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Research Article

THE VALUE OF NEEDLE BIOPSY IN THE CHEMICAL ESTIMATION OF LIVER LIPIDS IN MAN [1]

By BARBARA H. BILLING, HAROLD J. CONLON, DAVID E. HEIN, AND LEON SCHIFF WITH THE TECHNICAL ASSISTANCE OF CHARLENE STARR AND EILEEN McGREEVY

(From the Departments of Medicine and Pathology, University of Cincinnati College of Medicine, and the Gastric Laboratory, Cincinnati General Hospital, Cincinnati, Ohio)

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INTRODUCTION

Specimens of liver tissue obtained by needle biopsy may be subjected to various forms of study. Our interest in the fatty liver has led to the development of micro-chemical methods based on standard techniques for the quantitative analysis of lipids in the liver specimens in order to supplement the histological information obtained. The value of such determinations is dependent on the extent to which the specimen of liver obtained by the needle method is representative of the liver as a whole in respect to lipids. Some workers have questioned the validity of the lipid values obtained on analysis of a single liver specimen and have felt that particularly in cirrhosis marked variation in lipid content can occur. Waldstein and Szanto (1) using the needle technique have, however, reported that histological examination of specimens obtained at necropsy from 9 patients with cirrhosis, 2 cases of toxic hepatitis and 4 "normal" patients, indicates that fatty metamorphosis is uniform throughout the human liver. Rourke and Stewart (2), and Theis (3) have also shown by chemical analysis of 1 gram samples taken from different sites in the liver that the distribution of lipids is relatively uniform.

In the present study, specimens of liver have been taken from 18 different sites in each of 25 livers and examined chemically and histologically for lipids. The variation in lipid content in the different specimens is reported and the influence of cirrhosis on the interpretation of the findings is discussed.

TECHNIQUES

Material

The livers used in this study were obtained from patients at the Cincinnati General Hospital who had died from a variety of diseases (Table I). In the selection of material preference was given to livers showing gross evidence of cirrhosis and/or fatty vacuolization, so that of the 25 livers examined there were 13 non-fatty livers (Group I), 5 of which were cirrhotic, and 12 fatty livers (Group II), 4 of which were cirrhotic. Necropsies were performed within 6 hours of death, and the liver was then removed for immediate analysis. Ralli and her associates (4) have shown that in dogs autolysis during the first 6 hours after death does not cause appreciable change in the lipid content of the liver.

Using the Vim-Silverman needle, specimens of liver tissue were obtained from approximately 18 different sites as indicated in Fig. 1. Position 5 was that from which biopsy samples are usually obtained in living subjects using the intercostal approach. The sample was immediately removed from the needle with forceps, dried with gauze to remove adherent blood and cut into two; one half used for histological examination and the other, weighing 5 to 8 mg., for chemical analysis.

Weight measurements

In the present work all weighings were made using a single beam quartz fibre balance as described by Schmidt-Nielsen and Taylor (5). This was constructed to take a maximum load of 12 mg. Calibration showed that there was a linear relationship between the dial reading and the load and that 280 scale divisions were equivalent to a load of 1 mg. An error of less than 1 per cent was ob-

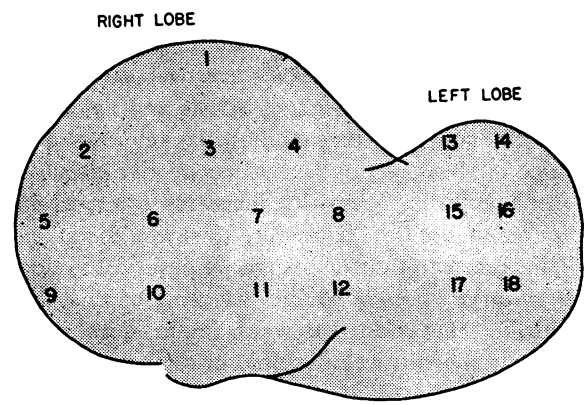

FIG. 1. POSITIONS IN LIVER FROM WHICH NEEDLE SPECIMENS WERE TAKEN

[1] Aided by grants from Samuel Freedman, the Robert Gould Research Foundation, the Milton Brown Memorial Fund, and the John Eisman Memorial Fund.

served for samples weighing 1 mg. whereas with larger samples the error was even smaller. Reproducibility of weighings was found to be excellent, and the speed at which these could be carried out proved advantageous.

Wet weight: In the course of weighing specimens of liver tissue obtained by the needle method there is considerable evaporation of water. Schmidt-Nielsen and Taylor (5) showed that the rate of water loss from a section of fresh rat liver is constant for several minutes after it is cut. They obtained the wet weight of the fragment by making repeated weighings and then extrapolating back to zero time. The same method was found to be applicable to the determination of the wet weight of liver specimens obtained by the needle method.

As soon as the needle was opened for the removal of the specimen a stop watch was started and the portion to be used for chemical analysis transferred to a glass hook of known weight (approximately 1 mg.). The hook was then placed on the balance and weighings were made at 15 second intervals for the next 2 minutes. The results were plotted on graph paper and the straight line obtained was extrapolated back to zero time to give the wet weight (Fig. 2).

Dry weight: The liver specimen was frozen at −24°C and then dried in vacuum over anhydrous calcium sulphate for 48 hours after which there was no further loss in weight. When dry, the specimen adhered to the glass hook, which could then be used as a carrier in all subsequent operations. Repeated drying in an oven at 105°C did give further small losses in weight, attributable to the loss of volatile substances other than free water. It is not possible to obtain exactly comparable results using the two methods for obtaining dry weights. The vacuum drying technique, however, has the advantage that oxidation of the lipid matter is prevented.

