investigated in 40 litters. A few representative results are given in Table V. This table shows that thyroxine, triiodothyronine and thyrotrophic hormone alike increase the dry substance content of all brain parts. Triiodothyronine does not appear to have a more marked action than thyroxine.

Discussion. It has been shown in these investigations that the oxygen consumption of the brain of the newborn rat can be increased under the influence of thyroid hormones. Thus it is considerably more sensitive to this treatment than the brain of the grown-up animal. It appears feasible that this action is equivalent to an acceleration of a physiological maturation process taking place in the brain. It is known(3) that the oxygen consumption of the brain rises during the first weeks of life.

It would be interesting to know why thyroid hormone acts as described on the brain of the newborn animal and not on that of the adult. It has been recently shown that the blood brain barrier is not yet established in newborn rats against glutamic acid(4) or Cl^{36}, or Thiocyanate(5). It would be tempting to assume that, similarly, during the first few days of life, thyroid substances can more easily contact the brain cells, owing to the fact that the blood brain barrier is not yet fully developed during this time.

Summary. 1. Thyroxine, triiodothyronine and thyrotrophic hormone significantly raise the brain oxidation of newborn rats; the dry substance content is also raised. These changes were regarded as an acceleration of normal maturation processes. 2. It is assumed that the described action of thyroid hormones on the brain of newborn animals is due to a still incompletely developed blood brain barrier.

1a. Hoexter, Frieda M., *Endocrinology*, 1954, v54, 1.

1. MacLeod, L. C., and Reiss, M., *Biochem. J.*, 1940, v34, 820.

2. Reiss, M., *Z. f. exp. Med.*, 1930, v77, 345.

3. Tyler, D. B., and van Harreveld, A., *Am. J. Physiol.*, 1942, v136, 600.

4. Himwich, H. E., and Himwich, W. A., in *Biochemistry of the developing nervous system*, Academic Press, 1955, p202.

5. Waelsch, H. in *Biochemistry of the developing nervous system*, Academic Press, 1955, p187.

Received February 9, 1956. P.S.E.B.M., 1956, v93.

In vitro Effect of Soybean Phosphatides on Serum Lipoproteins of Normal and Hyperlipemic Subjects.* (22651)

BERNARD A. SACHS AND ETHEL DANIELSON.
Medical Division, Montefiore Hospital, New York City.

The oral administration of soybean lecithin has previously been reported to lower the serum cholesterol of man(1) and cholesterol-fed rabbits(2). The present study was initiated when preliminary experiments revealed that incubation of human serum, after addition of a fat emulsion prepared for intravenous administration, caused a shift of lipid from beta-globulin to alpha-globulin, as determined by paper electrophoresis. Because lecithin was used as a stabilizer in the fat emulsion, this phenomenon was investigated further using soybean phosphatides. The effects of not only the whole phosphatide complex, but also the alcohol-soluble and the alcohol-insoluble fraction were investigated.

Material and methods. 1. *Exp. A—Whole Phosphatide Complex.* A purified extract of natural soybean phosphatides† containing approximately equal quantities of lecithin, cephalin and lipositol was used as follows: Five mg of soybean phosphatides were dissolved in 1 or 2 ml of sera from 6 normal subjects in

* Supported in part by grants from the Lasdon Foundation, and the Office of the Surgeon General, Department of the Army.

† This compound, Asolectin, was kindly supplied by Mr. J. Eichberg of the American Lecithin Co., Inc.

