

Synthesis and activity in blood clotting systems of unsaturated racemic phosphatidyl serines

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SUMMARY The synthesis of racemic 1,2-dioleoyl and 1-oleoyl-2-stearoyl glycerol-3-phosphoryl serine was achieved by the phosphorylation of unsaturated diglycerides with phosphorus oxychloride, and combination with the phthalimidomethyl ester of carbo-*t*-butoxy-DL-serine.

The synthetic phosphatidyl serines were tested in blood clotting systems *in vitro*. They have activity qualitatively similar to that of naturally occurring phosphatidyl serines: anticoagulant activity when well solubilized and weak to moderate thromboplastic activity in simple suspension.

PHOSPHATIDYL SERINE (PS) fractions extracted from natural sources have anticoagulant activity when solubilized and administered intravenously to animals (1–3). Such activity has also been shown in clotting tests *in vitro*, and the degree of anticoagulant activity appears to be related to the solubilization of the phospholipid (4, 5). Solubilization depends in part on the presence of unsaturated fatty acids. Phosphatidyl serines containing only saturated acids are too insoluble to test.

All naturally occurring phosphatidyl serines that have been studied contain both unsaturated and saturated fatty acids. No separation of phosphatides containing particular acids has ever been achieved, and the naturally occurring phosphatides are still lipid classes rather than molecular species.

The present investigation was undertaken to synthesize chemically pure phosphatidyl serines with specific unsaturated fatty acids, to test their activities in various blood coagulation systems, and to compare their activities with those of naturally occurring PS.

Synthetic Procedures

The earlier synthesis of saturated phosphatidyl serines by Baer and Maurukas (7) and by Bevan, Malkin and

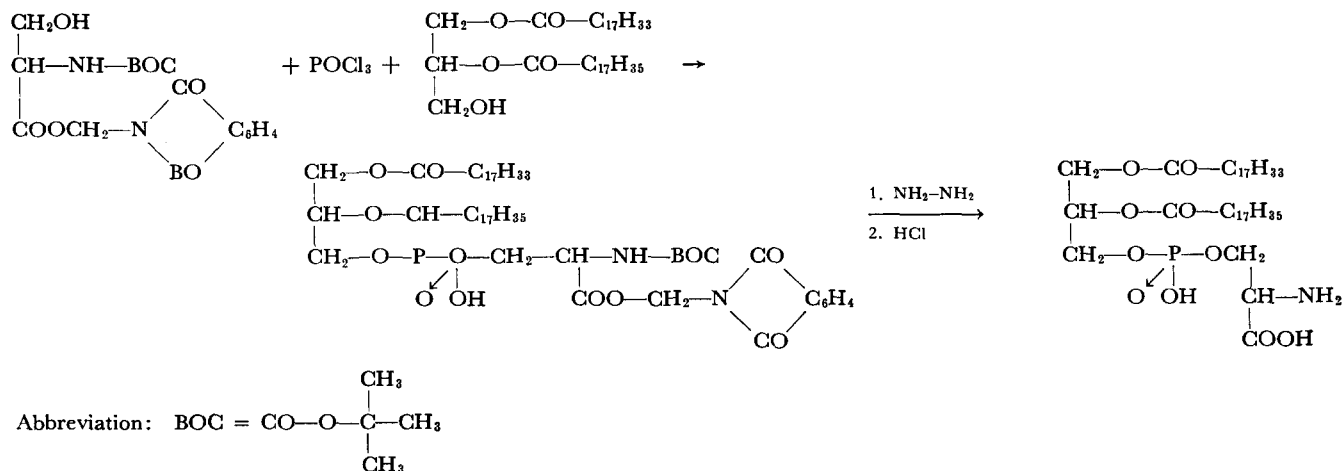
Tiplady (8) involved phosphorylation of the benzyl ester of carbobenzoxyserine with phenyl phosphorodichloride. De Haas and van Deenen (9) have synthesized saturated phosphatidyl serines using the *p*-nitrobenzyl group instead of the phenyl group on the phosphorylating reagent. However, removal of the protecting groups in all of these syntheses requires hydrogenolysis, which precludes their use for the synthesis of unsaturated phosphatidyl serines.

Protecting groups that do not require hydrogenolytic removal have been devised recently, and the use of these groups permits the synthesis of unsaturated phosphatidyl serines.