Total lipid content: Directly after weighing, the hooks

TABLE I

Summary of clinical and histological data

Group	Patient	Cause of death	Weight of liver g.	Histological classification of liver	Histological estimation of fat	Total lipids Mean ± S.D. g./100 g. liver
I(a)	H. B.	Carcinoma prostate	950	N.D.A.R.†	Absent	5.0 ± 0.8
	W. T.	Lobular pneumonia	1050	N.D.A.R.	Absent	5.1 ± 0.8
	M. D.	Subdural hematoma	1500	N.D.A.R.	Slight	6.5 ± 0.8
	G. S.	Gunshot wound	—	N.D.A.R.	Slight	6.3 ± 0.5
	P. H.	Lobular pneumonia	1050	Acute passive congestion	Absent	5.2 ± 0.4
	A. W.	Carcinoma prostate	1005	Acute passive congestion	Slight	6.1 ± 0.5*
	F. R.	Myocardial infarction	1205	N.D.A.R.	Absent	4.3 ± 0.3*
	F. C.	Gunshot wound	1525	Chronic nonspecific hepatitis	Slight	6.9 ± 0.6
I(b)	D. W.	Lung abscess	1600	Portal cirrhosis	Slight	7.0 ± 0.9
	C. H.	Myocardial infarction	1275	Portal cirrhosis	Slight	5.0 ± 0.7
	P. T.	Cerebral softening	1660	Portal cirrhosis	Absent	4.8 ± 0.6
	G. S.	Peritonitis	1875	Portal cirrhosis	Slight	4.3 ± 1.0
	H. D.	Perforated peptic ulcer	—	Portal cirrhosis	Absent	6.2 ± 0.5
II(a)	R. R.	Cerebral thrombosis	2520	Acute passive congestion	Moderate	13.8‡
	J. R.	Myocardial infarction	1975	Fatty vacuolization	Severe	20.3 ± 1.7
	W. D.	Lobar pneumonia	1500	Fatty vacuolization	Moderate	9.4 ± 0.7
	W. G.	Lobar pneumonia	1630	N.D.A.R.	Slight	8.9 ± 0.7
	E. C.	Intracerebral hemorrhage	2200	Severe fatty vacuolization	Severe	33.5 ± 1.8*
	E. N.	Acute liver necrosis	2355	Fatty vacuolization. Massive necrosis	Moderate	12.9 ± 1.3*
	J. B.	Diabetic coma	2000	Fatty vacuolization	Moderate	9.5 ± 0.7
	C. C.	Lobar pneumonia	—	Severe fatty vacuolization	Severe	29.0 ± 2.9
II(b)	V. M.	Portal cirrhosis	3175	Portal cirrhosis	Severe	28.3 ± 4.1
	J. Y.	Portal cirrhosis	2000	Portal cirrhosis	Severe	20.0 ± 3.5
	M. T.	Portal cirrhosis	4080	Portal cirrhosis	Severe	30.1 ± 2.9
	G. T.	Septicemia	3900	Portal cirrhosis	Moderate	14.9 ± 2.2

* Determined on dry tissue only.
† N.D.A.R.: No diagnostic abnormalities recognized.
‡ Insufficient chemical analyses for statistical analysis and inclusion in Fig. 6.

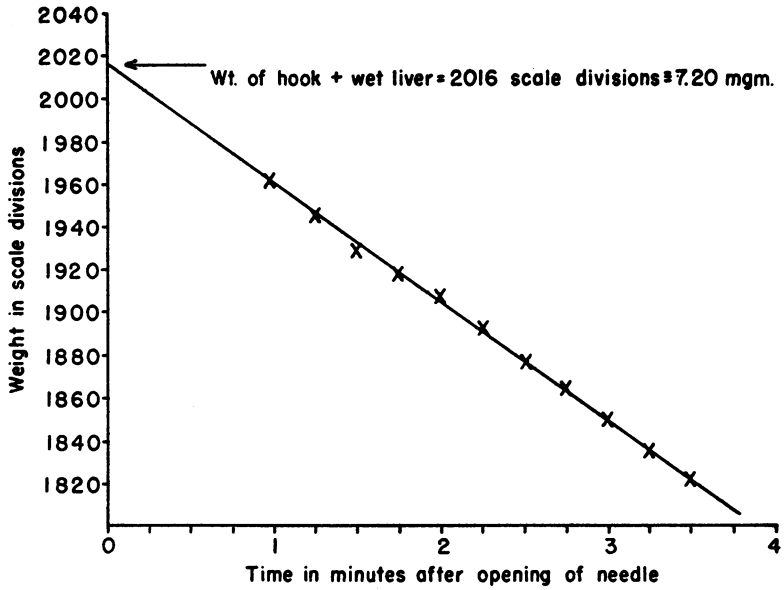

FIG. 2. DETERMINATION OF WET WEIGHT OF NEEDLE SPECIMEN OF LIVER BY MEASUREMENT OF THE RATE OF LOSS OF WATER

with the dried liver specimens were placed in glass tubes (80 mm. long and 4 mm. diameter), sealed at one end. Five hundred μl. ethyl alcohol (or Bloor's Reagent, containing 3 volumes alcohol to 1 volume ethyl ether) were added and the open end of the tube was sealed. The glass vials were then kept in an incubator for 48 hours at 60°C and shaken at intervals. After cooling, aliquots of the solvent were used for lipid analysis. The liver specimens were dried and reweighed and the difference between this weight and that of the dried tissue was taken to be the total lipid content.

Preliminary experiments showed that further extraction with fresh alcohol produced no appreciable change in the weight of the lipid free residue even with the very fatty livers, and that under the above conditions ethyl alcohol and Bloor's Reagent were equally satisfactory extracting agents. In several instances 10 g. specimens of liver from which needle specimens had been taken were dried and extracted with ethyl alcohol in a Soxhlet apparatus and the results obtained compared favorably with those of the needle specimens. The macro-method in these cases gave results of 16.5, 19.4, 16.1, 35.8, 11.8, 15.8 and 11.4 g. per 100 g. dry liver respectively as compared with 15.6, 19.0, 15.3, 36.0, 10.8, 15.9 and 11.4 g. by the micro-method.