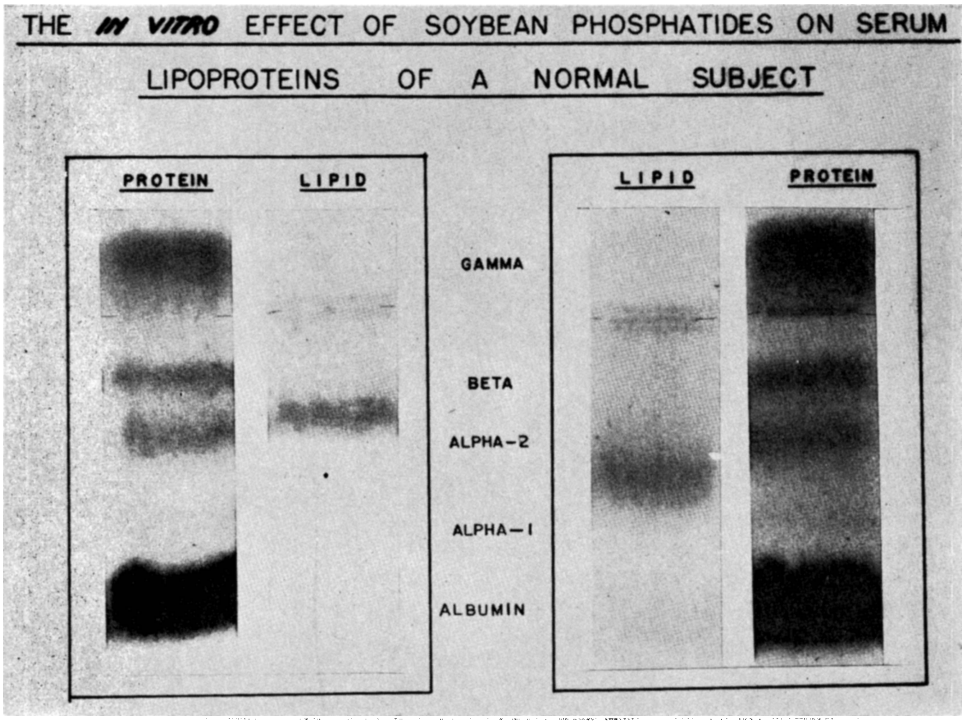

FIG. 1. Effect of incubating the whole phosphatide complex with serum from a normal subject. Lipid stain is red; black and white reproduction does not permit good visualization of the faint alpha lipoprotein band.

the post-absorptive state. In a similar manner, 10 or 20 mg of the phosphatide complex were dissolved in the sera of 5 patients with hyperlipemia.‡ Serum aliquots without added phosphatide served as controls. The control sera and sera with added phosphatide were immediately streaked on filter paper strips for electrophoretic analysis, which was promptly performed with Flynn and deMayo's modification of Durrum's apparatus(3). The serum aliquots of 0.01 or 0.02 ml were streaked for protein or lipid staining respectively. The remaining control and experimental sera were incubated at 38°C for 24 hours, before being subjected to paper electrophoresis. Protein was stained with naphthalene black 12B200 and lipid with oil red O by technics previously described(4). Total lipids were determined gravimetrically (4), and lipid phosphorus by a modified Fiske-SubbaRow procedure(5) in aliquots of serum extracted with Bloor's reagent (alcohol 3 parts/anhydrous ether, 1 part). Cholesterol was determined by Bloor's method(6) on the total lipid residue.

2. Exp. B-Alcoholic Fractions of the Phosphatide Complex. The effects of the alcohol-soluble fraction of the soybean phosphatides, consisting of approximately ⅔ lecithin and ⅓ cephalin, and the alcohol-insoluble fraction, consisting of approximately ⅔ lipositol and ⅓ cephalin, on the serum proteins and lipoproteins of 10 normal and 9 hyperlipemic subjects were studied. In 1 ml of each normal serum, 5 mg of each fraction were separately dissolved. Similarly, in 1 ml of each hyperlipemic serum, 10 mg were dissolved. Samples of the resulting 4 mixtures and untreated control sera then were subjected to electrophoretic analysis both before and after incu-

‡ Early in these studies it was found that incubation with 5 mg of the phosphatide had no effect on migration in markedly hyperlipemic sera. When the phosphatide added was increased to 10 or 20 mg, changes in lipoprotein similar to that demonstrated in the normals were noted.

