Determination of Xanthine Oxidase in Insects with Tetrazolium Salts

Xanthine oxidase is the enzyme that catalyzes the oxidation of the purine derivatives xanthine and hypoxanthine to form uric acid. This is a vital reaction in uricotelic animals such as insects. The flavin prosthetic group of xanthine oxidase is reoxidized by oxygen, by methylene blue, by the cytochrome system, and by other readily reduced chemicals. The experiments described here were performed to establish the principal sites of xanthine oxidase activity in insects and the desirability of substituting one of the several tetrazolium salts for the standard Thunberg technique using methylene blue in vacuum for the determination.

Samples of insect tissue were taken from nymphs of the American cockroach (*Periplaneta americana*), the yellow mealworm (*Tenebrio molitor*) in the larval stage, and 6th instar larvae of the southern army worm (*Prodenia eridania*). The tissues were dissected and washed free of adhering particles of other tissues or contained material and homogenized in a 2-percent solution of NaF in a suitable buffer with a detergent such as sodium lauryl sulfate or Triton X-100 added to liberate the enzyme from lipid particles. Pooled tissues from two to four insects were used for each experiment. Sodium fluoride at the 2-percent level proved to be the most effective bacterial inhibitor. The homogenates were either filtered or centrifuged, and the filtrate or supernatant was divided into two equal aliquots, diluted with equal volumes of buffer containing 800 μg of neotetrazolium chloride [p,p′-diphenylenebis-2-(3,5-diphenyl tetrazolium chloride), or NTC]. The paired sets of tubes with and without xanthine substrate were placed in a suction flask and evacuated through the side arm. An incubation period of 1½ hr at 30°C gave satisfactory reduction with NTC. Tissue preparations incubated with tetrazolium chloride [(2,3,5-triphenyl tetrazolium chloride) or TPTZ] required an incubation period of 20 hr.

At the end of the incubation period the samples with NTC were extracted with a constant volume of water saturated n-butanol. The butanol layer was separated and the optical density for wavelength 520 mμ was measured spectrophotometrically. With TPTZ, reduction was stopped with trichloracetic acid, and the red formazan was extracted with acetone, diluted to constant volume and determined for 480 mμ. The results indicate that tissue from the fat body and the gut reduced the dye 2 to 5 times faster than the controls, while tissues from the head and muscles showed an insignificant difference. These results are in general agreement with those of H. Liefert (*Zool. Jahrb. Physiol.* 55, 131 (1935)] who used a different analytic technique to examine the tissues of *Antheraea pernyi*. These data strengthen the hypothesis that the fat body of insects constitutes a principal site of intermediary metabolism and as such resembles the liver of vertebrates.

The tetrazolium salts TPTZ and NTC used in vacuum were demonstrated to be satisfactory receptors in this reaction. The fact that these form stable colored compounds which can be extracted quantitatively gives their use distinct advantages over the methylene blue technique. Because of the shorter incubation period, the neotetrazolium chloride is the more satisfactory.

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Simple Multiple-Sample Dialyzer

In connection with studies utilizing continuous paper electrophoresis, it is frequently necessary to dialyze large numbers of samples. Figure 1 shows a simple apparatus that has been in use in this laboratory for approximately 2 years and has been proved to be satisfactory in every respect.

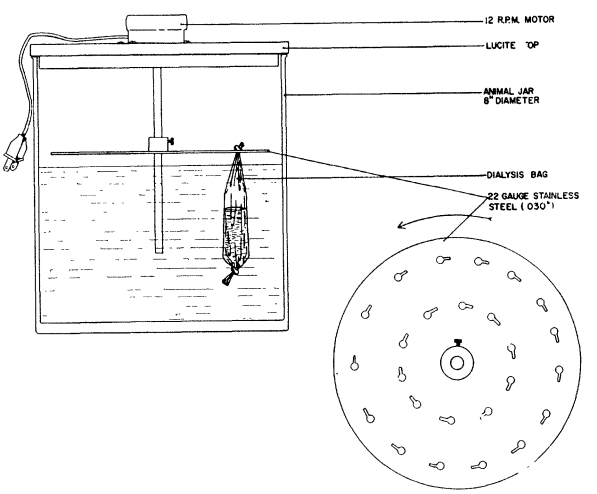

Fig. 1. Multiple-sample dialyzer.

Although Fig. 1 is self-explanatory, the following comments are offered. The knots that close the ends of the dialysis bags, made from the usual cellophane tubing, are passed through the "key holes" in the rotating plate and thus utilized to fasten the bag to it. A number has been stamped (not shown on figure) adjacent to each "key hole" for identification.

The amount of fluid, against which samples are dialyzed, can be varied and the height of the plate above the fiuid level is then adjusted accordingly.

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