K_A can be obtained from the "V" plots, K_C can be obtained from such a plot. Note that if K_m and K_v are known, the dissociation constant for the substrate K_s would be obtainable from the function K_s/K_mK_v. Previous use of product inhibition to determine K_s has however overlooked the potential effect of the K_p term.

Taking the reciprocal of the product-inhibition velocity equation, removing terms in p and p^3, and rearranging yields:

$$\frac{1}{v} = \frac{1}{V} + \frac{p^2}{V}\left(\frac{K_m}{sK_D} + \frac{1}{K_B}\right) \qquad (5)$$

A linear plot of $1/v$ vs. p^2 means that the p and p^3 terms are negligible in the region of p studied. The slope however is a complex function not easily evaluated.

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The Synthesis of α-Cephalins by a New Procedure*

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A new procedure for the synthesis of the racemic and optically active forms of both saturated and unsaturated α-cephalins has been developed. It is considerably shorter than any of the known procedures for the synthesis of cephalins because it does not require as starting materials α,β-diglycerides or derivatives thereof. The L-, D-, and DL-α-cephalins are obtained by phosphorylating D-, L-, or DL-acetone glycerol with phosphorus oxychloride and quinoline, esterifying the resulting acetone α-glycerylphosphoric acid dichlorides with 2′-hydroxyethylphthalimide, removing the acetone group by mild acid hydrolysis, acylating the barium salt of L-, D-, or DL-α-glycerylphosphoryl-2′-hydroxyethylphthalimide with a fatty acid chloride, and removing the phthaloyl group of the diacyl α-glycerylphosphoryl-2′-hydroxyethylphthalimides by hydrazinolysis. The preparation of L-α-(distearoyl)cephalin and L-α-(dipalmitoyl)cephalin is described.

The synthesis of cephalins possessing the α-structure and L configuration of the natural substances was first accomplished by Baer and co-workers (Baer *et al.*, 1951, 1952; Baer, 1957). The cephalins prepared by these authors possessed two identical saturated fatty acid substituents. Eight years later, Baer and Buchnea (1959) reported the first synthesis of an L-α-cephalin containing two identical unsaturated fatty acids.

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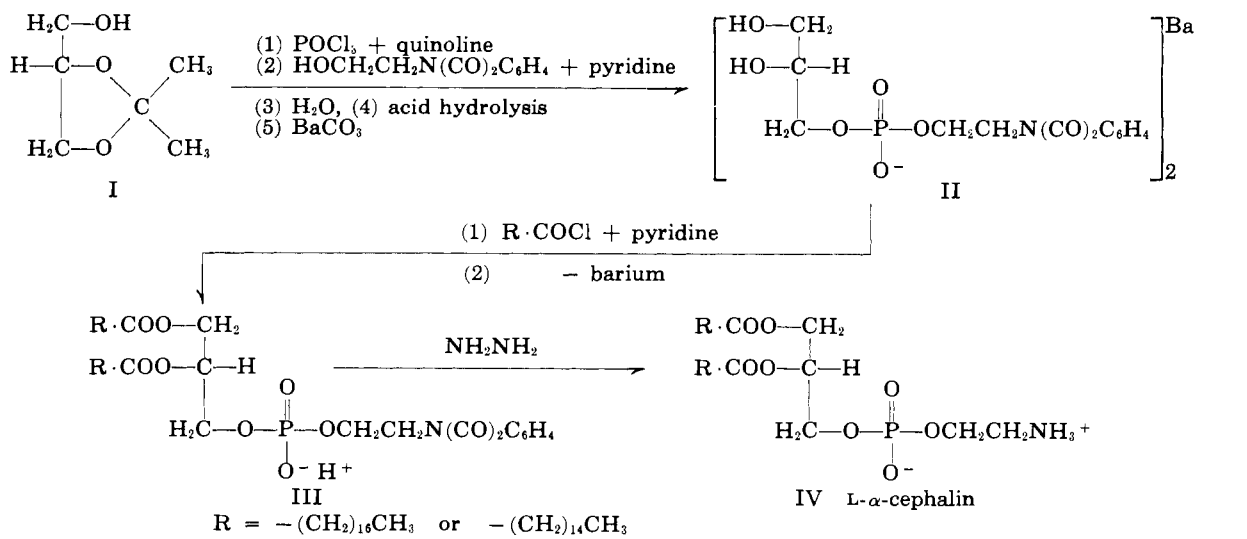
More recently, Baer and Buchnea (1961) described the first synthesis of two L-α-cephalins containing both a saturated and an unsaturated fatty acid. These (Baer *et al.*, 1951, 1952; Baer, 1957; Baer and Buchnea, 1959, 1961) and other procedures (Baylis *et al.*, 1958; Bevan and Malkin, 1951; Grün and Limpächer, 1927; Hirt and Berchtold, 1957; Hunter *et al.*, 1948; Rose, 1947; Shvets *et al.*, 1961) for the synthesis of α-cephalins require as starting materials α,β-diglycerides or suitably substituted derivatives thereof. Each diglyceride or derivative has to be prepared individually When one considers that the preparation of α,β-

