Observations on Naturally Occurring Phospholipid Systems.* (28634)

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The stability of fat particles in the blood following a meal containing fat is probably due largely to the presence in the chylomicron structure of a number of phospholipids (1). It seems likely that a successful fat emulsion for human intravenous use will contain phospholipids approximating in composition those naturally present in the chylomicron.

The precise quantitative determination of phospholipid mixtures is a lengthy procedure. In this note the use of thin-layer chromatography is described for rapid visual evaluation of naturally occurring phospholipid assemblies.

Methods. Blood was obtained from a normal human subject 6 hours after a meal of butter fat (200 g), and plasma separated. A chylomicron fraction was prepared by mixing plasma with saline of density 1.006 g/ml and spinning in a Spinco model L preparative ultracentrifuge for 8.6×10^5 *g*-min. The upper chylomicronous layer was separated.

Serum was obtained from fresh hog, sheep and steer blood.†

Lipids were extracted from the human plasma and chylomicrons, from the animal sera and from the yolk of a fresh hen's egg using the procedure of Folch *et al.*(2).

Thin-layer chromatography. Thin layers of Silica gel G (E Merck AG, Darmstadt, Germany) were spread on "Pyrex" glass plates (20 × 20 cm) and dried at 110° for 1 hour before use. Lipid equivalent to 20 μl of serum or plasma, or a convenient amount of egg-yolk lipids, was spotted on the plates which were developed in a mixture of chloroform:methanol:water 80:35:5 v/v/v. After drying the plates, the spots were visualized by spraying with 50% (v/v) sulfuric acid and heating at 250° on a hot plate.

Results and discussion. A thin-layer chromatogram illustrating the typical appearance of the phospholipid mixtures from the materials examined is shown in Fig. 1. It is clear that the phospholipids present in the chylomicron were generally similar in distribution to those of total human plasma. Phosphatidyl choline was the predominant component, with smaller amounts of sphingomyelins, phosphatidyl ethanolamine and lysophosphatidyl choline also present. This finding is in agreement with the report of Phillips(3) that the 4 major phospholipids of total human plasma are also present in the lipoprotein fraction of density less than 1.019.

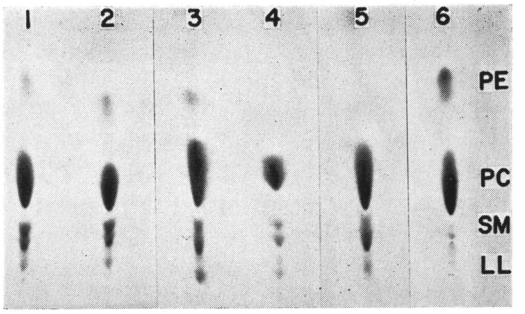

FIG. 1. Thin-layer chromatogram showing phospholipid distribution of: (1) total human plasma after fat-loading; (2) chylomicron fraction of human plasma after fat loading; (3) total hog serum; (4) total sheep serum; (5) total steer serum; and (6) hen egg-yolk. PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelins; LL, lysophosphatidyl choline. Other lipids present travelled close to the solvent front and are not shown in this figure.

Egg-yolk phospholipids contained the same phospholipid classes as chylomicrons, but clearly this material contained relatively more phosphatidyl ethanolamine and less sphingomyelin than was present in the natural particles.‡

The phospholipids of total hog serum resembled those present in human chylomicrons more closely than did those of sheep or steer

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† Kindly supplied by Armour and Co.

‡ "Intralipid," an emulsion stabilized with egg yolk phospholipid appears to be well tolerated in human subjects. This has been supplied through the kindness of the Riker Laboratories.

serum. In particular, the proportion of phosphatidyl ethanolamine in the two ruminants was apparently very low. This finding is consistent with the general impression that the hog is physiologically closer to man than are several other domestic animals, and may reflect the omniverous, non-ruminant mode of nutrition of the two species.

These results suggest that the phospholipids of hog serum may provide an efficient readily available and physiologically acceptable emulsifying agent for use in preparation of intravenous fat emulsions. An emulsion made with hog serum phospholipids as the only emulsifying agent is under investigation.

Summary. Thin-layer chromatography has been used to gain a roughly quantitative impression of the phospholipid distribution within the human chylomicron and of the phospholipid mixture present in various animal materials. The phospholipids of chylomicrons obtained following a fat meal were similar in distribution to those of total human plasma, and comprised phosphatidyl choline, sphingomyelins, phosphatidyl ethanolamine and lysophosphatidyl choline. Hen egg-yolk phospholipids showed a higher content of phosphatidyl ethanolamine but lower amounts of sphingomyelins. The sera of the sheep, steer and hog were examined, and of these the phospholipids of the last were closest in composition to those of the chylomicron. It is suggested that hog serum phospholipids may provide a physiologically acceptable emulsifying system for use in intravenous fat emulsions.

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Occurrence of (-)-Sympatol in Oranges.* (28635)

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p-Hydroxymandelic acid is a constant constituent of urine(1) and the amount excreted daily does not appear to fluctuate markedly. Octopamine (*p*-hydroxy-*α*-(aminomethyl) benzyl alcohol) was suspected of being an endogenous precursor for this acid, and was identified in tissues of animals which had been treated with monoamine oxidase inhibitors and in the urine of man and animals(2). In a more extensive survey of the phenolic amines in human urine *p*-sympatol, the N-methyl derivative of octopamine, was identified(3). It occurs only in the conjugated amine fraction and the amount present varies widely from day to day. For this reason it was suggested that it probably has a dietary source(3). However, Pisano *et al.*(4) suggested that *p*-sympatol might be of physiological significance, and Kraupp *et al.*(5) reported its occurrence in increased amounts in the urine of a patient with hypertension. Because of their reports and also because the presence in urine of increased amounts of *p*-sympatol or its metabolite, *p*-hydroxymandelic acid, might interfere with some of the tests used for diagnosis of pheochromocytoma the existence of a common dietary source of *p*-sympatol is reported here.

Human subjects maintained on rigidly defined diets(6) were observed to excrete markedly increased amounts of *p*-hydroxymandelic acid after they ingested oranges or orange juice. Further examination of their urine showed the presence of a large amount of a phenolic amine which had the chromatographic properties of *p*-sympatol. The results of an experiment made to extend this observation were as follows: A healthy adult male took a diet free of precursors of phenolic

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