materials stored for 2 days at 4° C., but it is readily inactivated by heating.

In a further similar experiment samples of lingcod flesh containing 5 γ per gram of chlorotetracycline, with and without 40 γ per gram of ascorbic acid or 200 γ per gram of sodium nitrite, were stored 2 days at 5° C. At the conclusion of this storage period the chlorotetracycline content of the various samples was: control 4.8, ascorbic acid–treated 3.6, and nitrite-treated 4.8 γ per gram. Thus, nitrite appears to have no effect and ascorbic acid a slight destructive effect on chlorotetracycline in fish flesh.

Antimicrobiological Effect of Chlorotetracycline, Puromycin, And Thiolutin

To test the antimicrobiological effect of added chlorotetracycline, puromycin, and thiolutin in ground beef and fish, a technique was used identical with that employed in previous work (*5*). Table VII shows that chlorotetracycline (1 γ per gram) suppressed bacterial development and permitted yeast growth, and that thiolutin (10 γ per gram) did not inhibit yeast development in the presence of chlorotetracycline to any important extent. In this respect thiolutin differed from rimocidin, which had been shown to exert a marked antiyeast activity (*5*). Puromycin was devoid of antibacterial activity.

Organoleptic Findings

During the investigation the experimental samples were subjected to occasional superficial organoleptic examinations. It was observed that fish iced with ordinary ice attained a state of obvious staleness about 4 or 5 days earlier than fish stored in the chlorotetracycline-containing ices. These organoleptic improvements in quality were even more obvious with fish stored in sea water containing chlorotetracycline, or briefly immersed in the stronger chlorotetracycline solutions.

No extensive tasting tests were made on the cooked fish, but the results of a few trials indicated that treated samples remained in an edible condition very much longer than those which were not treated.

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Quicker Method for Determining Fat in Liver

DETERMINATION OF LIVER FAT

Comparative Studies of Different Methods

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Fat is usually determined in liver by the rather lengthy ethyl ether extraction technique of the Association of Official Agricultural Chemists. The Roese-Gottlieb or Mojonnier technique of wet ether extraction is widely used in the dairy and food industry. Because this is shorter, it has been modified and adapted to the determination of fat in fresh liver tissues. The fat values obtained using this technique are consistently higher than the values obtained by dry ethyl ether extraction. When a mixed extracting solvent of ethyl ether, petroleum ether, and ethyl alcohol was used in the dry Goldfisch extractor, liver fat values approximated those obtained by the Mojonnier extraction. Alcohol may play a role in making the fat more easily accessible to ether extraction.

LIVER FAT IS PROBABLY most often determined by the lengthy technique of the Association of Official Agricultural Chemists for meat and meat products (*3*), using extraction with dry ethyl ether.

Only a few methods have been reported specifically for the determination of liver fat. Leites and Odenov (*7*) reported success with an alkaline digestion of liver, followed by a sulfuric acid extraction with petroleum ether, but,

their values appear to be low for normal tissue. Chavarelli (*5*) reported a rapid method for determination of fat in tuna fish liver by wet ether extraction, but the technique did not appear to be suitable for nutritional research.

Table I. Recovery of Added Liver Fat from Liver Homogenate by Mojonnier Extraction

Fat Added to Sample, G.	Fat Extracted from Sample, G.	Fat Recovered, G.	% Recovery
None	0.0271	...	...
0.05164	0.0793	0.0522	101
0.10328	0.1324	0.1053	102
0.15493	0.1837	0.1566	101
0.20666	0.2364	0.2093	101

The Roese-Gottlieb (*9*) or Mojonnier (*8*) technique of wet ether extraction is widely used in the dairy and food industry. This method or modifications of it have been successfully applied to the determination of crude fat in many food products (*2*). Soderhjelm and Soderhjelm (*11*) have reported an accurate and rapid method for determination of crude fat in feces using the Mojonnier extraction flask. Seeler and Dietrich (*10*) have also reported success with a modified Roese-Gottlieb method for determining fats in foods and fodder.

An extraction technique similar to the Roese-Gottlieb or Mojonnier method seemed likely to lend itself well to the determination of fat in fresh liver tissue. This paper reports application of this type of fat extraction to liver tissue, with a comparison of fat values obtained by several other methods of crude fat extraction.

Preparation of Liver Homogenate

The whole liver of an albino rat was removed from a sacrificed animal, weighed, homogenized in a Waring Blendor with water, and finally made to a definite volume with water. Suitable aliquots of this homogenate were taken for analysis.

Wet Ether Extraction

The general technique followed was a modification of the Roese-Gottlieb (*9*) and Mojonnier (*8*) methods. The Mojonnier extraction flask was employed with the Mojonnier tester, but the procedure can be carried out without the tester if a suitable hot plate, vacuum oven, and centrifuge are available.

Glass instead of aluminum dishes was used, as liver fat, especially when extracted from acid solution, had a tendency to react with the aluminum.

Materials and Apparatus Mojonnier fat-extraction flasks.

