Gas Chromatographic Analysis. Under the conditions described above, chlorpyrifos-methyl has a retention time of 1.98–2.05 min with a deflection of 34% for 2-ng samples and 72% for 120-ng samples at the proper attenuation as described above. In the cases of samples with low levels of chlorpyrifos-methyl, the extracts were evaporated to smaller volume prior to injection into the gas chromatograph. The conditions for the chromatographic analysis were such that no improvement in precision was noted when using the area of the peak instead of the height of the peak. Retention time and deflection percentage obtained seem to be convenient for a fast and precise analysis. Although the method described herein for extractions of samples containing low levels of chlorpyrifos-methyl was designed for aqueous solutions, it seems that with appropriate uses of solvents and clean-up procedure the method could be adapted for extraction and analysis of samples of biological nature. The simplicity of the manipulation for extraction of natural waters show that the method is adaptable for field uses. Well-protected extracts could be analyzed with accuracy in the laboratory up to 4 weeks after the extraction.

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Pierre-F. Blanchet
Département de Chimie-Biologie
Université du Québec à Trois-Rivières
Trois-Rivières, Québec, Canada G9A 5H7

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A Convenient Microfiltration Procedure for Purification of Bovine and Ovine Subcutaneous Tissue Lipid Extracts

A convenient and rapid microfiltration procedure for the extraction and purification of lipids in bovine and ovine subcutaneous tissues is described. The procedure is as efficient in the extraction of total lipid, free fatty acids, total sterols, and phosphorus-containing lipid as an extraction procedure employing an aqueous wash for purification. Using lipid calcium as a measure of nonlipid contamination, the microfiltration procedure is as effective as aqueous washing. Due to the avoidance of separatory funnel washing and lengthy phase separations, the microfiltration procedure is preferred if numerous samples are extracted.

Lipid extraction by chloroform–methanol mixtures followed by aqueous washing to remove nonlipid material is effective for many tissues (Johnston, 1971). Unfortunately, troublesome and persistent emulsions are frequently encountered during the aqueous wash. For example, during the extraction of butter and margarine it was suggested that after washing the systems be allowed to stand overnight for phase separation (Smith et al., 1978). This approach is suitable for the extraction of a few samples but is tedious when numerous samples are extracted. During the extraction of a considerable number of subcutaneous tissues from lamb and steer carcasses using the chloroform–methanol and aqueous washing procedure, stable emulsions were encountered. This prompted a search for a purification procedure other than aqueous washing and this paper is a description of an effective one using microfiltration.

The subcutaneous tissue samples studied were those taken over the longissimus muscle at the 12th rib. One sample of steer tissue and one of lamb tissue containing relatively high total lipid and one from each of the species containing relatively low total lipid were used for the comparison studies. Extractions and lipid determination for each of the four samples were triplicated. Analysis of variance was used to test differences of means between the two extraction procedures (Steel and Torrie, 1960).

The microfiltration procedure was performed in the following manner. About 1 g of tissue was weighed to nearest 0.02 g and transferred to glass homogenization flasks. Twenty-five milliliters of 2:1 (v/v) chloroform–methanol (CM) was added, and the contents were homogenized at full speed for 2 min in a Virtis "45" homogenizer (Virtis Co., Inc., Gardiner, N.Y.). The homogenate was filtered through a CM-washed folded filter paper (Schleicher and Schuell, Keene, New Hampshire, No. 588) into a 200-mL round-bottom flask. Another 25 mL of CM was used to rinse the homogenizer blades and flask, the filter paper and residue, and the funnel with the use of a dropping pipet. Visual examination of the filter and residue for oiliness after drying indicated little, if any, lipid present. The extract was evaporated in a rotary evaporator, using a 55 °C water bath to facilitate solvent removal. When it was apparent that water was being evaporated (solution becomes opaque), more chloroform was added to aid in water removal and prevent bumping. The lipid was extracted from the flask

