

tion for increasing the preliminary exposures to altitude beyond 30 days. It is suggested that such altitude rats be used for screening

of methods for the treatment of bacterial endocarditis in man.

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Endogenous O₂ Uptake of Intact Embryos, Homogenates and Cellular Constituents in Isotonic Sucrose and Ringer.* (19151)

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In the preparation of homogenates from vertebrate tissues, and especially liver, it has been shown that sucrose makes a more satisfactory suspension medium than isotonic balanced salt solution(1-3). This is especially

true when the activity of certain enzyme systems is being studied, for example, succinoxidase. Greater activity has been obtained when homogenates and cellular constituents are suspended in isotonic sucrose. For in-

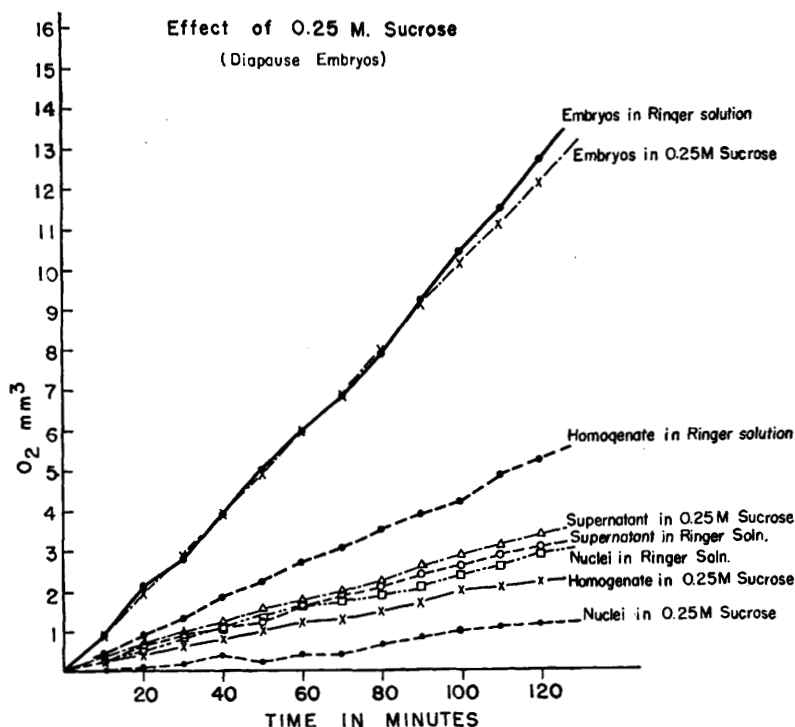


FIG. 1. Shows O₂ uptake of intact embryos, homogenates, supernatant (cytoplasm) and intact nuclei in .25 M sucrose and Ringer's solution. Arrow indicates time of emptying contents of side arm into respiration flask.

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1. Schneider, W. C., Claude, A., and Hogeboom, G. H., *J. Biol. Chem.*, 1948, v172, 451.

2. Lardy, H. A., *Respiratory Enzymes*, Burgess Publ. Co., Minneapolis, 1949, (Contains rather complete bibliography).

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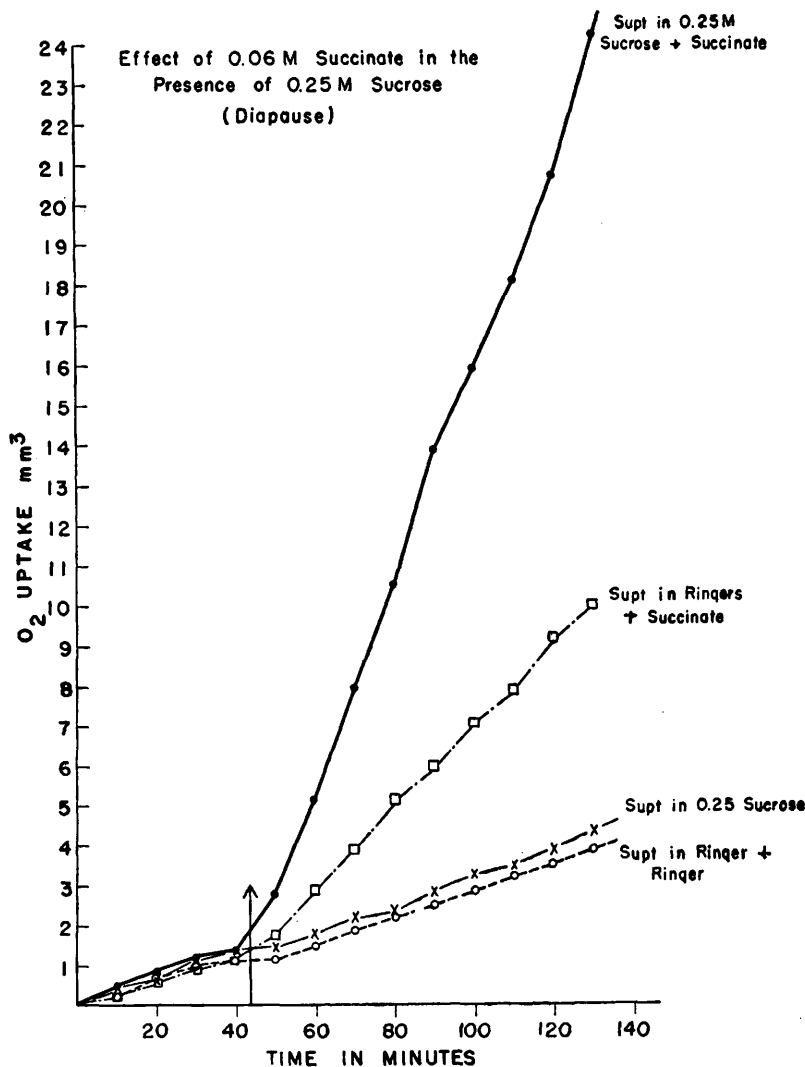


