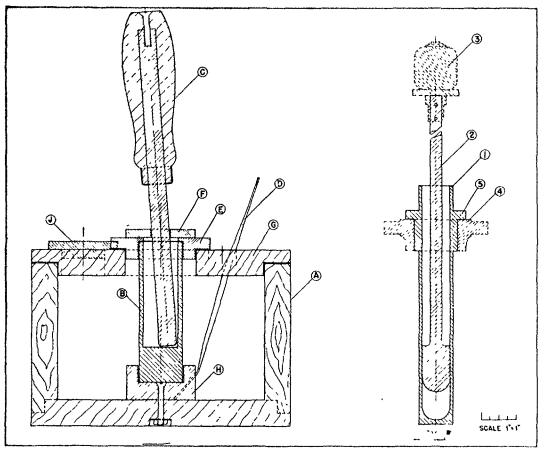
thawed sample in a measured volume of water by means of a stainless-steel cylinder and closely fitting rotating plunger. This method permits one to reduce any body tissue, with the exceptions of connective tissue and integument, to an acellular state with negligible losses of the original sample.

The apparatus shown in Figs. 1 and 2 was found to be satisfactory for the pulverization and homogenization of muscle samples weighing up to approximately 3 gm.[1]

The wooden box (A) in Fig. 1, with the stainless-steel mortar (B) in place in a steel anvil (H), is packed with dry ice and covered. The stainless-steel pestle with wooden handle (C), steel spatula (D), and a pair of forceps are inserted into the dry ice through a hole in the cover (G), and the box and its contents are allowed to reach a state of temperature equilibrium. The Lucite cover (E) and freely movable Lucite guard (F) are put into place and stoppered to prevent the accumulation of frost on and around the mouth of the mortar. The tissue sample is buried in the dry ice (under cover J) and also allowed to reach a temperature approximating $-70°$ C. After 20 min or so, the sample is rapidly transferred from the dry ice to the mortar with the prechilled forceps. The stopper used to close the Lucite guard is removed, and the



FIGS. 1 & 2

tissue is pounded with the pestle. The guard prevents brittle tissue chips from bouncing out of the mortar. After the first 5 or 10 blows, most of the muscle sample is shattered and free of its tendinous attachments, the greater portion of which can be removed as arborized units by means of the chilled forceps. After 20–40 more blows, the sample is reduced to a uniformly fine powder. A microscopic examination shows most of the powder to be cell free, although a few clumps of intact cells can be found. This preparation is adequate for qualitative extraction of the tissue.

For quantitative extraction, the sample is transferred to a glass boat for weighing. The weighed sample is then washed off the boat with a measured volume of distilled water or other extracting medium, into a Cori-type (*4*), stainless-steel, homogenizing tube (Fig. 2, 1), the upper portion of which has been machined with a collar for centrifugation (Fig. 2, 5).

The homogenizer tube is placed in a cup containing cracked ice; the plunger (Fig. 2, 2) is attached to a wall-mounted, 1,550-rpm, 1/30 hp motor (Fig. 2, 3). The contents of the tube are homogenized by moving the tube up and down to force the water and tissue suspension past the wall of the power-driven, rotating plunger and that of the tube. After the homogenate has been forced past the rotating plunger approximately 10 times, the tube containing the homogenate is inserted into a standard 50-ml #2 International centrifuge trunnion ring (Fig. 2, 4) and centrifuged. Microscopic examination of the precipitated muscle residue after centrifugation shows the absence of the occasional intact cells previously mentioned. The supernatant fluid may be used directly for quantitative estimations of extracted tissue components.

[1] Laurence H. Crisp, chief of Research Equipment Design and Fabrication, and John De Broske, chief instrument maker, assisted in the design and construction of this apparatus.

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A Microextraction Procedure for Phenol Determination[1]

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Difficulties were encountered in applying the Theis and Benedict (*4*) technique for phenol determination to fluids resulting from incubation of phenol with tissue slices and other tissue preparations. The greatest difficulty is due to the presence of interfering substances. In the case of slices, the concentration of such substances is rather low, and it is possible to determine phenol directly if proper precautions are observed. When mince, homogenate, acetone powder, or other similar tissue preparations are used, there are two main sources of error: (a) the presence of much higher concentrations of interfering substances, and (b) the adsorption of phenol to the protein precipitate in deproteinizing the sample. This second observation is in accord with the findings of Voinar and Babkin (*5*) regarding the recovery of phenol added to serum.

[1] This work was supported by a grant-in-aid from the U. S. Public Health Service.

[2] With the technical assistance of Marvin Bierenbaum.