diglycerides with two identical fatty acids from acetone glycerol requires three intermediates for saturated glycerides (Sowden and Fischer, 1941), four intermediates for the unsaturated ones (Baer and Buchnea, 1958), and eight for those containing two dissimilar fatty acids (Buchnea and Baer, 1960), it becomes obvious that much of the work involved in the synthesis of α-cephalins from α,β-diglycerides is spent on the preparation of the latter. It appeared worthwhile to seek a procedure for the synthesis of α-cephalins that would not require diglycerides as starting materials, and which would be applicable to the preparation of saturated as well as unsaturated α-cephalins in both of their optically active forms. In the following we describe such a procedure. It has the advantage of being considerably shorter than any of the known procedures for the synthesis of α-cephalins. The α-cephalins are obtainable by the new procedure in over-all yields that compare favorably with those reported for other procedures when the yields are calculated for a common starting material, i.e., acetone glycerol. Application of the new procedure to the synthesis of two known saturated α-cephalins is described. The synthesis of several unsaturated L-α-cephalins by this procedure has been accomplished and will be reported later.

Experimental Procedures

The essential features of the new procedure are the acylation, in the presence of pyridine, of the barium salt of α-glycerylphosphoryl-2′-hydroxyethylphthalimide with the chloride of the fatty acid to be introduced, and the removal of the phthaloyl group by hydrazinolysis (see the reaction scheme). Attempts to obtain the hitherto unknown L-α- and DL-α-glycerylphosphoryl-2′-hydroxyethylphthalimides (II) with phenylphosphoryl dichloride as phosphorylating agent were not successful. Although the acylation of D- and DL-acetone glycerol with phenylphosphoryl dichloride and quinoline, and esterification of the acetone L-α- and DL-α-glyceryl(phenyl)phosphoric acid chlorides with 2′-hydroxyethylphthalimide gave in good yields the acetone L-α- and DL-α-glyceryl(phenyl)phosphoryl-2′-hydroxyethylphthalimides (see Experimental, V and VR), removal of the phenyl group by catalytic hydrogenolysis and of the acetone group by mild acid hydrolysis yielded the barium salts of L-α- and DL-α-glycerylphosphoryl-2′-hydroxyethyl*hexahydro*phthalimides (VI and VIR). The removal of the phenyl group by catalytic hydrogenolysis, which consumed 8 moles of hydrogen, obviously was accompanied by a reduction of the phthaloyl group. Termination of the process at an earlier stage and removal of the acetone group by acid hydrolysis gave mixtures of α-glycerylphosphoryl-2′-hydroxyethylphthalimide and α-glycerylphosphoryl-2′-hydroxyethylhexahydrophthalimide which were difficult to separate. Acylation of the barium salts of VI and VIR with an excess of palmitoyl chloride and pyridine gave the corresponding *N-hexahydro*phthaloyl L-α- and DL-α-cephalins (VII and VIIR). These, however, could not be freed by hydrazinolysis from their protective hexahydrophthaloyl groups. Since the N-hexahydrophthaloyl-cephalins, which would be difficult to obtain by other methods, may possess interesting biological properties their preparation is described in the experimental part.

To obviate the necessity of removing the phenyl group with the concomitant reduction of phthalimide to hexahydrophthalimide, the phenylphosphoryl dichloride was replaced by phosphorus oxychloride. This change not only proved to be successful but also shortened the procedure by at least one step. The dipalmitoyl and distearoyl L-α-cephalins prepared by this procedure (see the reaction scheme) and by our earlier methods (Baer *et al.*, 1952; Baer, 1957) have identical properties. The D-α- and DL-α-cephalins are obtained by using as starting material L- and DL-acetone glycerol, respectively.