Chemical analysis of lipids

Constriction pipettes were used for all volumetric measurements made in the following determinations (Linderstrøm-Lang and Holter [6]).

Phospholipids: Three hundred μl. of the alcoholic extract were used for the determination of lipid phosphorus. The alcohol was removed by evaporation in a boiling water bath and 500 μl. 5N H_2SO_4 were added. The samples were heated at 90°C for 2 hours and then at 160°C until charring had occurred. Two hundred and fifty μl. 30 per cent hydrogen peroxide (superoxol) were added to the samples, the blank (500 μl. 5N H_2SO_4) and the standard (prepared in 500 μl. 5N H_2SO_4) and all were reheated for an hour at 160°C after charring had disappeared. When necessary more H_2O_2 was added to all the tubes, which were reheated for 1 hour at 160°C to remove excess H_2O_2. After cooling 3 ml. water, 1 ml. 10 per cent sodium molybdate and 1 ml. 0.5 per cent p-methyl aminophenol sulphate in 3 per cent sodium bisulphite were added and in 15 minutes the color developed was read using a Beckman Spectrophotometer at 660 mμ. The lipid phosphorus content was multiplied by 25 to give the value for phospholipid.

Total fatty acids and iodine number: The total fatty acids and their iodine number were determined on 140 μl. of the alcoholic exract using the micro-methods described by Schmidt-Nielsen (7–10). The extract was pipetted into a vial of thin glass (60 mm. × 3 mm.) and the alcohol immediately removed in a vacuum desiccator. Twenty μl. 50 per cent alcoholic KOH were used to saponify the lipids in the presence of 60 μl. toluene. The tube was sealed and placed in a water bath at 80°C for 30 minutes. After cooling, the vial was centrifuged for 1 minute and the alcohol and toluene removed in a vacuum desiccator containing paraffin shavings. Fifty-seven μl. N/2 HCl were added to acidify the mixture and 60 μl. toluene were pipetted for the extraction of the fatty acids. The tube was immediately sealed and shaken 30 to 50 times to obtain complete extraction. After centrifugation for 1 minute, aliquots of the toluene layer were taken for titration of the fatty acids and iodine number. The quantities of toluene extract used were adjusted so

that for the fatty acid determination not more than 100 μg. of fat was present, while the maximum amount possible was available for the iodine number determination. The latter was found to give reproducible results when there were 30 μg. fatty acids or more present; with smaller quantities the difference between the blank and the titration reading was too small to give accurate results. A solution of 284.5 mg. stearic acid per 100 ml. toluene was used for the standard and all readings for the fatty acids were expressed in terms of the equivalent amount of stearic acid.

Histological techniques

The portion of tissue taken for histological examination was fixed in 10 per cent formalin prepared with 2 per cent calcium acetate. Part of the specimen was sectioned in paraffin and stained with hematoxylin-eosin. Frozen sections were made of the remainder; these were stained with Sudan IV, Oil-Red O and Sudan Black.

Each section was graded for visible fat using the designations of "absent," "slight," "moderate" or "severe." If no vacuolization could be found, with hematoxylin-eosin staining, the designation of "absent" was used, even though these sections might be faintly positive for lipid with fat stains. When only spotty fatty vacuolization was noted, the section was graded as "slight." The fat infiltration was regarded as "moderate" when not more than one third of the cells contained fat. Sections in which the fatty vacuolization was greater than this were given the classification of "severe."

RESULTS

A liver has been designated "fatty" if the lipid content is greater than 8 g. per 100 g. liver, or if the fatty vacuolization was classified as "moderate" or "severe."

Percentage of dry matter

The amount of dry matter was determined in each of the specimens examined. In non-fatty livers the mean values ranged between 22 and 33 per cent with an average of 27.9 per cent (*i.e.*, 72.1 per cent water content). In fatty livers a rough linear relationship was found between the percentage of dry matter and the amount of lipid in 100 g. dried tissue; this finding confirms the observations of other workers using macro-techniques (Eger [11] and Halliday [12]) (Fig. 3). This relationship was not apparent in non-fatty livers.

Total lipids

The results obtained for the total lipids in 13 non-fatty livers and 12 fatty livers have been expressed as g. lipid per 100 g. wet liver and have been recorded in Figs. 4, 5, 6, 7 to show the varia-

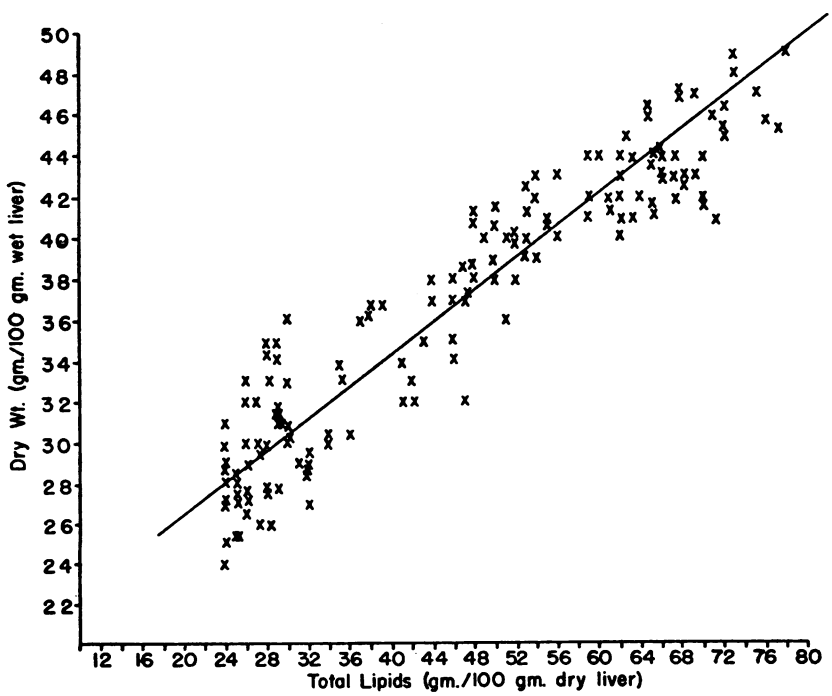

FIG. 3. THE RELATIONSHIP BETWEEN THE NUMBER OF GRAMS OF DRY MATTER PER 100 G. WET LIVER AND THE NUMBER OF GRAMS OF TOTAL LIPIDS PER 100 G. DRY LIVER