The solution to the first difficulty lies in the isolation of phenol. For this purpose Schmidt (*2*) has used continuous extraction with ethyl ether, and Deichmann and Schafer (*1*), extraction with ethyl ether followed by steam distillation. In both techniques the proteins were removed by precipitation with tungstic acid. Schmidt obtained only 76% recovery of phenol added to blood; Deichmann and Schafer obtained a higher recovery with their method.

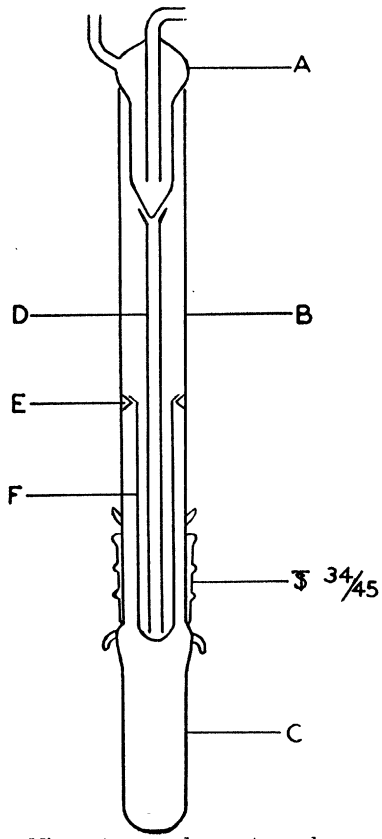

FIG. 1. Microextractor drawn to scale, one-fourth actual size.

For their extraction procedure, Schmidt and co-workers (*3*) used a modified Kutscher-Steudel extraction apparatus. We have devised a new type of microextractor based on the same principle (Fig. 1). The extractor consists of a finger-type condenser (A) resting within the opening of the middle piece (B) and centered by spikes. Inside this middle piece a thimble (F), which can be easily made from a test tube, hangs from two spikes (E). A long-stem funnel (D), with a dented lower end to permit passage of the solvent, rests on the bottom of the thimble. The thimble and funnel are introduced from the bottom of piece B and hung by rotation. The middle piece (B) is fastened with springs to the bottom part (C) by a 34/45 standard joint.

The dimensions of the thimble and funnel may be varied to accommodate the volume of fluid to be extracted. The specific gravity of the solvent used is an important factor to be considered.

For the extraction of phenol, the sample is placed in the thimble (F) and the funnel (D) introduced. This unit is then hooked inside the middle piece (B) and the condenser (A), so placed that the tip will keep the funnel centered. Two ml of approximately 0.1 N sodium hydroxide is placed in the bottom part (C), which is then attached to the middle piece and fastened with springs. The condenser is lifted slightly and petroleum ether[3] is poured through the funnel until it overflows from the thimble, after which an additional 5 or 6 ml is introduced. It is advisable to keep the level of the aqueous phase 1.5–2 cm below the rim of the thimble in order to avoid mechanical carrying over of the sample. The funnel and thimble must be loaded with solvent so that the circulation of solvent will begin as soon as it condenses and drops into the funnel; otherwise, the solvent will boil in the thimble and funnel, resulting in poor extraction.

To start the extraction, the bottom part of the extractor is immersed in a water bath at about 85° C.

We have found that 100% of the phenol is extracted in 3 hrs. The sodium hydroxide placed in the bottom part (C) binds the phenol extracted by the petroleum ether. This is necessary to achieve complete extraction, because some phenol volatilizes and circulates with the solvent. This probably accounts for the low recoveries obtained by Schmidt (*2*).

At the end of the extraction period the bottom tube (C) is disconnected, the remaining petroleum ether evaporated, and the sodium hydroxide solution, containing the extracted phenol, transferred to a graduated test tube. The sample is diluted to 7 ml with distilled water, 1 ml of 1% gum acacia is added, followed by 0.5 ml of 50% sodium acetate, and the contents of the test tube are stirred. Then 0.5 ml of a freshly diazotized *p*-nitroaniline reagent (prepared according to Theis and Benedict, *4*) and, after a minute, 1 ml of 20% sodium carbonate are added, with stirring after each addition. The density is read within 5 min against distilled water, using a cell of 1-cm thickness, at a wave length of 5,000 A in the Beckmann spectrophotometer.

The concentration is read from a graph obtained by plotting several known concentrations of phenol against the observed densities. The stock phenol solution was standardized iodometrically.

A straight line is obtained with quantities of phenol up to 50 μg, indicating conformity with Beer's law.

Recovery of phenol was within ±5% of theory for values between 50 and 200 μg, ±10% for values between 10 and 50 μg, and ±25% for 5 μg.