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Table I. Comparison of Extraction Efficiency of the Method Using Microfiltration (A) and the One Using an Aqueous Wash (B) (Each Value Represents an Average of Three Determinations with Standard Deviation)

subcutaneous tissue	total lipid[a]		free fatty acid[b] extraction		total sterol[c] procedure		lipid phosphorus[d]		lipid calcium[d]	
	A	B	A	B	A	B	A	B	A	B
bovine	87 ± 3	87 ± 4	40 ± 2	46 ± 5	0.57 ± 0.05	0.58 ± 0.06	71 ± 3	72 ± 2	3 ± 1	3 ± 1
bovine	83 ± 2	83 ± 3	32 ± 3	36 ± 3	0.86 ± 0.07	0.85 ± 0.07	86 ± 4	85 ± 3	2 ± 1	3 ± 1
ovine	89 ± 2	88 ± 1	13 ± 2	12 ± 2	0.53 ± 0.03	0.55 ± 0.04	116 ± 6	118 ± 7	1 ± 1	1 ± 1
ovine	76 ± 3	77 ± 4	16 ± 2	20 ± 4	0.73 ± 0.05	0.69 ± 0.04	130 ± 4	128 ± 6	1 ± 1	2 ± 1

[a] Expressed as percent of fresh weight. [b] Expressed as nM/g of lipid using oleic acid as standard. [c] Expressed as mg/g of lipid using cholesterol as standard. [d] Expressed as μg/g of lipid.

with several portions of hexane and each portion was passed through a microfiltration system into a 25-mL volumetric flask. The microfiltration system consisted of a 0.5-μm (pore size) solvent-inert membrane filter and a microfiber glass prefilter in a 25-mm micro-syringe filter holder (Millipore Corp., Bedford, Mass.) and was washed with hexane prior to use. A 25-mL glass syringe served as reservoir for the system and the glass plunger was used to speed filtration. After diluting to volume with hexane and mixing, appropriate aliquots of the 25 mL were used for the lipid analyses.

The aqueous wash procedure was performed in the following manner. The tissue and solvent was homogenized as for the preceding method and, after transferring to a 125-mL separatory funnel, 5 mL of deionized water was added and the contents were mixed. Phase separation was allowed to occur for 40 min, after which the lower phase was collected in a 200-mL round-bottom flask. Twenty-five milliliters of CM was used as a rinse as before and added to the separatory funnel. Another 5 mL of deionized water was added and the contents were mixed. Phase separation was obtained within 10 min and the lower phase was added to the round-bottom flask. After rotary evaporation as described in the previous method, the lipid was extracted from the flask with several portions of hexane and transferred to a 25-mL volumetric flask. After diluting to volume and mixing, this lipid solution served as a source of lipid for the analyses performed.

Aliquots of the hexane solutions were evaporated for the gravimetric analysis of total lipid and for the colorimetric analyses of free fatty acids (Lowry and Tinsley, 1976) and total sterol (Zlatkis et al., 1953). The lipid was saponified and the nonsaponifiables extracted prior to sterol analysis. Cholesterol served as a standard. Other aliquots of the hexane solutions, after evaporation, were ashed at 550 °C, dissolved in 1 mL of concentrated hydrochloric acid and diluted to 5 mL with deionized water. This solution was used for the atomic absorption spectroscopic analysis of calcium (Perkin Elmer Corp., 1968) and portions of the solution were evaporated and analyzed for phosphorus (Bartlett, 1959).

Data from all analyses appear in Table I. Statistical analysis did not show any differences at the 1% level in total lipid, lipid free fatty acids, total lipid sterols, or lipid phosphorus between the two procedures. Apparently, the relatively simple and rapid microfiltration procedure is as efficient in extracting polar and nonpolar lipids as is the more tedious aqueous wash procedure.

Calcium residue in the hexane solutions of total lipid was analyzed as a measure of contamination by nonlipid material. As can be seen in Table I, the levels of calcium in the lipids were low in all cases and there were no apparent differences between procedures. Statistical analysis was not attempted with the calcium values since all meter readings were very low and variable and some lipid samples indicated essentially no calcium present. The results suggest that aqueous washing did not provide any advantage with respect to the presence of calcium-containing nonlipid material.

On the basis of these data, the microfiltration procedure is an efficient, convenient, and relatively rapid method for the extraction and purification of subcutaneous lipid and is preferred when numerous samples are to be extracted.

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Glenn J. Miller*
Melvin L. Riley
Ray A. Field

Division of Animal Science
University of Wyoming
University Station Box 3354
Laramie, Wyoming 82071

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