The carbo-*t*-butoxy group has been used, particularly by Anderson and McGregor (10) and by Schwyzer and his co-workers (11), for the protection of the amino function, and valuable reagents for the easy introduction of this protective group have been devised by Carpino (12). The carbo-*t*-butoxy group can be removed with dry hydrogen chloride. Phthalimidomethyl esters described by Nefkens (13, 14) provide an easily removable protection for the carboxyl group.

Thus, it appeared advantageous to protect the amino group with the carbo-*t*-butoxy radical, and to mask the carboxyl function as the phthalimidomethyl ester. Difficulties were encountered in preparing the carbo-*t*-butoxy-DL-serine (BOC-serine) from free serine (cf. 10), but these were overcome by employing the methyl ester of serine (15). The methyl ester of BOC-serine obtained was then saponified to give the crystalline potassium salt, which was acidified with Dowex 50 (H⁺) in tetrahydrofuran. The solution of the free acid was then used directly to prepare the phthalimidomethyl ester. An alternative, less satisfactory, route involving a salt-acid complex analogous to the carbobenzoxyserine complex of

REACTION SCHEME



Brown and Wade (16) is described in this paper in order to clarify our preliminary publication (6).

The procedure of Baer and Buchnea (17, 18) for the synthesis of phosphatidyl ethanolamine by reaction of phosphorus oxychloride with a diglyceride and *N*-phthaloyl-ethanolamine was applied using the protected serine instead of the protected ethanolamine. By using DL-1,2-dioleoyl glycerol and DL-1-oleoyl-2-stearoyl glycerol, two protected racemic phosphatides were made as shown in the reaction scheme. Removal of the protecting groups gave the two racemic phosphatides, which were identical in infrared spectrum and in behavior on paper and thin-layer chromatography to natural PS. The yield was rather low; the losses were almost entirely the result of the hydrolytic effect of the hydrogen chloride used to remove the BOC group. The synthesis of the dioleoyl PS has been reported in our preliminary communication (6).

After the completion of our work, the total synthesis of L-phosphatidyl (oleoyl palmitoyl) DL-serine¹ was reported by De Haas and co-workers (19), who employed the *t*-butyl ester of BOC-serine and a different method of phosphorylation. Dr. De Haas kindly furnished a sample of his synthetic material before his method was disclosed. This sample has been tested in the work on blood coagulation described below, so that data are available in the

¹ Nomenclature. The following system is used. "Phosphatidyl" is defined as 1,2-diacylglycerol-3-phosphoryl, and where the nature and position of the acyl groups are known these are inserted in parentheses after "phosphatidyl" in order to define the radical more closely (rather than to imply further substitution of it). Thus "phosphatidyl (oleoyl stearoyl) serine" denotes 1-oleoyl-2-stearoyl glycerol-3-phosphoryl serine; the acyl radicals are always named in the order 1, 2 which refers to a Fischer projection of glycerol which shows C-1 on top and the hydroxyl at C-2 to the left of the carbon chain, cf. Hirschmann, *J. Biol. Chem.* **235**: 2762, 1960. In the abbreviation "oleoyl stearoyl PS," the same convention is implied, but the acyl radicals are named first in order to avoid separating the capital letters.

same tests for the activity of synthetic phosphatides obtained by very different synthetic procedures.

MATERIALS AND METHODS

SYNTHESIS

Oleic acid was purchased from Applied Science Laboratories Inc., State College, Pa., and was 99% pure by gas-liquid chromatographic analysis. Stearic acid of similar purity was purchased from Stearinerie Dubois Fils, Montreuil-sous-Bois, France; DL-serine from Winthrop Laboratories, New York, N. Y.; and L-serine from Mann Laboratories, New York, N. Y. Silicic acid was Unisil of the Clarkson Chemical Co., Williamsport, Pa. DEAE-cellulose was Selectacel from Bio-Rad Laboratories, Richmond, Calif. It was purified, converted to the acetate form, and used as described by Rouser et al. (20, 21). Dowex 50-X8, analytical grade, 200-400 mesh came from the California Corporation for Biochemical Research, Los Angeles, Calif. Chloroform was dried over molecular sieve 4A of the Linde Division of Union Carbide, Inc.

Thin-layer chromatograms on Adsorbosil (Applied Science Laboratories Inc.) were developed with chloroform-methanol-acetic acid-water (80:25:5:3). Chromatography was also performed on paper impregnated with silicic acid as described by Marinetti (22). Melting points were determined in capillary tubes, except for those marked K, which were determined on the Kofler Hot Bench. Infrared spectra were taken with the Perkin-Elmer model 137 spectrophotometer with sodium chloride optics. Nujol mulls were used, except for the final PS preparations, where potassium bromide disks were used.