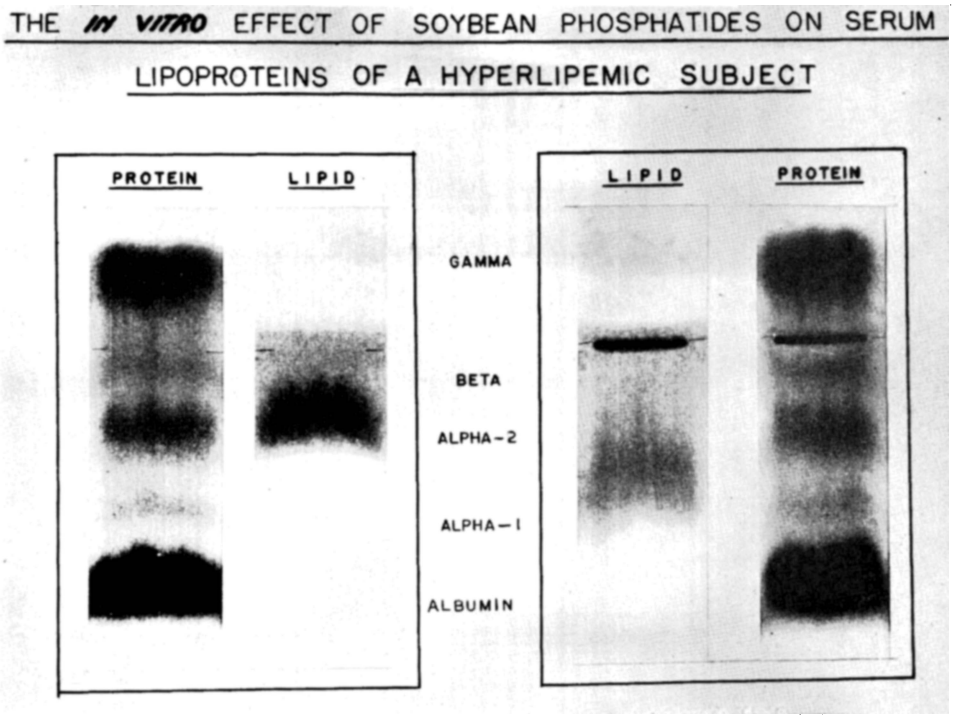

FIG. 2. Effect of incubating the whole phosphatide complex with serum from a hyperlipemic subject.

bation at 38°C for 24 hours. As in Exp. A. all sera were analyzed for total lipid, lipid phosphorus and total cholesterol prior to the experiment to determine degree of lipemia.

3. *Exp. C—Free Fatty Acids.* Free fatty acids were determined by the method of Grossman *et al.*(7) in aliquots of 7 sera incubated without phosphatide or with the alcohol-soluble or the alcohol-insoluble fractions. As in Exp. A, to 1 ml of each normal serum, 5 mg of either phosphatide were added whereas 10 mg were added to 1 ml of each of 2 hyperlipemic sera.

Results. 1. *Exp. A.* With the technic utilized, the lipid-staining band corresponding to beta lipoprotein normally is either associated with beta-globulin or is in an area between beta and alpha-2 globulin. This pattern was found in all the unincubated sera and in the phosphatide-free controls. However, in all of the phosphatide-containing incubated sera, both normal and hyperlipemic, the lipoprotein associated with beta-globulin migrated to the region between α-2 and α-1 globulin; *i.e.*, there was an increase in the migration velocity of beta-lipoprotein. (Fig. 1 and 2). In addition, in 5 of the 6 normal sera and 1 of the 5 hyperlipemic sera there was a definite but lesser increase in the migration of the lipid-staining band associated with α-1 globulin and albumin (alpha-lipoprotein) to an area on the strip immediately beyond albumin, after incubation with whole phosphatide complex.

2. *Exp. B.* The alcohol-soluble fraction produced little or no change in the serum lipoproteins after incubation. However, in all sera tested, incubation with the alcohol-insoluble fraction produced an increase in migration velocity similar to that demonstrated with the whole soybean phosphatide complex (Fig. 3).

3. *Exp. C.* Incubating sera with either the alcohol-soluble fraction or alcohol-insoluble fraction was associated with an increase in free fatty acid concentration, (Table I). However, no consistent differences between the 2 fractions on liberation of free fatty acids

were observed.

