In the Laboratory

Notes on the Possibility of a Histochemical Method for Localizing Adenosinetriphosphatase

FLORENCE MOOG and H. BURR STEINBACH
Washington University, St. Louis

In *Science* (1945, **102**, 429–430), Drs. Glick and Fischer presented a paper on "The histochemical localization of adenosinetriphosphatase in plant and animal tissues." Although an efficacious method for demonstrating this important enzyme histochemically will be of immense value in physiological research, our interest in this matter prompts us to point out that the invention of such a method is beset with difficulties which Glick and Fischer failed to mention.

First, it is by no means clear that the "ATPase" which the authors claim to demonstrate in their sections is in reality anything but ordinary phosphomonoesterase. That alkaline phosphomonoesterase working at about pH 9.0 will hydrolyze all three phosphate groups of ATP is well known (*3, 6*). Since this phosphomonoesterase can, of course, be demonstrated in acetone-fixed, paraffin-embedded sections (*2*) and must certainly be active in frozen sections, it is inevitable that this enzyme will register its presence by Glick and Fischer's method. Mouse heart, the test object reported on, possesses small but not inconsiderable alkaline phosphomonoesterase activity (*4*); one can say with assurance that the activity is great enough to produce a visible result in the 18- to 24-hour incubation period which Glick and Fischer found necessary for embedded sections. Consequently, the presence of ATPase[1] in either embedded or frozen material can be diagnosed only by precise comparison of tissue exposed to the ATP substrate solution with tissue treated identically except for exposure to glycerophosphate or other monoester of phosphoric acid as substrate.

Second, the notorious lability of ATPase makes it difficult to believe that this enzyme would survive the rigors of histological preparation. Every author who has reported on ATPase has remarked the necessity of working rapidly and at low temperature (see, for example, DuBois and Potter (*1*)), and in embryo homogenates the enzyme even begins to be inactivated within 10 minutes reaction at 37° (*5*). Singher and Meister (*7*) have similarly shown that purified myosin preparations tend to lose activity at 37°. Further, acetone fixation itself gradually destroys ATPase, as we have found in this laboratory by comparing the activity of a frog mesonephros kept in acetone at 5° C. for only 75 minutes with that of one held in a moist chamber during the same period: the former showed only 34 per cent of the activity of the latter. If acetone fixation is carried on longer, or at a higher temperature, and the tissue is then exposed to the heat of an embedding oven, it seems safe to assume that the ATPase activity will be entirely destroyed.

We feel, therefore, that any phosphate-liberating enzyme which may be localized in acetone-fixed tissue, whether frozen or embedded, and which requires long periods of incubation for its demonstration, cannot be accepted as ATPase unless proved to be such by the application of further tests. It may be possible to visualize the enzyme in fresh or fixed sections of highly active tissue, but the identification can be made in such cases only (a) by showing that the ATP substrate solution allows the appearance of a deposit in regions demonstrated to have little or no phosphomonoesterase activity, or (b) by showing that the ATP substrate allows deposition of calcium phosphate in a given region at a faster rate than does glycerophosphate.

[1] Since the enzyme cannot be readily identified in any case as the one which attacks only the terminal phosphate group of ATP, "adenylpyrophosphatase" would be a preferable term (see O. Meyerhof. *J. biol. Chem.*, 1945, **157**, 105).

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A Transparent Plastic Tank and Cover for the Warburg Manometric Apparatus

W. ROBBIE, P. J. LEINFELDER, and LEE ALLEN
College of Medicine, State University of Iowa

It is frequently desirable to observe the tissues or organisms that are being shaken in Warburg manometric flasks. Since the water bath and cover shown in the accompanying figures is made entirely of the transparent plastic, "Plexiglas," the contents of the flasks may be observed easily at all times. The material is much lighter in weight than glass, it is less easily broken, and since the corner joints are cemented

to form a continuous piece of material, there is less possibility of leaks developing than in a glass-metal water bath. The plastic is sufficiently nonconducting to make other insulation unnecessary. The cover helps to maintain the temperature equilibrium and also aids in keeping the water in the bath clean.

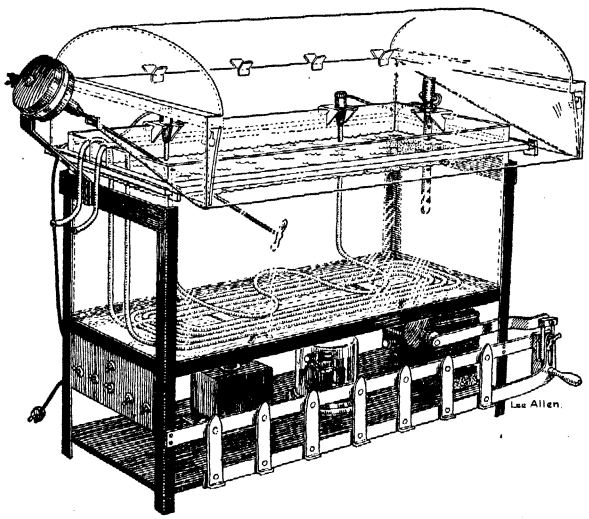

FIG. 1. Assembled transparent plastic tank and cover for Warburg manometric apparatus.