TABLE II

Variations in the dry matter, lipids and visible fat in needle specimens from a "normal" liver (F. C.). Group I(a)—Table I

Specimen No.	Dry matter *g./100 g. wet liver*	Total lipids *g./100 g. wet liver*	Total fatty acids *g./100 g. wet liver*	Phospho-lipids *g./100 g. wet liver*	Histological estimation of fat
1	31.6	6.4	—	2.6	slight
2	33.4	6.6	4.9	2.7	slight
3	33.9	7.1	4.8	2.9	slight
4	34.0	7.2	5.0	2.7	slight
5	33.3	7.8	5.2	2.9	absent
6	33.7	7.2	4.9	2.9	absent
7	33.2	6.0	4.5	2.7	absent
8	33.0	7.1	4.8	2.8	absent
9	34.7	6.2	4.2	2.8	absent
10	32.9	6.1	4.4	—	absent
11	32.5	7.5	4.9	2.8	slight
12	33.9	6.8	4.5	2.8	slight
13	33.3	6.9	4.8	2.9	slight
14	34.5	7.8	5.1	3.2	slight
15	31.4	6.2	4.6	—	slight
16	33.4	7.0	4.7	2.9	slight
17	33.0	7.4	5.0	3.0	slight
18	34.5	7.3	5.3	3.3	slight

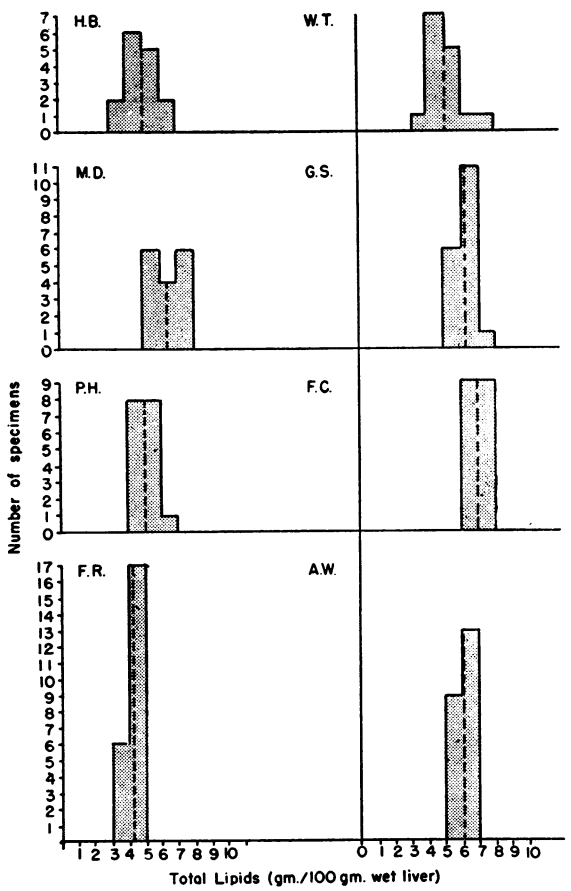

FIG. 4. GROUP I (A). THE DISTRIBUTION OF THE TOTAL LIPID CONCENTRATIONS IN SPECIMENS TAKEN FROM NON-FATTY, NON-CIRRHOTIC LIVERS

In this and subsequent figures the interrupted vertical line indicates the mean value for the analyses.

tion found in the different specimens from each liver. Tables II, III, IV, V give the detailed results obtained in a case representative of each group studied. In neither the non-fatty nor fatty livers was there a consistent difference between the lipid concentrations in the right or left lobes of the liver.

In 4 livers (marked with an asterisk—Table I) the lipid concentration of the specimens was determined in dry tissue only and approximate wet weights were calculated using Fig. 3 so that the results could be recorded in g. per 100 g. wet liver.

Non-Fatty Livers: Non-Cirrhotic (Fig. 4, Table II): Eight livers with no histologic evidence of intrinsic disease were examined. Values ranging from 3.5 to 7.8 g. lipid per 100 g. wet liver for individual specimens were recorded giving a mean value of 5.7 g. lipid per 100 g. wet liver for the group.

Cirrhotic (Fig. 5, Table III): Five of the livers studied were found on histological examination to be cirrhotic and to have only minimal fatty vacuolization. Values for individual specimens ranged from 2.3 to 8.7 g. lipid per 100 g. wet liver giving a mean value for the group of 5.5 g. per 100 g. wet liver. The specimens tended to show greater variance in their lipid content than was found in the non-cirrhotic group; this variance did not appear to be directly related to the amount of fibrosis present.