Discussion. In our previous unpublished preliminary investigation the same technic here described was utilized to test the effect of a fat emulsion containing 10% sesame oil and 4.5% dextrose stabilized with lecithin, on

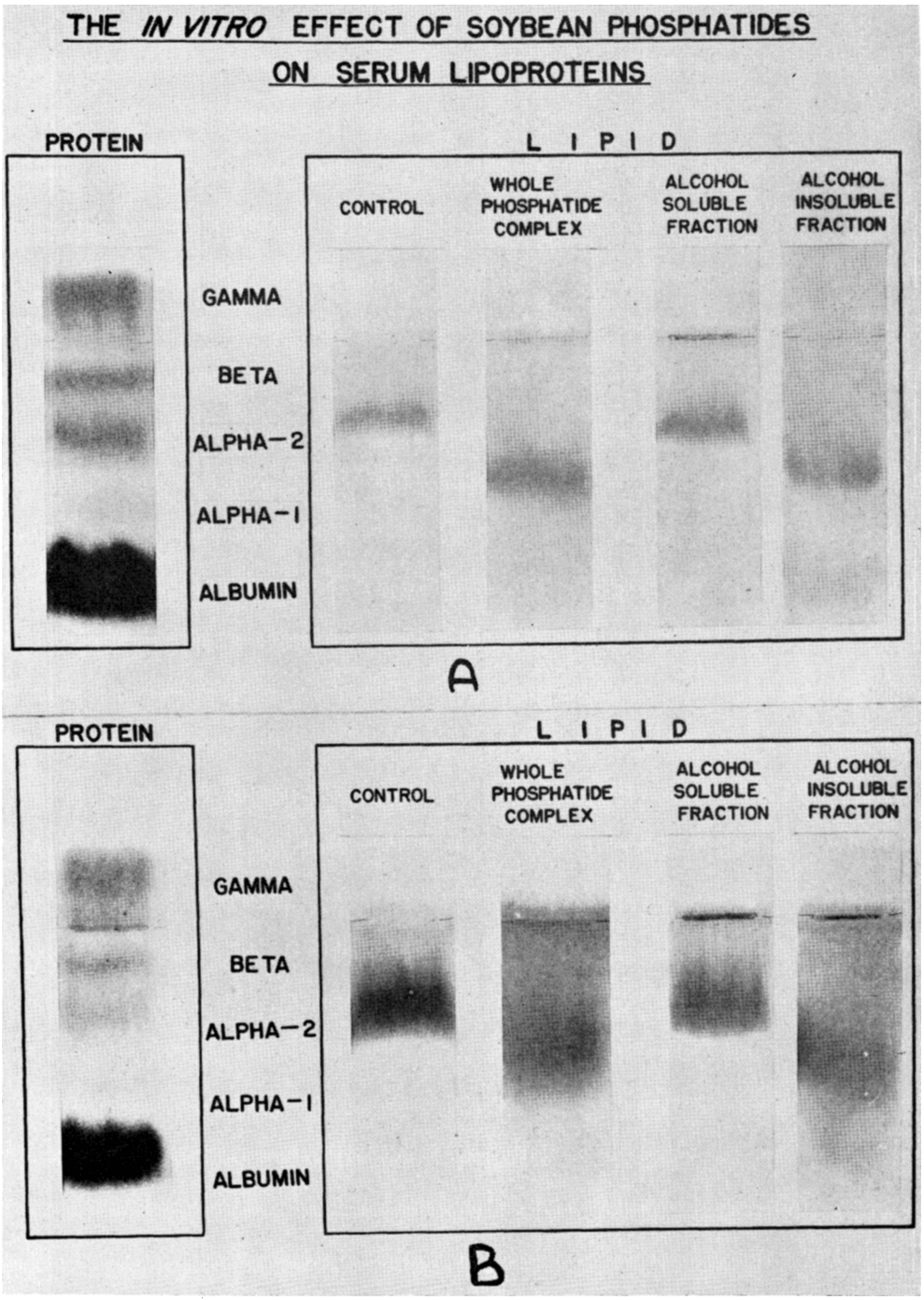

FIG. 3. Comparison of effects of incubating the whole phosphatide complex and its 2 alcohol fractions on serum from (A) a normolipemic subject and (B) a hyperlipemic subject.

