Extraction of lipids from blood serum and liver tissue

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» In order to arrive at a reliable method for the complete extraction of lipids, free from nonlipid impurities

TABLE 1. COMPARISON OF THE RESULTS OF THE EXTRACTION OF PIG SERUM BY THREE DIFFERENT METHODS

Serum No. →	I			II			III		
Extraction method*→	B	D	F_2	B	D	F_2	B	D	F_2
Amount of extract from 20 ml serum (mg)	89.2	95.9	88.4	80.1	70.4	80.4	70.6	71.6	74.6
P content of the extract (mg)	0.71	0.75	0.95	0.49	0.39	0.91	0.63	0.54	0.87
P content of the extract (%)	0.80	0.78	1.07	0.61	0.55	1.13	0.89	0.75	1.17

* B = modified Bloor's method (2). D = modified Delsal's method (4). F_2 = Folch's method (6).

from serum, the results of some modern extraction methods were compared — e.g., that of Mukherjee, Achaya, Deuel, and Alfin-Slater (1) who use Bloor's solvent mixture (2) (ethanol–ether 3:1); that of Fillerup and Mead (3), who use Delsal's solvent mixture (4) (methylal–methanol 4:1); and two procedures of Folch et al. (5, 6) who use a chloroform–methanol mixture (2:1). The last mentioned two procedures differ in the manner of purification — viz., F_1 with a tenfold amount of water (5) and F_2 with 0.2 volume of water (6).

These methods, except for F_1, were compared by applying them to equal amounts of a sample of fresh pig serum. The amounts, in mg, of extracted lipids and the content of the phosphorus in the lipids were determined, the latter by the method of Zilversmit and Davis (7). The results of this experiment are given in Table 1.

There was little difference in the amounts of lipid extracted by the three methods. The percentage and also the absolute amount of phosphorus extracted were highest when using Folch's method.

Chromatography according to Borgström (8) confirmed that more phospholipid was indeed extracted by the F_2 method than by the modified Bloor method (B). However, the phosphorus content of the phospholipid fractions obtained in this way (2.7% P) was lower than that to be expected (3.5–4.0%). Since from earlier results it seemed unlikely that the low phosphorus content was caused by contamination with "neutral lipid", contamination with nonlipid constituents was considered the most likely explanation, and a method was sought that would remove the nonlipid impurities completely.

Folch originally applied a different purification procedure (F_1) (5) for the extracted lipids from brain tissue. To remove nonlipid contaminants from the chloroform-methanol extract, he placed this mixture under a tenfold amount of water, with the result that the water-soluble constituents and methanol pass over into the upper phase. We compared the F_1 and F_2 procedures with regard to the lipids from serum. Extracts obtained were separated by chromatography into a "neutral lipid" fraction and a phospholipid fraction, and the phosphorus content of the latter was determined. The results are shown in Table 2. The same amount of phosphorus (mg) was extracted by both methods, but the phosphorus content of the phospholipids obtained from the F_1 extract approached the expected level (3.5–4.0%). To establish the character of the contaminant in the F_2 extract, the "phospholipid" fractions were investigated qualitatively by dissolving them in chloroform and extracting with an equal amount of water. The aqueous phase was analyzed for the presence of protein, amino acid, urea, sodium, chloride, and calcium. In the case of the "F_2 phospholipids," sodium chloride was found to be the major contaminant; tests for protein, amino acids, and urea were negative.

To establish whether all the lipids are fully extracted by Folch's method (F_1), the amounts of fatty acid and unsaponifiable constituents were determined in the serum and plasma as such, and in the F_1 extract, by using the following procedures:

1. Fifty ml serum (or plasma) was saponified with an excess of 0.5–1 N alcoholic potassium hydroxide; hydroquinone was added as anti-oxidant. The unsaponifiable constituents and, after acidification, also the fatty acids were extracted with light petroleum.
2. The lipid from 50 ml serum extracted according to method F_1 was saponified and treated in the same way.
3. The serum protein residue, removed by filtration after the addition of chloroform–methanol, was saponified and treated as above. To insure that no chloro-

TABLE 2. COMPARISON BETWEEN THE EXTRACTS OBTAINED WITH THE F_1 AND F_2 METHODS

Starting Material	Pig Serum	
	F_1	F_2
I. Amount of "lipid" from 50 ml serum (mg)	122.9	158.8
II. Amount of "neutral lipids" (mg)	78.4	79.6
III. Amount of "phospholipid" (mg)	46.9	78.3
Amount of phosphorus in III (mg)	1.55	1.58
Phosphorus content of III (%)	3.30	2.02