As much as 50% of added phenol was lost when the proteins of a 10% liver homogenate were precipitated with trichloracetic acid and the analysis carried out on the supernatant obtained by centrifugation.

It was necessary, therefore, to extract the homogenate or acetone powder without removal of protein. The thimbles containing the homogenate or acetone powder samples were placed, prior to extraction, in a boiling

[3] Purified by treatment with concentrated sulfuric acid; the portion distilling below 45° C is collected.

water bath for 5 min. This treatment served a double purpose: (a) to stop the enzymatic reaction and (b) to avoid emulsification of the solvent. If an emulsion is formed, a mechanical carrying over from the thimble ensues, and the extracted phenol is contaminated with interfering substances.

When using tissue slices (200 mg of rat liver), it is possible to precipitate the proteins with trichloracetic acid without loss of phenol. In this case it is necessary to neutralize the trichloracetic acid before extraction; otherwise, the phenol will not be completely extracted. The neutralization is carried out by addition of 20% potassium hydroxide until the fluid remains slightly acid and is completed by addition of approximately M phosphate buffer pH 7. At pH 7 the phenol is free and can be extracted, whereas at higher pH it may be partially or completely bound.

If, after extraction of free phenols, one desires to determine conjugated phenols, the thimble and funnel are removed. Conjugated phenols are hydrolyzed by placing the thimbles in a boiling water bath for 15 min after addition of 0.25 ml of concentrated hydrochloric acid. If no trichloracetic acid has been used, the thimble and funnel are replaced in the extractor, and the extraction and determination are carried out as indicated for free phenols. If trichloracetic acid was used, the sample must be neutralized as indicated above.

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A Convenient Apparatus for Recording the Blood Pressure of Small Animals

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For physiological and pharmacological investigations it is often more convenient to use small animals, such as rats and guinea pigs, since the food and housing problems are less than for larger animals. It is often desirable to measure the blood pressure of these animals, but application of currently used methods to small animals is sufficiently difficult that this aspect of many research problems is neglected. The Hamilton (*2*) optical manometer is a precision instrument that will measure the blood pressure of the smallest animals (*5*) and avoids the error introduced by the cuff methods sometimes employed for rats (*4*). However, the instrument is difficult to set up and maintain by unskilled personnel, it must be used in partial darkness, and, since it records on photographic paper, there is a considerable time lag between administration of a drug and observation of the results. Recently, the use of strain-gage manometers recording through a string-type electrocardiograph galvanometer has been suggested (*1*), but this also suffers the difficulties inherent in any method of photographic recording.

If only changes in mean blood pressure are desired, these can be obtained easily by the use of Lambert-Wood (*3*) strain-gage manometers,[1] with current supplied by a 7.5-v A battery[2] and recording in ink on a continuous strip chart by a General Electric photoelectric recording microammeter (Model 8CE1DJ15 or 8CE1DJ11). The apparatus is simple and rugged, and can be operated in any location with 110-v, 60-cycle current. The record is always visible, and annotations can be made on it in ink or pencil at any time (Fig. 1). It is not subject to destruction by folding, warmth, or scratching with fingernails or gritty substances, as are some of the electrolytic and plastic recording papers, and it can be mounted easily with glue or rubber cement. Although the GE instrument is a high-speed recorder, the electronic circuit is slow enough that damping of the systolic-diastolic pulse to mean blood pressure occurs. If systolic and diastolic blood pressure and contour slopes of the arterial pressure curves are needed for a given research problem, only the optical recording, small-diaphragm manometers of the Hamilton type are adequate; but if only information concerning mean blood pressure is desired, as is often the case in the bioassay of drugs, this apparatus provides an improvement over existing methods.

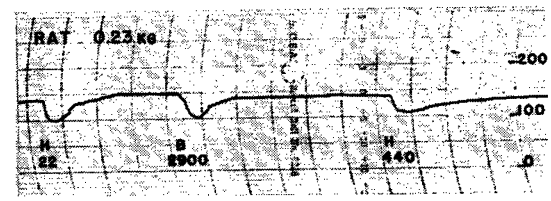

FIG. 1. Record of male rat: 250 mg of Na barbital/kg; 22-gage hypodermic needle in carotid artery. Injections of drugs made into external jugular vein. Blood pressure in mm Hg, ordinate; time, in minutes, abscissa. Twenty-two μg of histamine base/kg given at H, followed by 2,900 μg of diphenhydramine hydrochloride ("Benadryl")/kg at B, followed by 440 μg of histamine at second H. Record shows that this dose of diphenhydramine is able to antagonize the vasodepressor activity of 95% of a dose of histamine.

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[2] Burgess G5, Burgess Battery Company, Freeport, Illinois.