TABLE I. *In Vitro* Effect of Soybean Phosphatide Fractions on Serum Free Fatty Acids.

	—Free fatty acids/ml of .02 N NaOH—		
	Incubated serum		
Serum	+ Alcohol-soluble fraction	+ Alcohol insoluble fraction	Untreated control
A	.16	.12	.12
B	.20	.27	.12
C*	.22	.27	.11
D	.14	.13	.05
E	.13	.17	.07
F	.13	.13	.07
G*	.10	.12	0

* Hyperlipemic.

serum lipids and lipoproteins. The results were the same as the present observations with soybean phosphatide. As this paper was being prepared for publication, the lipid emulsion effect was confirmed *in vivo*(8). The observations on the effect of the lipid emulsion and the phosphatides are of particular interest because of the recent report by Lever and Waddell(9) that serum cholesterol falls following the intravenous administration of a fat emulsion, composed of 10% cottonseed oil and 1% of the same soybean phosphatide used in the present experiments. This preparation is the natural phosphatide complex, derived from soybean and consisting of 95% phosphatide with lecithin, cephalin, and lipositol present in approximately equal concentrations. It has been demonstrated that feeding soy lecithin produces significant falls in serum cholesterol levels of man(1,11,12), of cholesterol-fed rabbits(2) and, if soy sterols are also fed, of chicks(10). However, the effects of soybean phosphatides on serum lipoproteins have not been reported. The present comparison of the effects of the 2 fractions of the whole soybean phosphatide complex suggests that lipositol is probably the compound responsible for producing the lipoprotein shift. The alcohol-soluble fraction, (consisting of approximately ⅔ lecithin and ⅓ cephalin), produced little or no change in the serum lipoproteins after incubation. In contrast, the alcohol-insoluble fraction (consisting of ⅔ lipositol and ⅓ cephalin) produced a marked increase in migration velocity similar to that demonstrated with the whole soybean phosphatide complex. Simple addition of the whole phosphatide complex or either fraction, without incubation, produced little or no increase in the migration velocity. Lipositol is a lipid of unknown structural formula, which, on acid hydrolysis, yields inositol, phosphoric acid, oleic acid, saturated fatty acids, ethanolamine and tartaric acid. Alkaline hydrolysis of this inositol phosphatide liberates an amino-reducing carbohydrate, from which galactose may be obtained after mild hydrolysis(13). These effects produced by soybean phosphatide on the migration rates of serum lipoproteins are similar to those produced *in vivo* by heparin(14) or *in vitro* by "clearing factor"(15). *In vivo* heparinized plasma has been reported to cause a release of fatty acids from beta lipoproteins(16). Others(15,17) have found that fatty acids increase the electrophoretic mobility of alpha and beta lipoproteins *in vitro*. Therefore, soybean phosphatide might be increasing the migration velocities of lipoproteins by a similar mechanism; *i.e.*, by liberating fatty acids, too. However, the equivalent effects on free fatty acids following incubation with the alcohol-soluble as well as the alcohol-insoluble fractions suggest that some other mechanism must be responsible for the difference in the effects on lipoproteins produced by the two fractions.

Although several investigators(18-21) feel that an actual transformation of beta into alpha lipoprotein occurs, this position is disputed by others. Herbst *et al.* in recent studies,(22), which demonstrated that such an *in vivo* transformation does not occur, noted a pre-albumin component similar to that observed in some of the present sera and identified it as alpha lipoprotein. Sera used in their studies were obtained from subjects with either alimentary or idiopathic hyperlipemia. In the present series, the increase in migration velocities following soybean phosphatides was demonstrated on sera from fasting normolipemic as well as hyperlipemic patients.

