enzyme A in the β-oxidation of fatty acids by mitochondria. That fatty acyl CoA derivatives are intermediates in β-oxidation of fatty acids to acetyl CoA was demonstrated with soluble enzyme systems isolated from mitochondria(6). Several types of inhibition are dependent on the level of organization of the biological system studied(7). Also, the conversion of fatty acids to ketone bodies is a combination of the β-oxidation of fatty acids to acetyl CoA and the formation of ketone bodies therefrom.

In the liver acetyl CoA produced from fatty acids may undergo condensation to form acetoacetyl CoA plus coenzyme A(8). Mechanisms for subsequent formation of acetoacetate from acetoacetyl CoA also liberate coenzyme A(9,10). It is doubtful, however, that the added coenzyme A in the system reported herein is reversing acetoacetyl CoA formation from acetyl CoA or acetoacetate formation from acetoacetyl CoA since ketone body formation from caprylic acid was unaffected. A number of other differences in the metabolism of short-chain and long-chain fatty acids have been summarized(11). It is also unlikely that the added coenzyme A stimulated incorporation of added palmitate into phospholipids and glycerides sufficient to account for the reduction in ketogenesis since an acyl coenzyme A acceptor was not added. The actual mechanism by which coenzyme A decreased the conversion of palmitic acid to ketone bodies under these experimental conditions is undergoing further study.

Summary. A cofactor mixture containing ATP, cytochrome C and coenzyme A decreased ketone body formation from added palmitic acid in a rat liver homogenate system. The conversion of caprylic acid to ketone bodies, however, was not appreciably altered under these conditions. It was found that the decreased ketogenesis from palmitate was caused by coenzyme A. Other sulfhydryl-containing compounds tested did not affect ketone body production from palmitate in this system.

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Thin Layer Chromatography of Blood Lipids.* (29137)

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Thin layer chromatography (TLC) has proved to be a rapid, simple and versatile procedure for the separation of complex substances into their constituents. Although the technique was described as early as 1938(1), it has only recently been applied to the separation of lipids(2). The use of thin layer chromatography in the analysis of blood lipids has not been explored fully as yet. This report presents a simple application of a method originally described by Weicker(3)

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to the analysis of the blood lipids of man.

Materials and methods. Venous blood in the post-absorptive state was drawn from 10 normal subjects and 23 patients with disorders of lipid metabolism. The blood from the patients was collected in two ways: (1) as plasma, in a heparinized test tube which was immediately placed in an ice bath, centrifuged and sampled as rapidly as possible and (2) as serum, in a dry test tube, allowed to stand at room temperature for approximately 2 hours before being centrifuged and then sampled. The serum samples were analyzed for total cholesterol by the method of Abell *et al*(4), for lipid phosphorus by a modification of the Fiske-Subbarow procedure (5) on serum extracted with Bloor's reagent (alcohol:ether, 3:1) and for triglycerides by the Van Handel modification(6) of the procedure of Van Handel and Zilversmit(7). Serum protein and lipoprotein fractions were determined by paper electrophoresis.

Lipid extracts of serum and plasma for TLC were prepared by evaporating an aliquot of a Bloor's filtrate (1 cc of serum to 25 cc of Bloor's reagent) to dryness. The dried residue was washed 3 times with small portions of boiling petroleum ether, filtered through petroleum ether washed cotton plugs, and the combined washings evaporated to dryness. The washed residue was taken up in a volume of chloroform so as to effect a 10-fold concentration of the original aliquot.

Thin layer chromatograms were prepared as follows: 20 × 20 cm glass plates were coated with a 200-225 μ layer of Silica Gel G† and were allowed to air dry overnight. The plate was washed by allowing it to develop in a solvent system of chloroform: benzene (3:2) to the approximate height of 18 cm. This procedure eluted most of the stainable contaminants up beyond the zone of actual development. The plates were then activated at 120°C for 1 hour and stored in a desiccator until used.

