A Moist Chamber for Nerve-Muscle Experiments[1]

F. A. WATERMAN

Frederick Stearns & Company (Division of Sterling Drug, Inc.), Detroit

The moist chamber herein described was developed in order to save the student time in performing routine laboratory experiments involving the use of the nerve-muscle preparation. The standard moist chamber in use in most laboratories is rather cumbersome and requires considerable wiring prior to starting an experiment. If two muscles are used simultaneously in the chamber or if more than one pair of electrodes are used, the actual setting up is indeed time consuming.

This moist chamber (Fig. 1) weighs only $3\frac{1}{4}$ ounces, is $1\frac{3}{8}$ inch deep, $2\frac{1}{2}$ inches wide, and $3\frac{1}{8}$ inches high. It is made of $\frac{1}{16}$-

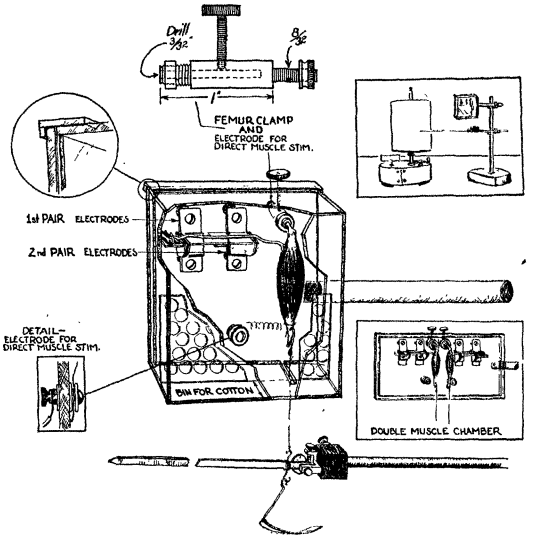

FIG. 1. Drawing of lucite moist chamber with lid partially cut away, showing a nerve-muscle preparation in place and attached to a muscle lever.

inch lucite. The femur clamps and aluminum electrodes are mounted near the top of the back panel, which is $\frac{1}{8}$ inch thick. The two pairs of electrodes are arranged 3 cm. apart, to the left and slightly below the femur clamp. Between these pairs is mounted a shelf of lucite so arranged that the nerve can be placed on it perfectly flat, thus forming a straight line for 3 cm. This group of electrodes is advantageous for experiments in which the student can determine roughly the speed of impulse conduction through the sciatic nerve.

Provision is also made for direct stimulation of the muscles. At the bottom of the back panel and near the level of the distal end of the muscle an electrode is introduced. This is an 8-32 roundheaded bolt with spaced washers and nuts around which a fine copper wire can be wrapped to conduct current to the lower part of the muscle (see detail, Fig. 1). On the back of the femur clamp is a binding post, forming a terminal for the opposite side of the electric circuit.

The cover is also of lucite and fits a flange that has been routed into the moist chamber base, forming a tight fit and thus preventing any appreciable moisture loss. The thread leading from the muscle tendon to the writing lever passes through a narrow saw cut in the lower side of the cover.

Near the front of the cover and extending for approximately two-thirds of its height is cemented a perforated plate of lucite, thus making a bin into which moist cotton can be placed. The perforations provide openings through which the moisture passes.

The electrodes, of thin sheet aluminum, are arranged so as to take up a minimum of space and are insulated from each other by any suitable nonconducting material such as mica or thin sheets of plastic, which are held together with De Kotinsky or plastic cement. All of the binding posts are equipped with standard 8-32 dry cell nuts.

The one disadvantage of this chamber is that it is not equipped with nonpolarizable electrodes. However, this type of electrode is rarely used in routine teaching of students. Furthermore, this disadvantage is more than offset by the speed with which the preparation can be put into use. A nerve-muscle preparation can be installed and all connections made in approximately two minutes. Moreover, with the humidity almost at the saturation point, a well-made nerve-muscle preparation will stay viable for several hours without any extra precautions being taken. Once the preparation is in the chamber, it need not be touched again for the duration of the experiment, thus eliminating any possibilities of mechanical or chemical damage which might give artifacts and other faulty results.

The lower right insert shows the back plate of a moist chamber in which two nerve-muscle preparations can be mounted simultaneously. This has a decided advantage over the single chamber, since it can be used on such experiments as the relative fatigability of the parts of the nerve-muscle preparations and on those in which one nerve is narcotized.

[1] This moist chamber was developed while the writer was teaching physiology at Georgetown University Medical School. A report was not published at that time because during the war years plastic material was not obtainable.

Homogenization of Sputum Specimens With a Paint-conditioning Machine

C. EUGENE WOODRUFF

William H. Maybury Sanatorium (Detroit Municipal Tuberculosis Sanatorium), Northville, Michigan

To obtain adequate digestion of sputum with either dilute alkali or dilute acid, forceful agitation of the specimen is necessary. Sputum specimens can be shaken mechanically in the ordinary Kahn shaking machine, but much more thorough and rapid agitation is obtained by the use of a paint-conditioning machine. The adaptation of these rugged machines to sputum work was first suggested by Steenken and Smith (*1*) in 1942. At that time the 1-gallon capacity machine was recommended, the gallon container being arranged to accommodate 10 individual sputum bottles. Recently it has been found that a machine similar in type[1] but of 1-quart capacity can be utilized.