Elementary analysis was performed by Schwarzkopf Laboratories, Woodside N. Y., and by Galbraith Laboratories, Knoxville, Tenn.

Potassium Salt of BOC-DL-Serine

The methyl ester of DL-serine was prepared as described by Guttman and Boissonnas (23) and converted to the BOC derivative by the method of Iselin and Schwyzer (15). The methyl ester was saponified with 1 N KOH in methanol at room temperature. The alkali was added in portions as it was consumed. The amount consumed was 95% of the theoretical. The resultant neutral solution was evaporated in vacuo. If the solution was not neutral, it was neutralized with 10% citric acid solution. The residue from the evaporation was crystallized from a mixture of methanol, ethyl acetate, and ether. Yield 90%, mp 206° (K).

Analysis: C₈H₁₄NO₅K; calculated: C, 39.49; H, 5.80; N, 5.76
found C, 39.5; H, 5.94; N, 6.14

Potassium Salt of BOC-L-Serine

This salt was prepared in the same manner as the DL-serine derivative. Mp 200° (K).

Analysis: C₈H₁₄NO₅K; calculated: C, 39.49; H, 5.80; N, 5.76
found C, 39.2; H, 6.24; N, 5.86

Potassium Salt-Acid Complex of BOC-Serines

The potassium salt of BOC-DL-serine was dissolved in water and treated with the theoretical quantity of 10% citric acid solution. The neutral solution was concentrated in vacuo to give crystals of the complex, mp 102° (K), yield 65% of theory. Attempts to recrystallize gave crystals of potassium salt. This is analogous to the behavior of similar complexes (Brown and Wade, 16).

Analysis: C₁₆H₂₉N₂O₁₀K; neutralization equivalent, 448; found, 450.

The potassium salt-acid complex of BOC-L-serine was made similarly and had mp 112° (K). It had the correct neutralization equivalent.

BOC-DL-Serine

The potassium salt of BOC-DL-serine was thoroughly dried and powdered. To 50 g of the salt suspended in 800 ml of dry tetrahydrofuran was added 50 g of dried Dowex 50-X8 in the hydrogen form. The mixture was stirred at room temperature until the suspended salt disappeared. The ion-exchange resin was removed by filtration and the solution was used directly for the preparation of the phthalimidomethyl ester. A portion was evaporated in vacuo to give a nearly quantitative yield of

BOC-DL-serine as a water-soluble oil. It had the correct neutralization equivalent and infrared spectrum: 2.9 μ (OH); 9.5 μ (primary OH); 5.95 μ (urethane); 5.75 μ (carboxyl); 8.0 μ (*t*-butyl, skeletal); and 7.34 μ (*t*-butyl, deformation).

Phthalimidomethyl Ester of BOC-DL-Serine

The tetrahydrofuran solution of the BOC-DL-serine was treated with the theoretical amount of dry triethylamine and equimolar phthalimidomethyl chloride. After standing for one week at room temperature, the mixture was heated for 15 min on a steam bath. It was cooled, and the triethylammonium chloride was removed. The solution was evaporated in vacuo and the residue was triturated with water until it crystallized. The crystals were dried in a high vacuum and recrystallized from ethyl acetate-petroleum ether, after which they had mp 121–122°; yield 60%. Identical material was made from the salt-acid complex using ethyl acetate as solvent for the reaction, as mentioned in our preliminary paper (6).

Analysis: C₁₇H₂₀N₂O₇; calculated: C, 56.04; H, 5.53; N, 7.69
found: C, 55.9; H, 5.34; N, 7.53

This was made as described for the DL-serine derivative, and had mp 125°. The yield was the same and the infrared spectrum identical.

Analysis: C₁₇H₂₀N₂O₇; calculated: C, 56.04; H, 5.53; N, 7.69
found: C, 56.31; H, 5.50; N, 7.93

Infrared Spectra of Phthalimidomethyl Esters and Salt Complexes

Four sharp bands in the carbonyl region were characteristic of the phthalimidomethyl esters: 5.56, 5.78, 5.65, and 5.95 μ. The first two bands were characteristic of the phthalimido group, as was a band at 13.8 μ (see Uhle, 24).