Standard reference solutions (1 mg/ml in chloroform) were prepared from the following compounds: cholesterol (California Foundation for Biochemical Research, recrystallized *via* the dibromide), cholesterol stearate (General Biochemicals, Chagrin Falls, Ohio), palmitic acid (recrystallized from heptane), monopalmitin, dipalmitin (Sigma Chemical Co., St. Louis, Mo.), tristearin (Eastman Organic Chemicals, Rochester, N. Y.), triolein and dipalmitoyl-L-lecithin (Mann Research Laboratories, N. Y.).

5 μl aliquots of the reference compounds and 10 μl aliquots of the serum or plasma lipid extracts were spotted on the washed, activated plates and developed to a height of 3 cm in n-propanol:NH_4OH (2:1). The plates were allowed to air dry for 2 hours to insure complete evaporation of the solvents, and then developed in a second solvent system of chloroform:benzene (3:2) to the 10 cm mark. The second development was done in a saturated atmosphere by lining the developing tank with filter paper as high as the 10 cm mark on the plate as it rests in the tank, and allowing it to equilibrate with the solvent system. After a few minutes of air drying to remove the solvents, the plates were thoroughly sprayed with a 10% solution of phosphomolybdic acid in ethanol. The plates were then heated in an oven for 10-20 minutes at 150°.

Results. 1. *Reference compounds.* (Fig. 1). The lipids appear in the following order of ascendancy on the silica gel plates: lecithin, palmitic acid, monopalmitin, dipalmitin, free cholesterol, tristearin, triolein and cholesterol stearate. The dipalmitin resolved into its 2 isomers.

2. *Serum.* (Fig. 2). The serum patterns, in ascending order appeared as phospholipids (2 or more components), fatty acids, monoglycerides (at first solvent front), diglycerides (2 fractions), free cholesterol, "saturated" triglycerides, "unsaturated" triglycerides, and cholesterol esters. The fatty acids and diglycerides could not be visualized in all of the normolipemic sera. Some of the normotriglyceridemic sera showed only the "unsaturated" triglycerides. There was excellent correlation between the chemically determined lipid fractions and the intensity and size of the corresponding spot on the thin layer chromatograph.

† Brinkman Instruments, Inc., Great Neck, N. Y.

3. *Plasma.* (Fig. 3). The migration of the lipid fractions in plasma extracts was similar to that observed for serum. However, a difference in the triglycerides between plasma and serum was noted. Except in the hypertriglyceridemic plasma where 2 triglyceride spots were found, chromatography of plasma lipids disclosed only the "unsaturated" triglyceride fraction. In addition, plasma showed a lower concentration of diglycerides and monoglycerides as compared to serum.

Discussion. Thin layer chromatography of blood lipids is a simple, reproducible and rapid technique. Excellent correlation between the intensity and size of the individual fractions with its chemically determined value was found. The increased concentrations of the various lipids in those patients with disorders of lipid metabolism was obvious on visual inspection. Specific changes in disease, such as the reversal of the normal free cholesterol:cholesterol ester ratio characteristic of biliary cirrhosis was evident. Of interest is the ready demonstration of mono- and diglycerides which were prominent in the hyperlipemic plasma and even more prominent in the hyperlipemic sera. The method may permit further investigation of these heretofore poorly evaluated blood constituents.

The finding of two triglyceride fractions is of interest. It should be noted that the method herein described does not characterize the triglycerides as to total chain length or degree of unsaturation. It would appear, however, that the separation is related more to differences in degree of unsaturation than to chain length(8). The difference observed between the migration of triolein (3 unsaturated bonds) and tristearin (completely saturated) would support this concept and led us to describe the triglyceride of greater mobility as "unsaturated" and the fraction of lesser mobility as "saturated." The quotes are used because closely related groups of compounds such as tristearin and tripalmitin migrate together and because each triglyceride spot consists of more than one triglyceride(9). It should be emphasized that the methodology is critical to effect separation of the triglyceride fractions in this system. For example, the thickness of the silica gel layer and the height of the filter paper liners must be as described. Of interest is a very recent

