Discussion. The foregoing procedure is recommended for the determination of aspartic acid in protein hydrolysates. Biological fluids in general cannot be measured because of possible interference by fumaric acid. Although the use of the chromatographic determination for fumaric acid(4) would improve the sensitivity, a prior analysis of fumaric acid in the sample would decrease the convenience of the method. Obviously, the precision, specificity and sensitivity of the final measurement can be no greater than that reported for the conversion(3) and the colorimetric procedure for fumarate(5).

Summary. A procedure is described for the determination of aspartic acid in quantities of 0.5 mg or above in the sample. The amino acid is converted to fumaric acid and this is measured colorimetrically.

Received May 4, 1950. P.S.E.B.M., 1950, v74.

Methylene Blue, 2,4-Dinitrophenol, and Oxygen Uptake of Intact and Homogenized Embryos.* (17935)

J. H. BODINE AND KIAO-HUNG LU

From the Zoological Laboratory, State University of Iowa, Iowa City.

That M.B. and 2,4-D.N.P. in suitable concentrations stimulate or augment the oxygen consumption of intact cells and tissues of a variety of biological forms seems well established(1,2,3). The exact mechanisms involved in such reactions, however, do not seem to be so well understood and many ideas have been advanced in attempting explanations of such phenomena. More recent investigators of the action of these chemicals upon the oxygen uptake of intact and homogenized cells emphasize the importance of factors associated with cell structure in conditioning the results(1,4). Marked effects upon intact cells have been pointed out while quite opposite and at times contrary results are described for homogenates and intracellular constituents of the same materials(1). Additions of various substrates to homogenates in certain instances seem to condition or at least influence the nature of the response(1,4).

Since much of the available data seems to have been obtained for mammalian tissues requiring the addition of substrates for the reaction it was thought desirable to extend these observations to the embryonic cells of invertebrates which normally do not require the addition of substrates for such studies. The intact embryo of the grasshopper, *Melanoplus differentialis,* which has proven of value for various cellular problems, has been used in the present investigation(2,3). Homogenates as well as intracellular constituents (nuclei, mitochondria, microsomes) prepared by fractional centrifugation techniques have also been employed. The suspension medium used throughout all experiments was .9% NaCl, buffered to pH 6.8 by .006M phosphates. No additional substrates were added so that oxygen uptake, as measured by standard Warburg manometers, represents that necessary for endogenous respiration. Respiration flasks, 5 ml capacity, were used and all experiments were conducted at 25°C. One hundred embryos per cc of suspension medium or their equivalent were used throughout. Homogenates were prepared by means of an all glass Potter-Elvehjem homogenizer (5). Nuclei, mitochondria and microsomes

* Aided by grant from National Institutes of Health. Acknowledgement is gratefully made to Etta Andrews for technical assistance in carrying out this investigation.

1. Peiss, C. N., and Field, J., *J. Biol. Chem.,* 1948, v175, 49. (Contains rather complete bibliography.)

2. Bodine, J. H., and Fitzgerald, L. R., *Physiol. Zool.,* 1949, v22, 283.

3. Bodine, J. H., *Physiol. Zool.,* 1950, v23, 63.

4. Tyler, D. B., *Arch. Biochem.,* 1950, v25, 221.

5. Potter, V. R., and Elvehjem, C. A., *J. Biol. Chem.,* 1936, v114, 495.

were separated by means of fractional centrifugation in plastic tubes at speeds of 600 to 1800 times gravity. All other details of technic were as previously indicated(3).

Results. Methylene blue. M.B. has previously been shown to markedly augment the oxygen uptake of actively developing intact grasshopper embryos(2) and as graphically shown in Fig. 1, such results have been confirmed in the present experiments. Maximum stimulation of oxygen consumption for the intact embryo occurs at concentrations of approximately 25 x 10^{-5} M. Homogenates, made from similar embroys, are also stimulated in their O_2 uptake to approximately the same degree by this reagent (Fig. 1). Intact nuclei, separated by fractional centrifugation and thoroughly washed, show a similar augmentation of O_2 uptake when treated with M.B. (Fig. 1). However, when granules (mitochondria and microsomes) are similarly treated, no reaction to M.B. can be detected (Fig. 1). It thus seems that intact embryos and nuclei, as well as the homogenate, respond to M.B. by an augmentation of oxygen consumption while cytoplasm constituents (mitochondria, microsomes) are not so affected.

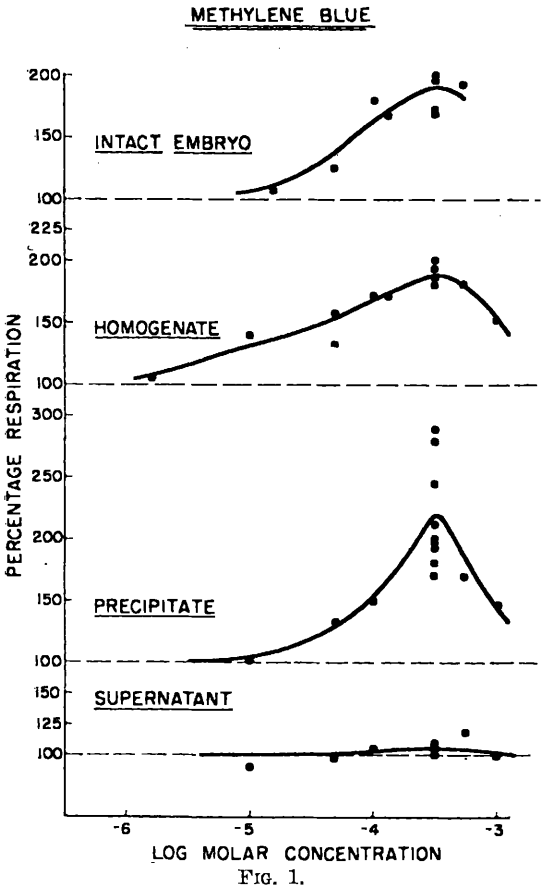

FIG. 1.