Materials.—D-Acetone glycerol, DL-acetone glycerol phenylphosphoryl dichloride (bp 103–106°/9 mm), and 2′-hydroxyethylphthalimide (mp 126.5–127.5°) were prepared as described by Baer (1952), Newman and Renol (1945), Zenftman and McGillivray (1951), and Rose (1947), respectively. Palmitoyl and stearoyl chloride were obtained as described by Fierz-David and Kuster (1939). The platinic oxide was prepared by the method of Adams *et al.* (1948), except that the sodium nitrate was replaced by an equimolecular amount of potassium nitrate (Cook and Linstead, 1934). The silicic acid was from Mallinckrodt (100 mesh powder, analytical reagent). It was sifted and all particles passing through a sieve of 150 mesh per linear inch were removed. Anhydrous pyridine was prepared from the best grade of pyridine by refluxing it for 8 hours over finely powdered barium oxide, and distilling it with the exclusion of moisture. Anhydrous quinoline was prepared from synthetic quinoline by shaking it for 2 days with barium oxide, and recovering the quinoline by vacuum distillation. The dimethyl-

formamide was dried with anhydrous potassium carbonate and distilled. Commercial hydrazine of a purity of not less than 95% was distilled from finely powdered sodium hydroxide. Anhydrous, ethanol-free chloroform was prepared for immediate use by distillation from phosphorus pentoxide. All distillations to remove solvents were carried out under reduced pressure (10–15 mm) from a bath at 30–40°.

Barium Salt of L-*α-Glycerylphosphoryl-2′-hydroxyethylphthalimide (II).*—Into a thoroughly dried 1.5-liter three-necked flask equipped with a dropping funnel, oil-sealed stirrer, and calcium chloride tube were placed 50 ml of anhydrous, ethanol-free chloroform and 9.20 ml (0.1 mole) of freshly distilled phosphorus oxychloride, and the flask was immersed in a mixture of crushed ice and sodium chloride at −12° to −15°. To the vigorously stirred solution of phosphorus oxychloride was added in the course of 90 minutes a solution of 13.2 g (0.1 mole) of D-acetone glycerol (I), and 14.1 ml (0.12 mole) of anhydrous quinoline in 250 ml of chloroform. The reaction vessel then was placed in a water bath at 25° which after 1 hour was raised to 35°. Thirty minutes later the temperature of the reaction mixture was lowered to 10°, and a solution of 19.1 g (0.1 mole) of 2′-hydroxyethylphthalimide and 32.2 ml (0.4 mole) of anhydrous pyridine in 300 ml of anhydrous chloroform was added over a period of 1 hour. The reaction mixture then was allowed to attain room temperature. Twenty hours later, 2.25 ml (0.125 mole) of distilled water was added with stirring, which was continued for 1 hour. The reaction mixture was evaporated under reduced pressure from a bath at 30–35°, and the residue was treated successively with three 250-ml portions of low-boiling petroleum ether and three 250-ml portions of anhydrous diethyl ether. The remaining material was extracted exhaustively with benzene. About six 250-ml portions of solvent were required, and the combined benzene extracts were evaporated to dryness under reduced pressure. The residue was dried in a vacuum of 0.1 mm at 30–35° for 4 hours.

This material was dissolved[1] in 500 ml of 80% methanol, the solution was passed through a column (5 × 40 cm) of Amberlite IR-120 (H-form), the column was rinsed with 1 liter of 80% methanol, and the combined effluents were evaporated under reduced pressure from a bath at 30–35°. The glasslike material was dissolved in 500 ml of distilled water, and the solution was allowed to stand at room temperature for 4 hours. It was then freed of traces of insoluble material by extraction with 250 ml of chloroform and 250 ml of ether. To the clear aqueous solution was added 25 g of lead carbonate, and the mixture was shaken for 1 hour. The insoluble lead salts were removed by centrifugation, the solution was freed of lead ions with hydrogen sulfide, and the filtrate was concentrated to a volume of approximately 250 ml by distillation under reduced pressure at 35–40°. To the concentrate was added 17.5 g of barium carbonate and the mixture was shaken for 1 hour. The excess of barium carbonate was removed by centrifugation, and the solution was evaporated to dryness under reduced pressure at 35–40°. The residue was taken up in 125 ml of methanol, the solution was freed of insoluble material, and the methanol was distilled off under reduced pressure. The residue was treated once more in the same manner. The remaining material was pulverized and was dried over phosphorus pentoxide at room temperature in a vacuum of 0.01 mm until its weight was constant. The barium salt of L-α-glycerylphosphoryl-2′-hydroxyethylphthalimide weighed 21.9 g (53.0% of theory). It was found to be readily soluble at room temperature in water, methanol, and dimethylformamide, and insoluble in ether, petroleum ether, benzene, or chloroform. $[\alpha]_D$ 0° in water, *c* 5; $[\alpha]_D$ ~ −0.5° in 2 N hydrochloric acid.

Anal. Calcd. for $C_{26}H_{30}O_{16}N_2P_2Ba$ (825.8): N 3.39, P 7.50, Ba 16.63. Found: N 3.05, P 7.41, Ba 16.22.

