material; the neck cannot be flamed, and leakage occurs occasionally.

A living culture of primary human amnion cells grown in a T-15 flask is shown in Fig. 1. Micrographs were taken with the Zeiss photomicroscope with 35 mm camera using the described optical combination and an ocular magnification of 4×. The outlines of the cells with thin cytoplasmic veils and extensions are easily seen. Conspicuous perinuclear granules, some of which show reversal of phase can be observed. The nucleus has the typical empty look of living nuclei; nucleoli are prominent. The nuclear membrane is clearly seen when not obscured by cytoplasmic granules.

Although more perfect optical conditions may be obtained with thin slides the contrast and the resolution achieved by the above method is satisfactory for most work. For micro-cinematography the water-immersion objective unfortunately is not well suited due to the rather rapid evaporation of the water. The system, of course, can be utilized in phase contrast microscopy with objectives of lower power without any immersion. If phase contrast is not essential the 40× water-immersion objective can be used for flasks with greater outside thickness than 20 mm provided the thickness of the floor does not exceed the working distance of the objective or 1.6 mm.

Summary. Optical equipment permitting the observation of finer structural details of living cells cultured in standard containers (T-flasks) has been described.

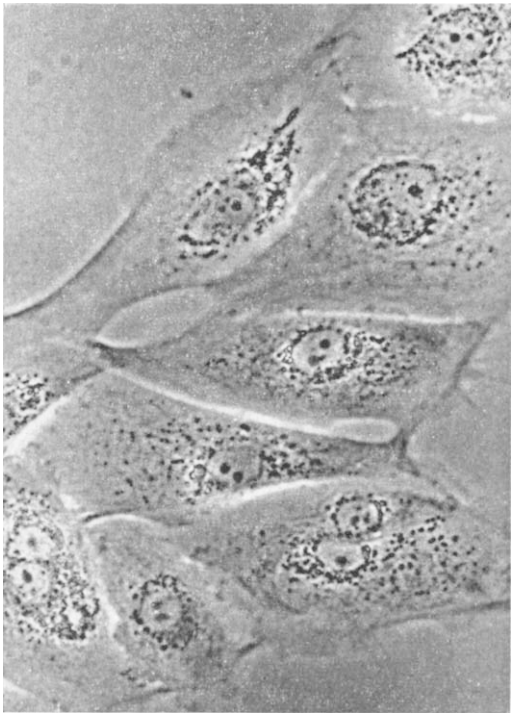

FIG. 1. Micrograph of primary human amnion cells in T-15 culture flask as observed in the Zeiss photomicroscope with Zeiss water-immersion phase contrast objective 40× combined with the adapted Wild special phase condenser with long focal distance. 560×.

1. Earle, W. R., Highouse, F., *J. Nat. Cancer Inst.*, 1954, v14, 841.

Received November 29, 1962. P.S.E.B.M., 1963, v112.

Simultaneous Distillation of Water and Extraction of Fat from Mouse Carcasses and Tissues.* (28094)

GEORGE L. WOLFF AND BOHDAN BAKAY (Introduced by J. A. Stekol)
Institute for Cancer Research, Philadelphia, Pa.

Total fat and water content of mouse livers and carcasses had to be determined for studies to be reported elsewhere. Previous work of this type(1) required drying of the tissue or carcass to constant weight to determine the water content before Soxhlet extraction of the fat could be undertaken. The method to be described eliminates the time required for this drying process. Distillation of body water and extraction of fat were performed in one simple operation based on the lower specific gravity (0.87) and higher boiling point (110.6°C) of toluene as compared

* This work supported in part by USPHS grant.

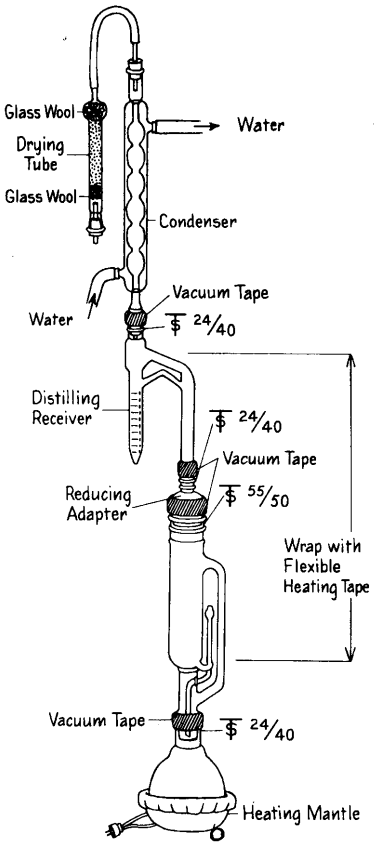

FIG. 1. Apparatus for simultaneous distillation of water and extraction of fat from mouse carcasses and tissues.

with water and the solubility of lipids in toluene.

