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The Effect of Ultracentrifuging on the Oxygen Consumption of Developing and Blocked Grasshopper Eggs and Embryos*

By Joseph Hall Bodine

Some effects of centrifugal force upon the egg of the grasshopper, *Melanoplus differentialis,* have been reported by Bodine and Boell (1936). It was thought advisable to extend these observations to the embryo of this species since with data on both the egg and contained embryo, conclusions might be derived as to which parts in the egg (embryo or extra-embryonic materials) were being most affected. The present paper, therefore, is based upon a study of the O_2 consumption of eggs and embryos subjected to various centrifugal forces as developed by the air-driven ultracentrifuge of Beams (1930).

Materials and Methods

The eggs used for all experiments were collected and handled in the manner described in a previous paper (Bodine and Boell, 1934). Each set of experiments was run in two ways as a check against each other.

Method 1—Eight micro-differential manometers were used. Into two manometers were placed 10-25 eggs (number depending on age of eggs) carefully selected with the aid of the dissecting microscope and the grasshopper embryo chart of Slifer (1932). An equal number of embryos was dissected from similarly selected eggs and placed in two other manometers in a phosphate buffered (pH 6.8) Belar solution. A third selected lot of eggs was centrifuged at given forces; one-half the number run in the intact egg stage in two manometers, and embryos dissected from the remainder and run in two other manometers. The set-up then consisted of four control manometers; two of eggs and two of embryos, and four experimental manometers of the same.

Method 2—Six to eight manometers of control eggs were run for a period of time and the oxygen consumption of the manometers checked against each other. The eggs running most closely together were then used for the day's experiment, and the others

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discarded. Embryos were dissected from one or two of the selected groups and their oxygen consumption determined. These determinations were then compared with the previously obtained oxygen consumption of the whole egg containing these same embryos. The remaining eggs, whose oxygen consumption had been determined, were centrifuged and run again. After this determination, the eggs were removed from the manometers and their embryos dissected out and again run. This made a check of the normal egg, centrifuged egg, and centrifuged embryo all from the same original egg.

It was found that these two methods gave practically identical results so they were used at the experimenter's discretion.

Nine complete sets of experiments involving three distinctly different physiological stages of the grasshopper egg—pre-diapause, diapause, and post-diapause—have been carried out. In each stage, determinations of the oxygen consumption of control eggs and embryos as well as of eggs and embryos after being subjected to forces of 21,000, 203,000 and 360,000 times gravity were made.

Results

The average results of typical experiments are given in table 1. In all cases, the respiration or oxygen consumption of the normal uncentrifuged egg is taken as 100%. By comparing the first column of the table we note that the rate of oxygen consumption of the diapause embryos is higher in relation to the oxygen consumption of the intact egg than either the pre- or post-diapause embryos. This does not mean that the diapause embryo consumes more oxygen than either the pre- or post-diapause ones, for the rate of oxygen intake of intact, diapause eggs is considerably less than that for pre- and post-diapause ones. But this does seem to indicate that the reduction in rate of oxygen consumption by the intact diapause or blocked egg is due more to the extra-embryonic constituents than to the embryo. When we compare the second column of the table, we again note a similarity between the reactions of the pre- and post-diapause eggs to centrifugal force and a vastly different reaction on the part of the diapause egg.

The pre- and post-diapause eggs are greatly affected by centrifugal forces, e.g., they show an approximate 50% reduction in the normal rate of oxygen consumption. The opposite reaction occurs in the diapause material. At a force of 21,000 times gravity diapause eggs are stimulated so that they now average twice their normal rate of oxygen consumption. And even at 360,000 times

gravity diapause eggs consume approximately 75% more oxygen than uncentrifuged normal ones.

The data show that after removing the embryos from the centrifuged eggs, their rate of oxygen consumption is proportionately greater than when removed from normal uncentrifuged ones. This is found to be the case for eggs subjected to any of the forces used. By taking the averages, however, the figures seem to indicate that all or almost all of the oxygen consumption of the centrifuged egg is due to the embryo. The averaged results of the comparison of the normal and centrifuged embryos seems to bear this out further, for the centrifuged embryo is shown to have been unaffected, and as there was a depression in the rate of oxygen consumption of the whole egg, this depression must have been due to the effect on the extra-embryonic parts.

