Proceedings of the Iowa Academy of Science

Volume 60 | Annual Issue Article 82

1953

Respiratory Quotients of Intact Egg, Isolated Embryo and Embryo Homogenate

Joseph Hall Bodine
State University of Iowa

William Lionel West
State University of Iowa

Respiratory Quotients of Intact Egg, Isolated Embryo and Embryo Homogenate*

By Joseph Hall Bodine and William Lionel West

Respiratory Quotients

The problem of the nature and type of chemical compounds utilized during the embryonic development of an organism has been studied for many biological forms and considerable data exist concerning the basic details of such phenomena (Needham, 1942; Spratt, 1952; Cleland and Rothschild, 1952). The terrestrial, cleidoic or "closed system" egg seems to be rather favorable material for study of this problem since the exchanges of materials from the outside to the inside of the egg and vice versa appear to be at a minimum. The egg of the grasshopper, *Melanoplus differentialis,* is unusually favorable material for investigating the problem since water, oxygen and carbon dioxide seem to be the only substances passing into and out of the egg during its entire course of development. The fact that the embryo can be completely isolated from the yolk for experimental purposes makes possible detailed quantitative studies of its metabolic activities during various phases of its development. During the normal course of embryonic development there occur periods of mitotic activity (pre-and postdiapause) and block (diapause), thus making possible studies on these phases of the cellular behavior of both the egg and embryo (Bodine and Boell, 1934). Food for the developing embryo is contained within the egg (yolk) and consists largely of lipids and lipid compounds (Slifer, 1930). Previous metabolic studies on the changes of food during the development of this form have indicated that respiratory quotients (R.Q.) are relatively low and suggest that fat conversions or transformations are largely responsible for the chief energy sources of the embryo during its entire developmental cycle (Boell, 1935; Hill, 1945).

The present problem has been concerned with an intensive study of the respiratory quotients of the intact whole egg, the isolated intact embryo and homogenates prepared from isolated embryos. Data obtained from such a study should aid in further elucidating the nature of the transformations taking place in the food materials

*Aided by grant from the National Institutes of Health. Acknowledgment is gratefully made to Etta Andrews for technical assistance.

(yolk) as well as those in the developing and growing cells of the embryo proper.

Material and Methods

The egg of the grasshopper, *Melanoplus differentialis,* and its isolated embryo, freed of yolk, have been used throughout this investigation (Bodine and Boell, 1934). Embryos were suspended in 0.9% sodium chloride, buffered with 1/15 M. phosphates (pH 6.8). Homogenates of embryos were prepared with lucite rods and pyrex tubes and handled as previously described (Bodine and Lu, 1950). Standard Warburg manometers were used for studying gas exchange and both direct and indirect methods of obtaining respiratory quotients were employed and results so obtained were checked against each other (Dixon, 1943). A minimum of 14 manometers was employed for individual experiments and data presented represent means taken from large numbers of determinations. Data have been treated statistically and only final results are given. Results typical for several developmental stages are presented, (1) 15-18 days prediapause, (2) diapause and (3) 1-5 days post diapause.

Results

Intact egg

Data for the respiratory quotient (R.Q.) of the intact egg are similar to those reported by Boell (1935) and show a value of approximately 0.7 throughout development (fig. 1). Such a value strongly suggests the utilization of lipids and this is further confirmed by the results of chemical analyses of Slifer (1930) for the fat changes of the egg during development. It should be strongly emphasized at this point that a value for the respiratory quotient suggesting lipid changes in the intact egg is in no way indicative of what goes on in the embryo contained within the egg. All food materials for the developing embryo are elaborated or prepared in the yolk. Since no organic connections between yolk and embryo have been described all metabolites elaborated in the yolk and utilized by the embryo must of necessity pass by diffusion into the embryonic cells.

Intact isolated embryo

The Respiratory quotient (0.90) for the isolated intact embryo is significantly higher than that for the intact egg and indicates the utilization of a different type of metabolite from that of the intact egg (fig. 1). Such a value has been assumed to be indicative of a carbohydrate metabolism and in the case of the grasshopper embryo considerable data of Hill's (1945) from chemical assays con-