Shows effect of M.B. on the oxygen uptake of intact embryos, homogenate, precipitate (nuclei) and supernatant (mitochondria and microsomes). Data expressed as % of normal in .9% NaCl suspension medium taken as 100%. 100 intact embryos/cc of suspension medium: 1 cc of homogenate equal to 100 embryos. Homogenization carried out at room temperature (22-24°C). Equilibration 15 minutes. Readings every 10 min. Values given are averages for 70 min. exposure to reagent and represent readings from a minimum of 12 manometers for each concentration of reagent. Note—All concentrations of M.B. employed give only stimulation. For precipitate (intact nuclei), at conc. of 25 × 10^{-5} M. Note range of results for large series of experiments. Centrifugation carried out at 600 times gravity for nuclei: 1200 times gravity for mitochondria: 1800 times gravity for microsomes. Supernatant used as substrate for mitochondria and microsomes. Washed intact nuclei contain inherent enzyme-substrate system.

2,4-dinitrophenol. As previously noted and confirmed by the present results, 2,4-D.N.P. stimulates oxygen uptake of intact embryos (3) (Fig. 2). Similar concentrations of this reagent when added to homogenates or intracellular constituents (nuclei, mitochondria, microsomes) produce no appreciable or significant effects on their oxygen consumption (Fig. 2). The intact cell or embryo thus seems necessary for the stimulating effects of 2,4-D.N.P. and this is in striking contrast to the action of M.B. However, in the case of M.B. the nuclei, as used, are intact and as such perhaps contribute to the response to the reagent. Since no satisfactory method for breaking the nuclei, without injury to enzyme-substrate system, is at hand this point must await for further elucidation.

Discussion. Present data for the augmentation of the oxygen uptake of the intact grasshopper embryo by M.B. and 2,4-D.N.P. confirm those for this and other biological forms. The marked differences in response of the homogenate and intracellular constituents to M.B. and 2,4-D.N.P. are of some interest in view of the results noted for similar experiments with rat cerebral cortex. Peiss

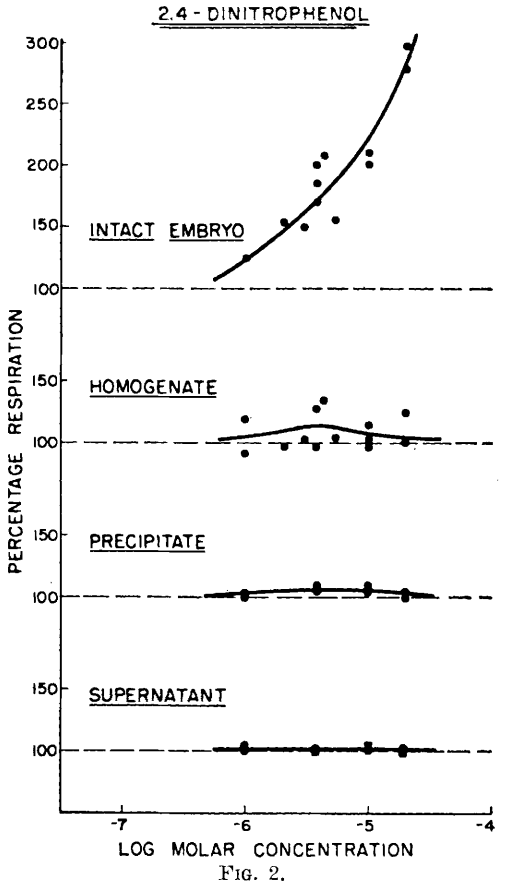

FIG. 2.

Similar to Fig. 1 except for 2,4-D.N.P. Note lack of stimulation of oxygen uptake in all except intact embryos.

and Field(1) have shown that brain slices respond to 2,4-D.N.P. by a marked augmentation of the oxygen uptake while homogenates made from similar tissues show no such reaction. As a matter of fact a rather marked inhibition of oxygen uptake of the homogenate is produced by doses of 2,4-D.N.P. which normally stimulate the intact brain slices. Tyler(4) more recently has pointed out that uninjured, intact nuclei of a rat brain homogenate are necessary for an augmenting effect of oxygen uptake by 2,4-D.N.P. For injured nuclei in the homogenate an inhibitory action is noted when similar concentrations of the reagent are employed. For the homogenate, intact nuclei and intracellular constituents of the grasshopper embryo, no augmenting action of 2,4-D.N.P. has been noted even though cytological examinations have revealed no apparent changes in the nuclei of the homogenate. From such observations one might well infer that for the grasshopper embryo the cell must be intact in order to produce significant augmenting effects of oxygen uptake by 2,4-D.N.P.

Although both M.B. and 2,4-D.N.P. may produce striking augmentation of oxygen uptake by the intact embryo they differ markedly in their action upon homogenates. The augmentating action of M.B. seems to be confined exclusively to the intact nuclei and does not affect the mitochondria or cytoplasmic constituents.

Summary. 1. M.B. and 2,4-D.N.P. produce augmentation of oxygen uptake of intact grasshopper embryos. 2. M.B. produces augmentation of oxygen uptake of homogenates made from embryos. 2,4-D.N.P. does not produce significant augmentation of oxygen uptake of homogenates. 3. M.B. produces augmentation of oxygen uptake of intact nuclei and not of mitochondria or cytoplasmic constituents. 4. 2,4-D.N.P. does not affect oxygen uptake of isolated nuclei or mitochondria or other cytoplasmic constituents.

Received May 22, 1950. P.S.E.B.M., 1950, v74.