Oxygen Uptake in Heat-Stressed Chick Embryos

W. C. Gunther, Valparaiso University

Introduction

The uptake of oxygen by the chick embryo has been extensively investigated by various workers. A review is given by Taylor (12). More recently, attention has been centered on the oxygen consumption of the embryo by whole egg techniques, measured in a modified Warburg as described by Csabay, et al. (1). Considerable research has also developed around the problem of separating respiring membranes (yolk sac, allantois, amnion) from the embryo and measuring the oxygen uptake of each (8, 9).

Gunther (2, 3) has reported various behaviorial anomalies and biochemical aberrations in chicks hatched from eggs subjected to non-optimally high temperatures during the first five days of incubation. Inasmuch as the results of some of these investigations indicated perhaps an involvement of oxygen, Warburg manometric pilot studies on the oxygen consumption of such heat-stressed embryos were initiated. The oxygen uptake of these embryos is compared with that of control embryos incubated at normal temperature, and the significance of the mean differences is evaluated by statistical methods.

Materials and Methods

One thousand eighty chick eggs (White Rock) were incubated for varying numbers of hours from July 23 through August 22. The eggs were placed in the incubators for periods ranging from 64 to 149 hours in lots of 72 at weekly intervals. Forty-eight eggs from each lot were incubated at 41° C. (called experimentals) and 24 were placed in the optimal temperature incubator, set at 37.5° C. (called controls). At the appropriate time the embryos were removed from the eggs and, after all membranes had been trimmed, were weighed to the nearest milligram. In the manometers only those embryos were used which showed strong heart beats and maximum development. While it was impossible with the techniques employed to trim each embryo exactly alike, the average weights agreed rather well with other series of weighed embryos (unpublished data).

For each particular age group (in hours) during the periods July 23 to July 25 and August 1 to August 8, three experimentals and 3 controls were placed one in each of 6 reaction vessels. The reaction vessels contained 10 ml. Spratt's (11) chick Ringer (without agar, for which the term maintenance medium appears appropriate) with 0.2 ml. potassium hydroxide in the center well. The vessels containing the embryos were attached to manometers and placed on a Bronwell Warburg with the bath water at 37.5° C. A seventh reaction vessel (con-

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taining only water) was attached to a seventh manometer, serving as a thermobarometer. The shaking frequency of the Warburg was reduced to 60 per minute by means of a special pulley. This slower speed was found desirable in order to keep the embryos intact.

For the period August 20 to August 22, two more reaction vessels and manometers were added, so that four experimental and four control embryos were run at the same time.

The manometers were read at 15-minute intervals over a two-hour period. The individual readings were converted to microliters of oxygen consumed by the whole embryo, and to microliters of oxygen consumed per milligram of wet weight of each embryo. The results are expressed both as the average microliters of oxygen consumed per hour per 3 (or 4) embryos without regard to weight (total oxygen consumed) and as the average microliters of oxygen consumed per hour per milligram wet weight per 3 (or 4) embryos.

For the period August 20 to August 22, the experimental set-up was identical with the above. However, 5 grams of sucrose were added to each liter of Spratt's Ringer solution.

Results

The results are summarized in table 1. Student's "t-tests" were

TABLE 1. Summary of O_2 consumption.

Maintenance medium (Spratt's)						
July 23—July 25						
	ul 0_2 uptake per hr.			ul 0_2 uptake per hr. per mg. wet weight		
Age	Control	Experimental	Significance (df = 5)	Control	Experimental	Significance (df = 5)
100hr.	25.640	22.981	none	.266	.262	none
125hr.	50.831	45.201	none	.252	.206	none
149hr.	70.075	65.283	none	.158	.133	none
August 1—August 2						
78hr.	9.376	14.781	P=<.05	.667	.422	none
98hr.	17.941	29.345	P=<.05	.261	.260	none
August 5—August 8						
64hr.	8.892	10.263	none	.735	.650	none
88hr.	11.513	17.031	none	.467	.463	none
112hr.	39.000	43.203	none	.303	.250	P=<.05
136hr.	56.160	63.729	none	.200	.144	P=<.05
Sucrose medium (Spratt's)						
August 20—August 22						
68hr.	14.256	12.037	none (df=7)	.758	.975	none (df=7)
87hr.	10.556	11.213	none (df=7)	.258	.270	none (df=7)
112hr.	22.456	23.675	none (df=7)	.235	.355	none (df=7)

made to test the significance of the mean differences of oxygen uptake between each lot of control and experimental embryos as indicated. Error variances were tested by the "F-max" procedure and were found to be homogeneous. It is seen that with increasing age both control and experimental chick embryos require an ever-increasing amount of oxygen when cultured on the maintenance medium (Spratt's Ringer without sucrose and agar). When sucrose is added to this medium, both controls and experimentals show a slight initial decline in oxygen consumption, followed by a rapid rise.