Fatty Livers: Non-Cirrhotic (Fig. 6, Table IV):

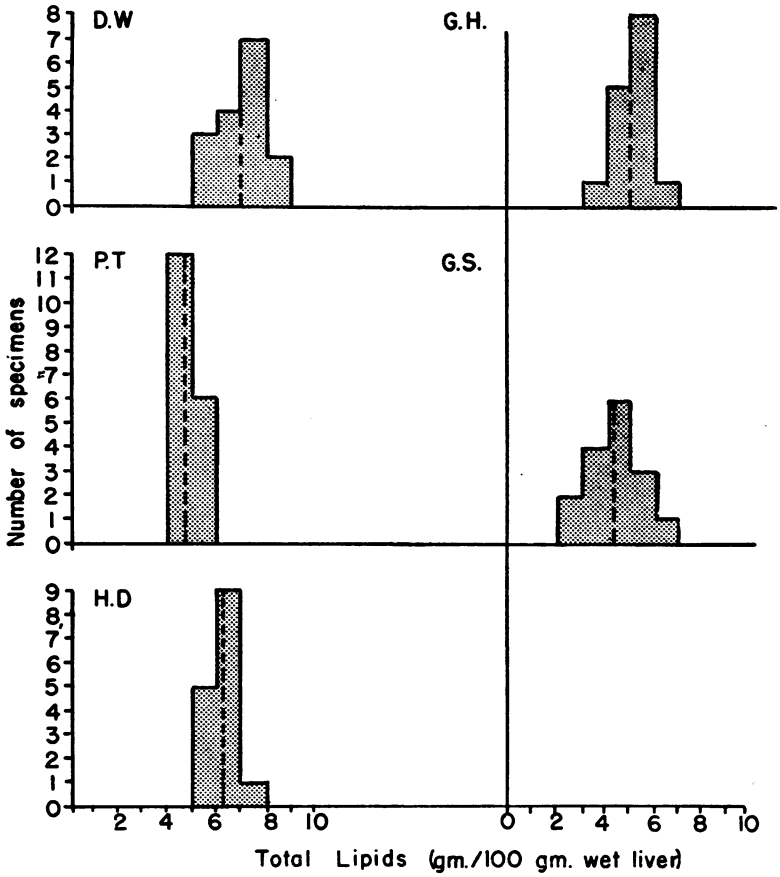

FIG. 5. GROUP I (B). THE DISTRIBUTION OF THE TOTAL LIPID CONCENTRATIONS IN SPECIMENS TAKEN FROM NON-FATTY, CIRRHOTIC LIVERS

TABLE III

Variations in the dry matter, lipids and visible fat in needle specimens from a non-fatty, cirrhotic liver (P. T.). Group I (b)—Table I

Specimen No.	Dry matter *g./100 g. wet liver*	Total lipids *g./100 g. wet liver*	Total fatty acids *g./100 g. wet liver*	Iodine No. of fatty acids	Phospho-lipids *g./100 g. wet liver*	Histological estimation of fat
1	22.8	5.3	2.3	135	2.4	absent
2	22.0	4.0	2.0	137	2.1	absent
3	21.9	4.4	2.7	—	2.3	absent
4	23.1	5.5	2.2	134	2.1	absent
5	23.4	4.5	2.1	137	2.0	absent
6	22.7	5.5	1.9	130	2.2	absent
7	23.0	4.3	2.2	—	2.3	absent
8	22.8	4.0	2.1	135	2.2	absent
9	21.7	4.0	1.9	—	2.2	absent
10	22.2	4.0	1.8	—	2.0	absent
11	22.3	4.8	2.0	133	2.1	absent
12	23.0	5.2	2.2	136	2.4	absent
13	23.9	5.6	2.2	139	2.4	absent
14	21.7	4.7	2.0	132	2.1	absent
15	23.0	4.7	2.1	138	2.2	absent
16	21.5	4.8	1.9	132	2.1	absent
17	21.7	4.8	2.0	138	2.0	absent
18	22.3	5.5	2.5	131	2.3	absent

This group consisted of 8 livers, mean values for the lipid content of which ranged from 8.9 to 33.5 g. per 100 g. wet liver (group mean 17.6 g. per 100 g.). The absolute variations between the lipid content of the specimens in this group were greater than those observed in the non-fatty liver groups and tended to increase as the lipid content of the liver increased. The variance per gram of lipid was, however, less than that found in the other groups.

Cirrhotic (Fig. 7, Table V): In 4 of the fatty livers, different degrees of fibrosis were encountered and there was considerable variation in the values for the lipid content of the specimens in a particular liver. This variation exceeded that found in the previous groups and was roughly pro-