In the cases of the salt complexes, the spectral changes in passing from acid to salt complex to salt were similar to those described by Brown and Wade (15) for carbo-benzoxyserine.

DL-Phosphatidyl (Oleoyl Stearoyl) DL-Serine

The preparation of DL-1-oleoyl-2-stearoyl glycerol was carried out from 1,3-benzylidene glycerol prepared as described by Johary and Owen (25), following a procedure described by Bogoslovskii, Samoshvalov, and Preobrazhenskii (26). The diglyceride was then combined with protected serine and phosphorus oxychloride as described by Baer and Buchnea (17, 18) in their synthesis of phthaloyl-phosphatidyl ethanolamine. This pro-

cedure differs from that of Baer and Buchnea in that the phthalimidomethyl ester of BOC-DL-serine was used in equivalent quantities in place of the *N*-phthaloyl ethanolamine. Following the reaction, the product was taken up in chloroform instead of ether and the chloroform solution was washed with ice-cold dilute sulfuric acid and water. Drying and evaporation gave an oil from which the protecting groups were removed without isolation of intermediates. The crude oil, presumed to be the phthalimidomethyl ester of the BOC-phosphatidyl serine, had close to the theoretical content of phosphorus but showed four spots on thin-layer chromatograms, all moving faster than PS, all staining for phosphate, and all ninhydrin-positive after heating. The yield of this intermediate from 23 g of the diglyceride was 38 g after drying in a high vacuum.

A portion (10 g) of the intermediate was dissolved in 250 ml of dry distilled 2-methoxyethanol and filtered. To the filtrate was added the theoretical quantity of a stock solution of hydrazine (prepared by dissolving 17.7 g of 95% hydrazine in 490 ml of 2-methoxyethanol and 10 ml of water). The required hydrazine was added in four portions, and after each addition the solution was allowed to stand in the dark under nitrogen for 2 days. The solvent was removed in vacuo. The residue was dissolved in 1200 ml of dry chloroform, and a brisk stream of dry hydrogen chloride was introduced for 30 min, followed by nitrogen for 2 hr. The chloroform was removed in vacuo under nitrogen, and the product was dried in high vacuum. The material was stirred with 300 ml of acetone, and centrifuged. The insoluble material was dissolved in 100 ml of chloroform and centrifuged, and the chloroform solution was purified by chromatography on silicic acid in two equal portions. Unisil (120 g) was deoxygenated by heating to 120° for 2 hr under a stream of nitrogen. It was cooled under nitrogen and introduced into a column as a slurry in chloroform. The material was introduced and eluted exhaustively with chloroform, which removed the greater portion of the product. The PS was then eluted with 12.5% methanol in chloroform. Elution was continued as long as the effluent was ninhydrin-positive (27, 28). The total yield from 10 g of intermediate was 2.4 g, nearly pure as judged by paper and thin-layer chromatography. This material was purified further using DEAE cellulose in the acetate form (20, 21). Using 50 g of the ion-exchange cellulose, it was possible to purify 500 mg of phosphatide. The pure phosphatide was removed with glacial acetic acid, which gave 300 mg of PS that showed only one spot on thin-layer and paper chromatograms. Its infrared spectrum was nearly superimposable on the infrared spectrum of natural PS (in the free acid form) from brain. The material used for comparison was made by purification of natural PS salt on a DEAE cellulose (acetate form) column.

Analysis: C₄₂H₈₀NO₁₀P; calculated: C, 63.85; H, 10.21; N, 1.77; P, 3.92
found: C, 63.5; H, 9.81; N, 1.61; P, 3.63

DL-Phosphatidyl (Dioleoyl) DL-Serine

This was made by the same method described for the mixed acid phosphatide, except that DL-(1,2)-diolein of Baer and Buchnea (29) was used. The characteristics of the product have been described in our preliminary communication (6).

Gas-Liquid Chromatographic Analysis of the Acids in the Synthetic Phosphatides

This was kindly carried out for us by S. F. Herb and F. Luddy of the Eastern Regional Research Laboratories of the U.S. Department of Agriculture, Wyndmoor, Pa., using the columns and techniques described by them (30-32). The acids in the dioleoyl PS were 97.8% oleic, 1.6% stearic, and 0.6% palmitic. The palmitic acid is an impurity in the oleic acid used, while the stearic acid originates from the reductive debromination of the tetrabromodistearin in the Baer and Buchnea (29) procedure. The diolein made by this method contains 10% of stearic acid before purification of the diglyceride on a column of silicic acid.