The tank now in use measures $9 \times 11\frac{1}{2} \times 26$ in. and is made of $\frac{3}{8}$-in. plastic sheets, with the corners both cemented and pegged with $\frac{1}{8}$-in. plastic dowels, as shown in Fig. 2, *e* and *f*. Very small holes were drilled the length of the dowels to allow escape of air and excess cement while they were inserted. The cover is constructed of $\frac{1}{8}$-in. material. A cement made by dissolving plastic scraps in chloroform was used for all seals where great strength was necessary. Other surfaces were joined by flowing chloroform from a pipette between the adjacent surfaces. The shelf around the top of the tank serves both as a brace and as a platform for the sidewalls of the cover. To accommodate the sidewalls a groove is cut around the back and two end pieces of this shelf (Fig. 2, *d, e,* and *f*). The upper, curved portion of the cover rests on the sidewalls, which have "Y"-shaped receptacle hinges (Fig. 2, *b*) along the top of the back edge. The $\frac{1}{8}$-in.-thick curved portion of the cover was bent by clamping the long edges between wooden 1×2's and playing a lazy gas flame under the sheet until it sagged nearly into shape. Final shaping was done by force, and the 1×2's were clamped in proper position with the curved sheet suspended between two tables of equal height. The "Y" hinges, and the hooked portion of the movable accessory brackets (Fig. 2, *g*) were softened by slowly heating the plastic over a Bunsen burner. When it could be bent

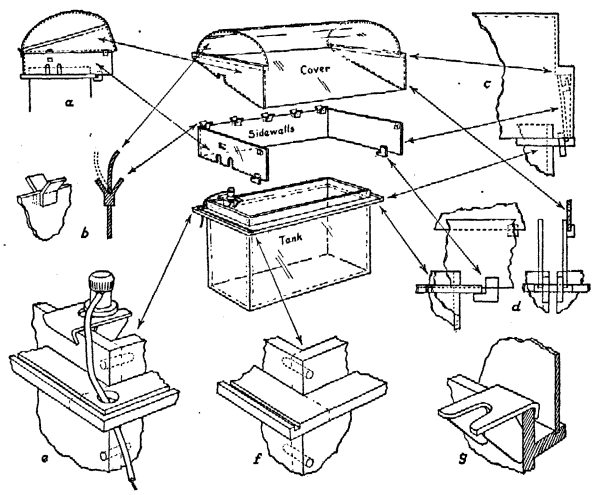

FIG. 2. "Exploded" view of "Plexiglas" tank, cover, and cover sidewalls with accompanying detail sketches: *a*, end view of assembled unit with cover lifted and resting on sidewall brackets; *b*, "Y"-shaped receptacle hinges; *c*, front view of closed cover showing how offset in cover rests on slightly displaced sidewall; *d*, solid hooks on sidewalls locking under shelf and locking device for supporting end of cover when lifted; *e*, construction of top rear corner with electrical accessory in its movable plastic bracket; *f*, construction of top front corner; *g*, view of accessory bracket on a cross-section of the top edge of tank.

with slight pressure, it was shaped over a form and held until cool.

In order to avoid strains and bubbles in the cemented joints of the tank it is advisable to allow 3 to 5 weeks for thorough drying.

A Simple Automatic Pressure-regulating Device for Use With Vacuum Lines

WALTER S. CLARK
Duke University

A reliable pressure-regulating valve for use with vacuum lines can easily be constructed from an ordinary drying tube and some glass tubing. The valve is designed to even off small variations in a vacuum line, rendering the partial vacuum very nearly constant. It is based on a regulator described by Asprey (*1*).

The valve (Figs. 1 and 2) consists essentially of a drying tube (b) with a side arm (a) blown into the body immediately below the bulb, a glass rod (r), and a sealed glass float (f) which is buoyed up by the mercury in the reservoir (m). The end of a glass rod which fits loosely in the tip (t) is heated in a Bunsen flame until a small, solid bead is formed with a diameter somewhat greater than that of the tip. Removed from the flame, the bead is carefully flattened and again revolved in the flame until it assumes an ellipsoidal form as shown (g). After grinding off the tip of the drying tube evenly, the rod (r) is in-