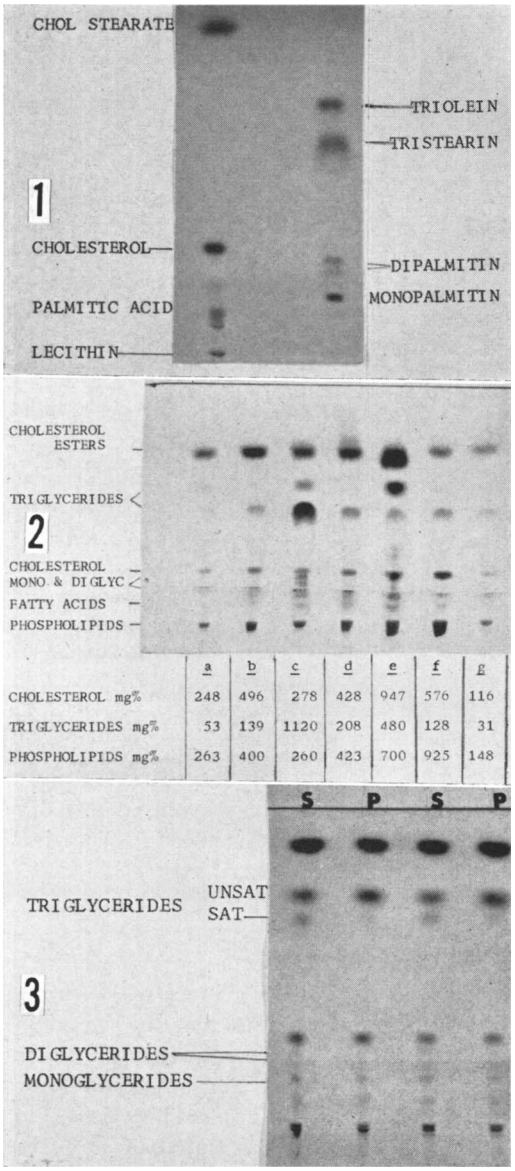

FIG. 1. Reference compounds. Compounds were applied in 2 columns for easier visualization.

FIG. 2. Thin layer chromatographic patterns of serum. Normal (a), familial hypercholesterolemia (b), hypertriglyceridemia (c), mixed hyperlipidemia (d), nephrotic syndrome (e), biliary cirrhosis (f) and multiple myeloma (g). Note correlation between size and intensity of the spots and chemically determined lipids.

FIG. 3. Difference between serum (s) and plasma (p) lipids in 2 subjects. In particular, note triglyceride fractions.

description of another technique which resolves only one triglyceride fraction in serum (10).

The reasons for the appearance of, or increase in, the "saturated" fraction in serum as compared to plasma are not apparent. The difference could be explained by the release of platelet triglycerides into serum, which would not occur in plasma(11), or by enzymatic hydrogenation of the unsaturated bonds. However, the latter has been demonstrated only in the case of methyl elaidate (12). Studies are currently under way which we hope will resolve this problem.

Summary. A simple method is described for the fractionation of blood lipids by thin layer chromatography. Lipid patterns for different disorders of lipid metabolism were demonstrated. A difference was noted in the triglyceride fraction between plasma and serum.

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Tests in Hamsters for Oncogenic Quality of Ordinary Viruses Including Adenovirus Type 7.* (29138)

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The oncogenic viruses of animal species exhibit ordinary properties of viruses, especially with regard to their structure, transmissibility, cell necrotizing capacity, and immunology. These attributes have provided support for the concept that ordinary viruses which infect the human species may, under appropriate circumstances, be responsible for certain malignancies in man.

In spite of extensive laboratory investigation, the direct attempts to recover viruses from human malignant tissue specimens have not succeeded in isolation of virus of proved etiologic relationship to neoplasia in man. Instead, the growing body of evidence has indicated that if viruses are responsible for human neoplasia, the virus may no longer be present in an infectious state, and only the significant genetic portion of the virus responsible for malignancy may remain. This has suggested the need for application of indirect approaches in human neoplasia such as the search for subviral components in malignant tissues and in examination of common viruses of human origin for their oncogenic potential in cell cultures and in animals.

The findings relating to tumor induction in newborn hamsters by SV_{40} virus(1,2) represented a breakthrough in studies of neoplasia in being the first demonstration of a malignant oncogenic quality for a virus of primate (monkey) origin. Even more striking were the findings of Trentin *et al*(3) and later Huebner *et al*(4) who showed an oncogenic capacity of human adenovirus types 12

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