Dipalmitoyl L-*α-Glycerylphosphoryl-2′-hydroxyethylphthalimide (IIIa).*—Into a dry flask were placed 1.5 g (1.82 mmoles) of thoroughly dried barium salt of L-α-glycerylphosphoryl-2′-hydroxyethylphthalimide, 1.47 ml (18.2 mmoles) of anhydrous pyridine, and 15 ml of anhydrous dimethylformamide. To the solution was added in one portion 2.5 g (9.1 mmoles) of freshly distilled palmitoyl chloride, and the closed flask was placed in an oven at 70° for a period of 40 hours. At the end of this time the reaction mixture was poured with stirring into 100 ml of a mixture of crushed ice and water. After 2 hours the mixture was centrifuged and yielded an upper and lower solid phase. The aqueous phase was removed by suction, and the combined solids were triturated successively with three 60-ml portions of water and four 60-ml portions of acetone, separating the mixtures by centrifugation. The remaining material was extracted with three 70-ml portions of anhydrous ether, and the combined ether extracts were cleared by centrifugation. The ethereal solution was washed as rapidly as possible with 200 ml of ice-cold 0.15 N sulfuric acid and two 200-ml portions of distilled water. To break the rather stable emulsions which formed on washing with water, 5 ml of ethanol was added, and the emulsions were separated by centrifugation or filtration under suction. The ethereal solution was dried with anhydrous sodium sulfate and filtered, and the ether was distilled off under reduced pressure at 30–35°. The residue was dissolved in 20 ml of a mixture of chloroform (U.S.P.) and benzene (1:1, v/v), the solution was passed through a column of silicic acid (3 × 30 cm), and the column was washed with the same solvent mixture until the effluent was free of solute (palmitic acid). The *N*-phthaloyl L-α-(dipalmitoyl)cephalin was obtained by eluting the column exhaustively with chloroform (U.S.P.). The eluate was evaporated under reduced pressure from a bath at 35–40°, and the residue was crystallized from methanol, yielding 1.0 g (33.6% of theory) of dipalmitoyl L-α-glycerylphosphoryl-2′-hydroxyethylphthalimide; mp 74–76°. The material was soluble in chloroform, ether, or benzene, only slightly soluble in methanol or ethanol, and insoluble in water; $[\alpha]^{24}_D$ +3.8° in chloroform (*c* 5).

Anal. Calcd. for $C_{45}H_{76}O_{10}NP$ (822): C 65.74, H 9.32, N 1.70, P 3.78. Found: C 65.76; H 9.29; N 1.60, 1.64; P 3.87, 3.78.

Distearoyl L-*α-Glycerylphosphoryl-2′-hydroxyethylphthalimide (IIIb).*—This compound was synthesized and purified as described for the corresponding palmitoyl compound. Three g of the barium salt of L-α-glycerylphosphoryl-2′-hydroxyethylphthalimide gave 2.1 g (32.9% of theory) of *N*-phthaloyl L-α-(distearoyl)-cephalin; mp 81.5–83° after two recrystallizations from methanol; $[\alpha]_D$ +1.5° in ethanol-free chloroform (*c* 3).

Anal. Calcd. for $C_{49}H_{84}O_{10}NP$ (878.2): C 67.02, H 9.64, N 1.60, P 3.53. Found: C 67.32; H 9.85; N 1.55, 1.61; P 3.57, 3.55.

L-*α-(Distearoyl)cephalin (IVb).*—To a stirred suspension of 1.0 g (1.14 mmoles) of *N*-phthaloyl L-α-(di-

[1] To prevent loss of glycerylphosphoryl-2′-hydroxyethylphthalimide by acid hydrolysis, the part of the procedure enclosed by asterisks should be carried out within the same day.

stearoyl)cephalin in 80 ml of 99% ethanol at room temperature was added 0.32 ml (2.5 mmoles) of an aqueous 25% solution of hydrazine. The temperature of the mixture was raised to the boiling point during 30 minutes, and the mixture was refluxed for 2 hours. The clear solution was cooled to room temperature, 1.0 ml of glacial acetic acid was added, and the solution was kept overnight at +4°. The precipitate was collected by filtration with suction, and was washed on the filter with two 15-ml portions of ice-cold ethanol, two 20-ml portions each of water and acetone, and six 25-ml portions of boiling ether. The remaining white solid was taken up in 17 ml of warm chloroform, the solution was filtered while hot, and 23 ml of boiling methanol was added to the filtrate. The solution was allowed to stand at room temperature for 4 hours and at +6° overnight. The precipitate was collected with suction, and was dried over phosphorus pentoxide at room temperature in a vacuum of 0.1 mm. The L-α-(distearoyl)cephalin weighed 485 mg (57% of theory); mp 182–184°; $[\alpha]_D^{24}$ +6.0° in a mixture of chloroform and acetic acid (9:1, v/v), *c* 2.5.