The eggs are completely stratified at a force of 21,000 times gravity, into three distinct parts; a lipoidal layer at one end, the embryo next, and finally a layer of water and proteins. Increasing the force to 360,000 times gravity compresses the embryo into a very small ball scarcely recognizable as an embryo. Although the embryos centrifuged at a force of 21,000 times gravity are undoubtedly affected, this effect seems only temporary as the embryos appear quite normal when removed from the egg and left for a time in Belar.

A force of 360,000 times gravity does not effect the rate of O_2 consumption of the egg or embryo any more than a comparatively much smaller force of 21,000. Bodine and Boell (1936) have shown similar results with forces less than 21,000 times gravity.

In dealing with diapause materials, at no time was there ever an initial depressive effect upon the rate of oxygen consumption of the eggs and only rarely upon the embryo. At a force of 21,000 times gravity, the rate of oxygen consumption of the diapause egg averaged 90% greater than normal; at 203,000 times gravity, 32% greater; and at 360,000 times gravity, 78% percent greater. This rise was apparently due to the effect upon both the embryo and the extra-embryonic parts of the egg. At the three forces, the stimulative effect upon the embryo varied from an average rise of 6.6% to 49% greater than normal. In the pre-diapause centrifuged material, the mean value for all the forces shows that 99.9% of the oxygen consumption of the egg was due to the embryo. In other words, the extra-embryonic parts seem to have been affected so greatly that they hardly consumed oxygen at all. In diapause, although the percentage of the centrifuged embryo to

the centrifuged egg was relatively high, it never reached such proportions as in prediapause.

In making the above statement that in the prediapause centrifuged egg, the extra-embryonic portions hardly consumed any oxygen, the writers took into account only the averaged percentages. But by taking the individual figures, it is noted that in many cases the embryo consumed *more* oxygen than the intact egg in which it had just been. This may be due to several factors. As stated before, centrifuging stratifies the egg and the embryo is quite compact within it. After removal, the embryo is washed carefully in Belar and loosens up or expands a great deal. This may result in an increase in activity which causes the increase in oxygen consumption. Also, while in the egg, the embryo may have more difficulty in obtaining oxygen due to the membranes and extra-embryonic materials surrounding it.

At forces of 21,000 and 203,000 times gravity in the post-diapause stages, we again find that the centrifuged embryo consumes more oxygen than the egg. Even though all eggs used for post-diapause experiments were chronologically 3 days out-of-diapause, it is probable that physiologically they had not completely reached the post-diapause or active condition, and the centrifuging affected them quite similarly as the diapause.

All centrifuged eggs used in Method 2 were returned to 25°C. after having their oxygen consumption rate determined and the effect of centrifuging on hatching noted. Not a single egg hatched nor developed further than before centrifuging, at the three forces used.

Summary

1. Ultracentrifuging eggs had a definite effect upon the rate of oxygen consumption; that of pre- and post-diapause eggs was reduced to about one half and increased almost 70% in diapause eggs.

2. Centrifuging of eggs usually affects the rate of O_2 consumption of the contained embryos.

3. The physical effect upon the embryos of centrifuged eggs was noted.

4. No embryos of centrifuged eggs, subjected to 21,000 times gravity force ever hatched or developed after the centrifuging.

Table 1

x-gravity	% respiration of normal egg due to embryo	% centrifuged egg respiration as compared with normal egg	% centrifuged egg oxygen uptake due to embryo	% respiration of centrifuged embryo compared to normal embryo
		Pre-diapause		
21,000	55.8%	52.2	99.7	96.2
203,000	55.8	50.5	101.2	98.1
360,000	55.8	49.2	98.7	97.4
		Diapause		
21,000	83.5	190.2	83.0	106.6
203,000	83.5	132.4	87.6	149.2
360,000	83.5	178.4	86.0	113.8
		Post-diapause		
21,000	58.0	55.9	118.8	111.2
203,000	54.0	35.6	127.0	83.3
360,000	59.1	40.5	87.9	60.5

*Assumes that normal egg consumes 100 units of O_2 in a given time, the oxygen consumption of the normal embryo and the centrifuged egg and embryo are expressed as percentage of or above these 100 units.

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