On the maintenance medium, three lots of experimentals (July 23-25) consistently consumed less oxygen than did the controls, but the mean difference failed to attain statistical significance. At all other periods the experimentals consumed more oxygen than the controls, and for the period August 1-2 the difference in the means between the two groups attained significance ($P<.05$). With sucrose added to the medium the means between the two groups failed to attain significance at any time. Of interest in this latter group, however, is the fact that the 68-hour experimental embryos consumed less oxygen than did the controls, but the 87-hour and the 112-hour experimentals used more oxygen than did the controls.

When the microliters of oxygen consumed are calculated on the basis of milligrams wet weight per hour per 3 (or 4) embryos for each group, it is evident that on the maintenance medium all age lots in both groups (controls and experimentals) consume less oxygen with increasing age. In addition, experimental embryos indicate an uptake of oxygen which is less than the controls at all ages. The difference in the means in one group (August 5-8) attained significance with 112-hour and 136-hour embryos, the experimentals consuming less oxygen.

If sucrose is added to the medium, the average microliters of oxygen uptake per hour per milligram wet weight of 4 experimental embryos is higher than that of 4 control embryos at all ages. On this medium both the 87-hour controls and experimentals consume less oxygen than the 68-hour lot. However, the 112-hour experimentals reverse the downward trend in oxygen consumption. On the other hand, the controls continue to consume less oxygen at increased age levels.

Discussion

The fact of increased oxygen consumption of chick embryos with increasing age has been firmly established in the literature (1, 10, 12). Romanoff (10) has also found that the amount of oxygen consumed per hour per unit (gram) of body weight declines to a minimum at the day before hatching. The research reported herein appears to be in general agreement with these findings. No attempt has been made to compare absolute values with those reported in the literature. In the first place the methods used by other workers are so varied, with the results expressed in such a variety of ways, that it would be meaningless to make comparisons at the present time. Furthermore, of more importance to the overall relation of embryonic nonoptimally high temperature stress to what I hesitatingly call mental retardation in these chicks is the difference in oxygen consumption between the controls and experimentals.

By way of explanation it should be stated that whenever I undertake embryonic physiological study under these conditions, I permit the majority of the eggs from the same batch to hatch. This is done in order to establish that behavior and/or biochemical differences between control and experimental chicks do exist. Thus far, all evidence gained from these studies points to a definite positive relationship between the degree of temperature insult coupled with the length of such stress, and the behavior, weight, and certain physical and biochemical aberrations of the hatched birds (4, 5, 6, 7).

The differences in the oxygen uptake between the control and experimental embryos as reported above indicate at least tentatively that the metabolic rate of the experimentals has been disturbed by the heat treatment. The differences in the means were statistically significant among two lots (August 1-2) without regard to weight. When expressed as microliters of oxygen consumed per hour per milligram wet weight of the embryo the means were likewise significantly different among two other lots (August 5-8). In the former case the metabolic rate between controls and experimentals expressed as microliters of oxygen uptake per milligram wet weight, although clearly lower for the experimentals, was not significantly different. In the latter case the oxygen consumption without regard to embryonic weight was higher in the case of the experimentals. Were it not for the fact that in three lots (July 23-25) the experimentals consumed less oxygen than did the controls, it would be tempting to theorize that these data all indicate a more rapid development of the embryo undergoing heat treatment. In other words, if the experimental embryos are developmentally older than the controls, one would expect to find a higher oxygen consumption over the controls without regard to body weight, and a lower oxygen consumption when these results are expressed as microliters per unit of body weight. However, in view of the conflicting nature of these data, it would be premature to accept this explanation without reservation. Data for more embryos per lot should be obtained, with oxygen consumption determinations being made from the same batch of eggs over longer periods of time at shorter intervals. Refinements of technique, particularly with regard to trimming the membranes and weighing the embryos, should be introduced. Such work is presently being continued in our laboratory.