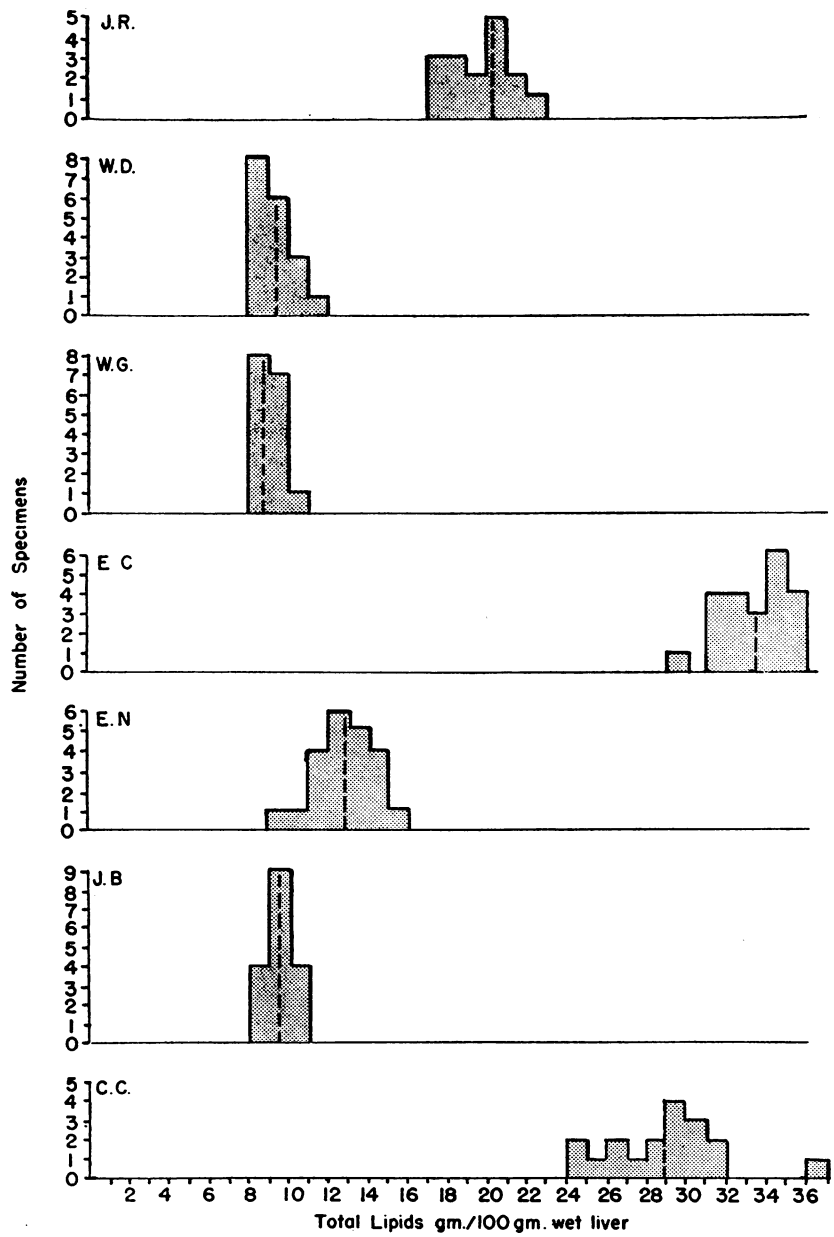

FIG. 6. GROUP II (A). THE DISTRIBUTION IN THE TOTAL LIPID CONCENTRATIONS IN SPECIMENS TAKEN FROM FATTY, NON-CIRRHOTIC LIVERS

TABLE IV

Variations in the dry matter, lipids and visible fat in needle specimens from a fatty, non-cirrhotic liver (J. R.). Group II(a)—Table I

Specimen No.	Dry matter g./100 g. wet liver	Total lipids g./100 g. wet liver	Total fatty acids g./100 g. wet liver	Iodine No. of fatty acids	Phospho-lipids g./100 g. wet liver	Histological estimation of fat
1	36.8	17.5	—	—	3.4	severe
2	38.8	18.3	15.3	64	—	severe
3	38.8	17.9	14.9	—	3.0	severe
4	39.4	19.0	15.7	61	3.0	severe
5	40.7	19.8	—	—	3.0	severe
6	40.9	21.5	18.7	63	3.0	severe
7	41.8	21.2	—	—	2.9	severe
8	40.8	21.9	14.0	64	3.2	severe
9	38.4	17.5	14.5	65	—	severe
10	38.3	18.7	14.8	68	2.9	severe
11	40.3	22.0	18.2	67	3.0	severe
12	40.9	20.0	16.7	65	—	severe
13	40.4	22.2	16.3	67	—	severe
14	42.0	20.7	17.6	64	—	severe
15	40.2	21.3	17.7	64	3.2	severe
16	41.5	21.5	18.9	66	—	severe
17	38.7	20.5	—	—	—	severe
18	42.9	23.5	18.0	68	3.0	severe

portional to the lipid content of the liver. Mean values for the lipid content in this group ranged from 14.8 to 30.1 g. per 100 g. liver (group mean 23.3 g. per 100 g.).

Total fatty acids

As seen in Tables II to V, the values for the total fatty acids generally paralleled those for the total lipids. Variations in total lipids for individual specimens particularly in the fatty livers appear, therefore, to be due mainly to variations in the neutral fat content.

Iodine number

Determination of the iodine number of the fatty acids present in the different specimens gave consistent findings for any particular liver. Tables V and VI show that this constancy is maintained

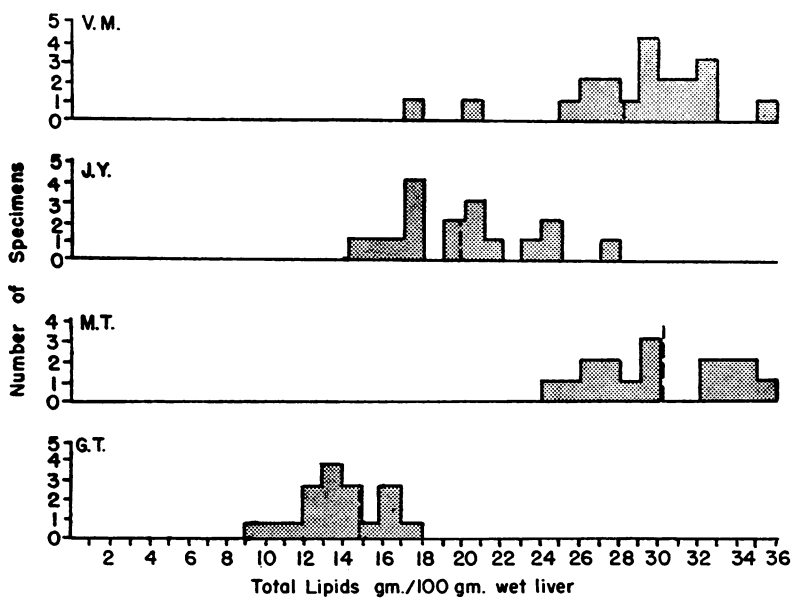

FIG. 7. GROUP II (B). THE DISTRIBUTION IN THE TOTAL LIPID CONCENTRATIONS IN SPECIMENS TAKEN FROM FATTY, CIRRHOTIC LIVERS

TABLE V

Variations in the dry matter, lipids and visible fat in needle specimens from a fatty, cirrhotic liver (M. T.). Group II(b)—Table I