Glass fat dishes (75 × 50 mm. crystallizing dishes).

Mojonnier tester or suitable hot plate, vacuum oven, and centrifuge.

Reagents Ethyl alcohol, 95% U.S.P.

Acidified ethyl alcohol, 100 ml. of 95% ethyl alcohol plus 1.0 ml. of 36% hydrochloric acid.

Ethyl ether, U.S.P.

Petroleum ether (Skellysolve F).

Procedure

1. Pipet a quantity of liver homogenate equivalent to 1.0 to 1.5 grams of wet liver into a Mojonnier flask. Add water so that the combined volume of homogenate and water is approximately 12.5 ml.
2. Hold the flask so that the homogenate is in the larger part, quickly add 10 ml. of acidified alcohol, and mix immediately. A practically clear mixture results.
3. Add 25 ml. of ethyl ether and shake 1 minute.
4. Add 25 ml. of petroleum ether and shake 1 minute.
5. Centrifuge at slow speed for several minutes.
6. Decant the ether layer into a weighed fat dish and evaporate on a hot plate at 110° C.
7. Subject the contents remaining in the flask to a second extraction, adding 5.0 ml. of alcohol in place of the acidified alcohol, and repeat steps 3, 4, 5, and 6.
8. Dry the dish and fat in a vacuum oven at 135° C. and 28-inch vacuum for 10 minutes.
9. Cool the disk in a desiccator until room temperature is reached.
10. Weigh the dish plus fat, and obtain the weight of the fat.

Table II. Liver Fat Values

(Nine groups of six rats each)

Average Fat Found in Fresh Liver, %		
Mojonnier	Goldfisch	Av. difference
5.8	3.2	2.6
5.8	3.1	2.7
6.8	4.4	2.4
7.0	5.0	2.0
5.8	3.8	2.0
5.3	4.0	1.3
4.3	3.2	1.1
8.3	6.3	2.0
8.8	6.6	2.2
Av. difference, all animals		2.0

11. Run a blank determination on the reagents.
12. Compute the fat content as per cent of the wet liver.

Extraction by Dry Ethyl Ether

Modified AOAC Procedure (*3*) An aliquot of liver homogenate containing from 2 to 3 grams of wet liver was placed in an evaporating dish and dried in a 100° C. oven for 12 to 24 hours. The dried sample was then ground to a fine powder with a pestle, transferred to a fat-extraction thimble, and extracted for 14 to 16 hours in a Goldfisch continuous extraction apparatus. The tared ether flask was removed, the ether was evaporated on a sand bath, and the flask and contents were dried in an oven at 100° C. for 2 hours.

Soxhlet Extraction This procedure is known as the Adams paper coil technique (*7*). It was employed with the purpose of leaving the solid dried liver residue in a more porous condition, so that the fat would be more accessible to the ether.

A strip of fat-free filter paper (Whatman No. 1 chromatographic), 6 cm. wide and 110 cm. long, was suspended like a hammock by its two ends. Ten milliliters of homogenate equivalent to 1.0 to 1.5 grams of wet liver was placed on this paper, so that it was equally distributed. It was allowed to dry in air and then was further dried for 2 hours in an oven at 100° C. The strip was then rolled into a coil, placed in a fat-extraction thimble, and extracted 14 to 16 hours in a Soxhlet extractor. A blank determination was run on filter paper containing no added liver. The drying and weighing of the ether extract were carried out as in the modified AOAC procedure, and the per cent of liver fat was calculated in the usual manner.

Application of Mojonnier Extraction Methods to Liver Tissue

In the first attempt to apply the Mojonnier technique to a liver homogenate, the procedure was followed as outlined for fresh milk. The liver proteins had a tendency to coagulate when ammonium hydroxide was added, but this was overcome by mixing the ammonia solution with the alcohol before using. It was found that the greater the ammonium hydroxide addition, the less the amount of crude fat extracted; in fact, an extraction containing no added ammonia gave the largest amount of extractable crude fat in spite of the fact that the liver proteins coagulated upon addition of the alcohol.

The next approach was to attempt extractions from samples acidified with hydrochloric acid. When the acid was previously mixed with the alcohol and then added to the sample, protein coagulation could be completely prevented when small amounts of acid were added and kept to a minimum when large amounts were added. Both weak and strong acid extractions yielded approximately the same amount of crude extractable fat as a neutral extraction. Further tests also indicated that an acid hydrolysis of the sample previous to extraction was not effective in increasing the amount of extractable crude fat.

It was apparent that the maximum amount of crude extractable fat could be obtained with the least difficulty from a weakly acidified sample containing approximately 0.1 ml. of 36% hydro-

chloric acid in the 10-ml. alcohol addition.

Evidence of the reliability of the Mojonnier extraction is presented in Table I. The crude liver fat was saved from previous extractions, known amounts were added to samples of liver homogenate, and fat recoveries were determined after extraction. The results of this experiment indicate that recoveries of added crude fat are approximately 100%.

