A Photoelectric Drop Counter[1]

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A photoelectric counter (Fig. 1) has been devised which, it is hoped, will eliminate the numerous operating difficulties presented by currently used instruments designed for counting drops. This instrument has been found useful in the study of the action of cholagogues and of diuretics and can be equally useful in the study of other physiological and pharmacological processes.

In this instrument, light reflected from the surface of the drop is used to initiate an electrical impulse which is amplified and recorded on the usual kymograph. As the drop falls through the beam of light there will be a unique point in the trajectory of the drop at which the reflected beam passes through the slits S_1 and S_2 (see Fig. 1).

The source of light is a 150-watt projection bulb (GE type T-8) placed just outside the focal point of a lens, the focal length of which is about 6 cm. This gives a converging beam with an angle of convergence of 35° for outside rays. The photocell is a high-vacuum GE type

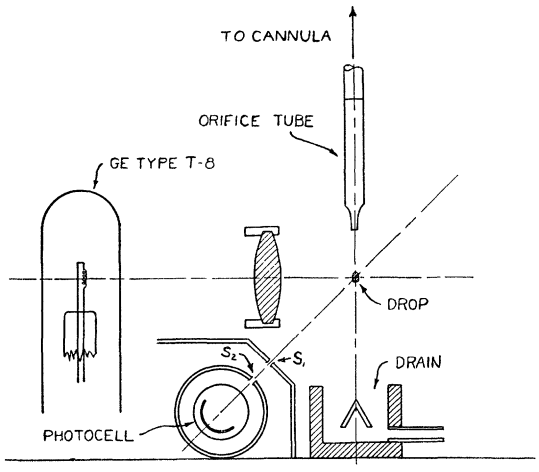

FIG. 1

FP-22 with S-1 response operated at 250-v anode potential and coupled to the amplifier with a 10-megohm load resistor.

The amplifier (Fig. 2) is a conventional resistance-capacitance coupled, single-sided circuit utilizing two 6F5-GT tubes with a total voltage gain of about 5,000. The output stage is a 6C5 tube with a relay in the plate circuit. By means of a variable bias from the power supply, the 6C5 is biased above cut-off, and this variable bias provides a means of varying the over-all sensitivity of the amplifier. The photocell is coupled to the amplifier in a forward circuit arrangement. The relay in the 6C5 plate circuit is a four-pole, single-throw type closing at 2.5 Ma and can be used to close any type of recording circuit. In the present instrument it closes the 115-v, a-c primary circuit of a transformer, the output of which is 8 v and is used to drive a solenoid timer.

This instrument is very simple in operation, requiring only turning on the switch and clamping in the orifice tube. The latter is removable to permit cleaning. As the beam of light is about 4 mm in diameter at the place at which the drop passes, the adjustment of the orifice tube is not critical.

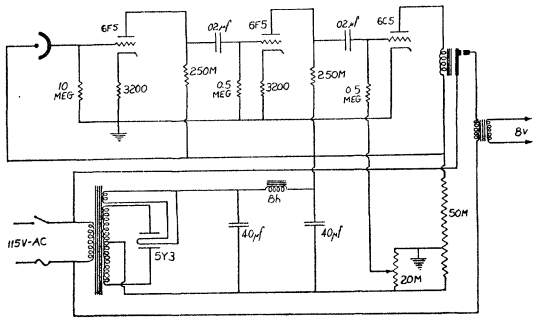

FIG. 2

With an orifice of 2 mm, this instrument is capable of handling some 30 ml of fluid/min, which is broken into discrete drops. There is no lower limit, and the instrument is capable of being run for an indefinite period. Because the amplifier is R-C coupled, a solid stream will not be recorded. At a capacity of 30 ml/min the drop rate is about 500/min. Using larger drops at this counting rate, a larger volume of fluid may be recorded. The size of drop is variable over a wide range, and operation is in no way dependent on viscosity, color, or opacity of the fluid.

Preparation of Acellular Homogenates From Muscle Samples

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The preparation of acellular homogenates from tissue samples is required in certain biochemical experiments. For softer tissues, this problem has been met with varying degrees of success by such methods as grinding with sand or glass powder, forcing the tissue past the wall of a cylinder and a tight-fitting, motor-driven plunger, grinding the tissue after mincing and freezing it, or homogenizing it in a Waring blendor (*1–4*).

None of the individual methods listed is adequate for preparing acellular homogenates of tougher tissues, such as skeletal or cardiac muscle, which can be assayed quantitatively.

The method herein described is based upon the complete pulverization of a tissue sample at a temperature of approximately −70° C by pounding and the subsequent homogenization of any desired amount of the pulverized,

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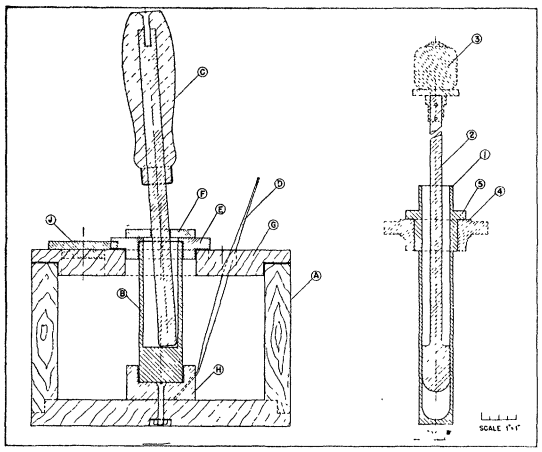
thawed sample in a measured volume of water by means of a stainless-steel cylinder and closely fitting rotating plunger. This method permits one to reduce any body tissue, with the exceptions of connective tissue and integument, to an acellular state with negligible losses of the original sample.

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.

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[2] With the technical assistance of Marvin Bierenbaum.