The direct determination of liver triglycerides

W. M. Butler, Jr., H. M. Maling, M. G. Horning, and B. B. Brodie

National Heart Institute
Bethesda 14, Maryland

[Received for publication August 19, 1960]

► The level of tissue triglycerides is generally calculated by subtracting the sum of phospholipids, cholesterol, and cholesterol esters from total lipids. The various procedures are usually tedious, and can give rise to considerable error since the triglycerides may constitute only a small fraction of the total lipids. Accordingly, there is need for a simple method for the direct assay of tissue triglycerides.

Recently, Van Handel and Zilversmit (1) published a method for the direct determination of plasma triglycerides. In this method, phospholipids are separated from triglycerides by adsorption on a synthetic zeolite. The triglycerides are then extracted into chloroform and estimated from the content of esterified glycerol.

This paper describes an adaptation of the method of Van Handel and Zilversmit to the direct estimation of liver triglycerides. This simple and accurate method makes it possible to analyze a large number of tissue samples in a single day.

REAGENTS

Phosphate Buffer, M/15, pH 7.0. Mix M/15 Na_2HPO_4 solution and M/15 KH_2PO_4 solution in the proportion of 61.1 to 38.9. Other reagents are the same as those described by Van Handel and Zilversmit (1), except for a higher concentration (2.0 M) of sodium arsenite. This increase in concentration of arsenite lowers the optical density of the unsaponified blank in determinations on liver homogenates.

PROCEDURE

The procedure consists of five steps: (*a*) homogenization of tissue, (*b*) the adsorption of phospholipids onto zeolite, followed by the extraction of triglycerides into chloroform, (*c*) hydroylsis of triglycerides to fatty acids and glycerol, (*d*) oxidation of glycerol with $NaIO_4$ to formic acid and formaldehyde, and (*e*) the formation of a colored complex of formaldehyde and chromotropic acid. The last four steps are carried out essentially as described by Van Handel and Zilversmit (1) for plasma.

Homogenize 2 to 10 g tissue with 9 volumes (9 ml/g tissue) of phosphate buffer for 90 seconds in a Waring blendor, using a semi-micro container. Immediately after homogenization, transfer 1 ml of the homogenate into a 25 ml glass-stoppered, graduated cylinder containing about 4 g of activated zeolite moistened with 2 ml of chloroform. Add 18 ml of chloroform and shake by hand intermittently for about 10 minutes (the tissue triglycerides are now diluted 1:200). Filter through coarse, fat-free paper. Pipette an aliquot of filtrate, containing about 0.05 mg of triglycerides (usually 0.125 to 1 ml), into each of three glass-stoppered tubes. Then pipette 1 ml of the standard corn oil solution (0.05 mg/ml) into each of three additional glass-stoppered tubes. Evaporate the chloroform from all tubes by placing in a water bath maintained at about 80°. To two out of each three standards and unknowns, add 0.5 ml of alcoholic KOH (saponified sample); to the third standard and unknown, add 0.5 ml of 95% alcohol (unsaponified sample). Maintain tubes at 60° to 70° for 20 minutes. Add 0.5 ml of 0.2 N H_2SO_4, and remove alcohol by placing tubes in a gently boiling water bath for about 15 minutes. Cool the tubes and add 0.1 ml of periodate solution. After 10 minutes, add 0.1 ml of sodium arsenite solution. Several minutes later, add 5 ml of chromotropic acid reagent. Mix and then heat for one-half hour by placing in a boiling water bath in the absence of excessive light. After cooling, determine the optical density (O.D.) at 570 $m\mu$.

CALCULATION OF RESULTS

Let R =

$$\frac{\text{O.D. sapon. unknown} - \text{O.D. unsapon. unknown}}{\text{O.D. sapon. corn oil stand.} - \text{O.D. unsapon. corn oil stand.}}$$

and A = volume of aliquot of chloroform extract in ml. Then triglyceride contents in milligram per gram tissue =

$$\frac{200}{A} \times R \times 0.05 = 10\,\frac{R}{A}.$$

RESULTS

Recoveries. Recoveries were determined by adding known amounts of corn oil to the liver homogenate, rehomogenizing the mixture for 90 seconds, and measuring the triglyceride content of aliquots of the resulting suspension. Recoveries from livers of dogs, rats, and squirrel monkeys averaged 85% to 105%.