The acids in the oleoyl stearoyl PS were equally divided between stearic and oleic acid with a trace of palmitic acid, the latter an impurity in the acids used as starting material.

ACTIVITY IN BLOOD COAGULATION TESTS

The racemic dioleoyl PS, racemic oleoyl stearoyl PS, the L-phosphatidyl (oleoyl palmitoyl) DL-serine provided by Dr. De Haas, and beef brain PS were tested for their effects on the coagulation of human blood plasma in the modified Hicks-Pitney test, the antithromboplastin test, and the recalcification test, using the methods exactly as described by Silver et al. (5). The PS from beef brain was prepared as described (33, 34), and differs from the synthetic phosphatides in being in the salt form rather than in the free acid form, in being one of four stereochemical enantiomorphs, and in containing at least 27 fatty acids and a trace of phosphatidyl serine (5).

Solubilization of the Phosphatides

Solubilization was effected with the aid of sodium desoxycholate or human albumin exactly as described by Silver et al. (5). *Suspensions* in buffered saline solution were also prepared as described (5). The importance of the solubilization achieved is discussed below.

TABLE 1 EFFECTS OF FOUR DIFFERENT PHOSPHATIDYL SERINES IN THE HICKS-PITNEY TEST
Substrate clotting time after 6 min of incubation

µg PS in Inc. Mix.	PS Solubilized in Desoxycholate				PS Solubilized in Albumin				PS Suspended in Buffered Saline				Controls			
	OSPS	OPPS	OOPS	BBPS	OSPS	OPPS	OOPS	BBPS	OSPS	OPPS	OOPS	BBPS	BA 15 µg	Alb. 25 µg	Nades 25 µg	Saline
25	>100	>100 ^{sec}	>100	>100	>100	>40 ^{sec}	>100	>100	21	40 ^{sec}	40	12	8	70 ^{sec}	45	60
6	70	>100	80	60	42	>40	>100	>100	15	40	37	26				
1.5	30	>100	23	24	37	>40	62	88	28	40	52	47				

Abbreviations employed: PS = phosphatidyl serine; Inc. Mix. = incubation mixture; OSPS = oleoyl stearoyl PS; OPPS = oleoyl palmitoyl PS; OOPS = dioleoyl PS; BBPS = beef brain PS; BA = Bell and Alton extract [crude brain phosphatides (32)]; Nades = sodium desoxycholate; Alb. = albumin; and Saline = buffered physiological saline solution.

Nature of the Solutions or Suspensions of Each PS

1. *Beef brain PS.* Solubilization of beef brain PS with albumin or sodium desoxycholate produced water-clear sols (5). Stable suspensions were obtained with buffered saline solution.

2. *Oleoyl Stearoyl PS.* Partial solubilization was obtained with albumin as solubilizing agent. The particulate matter settled rapidly and only the slightly turbid supernatant solutions were tested. Water-clear solutions were obtained with sodium desoxycholate as solubilizing agent. Stable suspensions were obtained with buffered saline solution.

3. *Dioleoyl PS.* Partial solubilization was obtained with albumin. The particulate matter was allowed to settle out and the supernatant solutions were tested. The degree of solubilization of the dioleoyl PS was greater than that obtained for the oleoyl stearoyl PS. Water-clear solutions with only a few visible particles were obtained with sodium desoxycholate. Stable suspensions were obtained with buffered saline solution.

4. *Oleoyl Palmitoyl PS.* A turbid suspension was obtained with albumin. Particles did not settle out. The whole suspension was tested. Sodium desoxycholate produced almost clear solutions. Slight turbidity was verified by the detection of a few scattered particles (0.5 to 2 µ in

TABLE 2 EFFECTS OF FOUR DIFFERENT PHOSPHATIDYL SERINES IN THE ANTITHROMBOPLASTIN TEST

Method of Solubilizing or Suspending	µg in Test	Clotting Time					
		Phosphatidyl Serine Tested				Controls	
		OSPS	OPPS	OOS	BBPS	Nades	Saline
Sodium desoxycholate	200	118	113 ^{sec}	170	250	23	
	100	77	69	90	141	22	
	50	55	46	50	64	21	
	25	—	40	45	—	20	
	5	30	28	29	39	30	
	2.5	25	24	26	32	20	
	1.2	23	—	22	26	20	
	0						20
Albumin	100	—	52	69	170	Alb. 20	Water
	50	41	40	48	108	20	
	25	—	35	40	—	20	
	5	25	25	27	41	20	
	2.5	24	24	25	33	20	
	0						20
Suspension in Buffered Saline	100	22	23	21	33		Saline
	50	21	22	20	28		
	25	20	21	20	—		
	5	20	20	20	20		
	2.5	20	20	20	20		
	0						20

Abbreviations as in Table 1. Test System: 0.1 ml each of human citrated plasma, test solution or control, human brain thromboplastin, and CaCl₂ solution (0.02 M).