Anal. Calcd. for $C_{41}H_{82}O_8NP$ (748.1): C 65.82, H 11.04, N 1.87, P 4.14. Found: C 65.48, H 10.89, N, 1.83, P 4.19.

The combined mother-liquors and wash-liquors were evaporated under reduced pressure from a bath at 35–40°, and the residue was dried in a vacuum of 0.02 mm. This material, on reprocessing with hydrazine as described above, yielded 202 mg (23.7% of theory) of L-α-(distearoyl)cephalin; mp 182–184°; $[\alpha]_D$ +5.9°.

Thin-layer chromatography of both batches of cephalin on silicic acid with a mixture of chloroform–methanol–acetic acid–water (50:25:8:4), and staining of the chromatograms with ninhydrin or Rhodamine 6G indicated that the first product was a homogenous substance, while the second product contained a minor amount of a slower moving component, presumably lysocephalin. This could not be removed by recrystallization from chloroform and methanol. However, purification was achieved by column chromatography on silicic acid. The cephalin was eluted with chloroform containing 2% methanol giving a 69% recovery of pure cephalin. The total yield of pure L-α-(distearoyl)cephalin was 625 mg (73.4% of theory).

L-α-*(Dipalmitoyl)cephalin (IVa)*.—The hydrazinolysis of N-phthaloyl L-α-(dipalmitoyl)cephalin (1.0 g, 12.2 mmoles) and the purification of the reaction product was carried out as described for the corresponding stearoyl compound and yielded 475 mg (56.4% of theory) of chromatographically pure L-α-(dipalmitoyl)cephalin; mp 186–188°; $[\alpha]_D^{24}$ +6.3° in a mixture of chloroform and acetic acid (9:1, v/v), *c* 3.

Anal. Calcd. for $C_{37}H_{74}O_8NP$ (692): C 64.22, H 10.78, N 2.02, P 4.48. Found: C 64.52, H 10.94, N 2.07, P 4.40.

The combined mother-liquors and wash-liquors contained mainly unreacted phthaloylcephalin and a small amount of cephalin. Reprocessing of this material with hydrazine and purification of the reaction product by column chromatography on silicic acid gave a further 125 mg (14.9%) of pure L-α-(dipalmitoyl)-cephalin. The total yield of pure cephalin was 600 mg (71.3% of theory).

Acetone L-α-*Glyceryl (phenyl) phosphoryl-2'-hydroxyethylphthalimide (V)*.—Into a dry three-necked 500-ml flask equipped with an oil-sealed stirrer, a calcium chloride tube, and a dropping funnel were placed 21.1 g (0.10 mole) of monophenylphosphoryl dichloride and 15 ml of anhydrous, ethanol-free chloroform. The flask was immersed in a bath of crushed ice and salt kept at −5°, and to the vigorously stirred solution of monophenylphosphoryl dichloride was added dropwise in the course of 30 minutes a solution of 13.2 g (0.10 mole) of D-acetone glycerol and 12.9 g (0.10 mole) of anhydrous quinoline in 15 ml of chloroform. The cold bath then was replaced by a water bath which was kept for 15 minutes at 25° and for 30 minutes at 35°. The water bath then was removed, and to the reaction mixture was added with stirring over a period of 30 minutes a solution of 15.8 g (0.2 mole) of anhydrous pyridine in 50 ml of chloroform, followed by the addition of a solution of 19.1 g (0.10 mole) of 2'-hydroxyethylphthalimide in 70 ml of pyridine.

After standing for 20 hours at room temperature, the reaction mixture was evaporated to dryness under reduced pressure (10–15 mm) from a bath at 35–40°, care being taken to remove as much as possible of the pyridine. The residue was extracted with four 150-ml portions of low-boiling petroleum ether. The remaining material was taken up in 500 ml of benzene, the insoluble pyridine and quinoline hydrochlorides were filtered off with suction and washed with 100 ml of benzene, and the combined filtrates were distilled under reduced pressure from a bath at 30–35°. The remaining viscous oil was dissolved in 50 ml of benzene and reprecipitated by the addition of 250 ml of low-boiling petroleum ether. The mixture was separated by centrifugation and the precipitate was treated twice more in the same manner. After the last mother liquor was decanted, the substance was transferred to a distilling flask by means of 50 ml of benzene, the benzene was distilled off under reduced pressure, and the residue was kept for 8 hours at 35–40° under a pressure of 0.03 mm. The acetone-L-α-glycerylphenylphosphoryl-2'-hydroxyethylphthalimide, a viscous pale yellow oil, weighed 38.2 g (82.8% of theory). It is readily soluble at room temperature in ethanol, acetone, chloroform, dioxane, and benzene, slightly soluble in ether, and insoluble in petroleum ether or water; n_D^{27} 1.5401; $[\alpha]_D^{27}$ +15.0° in dry and ethanol-free chloroform (*c* 5).