Specimen No.	Dry matter *g./100 g. wet liver*	Total lipids *g./100 g. wet liver*	Total fatty acids *g./100 g. wet liver*	Iodine No. of fatty acids	Phospho-lipids *g./100 g. wet liver*	Histological estimation of fat
1	47.2	34.4	29.9	—	2.2	severe
2	44.2	27.9	24.1	—	2.4	severe
3	42.7	26.3	23.2	70	2.1	severe
4	—	—	—	—	—	severe
5	45.1	34.9	29.5	70	2.3	severe
6	41.2	26.2	25.3	68	2.1	severe
7	41.1	25.8	23.2	67	2.1	severe
8	43.5	29.0	—	—	2.1	severe
9	45.3	32.7	28.1	66	1.7	severe
10	43.4	27.0	25.7	66	2.2	severe
11	46.6	35.9	29.3	67	2.1	severe
12	46.7	33.8	30.6	67	1.9	severe
13	43.5	28.4	25.4	67	2.2	severe
14	41.2	24.5	20.8	69	2.3	severe
15	44.6	29.5	23.0	74	2.1	severe
16	43.3	29.6	27.3	67	2.1	severe
17	45.0	32.5	28.5	68	1.8	severe
18	47.0	33.4	29.0	67	2.1	severe

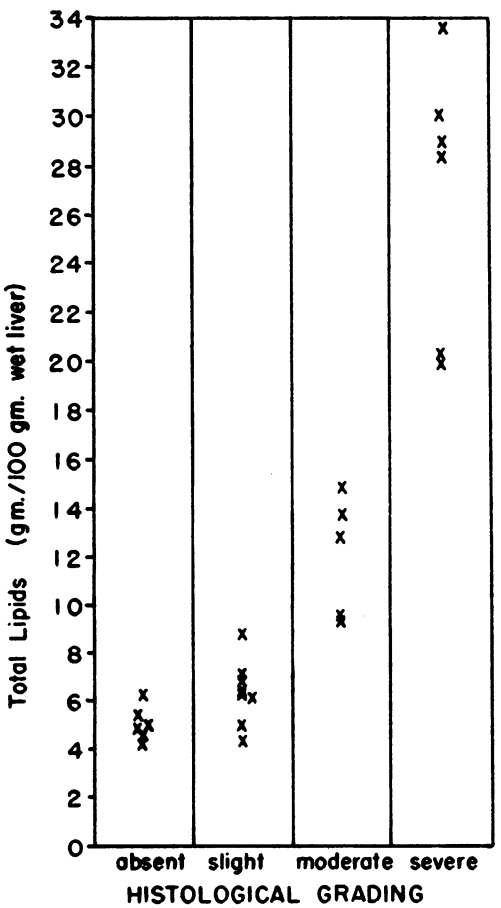

FIG. 8. THE RELATIONSHIP BETWEEN HISTOLOGICAL GRADING AND THE CHEMICAL DETERMINATION OF TOTAL LIPIDS (MEAN VALUE FOR LIVER)

even when the total lipid content shows some variation.

Phospholipids

Phospholipids appeared to be fairly uniformly distributed throughout both non-fatty and fatty livers. Slightly lower values were obtained for cirrhotic than for non-cirrhotic livers. The values recorded tended to be somewhat higher than those reported by other workers (4) possibly due to the method of extraction, which did not involve the use of petroleum ether.

Histological examination

Individual grading of the sections revealed relative uniformity in the amount of fat in the various

TABLE VI

Limits of error in the prediction of the lipid content of the whole liver from a single biopsy specimen and minimum change in lipid content of specimen indicative of a significant decrease of lipid content of whole liver upon repeat biopsy

Classification of liver	Approximate limits	Minimum differences indicative of significant decreases	
		Single sample	Paired samples
Non-fatty, non-cirrhotic	x ± 25%	0.39x	0.22y
Non-fatty, cirrhotic	x ± 35%	0.50x	0.30y
Fatty, non-cirrhotic	x ± 18%	0.31x	0.17y
Fatty, cirrhotic	x ± 30%	0.47x	0.28y

x = lipid content of specimen in g./100 g. liver.
y = mean lipid content of control pairs of samples in g./100 g. liver.

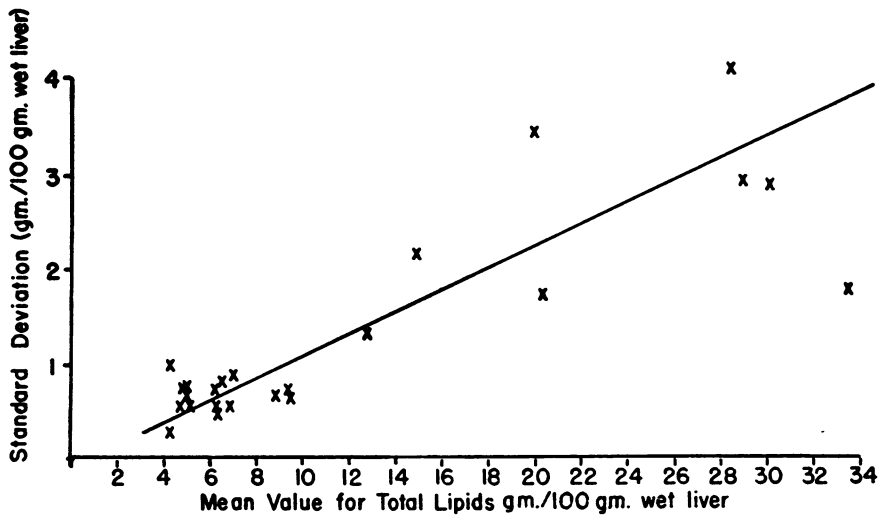

FIG. 9. THE RELATIONSHIP BETWEEN THE VARIANCE IN THE SPECIMENS AND THE MEAN TOTAL LIPID CONCENTRATION

portions of the liver. The impression gained of the amount of fat present by study of the hematoxylin-eosin stained sections, supplemented by the various fat stains, showed good correlation with the amount of fat found by quantitative methods (Fig. 8). With one exception (W. G.) livers graded for lipids as "absent" or "slight" had lipid contents of less than 8 g. lipid per 100 g. wet liver. Those graded "moderate" tended to have values ranging between 8 and 16 g. lipid per 100 g. wet liver, while those with greater amounts of lipid were all classified as "severe."

