lowed by rise to a peak of 133% of the initial level, reached after 4 hours and tapering off in the next 20 hours. A sharp decrease was observed thereafter. Less than 10% of the original concentration was found after 24 days of the 6 mg regimen. No ACTH could be detected after 55 days, at the 3 mg dosage level. This regimen (3 mg) induced an exponential reduction of the adrenal weight to hypophysectomy level within 32-55 days. From available indices of ACTH release under comparable conditions, it is inferred that the initial post-injection fall of pituitary ACTH is related to the ACTH-releasing effect of the injection *per se,* and its subsequent rise, to the inhibitory effect of the steroid on further release. Sustained and complete blockade of release during the chronic phase of the treatment is suggested by the rate of adrenal weight loss. The concomitant disappearance of pituitary ACTH is imputed to inactivation of the stored hormone in the absolute or relative absence of synthesis.

1. Fortier, C., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 13.

2. Saffran, M., Schally, A. V., *Endocrinology,* 1955, v56, 523.

3. Bliss, C. I., *Statistics of Bioassay,* Acad. Press, N. Y., 1952.

4. Harris, G. W., Jacobsohn, D., *Proc. Roy. Soc. B,* 1952, v139, 263.

5. Ingle, D. J., *J. Clin. Endocrinol.,* 1950, v10, 1312.

6. Ingle, D. J., Prestud, M. C., Nezamis, J. E., *Am. J. Physiol.,* 1951, v166, 171.

7. Farrell, G. L., Laqueur, G., *Endocrinology,* 1955, v56, 471.

8. Michailova, N. V., *Probl. Endocrinol. and Hormonotherap.,* (Moscow), 1956, v2, 79.

9. Kitay, J. I., Holub, D. A., Jailer, J. W., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 165.

10. Fortier, C., Yrarrazaval, S., Selye, H., *Am. J. Physiol.,* 1951, v165, 466.

11. Porter, J. C., Jones, J. C., *Endocrinology,* 1956, v58, 62.

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Lipid Composition of Human Serum Lipoprotein Fraction with Density Greater than 1.210.* (24508)

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On ultracentrifugation of samples of untreated human serum, Turner *et al.*(1) found that the fraction of high density at the bottom of the tube contained phospholipid and neutral fat in concentrations approximating those of whole serum, but little cholesterol. Subsequently, Turner *et al.*(2) estimated that approximately 65% of the serum phospholipid was present in a lipoprotein containing no free cholesterol. Hillyard, Entenman, Feinberg and Chaikoff(3) reported that on ultracentrifugation of human serum, the density of which had been raised to 1.220 by addition of potassium bromide, a fraction sedimented which contained an average of 13% of the total serum phospholipid and small quantities of cholesterol. Using more vigorous centrifugation of serum with density raised to 1.21, Havel, Eder and Bragdon(4) sedimented a fraction which contained lipid phosphorus but practically no cholesterol. They estimated that such a lipoprotein, which varied little in concentration between healthy subjects and patients with hyperlipemia, could account for no more than 15% of the total serum phospholipid. The lipid (ethanol-acetone soluble) phosphorus, which was not dialyzable against 0.15 M sodium chloride, was precipitable with serum proteins by zinc hydroxide, soluble in water, insoluble in petroleum ether, and migrated in starch electrophoresis largely with the a_1 globulin-albumin fraction. Similarities of this phosphorus fraction and a serum phos-

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TABLE I. Phospholipid Composition of Lipoprotein of Density Greater than 1.210.

Patient	Sex	Age	% total lipid P				% of total serum lyso-lecithin in 1.210 fraction	% of total lipid P in 1.210 fraction	Total serum lipid P, μMole/ml
			"Cephalin"	Lecithin	Sphingo-myelin	Lyso-lecithin			
E.R.	♂	22	3	23	7	68			2.90
N.R.	♂	23	5	31	15	49	52	9.0	2.58
H.D.	♂	24	7	21	17	55	66	7.5	2.70
A.G.	♂	39	3	22	6	70	61	8.3	3.06
R.D.	♀	31	9	30	18	43	48	8.0	4.01
A.R.	♀	31	11	20	19	50	50	6.0	3.30
Mean			6	25	14	56	55	7.8	3.09
S-1*	♂		5	20	13	63			
S-2*	♂		2	22	5	70			
S-3*	♂		3	27	9	60		9.3	3.31
S-4†	♂		6	33	14	48		5.7	3.07

* Nonfasting sample from healthy young adult male.
† Pooled nonfasting sample from 2 healthy young adult males. Density, 1.262.

phopeptide (containing no phospholipid) described by Hack(5) were pointed out, and Bragdon, Havel, and Boyle(6) postulated that the serum fraction sedimented at density 1.21 contained no true lipid other than unesterified fatty acids. In the present study, the ultracentrifugal method of Havel, Eder, and Bragdon(4) was used to isolate the serum fraction with density greater than 1.210, and the lipid extract of this fraction was chromatographed on silicic acid(7,8). Evidence was found for the presence of phospholipid and triglyceride, but little, if any cholesterol. The phospholipid, which comprised about 8% of the total serum phospholipid of 5 healthy young adults, was composed predominantly of lysolecithin.