Anal. Calcd. for $C_{22}H_{24}O_8NP$ (461.4): N 3.04, P 6.71, acetone 12.59. Found: N 3.08, P 6.65, acetone 12.82.

Acetone DL-α-*Glyceryl(phenyl)phosphoryl-2'-hydroxyethylphthalimide (VR)*.—The compound was prepared by virtually the same procedure as described for the optical isomer, except that DL-acetone glycerol was used as starting material; n_D^{21} 1.5467.

Anal. Calcd. for $C_{22}H_{24}O_8NP$ (461.4): C 57.26, H 5.24, N 3.04, P 6.71, acetone 12.59. Found: C 57.72, H 5.27, N 3.17, P 6.71, acetone 11.71.

L-α-*Glycerylphosphoryl-2'-hydroxyethylhexahydrophthalimide (VI)*.—Lead Salt.—Into an all-glass hydrogenation vessel of 250-ml capacity were placed a solution of 5.0 g of acetone L-α-glyceryl(phenyl)phosphoryl-2'-hydroxyethylphthalimide (V) in 80 ml of 99% ethanol, and 1.0 g of platinic oxide (Adams catalyst), and the mixture was shaken vigorously in an atmosphere of pure hydrogen at an initial pressure of 50 cm of water until the absorption of hydrogen ceased (approximately 4 hours). The consumption of hydrogen amounted to 1.90 liter, i.e., 7.8 times the amount of hydrogen required for the hydrogenolysis of the phenyl ester. After the hydrogen was replaced with nitrogen, the contents of the reduction vessel were transferred to a centrifuge tube, and the catalyst was centrifuged off and washed with three 20-ml portions of 99% ethanol. The ethanolic solutions were combined, and distilled under reduced pressure from a bath at 35–40°. To the residue was added 10 ml of distilled water, and the solution was allowed to stand at room temperature for 16 hours. To remove traces of potassium, the solution was treated with 10 ml of Amberlite IR-120 (H form)

for 5 minutes. The Amberlite was filtered off and washed with distilled water, and the combined filtrates were extracted with two 30-ml portions of ether. To the clear aqueous solution was added 5 g of lead carbonate, the mixture was shaken for 1 hour, and the excess of lead carbonate was removed by centrifugation. The solution of the lead salt was evaporated under reduced pressure at 35–40°, the residue was taken up in 100 ml of 99% ethanol, the solution was cleared by centrifugation, and the ethanol was distilled off under reduced pressure at 35–40°. The residue was treated once more in the same manner but using 100 ml of acetone. The lead salt was dissolved in 50 ml of distilled water, the solution was extracted with two 50-ml portions of a mixture of 1 volume of benzene and 1 volume of ether, the water was distilled off under reduced pressure at 35–40°, and the remaining material was dried thoroughly at room temperature under a pressure of 0.01 mm. The lead salt of L-α-glycerylphosphoryl-2′-hydroxyethylhexahydrophthalimide, a colorless glasslike material, weighed 3.6 g (73.2% of theory); $[\alpha]_D$ +1.2° in water (c 4.3). It is readily soluble at room temperature in water, acetone, or methanol, but insoluble in ether, benzene, or chloroform.

Anal. Calcd. for $C_{26}H_{42}O_{16}N_2P_2Pb$ (907.2): N 3.09, P 6.38. Found: N 3.28, P 6.69.

Barium Salt.—To convert the lead salt into barium salt, 3.4 g of the former was dissolved in 50 ml of water, the lead ions were removed with hydrogen sulfide, the lead sulfide was filtered off, the filtrate was concentrated under reduced pressure at 35–40° to about one-fifth of its volume, and to the concentrate were added 50 ml of water and 1.8 g of barium carbonate. The mixture was shaken for 1 hour, the excess of barium carbonate was removed by centrifugation, and the aqueous solution was evaporated to dryness under reduced pressure at 35–40°. The residue was taken up in 30 ml of methanol, the solution was cleared by centrifugation, and the methanol was distilled off under reduced pressure. The amorphous residue was pulverized and dried at room temperature over phosphorus pentoxide under a pressure of 0.01 mm until its weight was constant. The barium salt of L-α-glycerylphosphoryl-2′-hydroxyethylhexahydrophthalimide weighed 3.0 g (95.6% of theory). It is readily soluble at room temperature in water, methanol, or dimethylformamide, but insoluble in ether, benzene, or chloroform; $[\alpha]_D$ 0° in water (c 7.5).