TABLE 3. AMOUNT OF FATTY ACID AND UNSAPONIFIABLE MATERIAL (MG) OBTAINED IN VARIOUS WAYS FROM 50 ML SERUM OR PLASMA OR FROM THE CORRESPONDING PROTEIN RESIDUES

Serum or Plasma	1		2		3		4	
	Fatty Acid	Unsap.	Fatty Acid	Unsap.	Fatty Acid	Unsap.	Fatty Acid	Unsap.
Human	123.7	89.4	121.0	86.2	7.3	8.1	4.0	4.0
Pig 1	80.8	52.6	79.1	51.0	3.8	3.2	...	...
2	91.6	58.9	89.3	55.0	4.1	6.0	2.2	3.0

form was present during the saponification (development of formic acid), the protein residue was first washed with ether.

4. The protein residue (3, above) was first extracted by method F_1 and was then saponified as before.

From the results, shown in Table 3, it may be concluded that 97–98% of the lipid is extracted from serum or plasma by the F_1 method. For complete extraction, it is essential that the protein residue is extracted once more.

Fatty acids obtained from the protein residue (see 3) appeared to be impure for, on titration of 10.1 mg "fatty acid" obtained from the residue of 100 ml serum, only 0.0125 mEq was found — a figure that corresponds to 3.5 mg fatty acid (assuming a mol wt 277 (9)). On direct saponification of 100 ml of this serum, 161.6 mg fatty acid was found, so about 2% of the lipid remained in the protein residue.

To learn whether or not the phospholipids were completely extracted from the serum or plasma, the amount of phosphorus bound to lipids in the original serum (or plasma) and in the phospholipid fraction obtained from the extract was determined by the method of Zilversmit and Davis (7). Since just as much phosphorus was found in the "phospholipid" fractions obtained from an extract of serum or plasma as in the chloroform–methanol extract of the material precipitated with trichloroacetic acid, it appears that the phospholipids are almost entirely extracted by the F_1 procedure.

TABLE 4. AMOUNTS OF LIVER LIPID (MG) EXTRACTED BY THE F_1 AND F_2 METHODS

Extraction Method	Kind of Liver	Crude Extract	"Lipid"
		ml	*mg*
F_2	Rabbit	50	150.4
F_1	Rabbit	50	176.0
F_2	Ox	100	307.6
F_1	Ox	100	345.9

TABLE 5. TOTAL FATTY ACID AND PHOSPHORUS CONTENTS OF THE EXTRACTED LIPIDS AND OF THE TOP PHASE

	50 ml Crude Extract from Rabbit Liver Purified According to	
	F_1	F_2
I. Lipid (mg)	177.9	150.4
Total fatty acids from I (mg)	84.0	83.0
II. Phospholipid in I (mg)	129.2	102.7
Phosphorus in II (%)	2.89	3.60
III. Substance in top phase (mg)	50.1	82.0
Fatty acids in III (mg)	0.7	0.9

In view of the favorable results obtained with the F_1 method for extraction of lipids from blood serum, we wanted to confirm that in the case of liver tissue the modified method F_2 gives satisfactory results. A comparison of both methods applied to livers of different animals gave the results shown in Table 4. The data indicate that more lipid is extracted by the F_1 method than by the F_2 method, which is precisely the opposite of the result obtained with blood plasma (see Table 2). Thus, either the F_1 extract is contaminated with nonlipid constituents or lipids are lost in the top phase when applying the F_2 method. In order to ascertain the cause, the lipids obtained, as well as the top phases from the F_2 extract, were further investigated. The extracts were separated by chromatography (8) and phosphorus was determined in the phospholipid fractions. The total fatty acids in the lipids obtained by the F_1 and F_2 methods and in the top phases of both F extracts were also determined to check whether lipid diffuses over into this phase (Table 5). The data suggest contamination of the F_1 extract with nonlipid constituents. Positive tests for carbohydrate in "substance in top phase" obtained in the F_2 extraction, as well as in the "F_1 lipid," showed the contaminant to be a carbohydrate-containing nonlipid component. Thus, for the extraction of lipid from liver, method F_2 must be preferred to F_1.

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