When sucrose is added to the maintenance medium, the metabolic rate is apparently even more disturbed and interpretation of the data becomes increasingly difficult. Contrary to expectations based on developmental rather than chronological age, the 68-hour heat-treated embryos consumed less oxygen than did the controls. However, in the 87-hour and 112-hour heat-stressed embryos the oxygen uptake was greater, as could be expected. Puzzling is the fact that both the controls and experimentals in the 87-hour lot consumed less oxygen than did those of the 68-hour lot.

When the metabolic rate is expressed per unit of body weight for the same embryos cultured on the sucrose medium, all experimentals consumed more oxygen than did the controls. If the experimentals were developmentally older by reason of accelerated growth because of the nonoptimally high temperature, they should consume less oxygen than

the controls. The experimentals may be farther along in development, but may not be able to make as effective use of nutrients as do the controls. Clearly, here also the oxygen uptake of more embryos from the same batch over longer periods of time at closer intervals should be determined. It might be interesting to use several different sugars (including those which the normal chick embryo cannot metabolize) in an effort to determine whether there is some difference in the ability of the heat-stressed animals to make effective use of these. Our investigation continues along these lines, also.

Summary

The oxygen uptake of chick embryos from different hatches subjected for varying numbers of hours to the optimal temperature of 37.5° C. was compared with that of embryos from the same hatches treated for similar periods but incubated at the nonoptimally high temperature of 41° C. Determination of oxygen consumption was made by the standard Warburg technique, and expressed as average microliters consumed per hour per embryo without regard to weight, and as average microliters per hour per embryo per milligram wet weight.

With increasing age and on a maintenance medium both control and experimental embryos consume an ever-increasing amount of oxygen. When these results are expressed in terms of microliters of oxygen consumed per hour per embryo per milligram wet weight, the oxygen consumption declines with inceasing age.

Statistically significant differences in oxygen consumption were recorded between control and heat-stressed embryos among 4 age groups. At ages other than these, differences were noted, but these failed to attain statistical significance.

When sucrose was added to the maintenance medium, differences in oxygen consumption among three lots were again noted, but these failed to attain significance. Of particular interest was the fact that the difference between control and experimental embryos in the amount of oxygen consumed in the sucrose medium was of a different order than those differences noted between controls and experimental embryos in the maintenance medium.

Literature Cited

1. CSABAY, L., L. HORVATH, and A. FERENCZ. 1960. Studies on the O_2 consumption of chick embryos by a modified Warburg method. Acta Biologica **11**:1-5.
2. GUNTHER, W. C. 1958. Effect of abnormal incubating temperature on chick behavior. Proc. Ind. Acad. Sci. **68**:363-366.
3. GUNTHER, W. C. and R. K. JONES. 1963. Some effects of nonoptimally high incubation temperatures on chicken blood chemistry. Proc. Ind. Acad. Sci. **72**:353-361.
4. ————. 1963. Effect of nonoptimally high incubation temperature on frequency of pecking and on color preferences in the chick. Proc. Ind. Acad. Sci. **72**:290-299.
5. ————. 1962. Effect of environmental stress on chick weight. Proc. Ind. Acad. Sci. **71**:385-398.
6. ————. 1962. Effect of nonoptimally high incubation temperatures on T-maze learning in the chick. Proc. Ind. Acad. Sci. **71**:327-333.

7. GUNTHER, W. C., ROBERT K. JONES, and PAUL MANSKE. 1961. The effect of high and low incubating temperatures on chick behavior. Proc. Ind. Acad. Sci. **70**:285-292.

8. NEEDHAM, J. 1932. On the metabolic rate of the chick embryo and the respiration of its membranes. Proc. Roy. Soc. London (B) **110**:46-74.

9. ————. 1932. A manometric analysis of the metabolism in avian ontogenesis. II. The effects of F-iodoacetate and other reagents on the respiration of blastoderm, embryo, and yolk sac. Proc. Roy. Soc. London (B) **112**:114-138.

10. ROMANOFF, A. L. 1941. The study of the respiratory behavior of individual chicken embryos. J. Cell. and Comp. Physiol. **18**:199-214.

11. SPRATT, N. T. 1949. Nutritional requirements of the early chick embryo J. Exp. Zool. **110**:273-298.

12. TAYLOR, LEWIS W. (Ed.). 1949. Fertility and hatchability of chicken and turkey eggs. John Wiley & Sons, Inc., New York.