Comparison of Mojonnier and Dry Ether Extraction

For comparison of liver fat values obtained by Mojonnier extraction and by dry ether extraction, livers from nine groups of white rats containing six animals each were analyzed. These results, presented in Table II, indicate that values obtained by Mojonnier extraction are consistently and significantly higher than values obtained by dry Goldfisch ether extraction.

A further comparison of liver fat values was made employing a mixed solvent containing 5 parts of ethyl ether, 5 parts of petroleum ether (Skellysolve B), and 2 parts of ethyl alcohol in the Goldfisch extractor. This solvent approximates the composition of the solvent used in the Mojonnier extraction. The solubility in ethyl ether of the fat residue obtained using this solvent was also determined by redissolving it in ethyl ether, and filtering through Whatman No. 40 filter paper, with final redrying and reweighing. These results, presented in Table III, indicate again that the Mojonnier technique extracts more ethyl ether–soluble material than the Goldfisch extraction in dry ethyl ether. When the mixed solvent was used, however, the Goldfisch technique extracted approximately the same amount of ether-soluble material as the Mojonnier method.

Tests were also made using the Adams paper coil procedure with a Soxhlet extractor (Table IV). Only a few livers were analyzed using this technique, but crude fat values obtained were still less than those obtained by Mojonnier extraction and only slightly higher than values obtained by dry ethyl ether extraction with the Goldfisch continuous extractor.

Table III. Liver Fat Values

Sample No.	Fat Found in Fresh Liver, % Mojonnier	Ethyl ether Goldfisch	Mixed solvent Goldfisch
1	5.6	3.5	5.5 (99)[a]
2	6.3	4.5	6.5 (96)
3	5.4	3.8	5.6 (98)
4	5.4	3.4	5.3 (99)
5	4.8	2.6	5.2 (99)
6	7.1	5.4	7.4 (99)
7	6.6	5.1	6.7 (98)
8	6.5	5.2	7.2 (97)
9	6.1	4.5	6.3 (97)
10	4.4	2.8	4.9 (97)
11	4.3	2.7	4.8 (95)
12	4.1	2.5	4.5 (96)
13	4.3	2.7	4.8 (98)
Av.	5.5	3.7	5.7 (98)

[a] Figures in parentheses refer to per cent ethyl ether solubility of fat residue from mixed solvent Goldfisch extraction.

Table IV. Liver Fat Values

Sample No.	Fat Found in Fresh Liver, % Mojonnier	Goldfisch	Adams paper coil
1	5.2	2.7	3.1
2	5.8	2.9	3.3
3	5.2	2.7	3.1
4	6.4	3.9	3.1
5	6.3	3.8	4.6
6	6.1	3.4	4.5

Discussion

A wet ether extraction technique using the Mojonnier flask can be successfully applied to the determination of crude fat in fresh liver tissue. Agreement between duplicate samples is excellent, and the recovery of liver fat added to a liver homogenate is approximately 100%. If the Mojonnier tester is employed, eight samples can be tested in about 2 hours. The solids content of the liver homogenate can also be determined with the Mojonnier tester.

Liver fat values obtained by Mojonnier extraction are consistently from 1.1 to 2.8% higher than values obtained by dry ether extraction, but dry extraction employing a solvent mixture of ethyl ether, petroleum ether (Skellysolve B), and ethyl alcohol gives values that approximate those found by Mojonnier extraction.

In the drying of the homogenate for dry extraction some of the fat may be made inaccessible to ethyl ether. Traces of moisture are probably still present in the dried homogenate. Because water and ether are only slightly soluble in each other, the moisture could act as an ether barrier around each dried particle, thus interfering with complete fat extraction. The higher fat values obtained with dry extraction in mixed solvent could be explained on the basis that alcohol is miscible with both water and ether in all proportions and could act as a medium permitting the ether to extract fat from within the dried particles. In both wet and dry extractions alcohol might be effective in releasing ether-soluble materials that are in combination with proteins or other substances.

Budde (*4*) and Hoffmann (*6*) have reported that dry ether extraction of baked dog food gives fat values that are too low, while Mojonnier-type procedures of wet acid hydrolysis extract materials that are not soluble in pure ethyl ether. Hoffmann proposes redissolving the dried fat residue from wet extraction in ethyl ether and filtering through fat-free filter paper before final drying and weighing.

This proposed extra filtration technique was applied to 12 samples of liver fat residue from Mojonnier extraction as well as to 13 samples from mixed-solvent Goldfisch extraction. The original fat residue in all samples was from 95 to 100% soluble in ethyl ether, indicating that little or no material is extracted that is not soluble in ethyl ether.

Petroleum ether is added in the Mojonnier extraction not as a fat extractant but to reduce the amount of alcohol and water included in the ether layer. The role that it plays when added to the Goldfisch solvent has not been studied. The amount and type of petroleum ether added, however, would influence the boiling point of the mixture and in this respect would influence the amount of alcohol coming into contact with the dried sample in the thimble.

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