FIG. 2. Shows O₂ uptake of supernatant (cytoplasm) in .25 M sucrose and Ringer's solution with and without addition of .06 M sodium succinate.

vertebrate tissues little such data seem to exist. The present paper, therefore, is concerned with the effects of isotonic sucrose and Ringer solution upon the endogenous O₂ uptake of intact grasshopper embryos, their homogenates and intracellular constituents.

The embryo of the grasshopper, *Melanoplus differentialis*, dissected from the egg and freed of yolk has been used as experimental material. Details of procedure have been repeatedly described and will be omitted

here(4). Oxygen uptake was measured with standard Warburg manometers at 25°C using respiration flasks of 5 ml capacity. Sucrose (0.25 M) and Ringer solution, both at pH 6.8 were used as suspension media. One hundred embryos per ml of medium have been used throughout. Homogenization and fractional centrifugation were carried out as previously described(4).

Results of typical experiments are graphically shown in Fig. 1 and 2. Fig. 1 shows that intact diapause embryos consume similar amounts of O₂ in either sucrose or Ringer

4. Bodine, J. H., and Lu, K., *Physiol. Zool.*, 1951, v24, 120.

over the course of the experiment. Intact embryos homogenized in Ringer solution show a considerably higher O_2 uptake than for similar embryos when homogenized in 0.25 M sucrose. When homogenates are fractionally centrifuged and supernatants (cytoplasm) and precipitates (nuclei) tested, the endogenous O_2 uptake of supernatants whether in sucrose or Ringer is similar. Nuclei suspended in Ringer have much higher O_2 consumption than those in sucrose. It seems reasonable from this to assume that low O_2 uptake for homogenates in sucrose must have been conditioned by the lowered O_2 uptake of the contained nuclei. As a matter of fact, nuclei when suspended in Ringer, appear quite distinct and show no visible signs of injury. On the other hand, nuclei in isotonic sucrose become sticky, clumped and practically impossible to handle with pipettes. They lose their shape, break up and appear as a sticky mass. It would thus seem that nuclei from grasshopper embryos are markedly affected when suspended in isotonic sucrose solution. That other parts of the cell are not so affected by the sucrose seems of interest and especially so when various substrates such as succinate are added to them.

Supernatants (cytoplasm) free of nuclei, and suspended in sucrose and Ringer show rather striking differences in O_2 uptake when sodium succinate is added to them. Results of typical experiments are graphically shown in Fig. 2. An inspection of this figure shows

that supernatants suspended either in Ringer or sucrose respire at approximately the same rate. When sodium succinate (0.06 M) is added, however, supernatants in sucrose show increased O_2 uptake, markedly in excess of that shown by those suspended in Ringer. No apparent reasons for this marked difference in response to the succinate are evident. Since succinic dehydrogenase seems to be associated largely with mitochondria it seems reasonable to assume these differences may be due to some effect of the sucrose upon them, possibly a softening or splitting effect making the dehydrogenase more readily available than in the Ringer solution.

Summary. 1. Intact embryos of the grasshopper, *Melanoplus differentialis*, show similar rates of endogenous O_2 uptake when suspended in isotonic, Ringer or sucrose solution. 2. Homogenates of embryos suspended in Ringer solution show much higher O_2 uptake than those suspended in isotonic sucrose solution. 3. Isolated intact nuclei suspended in Ringer solution show much higher O_2 uptake than those suspended in isotonic sucrose solution. 4. Isotonic sugar solution affects the endogenous O_2 uptake of only nuclei and no other parts of the embryonic cell. 5. Supernatants (cytoplasm minus nuclei) suspended in isotonic sucrose show greater stimulation of O_2 uptake by addition of succinate than when suspended in Ringer solution.

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Excretory Function of the Skin in the Squid.* (19152)

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It is general knowledge that the cephalopoda have a special excretory organ, a kind of nephron for the elimination of waste products. The epidermis of this group of animals contains glands, which in some species eliminate

a fluid rich in calcium salts, and in some mollusks produce certain coloring matter. The excretory function of the skin of squids is unknown, therefore the following observations are significant.

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One to 4-day-old squids showing pronounced phototaxis and aerotaxis were