Conclusions. Incubation of 6 normal and 5 hyperlipemic sera with a purified extract of natural soybean phosphatides produced an increase in migration velocity of beta lipoprotein, determined by paper electrophoresis. In 5 of the normal and one of the hyperlipemic

sera increases in the migration velocity of alpha lipoprotein also were observed. Incubation of 10 normal and 9 hyperlipemic sera with the alcohol-soluble fraction of the phosphatide complex (which consists of ⅔ lecithin and ⅓ cephalin) produced little or no change in the migration velocities of the serum lipoproteins. Incubation of these sera with the alcohol-insoluble fraction, (which consists of ⅔ lipositol and ⅓ cephalin) produced an increase in the migration velocities similar to that noted with the whole phosphatide complex. Therefore, lipositol may be the compound responsible for the change observed. Determination of free fatty acids after incubation with either fraction failed to disclose any differential liberation of free fatty acids by the two fractions. The mechanism by which the alcohol-insoluble lipositol fraction increases the migration velocity of beta lipoprotein remains unexplained.

1. Adlersberg, D., and Sobotka, H. J., *J. Mt. Sinai Hosp.*, 1943, v9, 995.

2. Kesten, H. D., and Silbowitz, R., PROC. SOC. EXP. BIOL. AND MED., 1942, v49, 71.

3. Flynn, E. V., and deMayo, P., *Lancet*, 1951, v2, 235.

4. Sachs, B. A., Cady, P., and Ross, G., *Am. J. Med.*, 1954, v17, 662.

5. Fiske, B. H., and SubbaRow, Y., *J. Biol. Chem*, 1925, v66, 375.

6. Bloor, W. R., *ibid.*, 1916, v24, 227.

7. Grossman, M. I., Palm, L., Becker, G. H., and Moeller, H. C., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 312.

8. Herbst. F. S. M., Lever, W. F., and Waddell, W. R., *Science*, 1956, v123, 843.

9. Lever, W. F. and Waddell, W. R., *J. Invest. Dermatol.*, 1955, v25, 233.

10. Peterson, D. W., Shneour, E. A., Peek, N. F., and Gaffey, H. N., *J. Nutrition*, 1953, v50, 191.

11. Steiner, A., and Domanski, B., PROC. SOC. EXP. BIOL. AND MED., 1944, v55, 236.

12. Pottenger, F. M., Jr., and Krohn, B., *Am. J. Digest. Dis.*, 1952, v19, 107.

13. Deuel, H. J., *The Lipids*, Interscience Publishers, 1951, v1, 450.

14. Rosenberg, I. N., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 751.

15. Laurell, S., *Scand. J. Clin. and Lab. Invest*, 1955, v7, 28.

16. Shore, B., Nichols, A. V., and Freeman, L. W., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 216.

17. Gordon, R. S., *J. Clin. Invest.*, 1955, v34, 477.

18. Boyle, E., Bragdon, J. H., and Brown, R. K., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 475.

19. Brown, R. K., Boyle, E., and Anfinsen, C. P., *J. Biol. Chem.*, 1953, v204, 423.

20. Williams, G. R., *Biochim. et Biophys. Acta*, 1954, v13, 72.

21. Nikkila, E., *Scandinav. J. Clin. and Lab. Invest.*, 1953, v5, Suppl. 8.

22. Herbst, F. S. M., Lever, W. F., Lyons, M. E., and Hurley, N. A., *J. Clin. Invest.*, 1955, v34, 581.

Received June 12, 1956. P.S.E.B.M., 1956, v93.

Method for Study of Electrocardiogram of Early Chick Embryo within the Shell. (22652)

ABEL A. LAZZARINI, JR.* AND J. WELDON BELLVILLE.†
(Introduced by L. Corsan Reid.)
Deparments of Surgery and Surgery (Anesthesiology), New York Hospital.

The electric activity of isolated heart muscle of the chick embryo as an organ, or as a portion in tissue culture, has been studied by a number of investigators(1,2,4,5,6,7,10,11). Lagen and Sampson(8) employed this preparation, and Friedman and Bine(3) dissected the embryonic duck heart to study electrocardiographic changes in evaluating drugs. Today, isolated muscle from a number of animals is used in physiologic and pharmacologic studies for standardization of drug action upon the myocardium. All these technics involve considerable trauma and the use of artificial

* Present address: New York University Post-Graduate Medical School.

† Present address: Memorial Hospital, New York City.