The apparatus shown in Fig. 1, consists of a 300 ml or larger short neck round bottom flask (AHT 5341)† to which a Soxhlet extractor (AHT 4987-C)‡ is fitted. A piece of stainless steel wire cloth, 20 mesh, is cut to fit snugly into the extractor just above the syphon opening. Pyrex glass wool is placed on this screen to the level of the syphon bulb. If properly packed, glass wool will retain even uncoagulated blood and prevent it from entering the flask.

A 25 ml graduated Stark-Dean distilling receiver with a return tube (AHT 7618-P2) is attached to the Soxhlet extractor by means of a straight adapter. A 300 mm Allihn condenser (AHT 3908-A) is joined to the distilling receiver and connected to the water supply. A drying tube, containing Tel-Tale Silica Gel, is attached to the top of the condenser by a short piece of plastic tubing. Heat is supplied by an electric flask heater (AHT 6134-N) and a 6 feet × ½ inch flexible heating tape (AHT 6148-A) which is wrapped around the extractor and connecting arm of the distilling receiver. Solid flint glass beads, 4 mm in diameter, are placed in the flask to reduce bumping of the toluene at boiling.

The mouse carcass, from which the liver had been previously removed, is cut into 5 pieces with scissors directly into the extractor. Sufficient toluene reagent is poured into the extractor to activate the syphon. As soon as the extractor is empty, it is filled with toluene up to the bulb of the syphon. The distilling receiver is also filled with toluene and connected to the extractor. All ground glass joints are checked for tightness and sealed by winding with vacuum tape (AHT 1042-S4). The heating tape is wound around the extractor and connecting arm of the distilling receiver. Wherever heating tape and vacuum tape are in contact, they are separated by asbestos tape (AHT 1199-C).

The toluene in the flask is brought to boiling and allowed to reflux for 20 hours. At this temperature (110°C) the tissues are gradually dehydrated, the vapors of toluene and water condense, and the water is collected under the toluene layer in the distilling

TABLE I. Mean Values of Percentage of Water and Fat in Mouse Carcasses and Livers Determined by the Method Described.

	6 yellow ($A^y a$) ♀♀ Mean ± S.E.	6 non-yellow (aa) ♀♀ Mean ± S.E.
Body wt minus liver wt	47.6 ± 2.8 g	23.4 ± .7 g
% water	38.4 ± 1.2%	61.6 ± 1.6%
% fat	47.7 ± 1.8%	15.6 ± 2.0%
Water as % of fat-free tissue wt	73.5 ± .6%	73.0 ± .4%
Liver wt	3.4 ± .6 g	1.3 ± .05 g
% water	59.2 ± 1.9%	58.3 ± 3.1%
% fat	16.2 ± 2.6%	6.0 ± .9%
Water as % of fat-free tissue wt	70.8 ± 2.2%	62.0 ± 2.9%

† Catalog, Arthur H. Thomas Co., 1962.

‡ For adult mouse carcasses a Soxhlet extractor with a 55/50 Standard Taper top opening is needed. For single tissues, however, smaller extractors are satisfactory.

receiver. The toluene condensate overflows from the distilling receiver and returns to the Soxhlet extractor. Here the body fat is extracted and is carried down into the flask by the reflux action of the Soxhlet apparatus. A 20 hour period from the beginning of heating has been found to yield approximately 98% of the extractable fat. Extraction for an additional 8 hours with fresh toluene removed an amount of fat equivalent to 1-2% of total yield. Complete distillation of water was obtained in less than 6 hours.

The volume of water collected is read directly from the graduations on the distilling receiver and converted to weight. To obtain the weight of the extracted fat, the contents of the round bottom flask are heated until all of the toluene is distilled off. The residue is then dissolved in reagent petroleum ether (B.P. 40.4-54.8°C) and transferred into a tared weighing dish. One-tenth milliliter of a 2% aqueous solution of Antifoam B§ is added to the residue in the weighing dish to minimize foaming. The material is then dried *in vacuo* over vacuum pump oil and phosphorus pentoxide for 48 hours and weighed. Table I shows some of the results obtained by use of this method.

The advice of J. J. Kolb and N. F. Floyd during the development of this method is gratefully acknowledged.

1. Mirone, L., *Agric. and Food Chem.*, 1953, v1, 519.

§ Kindly provided by Dow Corning Corp.

Received December 3, 1962. P.S.E.B.M., 1963, v112.