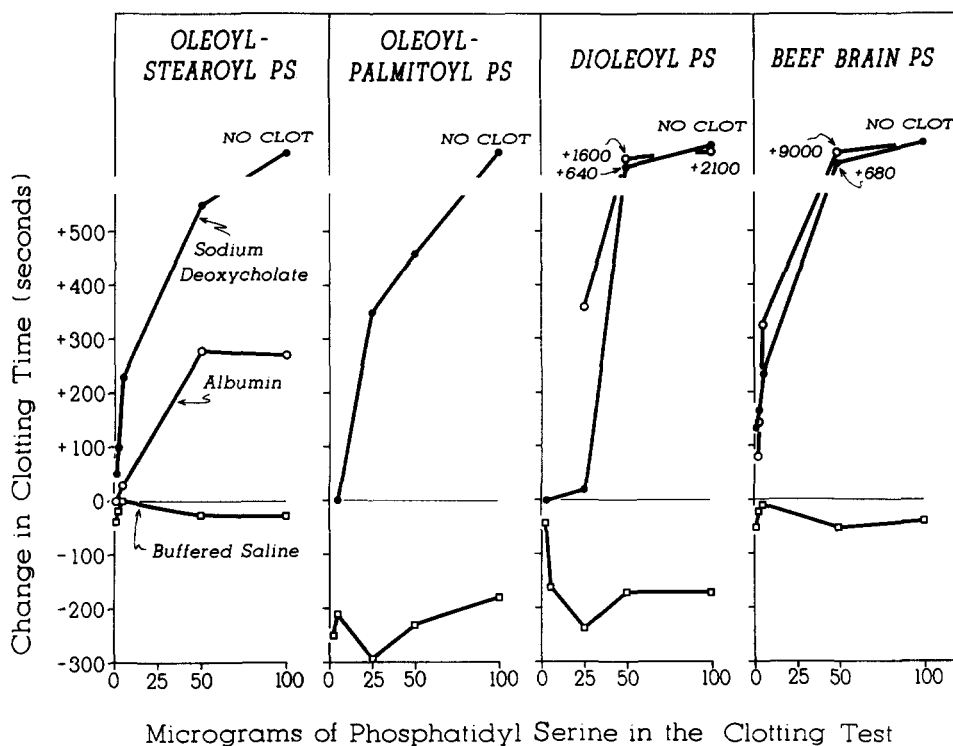


FIG. 1. Effects of four different phosphatidyl serines in the recalcification test. Phospholipids solubilized with sodium deoxycholate or albumin solutions or suspended in buffered saline solution. ●—●—● = PS solubilized with sodium deoxycholate; ○—○—○ = PS solubilized with albumin solution; □—□—□ = PS suspended in buffered saline solution. Test systems: 0.1 ml each of human citrated plasma, test solution or control, and CaCl_2 solution (0.02 M). Control clotting times: buffered saline, 430 sec; albumin, 300 sec; deoxycholate, 340 sec.

size) under the light microscope. Stable suspensions were obtained in buffered saline solution

RESULTS

Activities of the Various PS Preparations in the Hicks-Pitney Test

Table 1 shows the salient results obtained in this clotting test. The crude mixed phosphatides of Bell and Alton (35) (BA) used as a comparative acceleratory control (see ref. 5, p. 169) had a clotting time of 8 sec at six min incubation. This suggested the following criterion for good acceleratory activity (due to rapid plasma thromboplastin formation): the preparation being tested should produce substrate clotting times of 10 sec or less after short periods of incubation (maximum 6 min). By this criterion none of the preparations of PS had good acceleratory activity (Table 1). On the contrary, most of the preparations tested exhibited prolonged substrate clotting times, indicating interference with rate of plasma thromboplastin formation. All the data obtained in these tests have not been presented here because limitations of space make this impractical. However, in 7 of the 36

series of tests that were run there was indication of complete thromboplastin formation (substrate clotting in 10–12 sec) after prolonged periods of incubation (8–14 min). The clotting times at the 6-min incubation period have been presented here because they clearly indicate whether or not complete thromboplastin formation has occurred at a rapid rate.