Reproducibility. Replicate estimations were made of the liver triglyceride content of normal rats and of animals pretreated with alcohol, ethionine, and carbon tetrachloride (2). Although the triglyceride values ranged from 5 to 100 mg/g, the coefficients of variation were between 3.5 and 6.5.

Specificity. Estimation of the triglyceride content of four rat liver homogenates yielded values which agreed satisfactorily with those obtained on the same homogenates by silicic-acid chromatography after ethanol-ether extraction (3) (Table 1). The possibility was investigated that mono- and diglycerides, if present in appreciable amounts, might be included also in the direct assay of triglycerides. The α-monoglycerides would increase the optical density of the unsaponified blank, since the periodate oxidation yields an almost stoichiometric amount of formaldehyde. However, the typical α-monoglycerides, 1-monopalmitin and 1-monostearin, were found to be largely (more than 80%) retained by the zeolite. Since the extraction of the monoglycerides from the zeolite is largely incomplete, it may be concluded that the triglyceride content of tissues would not be significantly influenced by the monoglyceride content unless abnormally large amounts were present.

TABLE 1. TRIGLYCERIDE CONTENT OF NORMAL RAT LIVER*

Rat	Liver Triglyceride	
	Proposed Method	Silicic-Acid Chromatographic Method
	mg/g	*mg/g*
1	7.2	7.3
2	10.6	10.2
3	4.0	3.6
4	6.5	6.3

* A comparison of values of triglyceride content of normal rat livers obtained by the method proposed in this paper and by silicic-acid chromatography after ethanol-ether extraction (3). Each rat liver was homogenized with phosphate buffer, 1 ml removed for the colorimetric determination, and the triglycerides in the remaining homogenate were determined gravimetrically after extraction with ethanol-ether and isolation by silicic-acid chromatography.

In contrast, 1-3-dipalmitin, a typical diglyceride, was not adsorbed onto zeolite, but was extracted into chloroform along with the triglycerides. Values for liver triglycerides will therefore include any diglycerides present in the tissue. In normal rat liver these generally do not exceed 1 mg/g of liver.[1] We tested the possibility that a fatty liver might contain a relatively high concentration of diglycerides. A fatty liver was obtained from a rat treated with carbon tetrachloride. A portion (1.8 g) of the liver was homogenized and treated with zeolite and chloroform to remove the phospholipids. The chloroform supernatant from the zeolite was separated into the neutral lipid classes by silicic-acid chromatography (3). Only 5 mg/g of phosphate-free, nontriglyceride lipid was obtained. Analysis of this material for glycerol (steps *c* through *e* under Procedure) indicated that it consisted of glycerides. Calculated as diglyceride, the colorimetric estimation yielded a value of 4.2 mg/g, about the same as the actual weight of the analyzed lipid. In this markedly fatty liver, the diglyceride content, although elevated, was less than 4% of the triglyceride content (4.2 mg/g diglyceride; 119 mg/g triglyceride).

The diglyceride content of liver can account for the slightly higher triglyceride values obtained by the colorimetric procedure than by silicic-acid chromatography.

The 1,3-dipalmitin and 1-monopalmitin were generously supplied by Dr. D. M. Turner and had been obtained from Dr. F. Mattson. The 1-monostearin was purchased from Eastman Chemical Co.

REFERENCES

1. Van Handel, E., and D. B. Zilversmit. *J. Lab. Clin. Med.* **50**: 152, 1957.
2. Maling, H. M., M. G. Horning, W. M. Butler, Jr., B. Highman, and B. B. Brodie. *Federation Proc.* **19**: 229, 1960.
3. Horning, M. G., E. A. Williams and E. C. Horning. *J. Lipid Research* **1**: 482, 1960.

[1] D. Turner, personal communication.