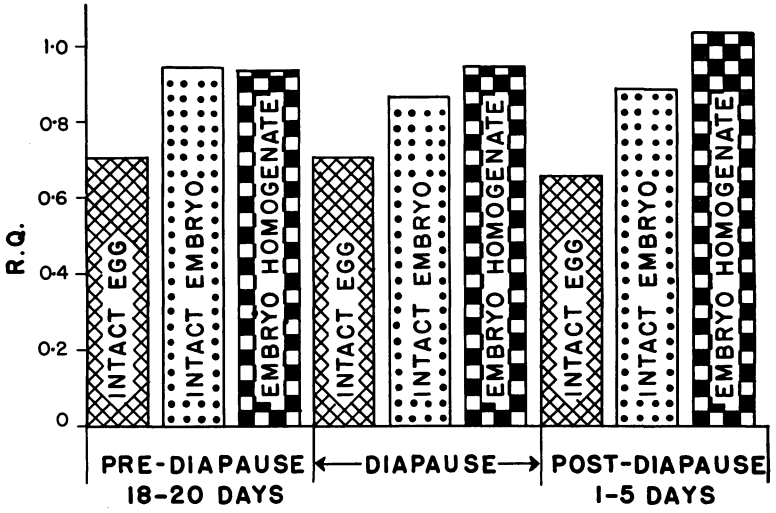

Figure 1

firm such an assumption. During the entire course of embryonic development the R. Q. values of the isolated intact embryo remain higher than those for the intact egg. (fig. 1) Such a situation would lead one to suspect marked changes or transformations in the yolk before being passed by diffusion to the cells of the growing embryo. Results of recent experiments, to be reported elsewhere, would lead one to suspect that phosphorlation of carbohydrates produced from yolk takes place either in the yolk itself or at the embryonic cell membrane. No phosphatase has been found in the embryo proper (Fitzgerald, 1949).

Homogenized embryo

Homogenates of embryos give values for R. Q. somewhat similar to those for the intact embryo and are of such magnitude (0.9-1.0) to suggest the utilization largely of carbohydrate, (fig. 1). No marked statistical difference between the R. Q. of the embryo and homogenate exists for the prediapause and diapause stages (Table 1). However, a statistically significant difference seems to exist between the R. Q. of the postdiapause embryo and homogenate (see table). No reason is at hand to explain such a situation. Apparently carbohydrate splitting enzymes seem active in the organized as well as in the unorganized (homogenate) states of the embryo.

Table 1

Summary of R. Q. readings.

	mean average	std. dev.	std. error of mean			
predia.	—H = 0.93	0.204*	.0645	insignificant	insignificant	insignificant
	E = 0.938	0.087	.048			
diapause	—H = 0.950	0.1513	.0375	insignificant		
	E = 0.868	0.130	.049			
postdia.	—H = 1.02	0.1345	.0326	significant		
	E = 0.876	0.113	.0545			

H = homogenate.
E = egg.
*a rather marked diversity exists for endogenous O_2 uptake of homogenates.

Discussion

The fact that an intact egg can give a value for its R. Q. quite different and distinct from that given by its contained growing embryo seems significant and especially so when one considers the rather large amount of data recorded without distinction as to either the embryo or egg (Needham, 1942). A consideration of the detailed morphology of the internal egg and embryo relations for the grasshopper shows that no organic connections such as blood vessels exist between embryo and yolk upon which it feeds. The yolk cells apparently break down or transform the large lipid containing molecules into those readily diffusible through and into the embryonic cells which they undoubtedly nourish. The major work of the egg consists essentially of a catabolizing of the large yolk supply into building stones which are used almost exclusively in the anabolic metabolism by the embryo. The energy for development or activation of these molecules prepared by the yolk is brought about by a phosphorlation occurring in the yolk or during the passage through the embryonic cell membrane since no phosphatase has been demonstrated in the embryo proper. Perhaps the one significant point of this discussion might well be the pointing out of some sources of errors involved in studies on egg metabolism, i.e. to infer the type of metabolism of the embryo proper from data on the intact egg alone.

Summary

1. An intensive study has been made of the respiratory quotients (R. Q.) of the intact egg, isolated embryo and homogenate of embryo of the grasshopper, *Melanoplus differentialis.*

2. The R. Q. of the intact egg remains low (approximately 0.7) throughout the period of development.

3. The R. Q. of the intact isolated embryo and its homogenate (approximately 0.9-1.0) are significantly higher than that for the intact egg.

4. Values obtained for R. Q. indicates a quite different type of metabolism for the intact egg than for its contained embryo.

Literature Cited

Bodine, J. H., and E. J. Boell, 1934. Respiratory mechanism of normally developing and blocked embryonic cells. Jr. Cell. and Comp. Physiol. 5:97-113.

Bodine, J. H., and K. Lu, 1950. Methylene blue, 2, 4-dinitrophenol and oxygen uptake of intact and homogenized embryos. Proc. Soc. Exp. Biol. and Med., 74:448-450.

Boell, E. J., 1935. Respiratory quotients during embryonic deveolpment. Jr. Cell and Comp. Physiol., 6:369-385.

Cleland, K. W., and Lord Rothschild, 1952. The Metabolism of the sea-urchin egg. Oxidation of carbohydrate. The Jr. of Exp. Biol., 29:416-428.

Dixon, M., 1943. Manometric Methods. MacMillan, N. Y.

Fitzgerald, L. R. 1949. The alkaline phosphatases of the developing grasshopper egg. Jr. Exp. Zool., 110:461-487.

Hill, D. L., 1945. Carbohydrate metabolism during embryonic development (Orthoptera). Jr. Cell. and Comp. Physiol., 25:205-216.

Needham, J., 1942. Biochemistry and Morphogenesis, Cambridge.

Slifer, E. H., 1930. Insect development. I. Fatty acids in the grasshopper egg. Physiol. Zool., 3:503-518.

Spratt Jr., N. T., 1952. Metabolism of the early embryo. Ann. of the N. Y. Acad. Sc., 55:40-49.

Zoological Laboratories
State University of Iowa
Iowa City, Iowa