[1] Made by Landon P. Smith, Inc., Irvington, New Jersey.

The container is an ordinary 46-ounce tomato juice can cut down to a height of 13 cm. A tin cover is fabricated just large enough to telescope over the top of the can (Fig. 1). Also illustrated is the separator which slips inside the can and which consists of a central tin cylinder, with an inside diameter of 31 mm., surrounded by 7 vanes, each 3 cm. in width. The cylinder and vanes are 14 cm. in length.

A 50-cc. centrifuge tube, containing the sputum-sodium hydroxide mixture, is slipped into each compartment. Thus, 8 specimens can be shaken at one time. Two wide rubber bands (cut from an automobile inner tube) encircle the separator to help keep the tubes from rattling; a disc cut from rubber belting covers the bottom of the can.

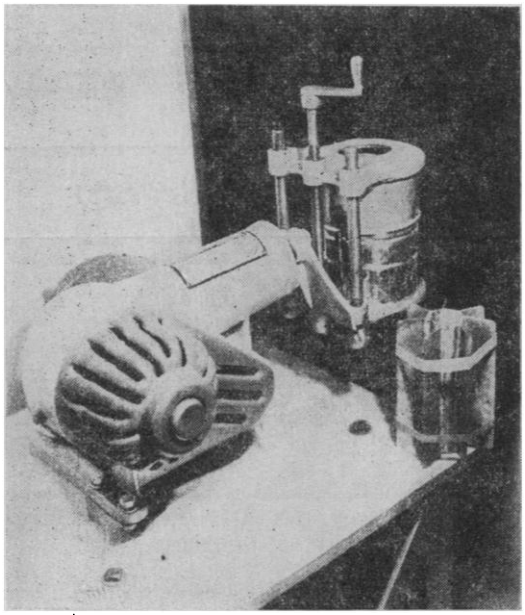

FIG. 1

The centrifuge tubes are of the round-bottom variety. They should be straight edge with no "pourout" lip. The tubes are closed with cork stoppers; rubber corks are not satisfactory. There should be an air space of at least 1 cm. between the top of the sputum-sodium hydroxide mixture and the bottom of the cork. When the corked tubes are in place in the can, there is always some variation in the total height of the various tubes with their corks. To compensate for this, a supply of cork discs of varying thickness is kept on hand, a disc of proper thickness being laid on top of each of the lower tubes. This leveling process does not have to be exact, since the telescoping cover, when screwed down tightly, will take up slight unevenness.

When the specimens are shaken in the paint-conditioning machine, complete homogenization is secured in 5–10 minutes. The same centrifuge tubes are used for spinning down the sediment.

Reference

STERNKEN, W., and SMITH, M. M. *J. lab. clin. Med.*, 1942, **27**, 1582.

Estimation of Coenzyme I Through the Uptake of Oxygen

P. S. KRISHNAN[1]

Department of Biochemistry,
New York State College of Agriculture,
Cornell University, Ithaca, New York

A new method for the estimation of Coenzyme I has been worked out in which washed, dried brewer's yeast is used as the source of the enzymes, aldolase and triose phosphate apodehydrogenase. The substrate is hexose diphosphate, in the form of the potassium salt. Aldolase splits the hexose diphosphate into dihydroxyacetone phosphate and 3-phosphoglyceric aldehyde; the latter reacts with inorganic phosphate to give 1,3-diphosphoglyceric aldehyde. When Coenzyme I and methylene blue are now added, oxidation to diphosphoglyceric acid takes place, resulting in oxygen absorption, which is measured in the Warburg manometer. Oxygen absorption is steady for a period of 15–20 minutes. When different concentrations of Coenzyme I are employed, we find that if oxygen absorption in microliters during the first 15 minutes is plotted against the concentration in gamma of the coenzyme, the points lie more or less on a straight line (Fig. 1).

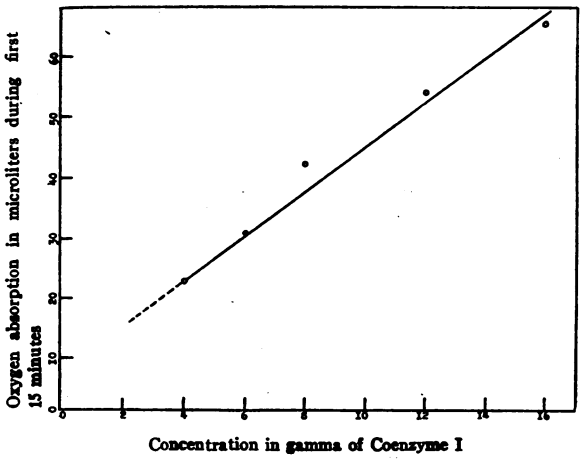

FIG. 1

It has not been possible to wash out the coenzyme completely from dried bottom yeast, but by the use of alkaline phosphate buffer we have succeeded in reducing blank oxygen absorption to a minimum, at the same time retaining maximal potency. There is no induction period, and the reaction is not altered appreciably by the absence of magnesium and manganese ions.

[1] The author's thanks are due to Prof. J. B. Sumner for his interest in the investigation and to the Haberle Brewing Company, Syracuse, New York, for generous supplies of bottom yeast.