Anal. Calcd. for $C_{26}H_{42}O_{16}N_2P_2Ba$ (837.8): N 3.34, P 7.40. Found: N 3.48, P 7.28.

DL-α-Glycerylphosphoryl-2′-hydroxyethylhexahydrophthalimide (VIR).—Lead Salt.—It was obtained from acetone DL-α-glycerylphenylphosphoryl-2′-hydroxyethylphthalimide (VI) by the procedure described for the preparation of the lead salt of the corresponding optically active compound. Yield, 77% of theory. The lead salt, a colorless glasslike material is readily soluble at room temperature in water or acetone, slightly soluble in 99% ethanol, and insoluble in ether, chloroform, benzene, or dimethylformamide.

Anal. Calcd. for $C_{26}H_{42}O_{16}N_2P_2Pb$ (907.2): N 3.09, P 6.83. Found:[2] N 3.15, 3.16, 3.24; P 6.64, 6.69, 6.65.

Barium Salt.—It was prepared from the lead salt of DL-α-glycerylphosphoryl-2′-hydroxyethylhexahydrophthalimide (VIR) as described for the corresponding optically active compound. Yield, 87% of theory. Its solubility properties are similar to those reported for the barium salt of the L-isomer.

Anal. Calcd. for $C_{26}H_{42}O_{16}N_2P_2Ba$ (837.8): N 3.34, P 7.40. Found: N 3.23, P 7.41.

[2] Analyses of several independent preparations.

Free Acid.—To obtain DL-α-glycerylphosphoryl-2′-hydroxyethylhexahydrophthalimide, 7.0 g of its lead salt was dissolved in 70 ml of distilled water, the lead ions were precipitated with hydrogen sulfide, the lead sulfide was filtered off, the filtrate was evaporated under reduced pressure, and the residue was kept for at least 12 hours at 35–40° in a vacuum of 0.01 mm. The free acid, a colorless glasslike material, weighed 4.6 g (85.2% of theory). It is readily soluble at room temperature in water, methanol, ethanol, or pyridine, but insoluble in ether, chloroform, or benzene.

Anal. Calcd. for $C_{13}H_{22}O_8NP$ (357.3): N 3.92, P 8.67. Found: N 3.92, P 8.52.

Identification of the Reduction Product of Phthalic Acid as Hexahydrophthalic Acid.—One gram of barium salt of the product obtained by the reductive cleavage and deacetonation of acetone DL-α-glycerylphenylphosphoryl-2′-hydroxyethylphthalimide, and 5 ml of concentrated hydrobromic acid (48%) were boiled under reflux for 5 hours. The reaction mixture was diluted with 30 ml of distilled water, and the solution was evaporated to dryness under reduced pressure from a bath at 45–50°. The remaining brown material was dissolved in 15 ml of water, the solution was decolorized with charcoal, and the filtrate was evaporated to dryness under reduced pressure. The pale yellow crystalline material thus obtained on repeated crystallization from hot water gave 0.30 g (73.0% of theory) of hexahydrophthalic acid; mp 185–188°.

Anal. Calcd. for phthalic acid ($C_8H_6O_4$): C 57.84, H 3.64; for hexahydrophthalic acid ($C_8H_{12}O_4$): C 55.81, H 7.03. Found: C 55.78, H 7.08.

Dipalmitoyl L-α-glycerylphosphoryl-2′-hydroxyethylhexahydrophthalimide (VII).—Into a dry flask were placed 2.6 g (3.1 mmoles) of thoroughly dried barium salt of L-α-glycerylphosphoryl-2′-hydroxyethylhexahydrophthalimide (VI), 2.5 ml (31 mmoles) of anhydrous pyridine, and 20 ml of anhydrous dimethylformamide, and to the solution was added in one portion 5.1 g (18.6 mmoles) of palmitoyl chloride. The closed flask was placed in an oven at 65° for a period of 40 hours. At the end of this time flask and contents were brought to room temperature, and 10 ml of crushed ice and water was added. Two hours later the mixture was poured with stirring into 200 ml of water, and the white precipitate was collected by centrifugation and was washed successively with three 200-ml portions of distilled water and three 200-ml portions of methanol; each time the mixture was separated by centrifugation. The semisolid material was dissolved in 100 ml of ether, the solution was cleared by centrifugation, the ether was distilled off under reduced pressure at 30–35°, and the residue was kept for 5 hours over phosphorus pentoxide under a pressure of 0.02 mm. The crude barium salt of dipalmitoyl L-α-glycerylphosphoryl-2′-hydroxyethylhexahydrophthalimide, weighing 3.2 g, was dissolved in 200 ml of ether, and the solution was washed successively with 200 ml of ice-cold 0.5 N sulfuric acid and two 200-ml portions of distilled water. To break the rather stable emulsions which form with water, 5 ml of 99% ethanol was added, and the mixtures were separated by centrifugation. The ethereal solution was dried with anhydrous sodium sulfate, and the ether was distilled off under reduced pressure. The residue was dissolved in 20 ml of benzene, the solution was passed through a column of silicic acid (3 × 30 cm), and the column was washed until the effluent was free of solute. The *N*-hexahydrophthalimide L-α-(dipalmitoyl)cephalin (VII) was recovered by washing the column exhaustively with a mixture of 5 volumes of methanol and 95 volumes of benzene. The eluate was concentrated under reduced

