constant oil rate. The addition of the distributors produced no noticeable pressure drop in the column.

ACKNOWLEDGMENT

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• *Letters to the Editor*

Direct Application of Serum to Thin-Layer Plates for Rapid Determination of Serum Lipids[1]

IN THIS LABORATORY it has been found in confirmation of earlier studies with silicic acid impregnated paper (1) and observations with thin-layer systems (2) that lipid chromatography may be carried out on thin-layer plates to which serum is applied directly. This direct application of serum to thin-layer plates is a useful technique for rapid qualitative and quantitative evaluation of serum lipids.

As a screening procedure for cholesterol and triglyceride, 20 μl of serum are applied to thin-layer plates coated with a 0.125 mm layer of silica gel. The serum spots are dried 3 to 4 min under an infrared lamp. The plates then are developed in n-hexane/diethyl ether/acetic acid (80:20:1.5 v/v/v) (3) and the separated lipid components charred with 0.6% $K_2Cr_2O_7$ in 55% H_2SO_4 (w/v) (4) at 180–190C for 40 to 60 min. There is excellent correlation between the spot size of the triglyceride fractions of serum samples chromatographed in this manner and the concentration of triglyceride as determined by a modified Carlson procedure (5). Total cholesterol levels (6) correlate with the spot size and intensity of the sum of the cholesterol ester and cholesterol fractions. Differences in the ratio of cholesterol to cholesterol esters are readily discernible on such thin-layer chromatograms.

Free cholesterol and the other slower moving lipids are not clearly separated by this screening technique. However, as illustrated in Figure 1, these constituents can be resolved by using a thicker layer, 0.25 mm, of adsorbent and developing three times to the same point (F), 5 or 6 cm from the starting line, with chloroform/methanol (2:1 v/v), prior to final development in the solvent system. The resulting chromatograms are virtually indistinguishable from those obtained when lipid extracts of the same sera are chromatographed.

Both screening techniques are particularly useful in fat tolerance tests to estimate the level of serum triglyceride and the time at which the concentration reaches a maximum. In addition, the procedures are helpful in selecting the size aliquot to be used in quantitative procedures for triglyceride and cholesterol.

For quantitative analysis of triglyceride an aliquot of serum containing from 25 to 75 μg of triglyceride is applied. The lipids are extracted and the chromatogram developed as previously described. After the lipid components are visualized with Rhodamine 6G, the areas of Silica Gel G containing triglyceride are extracted at room temperature with n-hexane/diethyl ether (50:50 v/v). Then the extracts are saponified, and the original triglyceride

TABLE 1

Mean Serum Triglyceride Concentrations by Thin-Layer and Modified Carlson Procedures [a]

Serum sample No.	Thin-layer[b] mg%	Modified[c] Carlson mg%
1	50	73
2	78	96
3	113	141
4	157	185
5	224	259
6	509	597

[a] Six serum samples analyzed on two occasions by both procedures. Standard error of the sample mean was 4 mg%.
[b] Eight analyses for each serum sample.
[c] Four analyses for each serum sample.

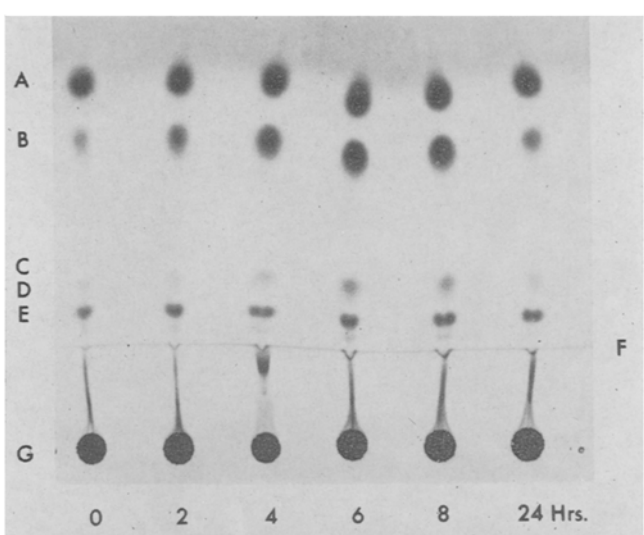

FIG. 1. Chromatogram of serum samples from a fat tolerance test: Samples were drawn at 0 (fasting sample), 2, 4, 6, 8, and 24 hr after ingestion of 100 g of corn oil. A, cholesterol ester; B, triglyceride; C, fatty acid; D, free cholesterol; E, diglyceride; F, front to which the chromatoplate was developed with chloroform/methanol; G, serum samples, 20 μl spotted. Serum triglyceride (5) concentrations, left to right: 209, 289, 566, 691, 587, 162 mg %.

