A method for concentration of biological fluids in large quantities

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EXTRACTION OF LIPIDS from biological fluids containing very small amounts of lipids (e.g., urine) is technically simplified if the extraction is preceded by a concentration of the proteins to which the lipids are bound. Ultrafiltration through semipermeable membranes is useful for this purpose, and several methods using this principle have been devised (1–4). The method described here comprises a dialysis through a semipermeable membrane and, simultaneously, an ultrafiltration. The apparatus is easy to make, and can be used for concentrating 300–500 ml of fluid down to 10–20 ml in 18–24 hr.

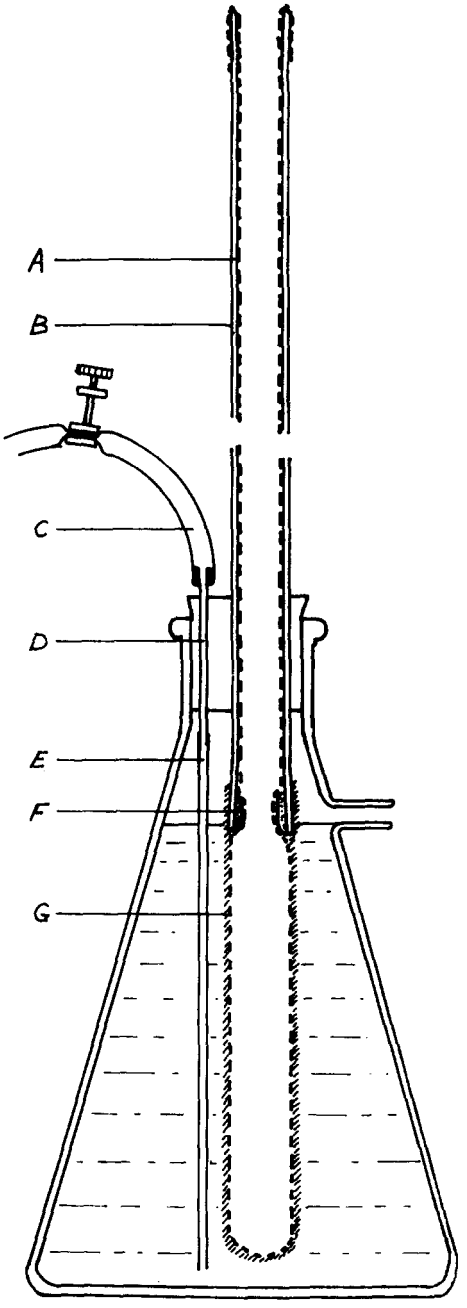

FIG. 1. Apparatus for the concentration of biological fluids. *A*, cellulose tube; *B*, glass tube; *C* & *E*, plastic tubing; *D*, cannula; *F*, foam rubber; *G*, Tubegauz.

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TABLE 1 LIPID VALUES OF PLASMA, PLASMA EXTRACTS, AND EXTRACTS FROM DILUTED AND THEREAFTER CONCENTRATED PLASMA

Expt. No.	Material*	Total Cholesterol	Free Cholesterol	Cholesterol Esters	Triglycerides	Phospholipids	Total Lipids by Chem. Assay	Weight of Extracted Lipids
1	A	270	95	293	178	248	814	
	B	277	90	313	182	236	821	806
	C	262	82	302	193	261	838	779
2.	A	219	67	255	175	229	726	
	B	221	67	258	173	227	725	757
	C	201	57	241	164	216	678	710
3.	A	183	35	247	158	185	625	
	B	181	33	247	155	184	619	646
	C	178	26	254	155	179	614	616
4.	A	241	63	297	191	220	771	
	B	241	61	301	189	222	778	771
	C	235	60	292	194	214	760	721
5.	A	200	38	271	161	179	649	
	B	200	39	269	159	178	645	633
	C	188	32	261	155	172	620	629
Mean of Nos. 1–5	A	223	61	273	173	212	717	
	B	224	58	278	172	209	718	723
	C	219	52	270	172	208	702	691

* Fraction A, plasma lipids analyzed before extraction; Fraction B, plasma lipids extracted according to the method of Folch et al. (5) and then analyzed; Fraction C, plasma diluted with water to 400 ml, concentrated by the method described, lipids extracted, and analyzed.

A 65 cm long glass tube with ground joints (B 24 sockets) is mounted in a rubber stopper suitable to fit a 5 liter vacuum flask (Fig. 1). The dialysis bag is prepared from Visking seamless cellulose tubing[1] with a flat width of 43 mm and a wall thickness of 0.025 mm. A piece of tubing is softened in water and introduced inside the glass tube with the help of string, avoiding twisting of the tubing during the process. The tubing should be long enough to make the free hanging part 50% longer than the distance from the rubber stopper to the bottom of the flask. The top of the tubing is pulled over the top of the glass tube and fastened with a rubber band. A thin layer of foam rubber is placed between the tubing and the bottom opening of the glass tube. Closing of the bottom part of the bag takes place as follows. Approximately 1 cm of the end of the tubing is folded upward twice. The sides are thereafter folded twice toward the middle in such a way that the bulge caused by the upward folding faces outward. The closed end so formed is tied tightly with twine. The bag alone cannot bear the pressure to which it will be exposed so it is covered with a supporting bag of cotton. This is done by carefully pulling a double layer of Tubegauz[2] No. 01 over the cellulose bag, and fastening it to the lower end of the glass tube with twine. The outer bag is pulled tight and closed with twine just under the inner bag. At the same place, a heavy object (e.g., a glass stopper) is attached to hold down the dialysis bag during testing while empty. A cannula is inserted through the rubber stopper and a length of plastic tubing connected to the bottom end of it. To the top of the cannula is fitted a piece of plastic tubing with a screw clamp attached; this enables a continuous changing of the water with only slight loss of vacuum. The completed bag is mounted in the vacuum flask, which is full of water, and maximum water suction is applied for $^1/_2$ hr to control the bag for possible leakage.

When the concentration is completed, the outer bag is removed and the remaining fluid emptied after untying the cellulose bag. The bag is rinsed twice with a few milliliters of water, and the last drops are squeezed out by wringing the bag, beginning at the top.

Control Experiments. Heparinized plasma from five normal fasting persons was treated in the following way. Ten milliliters was extracted for lipids according to the method of Folch et al. (5) (Fraction B in Table 1). Ten milliliters of the same plasma was diluted with water up to 400 ml (corresponding to a dilute biological fluid), concentrated by the method described above, and extracted for lipids (Fraction C). The two lipid extracts were weighed, and the extracts and a sample of the plasma (Fraction A) were chemically examined as follows. Free cholesterol, after precipitation with digitonin, and total cholesterol were estimated using the method of Huang et al. (6). Glycerides, expressed as tripalmitate, were measured according to

[1] Union Carbide International Company, New York.
[2] Seamless tubular gauze bandage, The Scholl Manufacturing Co., Ltd., London.

Carlson (7) and phospholipids were measured by the method of Baginski and Zak (8). The cholesterol esters were calculated as (total cholesterol − free cholesterol) × 1.67, using 277 as the average molecular weight of fatty acids in human plasma (9).

The column entitled "Total lipids by chemical assay" in the table represents the sum of free cholesterol, cholesterol esters, glycerides, and phospholipids.

Comparison between the weights of the lipids extracted from plasma, and those extracted from the concentrate show only a slight loss during the concentration process, averaging 4.2%. Good agreement was also found when the lipid contents were determined chemically, the average loss here being 2.2%.

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