pressure at 35–40°, and the residue was kept in a vacuum of 0.01 mm until its weight was constant. The *N*-hexahydrophthaloyl L-α-(dipalmitoyl)cephalin, a waxy material, weighed 2.24 g (43.6% of theory). It was found to be soluble at room temperature in methanol, ether, chloroform, or benzene, and insoluble in water.

Anal. Calcd. for $C_{45}H_{82}O_{10}NP$ (828.1): N 1.69, P 3.74. Found: N 1.81, P 3.66.

Dipalmitoyl DL-*α-glycerylphosphoryl-2′-hydroxyethyl hexahydrophthalimide (VIIR).*—This compound was obtained from the barium salt of DL-α-glycerylphosphoryl-2′-hydroxyethylhexahydrophthalimide(VIR) by virtually the same procedure as that reported above for the L isomer. Its solubility properties resemble closely those of the L isomer.

Anal. Calcd. for $C_{45}H_{82}O_{10}NP$ (828.1): N 1.69, P 3.74. Found: N 1.75, P 4.12.

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The Effect of Interstitial-Cell-stimulating Hormone on the Biosynthesis of Testicular Cholesterol from Acetate-1-C^{14}*

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The influence of interstitial-cell-stimulating hormone (ICSH) on the incorporation of acetate-1-C^{14} into cholesterol and testosterone by slices of rabbit testis was examined by the application of a method for rigorous purification of testicular cholesterol-C^{14}. When slices were incubated with ICSH, the cholesterol-C^{14} showed less radioactivity and lower specific activity than slices incubated without ICSH. On the other hand, slices incubated with ICSH showed more testosterone-C^{14} than those incubated in the absence of ICSH. When ICSH was administered *in vivo* and serial testicular biopsies were incubated *in vitro* with acetate-1-C^{14}, a fall in cholesterol-C^{14} and an increase in testosterone-C^{14} were observed. It is considered that these findings could be explained by assuming that cholesterol is an intermediate in the biosynthesis of testosterone, and that ICSH stimulates the biosynthetic pathway between acetate and testosterone at some point(s) after cholesterol.

It is generally accepted that cholesterol can be converted to steroid[1] hormones by those tissues which produce these hormones (Zaffaroni *et al.*, 1951; Werbin *et al.*, 1957; Ungar and Dorfman, 1953; and Bloch, 1945). However, it has not been possible to establish an *obligatory* role for cholesterol in the biosynthesis of steroid hormones (Samuels, 1960). Measurements of the specific activity of cholesterol-C^{14} formed from acetate-C^{14} during the biosynthesis of C^{14}-labeled steroids by endocrine tissue *in vitro* have revealed that the specific activities of the labeled steroids are higher than that of the cholesterol-C^{14} isolated from the same tissue (Hechter, *et al.*, 1953; Bligh, *et al.*, 1955). Brady (1951) observed increased incorporation of acetate-1-C^{14} into testosterone by slices of testis in the presence of human chorionic gonadotrophin without any demonstrable change in the radioactivity of the digitonin-precipitable material isolated from the same slices. Such findings have led to the suggestion that much of of the cholesterol in steroid-forming organs is excluded from the pathway to steroids. Moreover, in some cases meticulous purification of cholesterol-C^{14} to eliminate "high-counting companions" as described by Schwenck and Werthessen, (1952) has not been undertaken, with the result that the role of cholesterol in the biosynthesis of steroids remains uncertain.

It has been shown that interstitial-cell-stimulating hormone (ICSH) both *in vivo* and *in vitro* increases the incorporation of acetate-1-C^{14} into testosterone by slices of rabbit testis (Hall and Eik-Nes, 1962a). The present experiments were designed to examine the influence

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[1] Throughout this paper the word steroid is used for 19- and 21-carbon compounds as distinct from sterol, which is reserved for cholesterol and other 27-carbon compounds. Abbreviations used are: ICSH, interstitial-cell-stimulating hormone; PPO, 2,5-diphenyloxazole; POPOP, 1,4-2-(5-phenyloxazolyl)-benzene.