Materials and methods. Serum samples were obtained from 4 male and 2 female white adults in the fasting state and from 5 young male white adults 4 to 5 hours after a light breakfast (Table I). All 11 subjects were apparently healthy. Ultracentrifugation of serum was begun within 2 to 3 hours after withdrawal according to the method of Havel, Eder, and Bragdon(4). Samples were centrifuged at 105,000 x g at 15°C in the #40 rotor of a Model L Spinco ultracentrifuge. In the series of fasting subjects, 5 to 8 ml of serum were diluted to a density of 1.019 using a NaCl-KBr solution of density 1.085(4), centrifuged for 22 hours, and the top layer removed. A known amount of the remaining sample was then diluted to density 1.210 using NaCl-KBr solution of density 1.346(4) and centrifuged for 48 hours. The bottom layer, which contained lipoprotein of density greater than 1.210, was then extracted for lipid analysis. In one of the non-fasting subjects (S-1, Table I), 10 ml of serum was made to a density of 1.019, centrifuged for 22 hours, and the upper layer removed; the bottom layer was made to a density of 1.063, centrifuged for 22 hours, and the upper layer removed; the bottom layer was then made to a density of 1.210, centrifuged for 48 hours, and the bottom layer extracted. In the other subjects, 9 ml of serum was made directly to a density of 1.210 (S-2, S-3, Table I) or 1.262 (S-4, Table I) with the solution of density 1.346, centrifuged for 48 hours, and the bottom layer extracted. In all these samples, centrifugation resulted in a distinct layer at top and bottom of tube with a colorless zone, about one-half the length of the tube, in between. The sedimented serum fraction with density greater than 1.210 was always visibly clear. The fractions were separated by slicing the tube at approximately the center of the colorless zone with a Spinco tube slicer. Centrifuged serum samples were extracted with methanol-chloroform (1:1) and the extracts chromatographed on silicic acid columns (1 g), to determine concentrations of individual phospholipids, cholesterol, cholesterol esters, and triglycerides(8). In fasting

subjects, these same determinations were done on the centrifuged fractions with density less than 1.210 and on whole serum (to be published). In 2 (S-3, S-4, Table I) of the nonfasting subjects, only total serum lipid phosphorus was determined, to calculate the percentage of total lipid phosphorus present in the fraction with density greater than 1.210. The lipid extract of centrifuged serum was also chromatographed on silicic acid-impregnated filter paper (Whatman #3) using 20% methanol in chloroform (v/v) as solvent. In addition, column fractions were hydrolyzed in 6N HCl at 110° for 18 hours in a sealed tube and chromatographed on filter paper (Whatman #1) using butanol-acetic acid-water (4:1:4, using butanol layer) as solvent. These paper chromatograms were developed by (1) spraying with ninhydrin in butanol, followed by heating at about 110° for 5 minutes and (2) the phosphomolybdic acid stain of Chargaff, Levine and Green(9). The column fractions were analyzed for phosphorus by the method of Fiske and Subbarow(10), for cholesterol and cholesterol esters by the method of Schoenheimer and Sperry(11) as modified by Sperry and Webb(12) and for ester bonds by the method of Rapport and Alonzo(13). The quantities of phosphorus in the "cephalin" and sphingomyelin fractions were usually so small that the errors in the phosphrous determinations were of the order of $\pm$ 30%.

Results. In fasting normal subjects, lipid phosphorus of serum fraction with density greater than 1.210 comprised about 8% of total serum lipid phosphorus (Table I). About one-half of this lipid phosphorus appeared to be in the form of lysolecithin; and the lysolecithin in this fraction in turn accounted for about one-half of total serum lysolecithin. The other phospholipid constituents, identified according to mobility on column, were lecithin, sphingomyelin, and "cephalin," in order of decreasing concentration. Some variation in concentrations of these phospholipids between the subjects may be attributed to errors in determination of the small amounts of phospholipid chromatographed and, possibly, to the level at which the centrifuge tube was sliced. Centrifuging the serum at a density of 1.262 instead of 1.210 seemed to have little effect on phospholipid composition of this fraction (Table I).

The lysolecithin fraction in 2 samples tested had an ester-to-phosphorus ratio of 0.9 and had the same mobility as the lysolecithin of whole serum lipid extract when chromatographed on silicic acid-impregnated filter paper with methanol-chloroform elution. Acid hydrolysis of this material followed by filter paper chromatography with butanol-acetic acid-water elution gave rise to one spot with the phosphomolybdic acid stain with the mobility of choline and very small amounts of ninhydrin-reacting material. When 0.01 micromoles (on basis of phosphorus) of this fraction were incubated with washed fresh sheep red blood cells at 37.5°C for 2 hours, visible hemolysis was evident.

