edges carefully with the side of the carborundum disc. A 24″-long trough may be completed in an hour.

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A Simple Micromethod for Rapid Extraction of Lipids[1]

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Ever since Soxhlet's (1848–1905) extractor came into general use, many modifications of the fundamental method have been proposed. The problem has been how to extract all the lipids as completely as possible without undue expenditure of time and exposure of the tissue and extract to oxidation. Recently Bloor (*1*) recommended boiling 95% ethanol followed by ethyl ether as the most generally useful solvent in the microdetermination of lipids. The tissue is boiled in an Erlenmeyer flask and the extract separated from the tissue by filtration. Ernst (*2*) uses a sintered-glass plate fused into a separatory funnel for rapid and repeated extraction of fats from meat, combining extraction and filtration into one process. But the method suffers from being a cold extraction only. In the present method the tissue is extracted with boiling solvent, the processes of repeated extraction with fresh solvent and the final filtration are all combined into a single step in the procedure, and the apparatus used can be easily assembled in any laboratory.

The extractor (Fig. 1) consists of a cold finger, A, the end of which is drawn out into a hook and with a bulb blown near the other end in order to rest on and close the mouth of a 100-ml Kjeldahl digestion flask, B. A thin glass rod, C, with a hook on its upper end, is attached on the lower end of the cold finger and leads into a glass tube, E, placed inside an insect vial, D. The inner tube, E, is made from a 6-cm section of ordinary glass delivery tube with a coarse, sintered-glass plate fused onto its bottom. (The sintered-glass plate used here was made by pulverizing a piece of glass tube and fusing the powder onto one end of a 6-cm section of the same material.) The insect vial, D, has a round opening, F, blown out at its lower third.

For microextraction of lipids from tissue the latter is placed in the inner sintered-glass tube, E, which is placed inside the vial, D. The whole is introduced into the Kjeldahl flask with the help of a glass rod while the apparatus is in a horizontal position. Ten–20 ml of redistilled 95% ethyl alcohol is poured slowly into the flask. After clamping the flask and starting the circulation of water through the cold finger, the bottom of the flask is gently heated with a microflame. As the alcohol boils, its vapor is condensed on the cold finger and flows along the guiding rod, C, into the inner tube, E, and onto the tissue which is being boiled at the same time. Condensed alcohol will at first accumulate in the inner tube, E, extract the lipids, and be filtered into the outer tube, D, through the sintered-glass plate. As soon as the alcohol in tube D reaches the level of the opening, F, it flows out

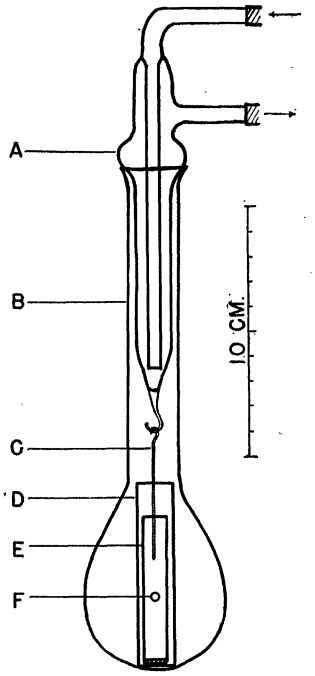

FIG. 1

into the flask. In this way the solvent in tube D is never higher than the level of F, while fresh condensate keeps on coming into the inner tube to extract the tissue. After a specified time the extract is removed from the tubes and the flask. A cork mounted on the end of a 25-cm-long glass rod is convenient for taking the outer tube, D, with its contents, out of the flask. The extraction is completed with ethyl ether in the same way.

In charging the tube with tissue and in removing the extract, precaution is observed against introducing foreign lipids from either the operator's hands or other objects. As some air is always trapped under the vial, D, which then serves as a boiling tube, there is no danger of bumping, for the solvent boils smoothly.