DISCUSSION

The present work has been designed to determine the possibility of estimating the lipid concentration of the entire liver from specimens obtained by needle biopsy. The error of this estimate will depend upon the accuracy of the chemical analysis and the distribution of lipids in the human liver, in various pathological conditions.

That there is no important systematic deviation between the analysis made by the micro-methods, described in this paper, on 5 to 8 mg. of tissue and by the accepted macro-methods is suggested by the agreement of our findings for the "normal" liver (5.7 ± 0.8 g. lipid per 100 g. wet liver for the mean $\pm$ standard deviation) and those of Ralli and her co-workers (4) who concluded that the concentration of total lipid in the normal human liver will probably not exceed 8 g. per 100 g. wet liver. Other workers have reported similar values for the normal human liver (Man and coworkers [13], Thannhauser and Reinstein [14], and Rourke and Stewart [2]).

Histological examination of needle specimens taken from various sites in the liver has shown that relative uniformity in the distribution of fat does exist even in cirrhotic livers. Chaikoff and Kaplan (15) reported that in depancreatized dogs the distribution of fat in the liver may be uneven and that fatty as well as normal portions may exist simultaneously in the same liver. Such a situation has not, however, been observed in our material.

Inspection of the results obtained by chemical analysis shows that the lipid content of the specimens obtained from any one liver (*1*) varies for each individual, (*2*) is dependent upon the histopathologic alteration, (*3*) shows greater variation in those livers with a high lipid content (Fig. 9) and that (*4*) this variation depends to some extent on the presence or absence of cirrhosis. Statistical analysis has shown, however, that the variances for the four groups are essentially homogeneous. It can therefore be concluded that it is as accurate, on a percentage basis, to estimate the lipid concentration of the entire liver from a single specimen, whether cirrhosis is present or absent, or the fat content high or low.

Using the data obtained in this study, it is possible to estimate from a needle specimen the lipid concentration of the entire liver with a determined

error. The best estimate is the concentration of lipid found in the specimen itself and its error may be conveniently expressed as the 5 per cent fiducial limits, which indicate the limits between which the lipid concentration of the liver will probably be 19 out of 20 times (16). In calculating these limits the variance of the group is used since this is the best estimate of the variance of the individual and the grouping is known from histological examination of the specimen. Thus, for a non-fatty, non-cirrhotic liver (*i.e.*, "normal") the lipid concentration in the whole liver is within the range of ± 25 per cent of the value found from the micro-analysis of a single sample. The limits for the remaining groups are summarized in Table VI. When two samples are analysed, a more precise estimate can be obtained.

If some therapy is given that is expected to modify the lipid content of the liver, it will be necessary to know whether any change observed is greater than that expected by chance. The "t" test (17) has been performed on the differences in the mean values, using the variance of the group as the best estimate of the variance for any sample shown by histological examination to belong to that group. In the third column of Table VI is given the minimum change in lipid (expressed as a fraction of the control sample) which is necessary to indicate a decrease, at the 5 per cent level of significance, in the second sample. For example, if the initial sample from a fatty non-cirrhotic liver had a lipid concentration of 20 g. per 100 g. liver, then a decrease of 6.2 g. per 100 g. liver or greater would be necessary to ensure that the change would not have been likely on the basis of sampling variation. If the analysis is carried out on two biopsy specimens, taken simultaneously, then a smaller difference between the first and second samples will be significant (Column 4, Table VI).

T. K. With (18) showed that in man, analysis of a small sample of liver tissue does provide values which can be regarded as representative of the average concentration of Vitamin A in the liver, particularly of the right lobe. Specimens of liver obtained by the needle biopsy technique can also be regarded as representative of the lipid content of the liver as a whole within the limits stated above. Recognition of these limits is necessary if a valid interpretation of the results obtained is to be made.

SUMMARY

1. Methods for the chemical analysis of the percentage of dry matter, total lipids, total fatty acids and their iodine number, and phospholipids in specimens of liver tissue obtained by the needle biopsy technique are described.

2. Using a Vim-Silverman needle, 18 specimens have been taken from different sites in each of 25 post-mortem livers (13 non-fatty and 12 fatty livers). The samples have been examined histologically and their total lipid concentration determined chemically. The variation in the different specimens is reported and the effect of cirrhosis on the findings is discussed.

3. Relative uniformity in the distribution of lipids throughout the human liver has been observed. A good correlation was found between histological and chemical findings.

4. Approximate limits of error in estimating the lipid concentration of the whole liver from analysis of a single biopsy specimen are given.

5. Factors are given which may be used to determine whether the value for the lipid concentration of a repeat biopsy specimen indicates a significant change in the lipid concentration in the whole liver.

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ANNOUNCEMENT OF MEETINGS

The 45th annual meeting of the American Society for Clinical Investigation will be held in Atlantic City, N. J., on Monday, May 4, 1953, with headquarters at the Chalfonte-Haddon Hall. The scientific session will begin at 9 a.m. at the Steel Pier Theater.

The annual meeting of the Association of American Physicians will be held at the Chalfonte-Haddon Hall on Tuesday, May 5, and the morning of Wednesday, May 6, 1953.