In the Laboratory

Notes on the Possibility of a Histochemical Method for Localizing Adenosinetriphosphatase

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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

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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