Activities in the Antithromboplastin Test

Table 2 shows the results obtained in this test. When solubilized with sodium deoxycholate all of the PS preparations showed marked anticoagulant activity over the whole range of concentrations tested. The clotting times obtained by the synthetic preparations at the higher concentrations (100 and 200 μg) are roughly one-half those obtained with the beef brain preparation at similar concentrations. Quantitatively similar results were obtained when the four different PS preparations were solubilized with albumin. This was also true for the oleoyl-stearoyl and dioleoyl phosphatidyl serines, notwithstanding the fact that only the supernatant solutions (see above) were employed in the clotting tests and therefore the amounts of phosphatidyl serine actually tested were lower than indicated in Table 1 and Fig. 1 for these

preparations. When simple suspensions (in buffered saline solution) of the four PS preparations were tested, only the beef brain PS exhibited significant anticoagulant activity at levels of 50 and 100 μg in the test.

Activities in the Recalcification Test

Results obtained in this test are expressed graphically in Fig. 1, which shows increases or decreases in clotting time of citrated, human, platelet-poor plasma. In this test it can be seen that there is correlation between the degree of solubilization and the anticoagulant activity. For example, beef brain PS, which is well solubilized by both albumin and sodium desoxycholate, causes significant increases in the clotting time of the plasma even at low concentrations. The anticoagulant activity of the oleoyl stearoyl PS solubilized with albumin is somewhat less than that obtained with dioleoyl PS in albumin. It will be recalled that the dioleoyl PS was solubilized by albumin better than the oleoyl stearoyl PS. When the phosphatidyl serines were simply suspended in buffered saline solution, small to moderate decreases in the clotting times of plasma were obtained.

DISCUSSION

The effect of phosphatides in clotting tests appears to be dependent on the size, shape, and electric charge of micelles present in the test preparations (5). Solubilized PS has been shown to have anticoagulant effects both in vivo and in vitro, while PS simply suspended in saline has weak to moderate clot acceleratory activity. Daemen (36) has reported similar findings for a suspension of a synthetic (linolenoyl palmitoyl) serine of De Haas.

If the composition and degree of unsaturation of the fatty acid side chains are of importance for the solubilization of PS, and consequently for its physiologic or pharmacologic role in blood coagulation, it is important to compare the effects of phosphatidyl serines containing well defined, unsaturated fatty acids on blood coagulation with those of naturally occurring phosphatidyl serines.

The activities of the synthetic phosphatides, prepared as described here, in three different coagulation tests were qualitatively similar to those of natural (beef brain) PS. This shows that the anticoagulant activity of naturally occurring phosphatidyl serines, when properly solubilized, is indeed an effect of the class of phosphatidyl serines and is not caused by unknown trace contaminants.

It is not as yet possible to interpret clearly the quantitative difference in activity between the optically active beef brain PS and the synthetic racemic phosphatides, but the explanation may be as follows. The fatty acid composition of the beef brain PS is complex (5), and a number of highly unsaturated fatty acids are present.

These various unsaturated fatty acids may contribute substantially to the easy solubilization of the naturally occurring PS and thus to its enhanced anticoagulant activity. This problem will be resolved when synthetic PS preparations with a variety of fatty acids (similar to the naturally occurring ones) become available for testing individually and in specific mixtures.

The effect of stereochemical configuration is also of interest. A racemic phosphatidyl (dioleoyl) ethanolamine (37) was found to be fully as active as the natural stereoisomer. The present work also suggests that stereochemical configuration is not a factor in the anticoagulant activity of PS, but this requires more investigation after all four enantiomers have been synthesized.

The exact physiological significance of phosphatidyl serine is still unknown; its presence in blood platelets suggests that it plays an important role in blood coagulation, although recent investigations seem to indicate the participation of lipoproteins rather than lipids as such. The availability of chemically pure and well defined synthetic phosphatidyl serines may enhance our understanding of this complex process.

The anticoagulant properties of solubilized PS suggest its use as a therapeutic anticoagulant.

Supported by PHS Research Grant A-533 from the National Institutes of Health, U.S. Public Health Service.

Manuscript received December 30, 1963; accepted May 11, 1964.

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