The total extracts recovered after evaporating the solvents under reduced pressure, extracted from different tissues for various length of time (5–20 min), are shown in Table 1. There is apparently no gain in increasing the

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time of extraction from 10 to 20 min. On the other hand, there seems to be an actual loss in total extract, as shown in the lower half of Table 2, when the time is greatly

TABLE 1

LIPID EXTRACTS FROM DIFFERENT TISSUES OF STARVING *Fundulus heteroclitus* AND *Tautoga onitis*

Tissue	Extraction time (min)	Wet wt. of tissue (mg)	Wt. of extract (mg)	%	Average %	Deviation from average (%)
Brain, *F. heteroclitus*	20	54.1	6.3	11.6	12.6	−1.0
	15	42.8	5.3	12.4		−0.2
	10	29.5	3.8	12.9		+0.3
	5	40.7	5.5	13.5		+0.9
Liver *F. heteroclitus*	20	83.4	13.1	15.7	16.4	−0.7
	15	75.3	12.2	16.2		−0.2
	10	55.3	8.9	16.1		−0.3
	10	46.9	7.7	16.4		0
	5	57.5	10.0	17.4		+1.0
Muscle, *F. heteroclitus*	20	246.6	6.7	2.7	2.8	−0.1
	15	149.5	4.7	3.1		+0.3
	10	173.9	5.1	2.9		+0.1
	5	260.8	6.8	2.6		−0.2
Liver, *Tautoga onitis*	20	130.6	7.3	5.6	5.55	+0.05
	15	92.8	5.0	5.4		−0.15
	10	74.5	4.5	6.0		+0.45
	5	97.7	5.1	5.2		−0.35

prolonged. This loss is probably due to the removal of volatile fatty acids during prolonged boiling. Extraction made for 10 min with this apparatus gives very consistent results, as shown in the upper half of Table 2, where the deviation in the four samples is not more than 0.3% from

TABLE 2

LIPID EXTRACTS FROM FISH LIVER

Tissue	Extraction time (min)	Wet wt. of tissue	Wt. of extract	%	Dev.
Tautog liver	10	275.1	30	10.9	+0.1
	10	238.3	25.8	10.8	0
	10	282.1	29.6	10.5	−0.3
	10	276.9	30	10.8	0
			Average	10.8	
Fundulus liver	10	34.5	7.6	22	
	20	39.0	7.4	19	
	40	46.3	8.5	18.3	
	80	37.9	6.0	16	

the mean value. For comparison, a piece of the liver from the same tautog was subjected to the classical method of Soxhlet extraction, and the following results were obtained:

Time of extraction in hours	1	2	3	4	5	6
Lipids recovered (%)	4.9	8.0	8.5	9.1	9.5	10.5

Compared with these data from Soxhlet extraction, the results obtained with the present apparatus show that the amount of lipid extracted is not less than the older method, but the saving of time and solvent is obvious.

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Use of the Freezing-Drying Technique for Study of Vasomotor Activity

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The freezing-drying technique has been adequately discussed by Simpson (*2*) and by Flosdorf and his collaborators (*1*), but it has not been used for preservation of vasomotor pictures in histological preparations. Direct microscopic observation of the smallest arteries and arterioles responding to stimulation was found possible by utilization of the method following intravenous administration of autonomic drugs with resultant vasomotor responses.

In these preliminary experiments 22 white rats under Nembutal anesthesia were used. The abdomen of each animal was opened sufficiently to allow extraction of a loop of duodenum. Following drug administration, a duodenal loop was immersed directly in a small Dewar flask filled with a mixture of dry ice and acetone at a temperature of −70° to −78° C. This rapidly froze the gut and preserved the vasomotor picture. While still frozen, a small piece of duodenum was cut off and placed in a test tube previously cooled in a freezing bath of dry ice and acetone. The test tube, still immersed in the freezing bath, was attached to a "Duo-Seal" vacuum pump and the tissue fixed by freezing and drying. Sections were cut in paraffin at 10 μ and stained with toluidine blue and eosin.

In small pieces of duodenum frozen after injection of atropine sulphate (1 cc of a 1: 1,000 solution) into the femoral vein, the arteries in the subserosa and the arterioles in the submucosa of the gut were constricted. The capillary beds in the villi contained a small amount of blood. Following administration of ergotoxine phosphate (0.5 cc of a 1: 1,000 solution), the arteries and arterioles were dilated, and the capillary beds in the villi were engorged with blood.

Quick freezing of blood vessels and fixation by freezing and drying preserves physiological pictures in histological preparations and is suggested as a method for use in studies of vasomotor activity and similar histophysiologic phenomena.

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