Concentrations of cholesterol and cholesterol esters, which were eluted initially off the column(8), were too small to be measured. Less than 0.2 mole of total cholesterol/mole of phosphorus appeared to be present. Total ester, however, presumed to be triglyceride, could be measured and fell within a fairly narrow range, averaging about one mole of triester per 2 to 3 moles of phosphorus.

Discussion. The evidence presented indicates that the fraction of centrifuged human serum with density greater than 1.210 contains phospholipid and non-phospholipid ester, presumably triglyceride, with little or no cholesterol, either free or esterified. The high density of this fraction, the low water solubility of most lipids present, lack of opalescence in this fraction, and the demonstration that the lipid migrates with α_1-globulin-albumin in starch electrophoresis(4,14) make it clear that the lipid in this fraction is bound to protein. The high density, moreover, suggests a low lipid-to-protein ratio. Whether all of these lipids are attached to one protein in the proportions found cannot be stated, although lack of discernible difference in lipid composition of a fraction of density greater than 1.262 as compared to one of density greater than 1.210 was suggestive of this possibility.

This lipoprotein fraction was unusual not

only in its content of little or no cholesterol. but because about one-half of the phospholipid appeared to be in the form of lysolecithin. The lysolecithin in this fraction in turn accounted for about one-half of the total serum lysolecithin. The properties of the ethanol-acetone soluble phosphorus found in this fraction by Havel, Eder, and Bragdon (4) are consistent with those of lysolecithin (15). The high density ethanol-ether soluble phosphorus described by Hack(5) as a phosphopeptide with its phosphorus in the form of serine phosphate, however, may be a different substance.

The percentage of total serum phospholipid in this fraction is in general agreement with the values of Havel, Eder, and Bragdon(4). It is conceivable, however, that the density of this lipoprotein is a function of its triglyceride content, since in this fraction the triester-to-phosphorus ratio varied little between samples. If this is the case, it is possible that more of this lipoprotein could be isolated by ultracentrifugation at a serum density somewhat less than 1.210 and greater than 1.125. as Havel, Eder, and Bragdon(4) showed that at 1.125 density other lipoproteins apparently sediment. If all serum lysolecithin were present in this type of lipoprotein, moreover, serum concentration of this lipoprotein would be double, as about one-half of serum lysolecithin was found in serum fractions with density less than 1.210, including fractions with densities less than 1.019. 1.019 to 1.063. and 1.063 to 1.210 (to be published).

Although no metabolic role can be ascribed as yet to this lipoprotein fraction, Turner *et al.*(2) reported that phosphorus turnover in the lipoprotein system without cholesterol appeared to be greater than in the system with cholesterol.

Summary. A human serum lipoprotein fraction with density greater than 1.210 has been separated by ultracentrifugation and found to contain about 8% of total serum phospholipid, non-phospholipid ester, presumably triglyceride, and little or no cholesterol. About one-half of the phospholipid was found to be in the form of lysolecithin, which in turn comprised about one-half of the total lysolecithin of serum.

1. Turner, R. H., Snavely, J. R., Goldwater, W. H., Randolph, M. L., Sprague, C. C., Unglaub, W. G., *J. Clin. Invest.*, 1951, v30, 1071.

2. Turner, R. H., Snavely, J. R., Goldwater, W. H., Randolph, M. L., *Yale J. Biol. and Med.*, 1952, v24, 450.

3. Hillyard, L. A., Entenman, C., Feinberg, H., Chaikoff, I. L., *J. Biol. Chem.*, 1955, v214, 79.

4. Havel, R. J., Eder, H. A., Bragdon, J. H., *J. Clin. Invest.*, 1955, v34, 1345.

5. Hack, M. H., *Fed. Proc.*, 1955, v14, 222.

6. Bragdon, J. H., Havel, R. J., Boyle, E., *J. Lab. and Clin. Med.*, 1956, v48, 36.

7. Phillips, G. B., *Proc. Nat. Acad. Sci.*, 1957, v43, 566.

8. ———, *Bioch. et Biophys. Acta*, 1958, v29, 594.

9. Chargaff, E., Levine, C., Green, C., *J. Biol. Chem.*, 1948, v175, 67.

10. Fiske, C. H., Subbarow, J., *ibid.*, 1925, v66, 375.

11. Schoenheimer, R., Sperry, W. M., *ibid.*, 1934, v106, 745.

12. Sperry, W. M., Webb, M., *ibid.*, 1950, v187, 97.

13. Rapport, M. M., Alonzo, N., *ibid.*, 1955, v217, 193.

14. Kunkel, H. G., Trautman, R., *J. Clin. Invest.*, 1956, v35, 641.

15. Hanahan, D. J., Redbell, M., Turner, L. D., *J. Biol. Chem.*, 1954, v206, 431.

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