[1] Based on results presented at the 56th Annual Meeting of The American Oil Chemists' Society, Houston, Texas, 1965.

content is determined by a periodate-chromotropic acid method of analysis for glycerol (5). Standard solutions of triglyceride, triolein/tripalmitin (2:1 w/w), containing 25, 50, and 75 μg are subjected to the entire procedure with each run. Recoveries of these standards have averaged 96%. Results of analyses of serum samples by the thin-layer procedure and a modified Carlson (5) procedure are given in Table I. The triglyceride levels obtained by the thin-layer procedure averaged 19% lower than those found by the modified Carlson (5) method. Experiments with model compounds showed that the modified Carlson (5) procedure determines total glycerides. Further experiments are in progress to determine whether the lower values obtained by the thin-layer procedure are due to the specificity of this method for triglyceride.

VIRGINIA S. WHITNER
O. T. GRIER
ANTOINETTE N. MANN
R. F. WITTER
U.S. Public Health Service
Communicable Disease Center
Heart Disease Control Unit
Lipid Standardization Laboratory
Atlanta, Georgia

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Dietary Myristate and Plasma Cholesterol Concentration

IN A RECENT COMMUNICATION Hegsted et al. (1) reported that the elevated serum cholesterol concentration effected by dietary saturated fats is due mainly to their myristic acid content. Also, it has been found by us that the ingestion by pigs of a triglyceride made up of myristic and lauric acids resulted in elevated incorporation of labeled acetate into liver and plasma cholesterol and bile sterols (2).

During a study in this laboratory in which the cholesterogenic and lipogenic responses to a series of simple triglycerides were determined (3), the plasma cholesterol concentrations were assayed but not reported. Previously unpublished data from that study (Table I) are herein presented in support of the observations (1,2) of the outstanding effects of myristic acid. The high plasma cholesterol response to dietary trimyristin is manifest.

The details of the experiment were given in the original publication (3). In brief the simple triglycerides were fed for two weeks to 200 g male rats as 10% or 30% of a semisynthetic diet, and plasma cholesterol assays were made (4).

TABLE I

Cholesterol concentration in blood plasma of 200 gram rats fed various fats as 30% or 10% of their diets for two weeks

Diet	Plasma Cholesterol	Diet	Plasma Cholesterol
	mg/100 ml		mg/100 ml
Basal	99	30% Trimyristin	158
	99		122
	112		129
	103		177
Average	103	Average	149
30% Tributyrin	75	30% Tripalmitin	86
	70		97
	73		90
	73		117
Average	73	Average	98
30% Tricaprylin	105	10% Triolein	112
	102		102
	105		112
	108		
Average	105	Average	109
30% Tricaproin	89	30% Trilinolein	120
	80		112
	85		93
	89		81
	89	Average	102
Average	86	30% Palmitoyl-olein	129
30% Tricaprin	93		138
	89	(1:2 mole ratio)	141
	96		116
	95		118
Average	93	Average	120
10% Trilaurin	96	10% Safflower oil	134
	116		112
	118		129
Average	110	Average	125

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RAYMOND REISER
MARY CARR WILLIAMS
MARY FRANCES SORRELS
N. L. MURTY
Deparment of Biochemistry & Nutrition
Texas A&M University
College Station, Texas

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• *Addendum*

JAOCS *42*, 775, 1965, R. J. VanderWal: "Semiquantitative Structural Analysis of Fats by Thin-Layer Chromatography of the Allyl Esters of the Products of vonRudloff Oxidation."

In section II, Paragraph 3, a small but important step was omitted. After the volume is reduced, and prior to extraction with chloroform, the mixture is acidified by addition of 1 ml of concentrated hydrochloric acid in 4 mil of water.