vc}-factor activity in % of untreated plasma.
Abscissa: calcium carbonate in mg/ml.
Curves A: adsorption from oxalated plasmas.
Curves B: adsorption from corresponding oxalated sera.
+, O and ×: three different experiments with three different chickens, all on diet No. S-07.3A.

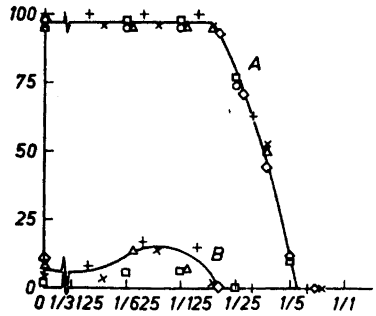


Fig. 7. φ_{Vc}-Factor and prothrombin activity in citrated sera.
Ordinate: activity in %.
Abscissa: concentration of the thromboplastin solution (log scale).
Curve A: φ_{Vc}-factor activity relative to the φ_2-factor activity of the corresponding oxalated plasma.
Curve B: prothrombin activity in % of the corresponding oxalated plasma.
The symbols +, ×, O, □, ◇, and △ refer to six different experiments (all chickens on diet No. S-07.3A).

tial adsorption of a serum calcium carbonate *non-adsorbable* φ_{Vc}-factor (φ_1-factor), because of the established competitive adsorption of the φ_1- and φ_2-factors by crystalline calcium oxalate [1]. The φ_{Vc}-factor activity of the oxalated as well as of the citrated sera must therefore be due to φ_2-factor alone. Consequently, φ_1-factor must be completely consumed during spontaneous coagulation of normal blood at 37°C.

The effect of addition of thromboplastin in varying concentrations to native blood is seen from Fig. 7. Concentrations up to 1/40 of that of the original extract had no effect on the residual φ_{Vc}-factor activity of the citrated sera, but reduced the residual prothrombin activity practically to zero. Higher concentrations of thromboplastin brought about increasing consumption of serum φ_{Vc}-factor activity (φ_2-factor). The φ_{Vc}-factor activity of sera was reduced to zero with thromboplastin concentrations of 1/3 of that of the original extract. The experiments show that prothrombin consumption does not parallel the consumption of φ_1-factor, nor that of φ_2-factor.

DISCUSSION

The adsorption procedures introduced for determination of the φ_1- and φ_2-factor concentrations in plasmas were suggested by previous observations [1]: (1) the φ_1- and φ_2-factors are adsorbed competitively on strontium carbonate by specific adsorption "sites" which are not engaged in the adsorption of other φ-factors, (2) specific adsorption "sites" on calcium carbonate allow a selective adsorption of φ_2-factor, and (3) the adsorbed activity is proportional to the concentration of adsorbent until complete adsorption of the activity. The present experiments demonstrated that all observed differences in the φ_1- and φ_2-factor activities of plasmas were associated with proportional differences in the minimum concentrations of the respective adsorbents required for com-

plete adsorption of the factors. The objection to the adsorption procedures discussed previously,[1] that variations in the concentrations of other, inert, plasma factors possibly adsorbed in competition with the φ_1- and φ_2-factors might be responsible for observed variations in the minimum concentration of adsorbent required for complete adsorption of the factor, can therefore be rejected. It is accordingly possible to express the plasma concentrations of the φ_1- and φ_2-factors in absolute terms (as the equivalent concentration of adsorbent). This allows a direct comparison of results obtained in different experiments carried out at different times with slightly different substrates and with solutions of assay accelerator differing in potency. A rational study of the dietary dependence of the φ_1- and φ_2-factors could then be initiated.

The preliminary results reported here show that the concentration of φ_1-factor depends on vitamin K, but probably not on additional, unidentified dietary factors. The φ_2-factor concentration is also vitamin K dependent. In addition, exclusion of certain nutrients from a diet adequate in vitamin K results in reduced levels of φ_2-factor. So far, work on the extraction and preliminary purification of the active principles from these essential nutrients has shown that fractions carrying the activity can be separated from the bulk of inactive material by conventional procedures. The activity of each essential nutrient thus appears to be due to the content of a special dietary *factor*. The active factor in corn oil is thus not found in comparable quantities in peanut oil. It is non-saponifiable, probably not a sterol, and its behavior on partition and chromatography indicates that it may be a hydrocarbon with a relatively small number of polar groups. The active factor in barley, oats and wheat bran is probably a common component of these nutrients. It is water-soluble, poorly soluble in ethanol, and forms a compound with barium salts which is insoluble in aqueous solutions at pH 9. Further studies on the purification of these dietary factors are in progress.

It should be noticed that the differences in the functional and physicochemical properties of $\varkappa$-factor, φ_1-factor and φ_2-factor, which permitted discrimination between these factors [1–3], are reflected in differences in the dietary requirements for maximum levels of the factors in chicken plasma (*cf.* Ref.[4]). The latter differences may be regarded as additional confirmation of the non-identity of the coagulation factors mentioned.

The dependence of φ_2-factor and of $\varkappa$-factor [4] on unidentified dietary factors, and the dependence of the $\varkappa$-, φ_1-, and φ_2-factors on vitamin K, appear to be representative cases of a more general problem, namely, the role of special dietary factors in the biosynthesis of specific coagulation factors (trace proteins). The analogy with the accepted role of vitamin K in the biosynthesis of prothrombin, factor VII (proconvertin) and other coagulation factors is striking. Studies on the biosynthesis of the labile coagulation factors of chicken plasma has revealed a whole family of related problems, as will be shown in forthcoming publications.

The relation of the φ_1- and φ_2-factors to coagulation factors presently recognized in mammalian plasmas is a matter of some interest for comparative coagulation research. A similarity between Stuart factor and φ_2-factor was indicated by the reported presence of Stuart factor in serum with unchanged [8] or slightly increased [9] activity. However, chicken plasma without demonstrable

φ_2-factor activity (calcium carbonate adsorbed normal plasma) has a coagulation rate only slightly inferior to that of untreated plasma. This contrasts with the markedly prolonged coagulation time of Stuart factor deficient plasma [10]. A similarity between φ_1-factor and prothrombin was indicated by the fact that both of these factors are consumed during spontaneous coagulation of normal blood. However, φ_1-factor consumption appears to be complete in sera in which more than 10 % of residual prothrombin activity can be demonstrated. Furthermore, adsorbed plasmas without demonstrable φ_1- or φ_2-factor activity are still able to clot with thromboplastin, indicating that at least some prothrombin is present in these plasmas. These facts seem to preclude an identification of the φ_1- and φ_2-factors with prothrombin and Stuart factor, respectively, if the two latter factors are to be regarded as homogeneous coagulation entities. The φ_1- and φ_2-factors may, therefore, represent coagulation factors which have not been described in mammalian plasmas. Their precise role in the physiological blood coagulation mechanism of chickens is unknown. The fact that chicken plasmas are still able to clot with thromboplastin after removal of the φ_1- and φ_2-factors by adsorption, indicate that these factors might function only as accelerators in the conversion of prothrombin to thrombin.

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