METABOLISM OF THE BLACK SNAKE EMBRYO

II. RESPIRATORY EXCHANGE

By HUGH CLARK
Department of Zoology, University of Connecticut

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Bohr (1904) reported CO_2 production of an embryo of *Natrix natrix*, weighing 0·46 g., to be 120 mm.^{3}/g./hr. at 14·2° C.; the CO_2 produced at 28° C. was 3 times that at 15° C., and production by a newly hatched snake at 15° C. was approximately 90 mm.^{3}/g./hr. Other data are given, but they are not related to stage of development, and oxygen utilization was not measured. Zarrow & Pomerat (1937) made rather exhaustive studies of the effect of temperature on various aspects of respiratory behaviour of the smooth green snake during the last week of development. It appeared profitable, therefore, to obtain details of respiratory exchange throughout the developmental period of snake embryos. Such data were available from the same embryos which served for the excretion studies reported elsewhere in this *Journal* by the author (1953).

MATERIALS AND METHODS

Data on oxygen consumption, CO_2 production and R.Q. were obtained by use of a special flask (Aminco 5-210) with a standard Warburg apparatus. Temperature was maintained at 23·9° C. during the determinations, although the environmental temperature between observation periods fluctuated between 21 and 30° C. The period of thermal adjustment of the egg prior to actual determinations was therefore various. CO_2 was absorbed by 20% KOH. After thermal equilibrium had been attained, the initial determination was made without KOH, giving a measurement of O_2–CO_2; KOH was then added to the flask, a second more brief period of thermal adjustment followed, and the determination of consumed O_2 was made. Subtraction of the former from the latter yields the value of CO_2 produced. The readings of CO_2 produced and O_2 consumed are therefore made within 1–3 hr., which interval in a developmental period of more than 2 months is not considered to be significant. The individual observations extended over periods of 1–3 hr. The values obtained were corrected for minor barometric and thermal fluctuations in the usual way.

RESULTS

Oxygen consumption. The relation of total consumed oxygen to growth of the embryo and its membranes is shown in Fig. 1. Parallelism between the embryonic growth and oxygen consumption is self-evident, but of particular note is the obvious divergence of the curves at the beginning of development. The explanation may lie partly in the fact that the first 3 weeks constitute the period of most rapid growth of the yolk-sac; it is also conceivable that during intra-uterine development an oxygen debt is created, which is repaid during the initial period of exposure to the atmosphere. Although there is a degree of increased vascularization of the uterus, the developing embryo is isolated from an oxygen source by its own shell and shell membrane, and must rely on diffusion rather than a special oxygen transport device

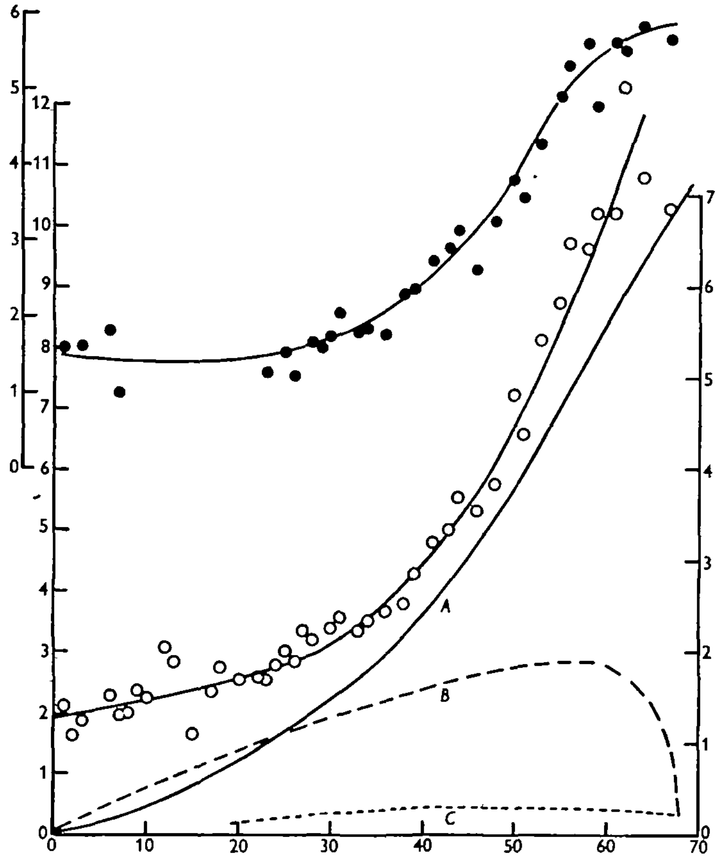

Fig. 1. Oxygen consumption (open circles) and carbon dioxide production (closed circles) throughout development. Smoothed growth curves of embryo, yolk-sac and chorio-allantoic membrane (*A*, *B* and *C*, respectively) are reproduced to make it easier to relate respiration to growth. Ordinate: right-hand scale, g.; left-hand scales, lower: oxygen consumption, mm.^{3}/hr. in hundreds; upper: CO_2 produced, mm.^{3}/hr. in hundreds. Abscissa: age in days after deposition.

for oxygenation of its tissues. The possibility of post-deposition oxidation of hydrogen acceptors in the yolk or elsewhere, or of other mode of oxidation, is not remote in light of the fact that newly laid infertile eggs, eggs from which only the embryo was removed, and isolated masses of yolk were observed to take up oxygen at the rate of 150 mm.^{3}/hr. (egg weight, $5 \cdot 0 \pm 0 \cdot 5$ g.). The question of bacterial contamination existed, and inoculation of a variety of media from several parts of eggs was carried out. These proved entirely negative. Such bacteria as were found were from the egg surface and were, of course, present on normal eggs. (The author is indebted to Dr R. A. Cleverdon for these findings.)

Further evidence of the high rate of initial metabolism of the intact egg is provided in Fig. 2. The metabolic rate calculated on the basis of embryonic weight alone, or combined embryonic and extra-embryonic tissues, during the first 5 days, is not greatly different from that of the chick, developing at a temperature of 39° C. Thereafter, however, the respiratory rate is much less than that of the chick (Beyer, 1952). The reference to combined weight of embryo plus membranes in the same figure presumes the same rate of metabolism for both, and in this respect may be somewhat misleading, since in fact no data are available on the separate rates of the embryonic and extra-embryonic tissues.

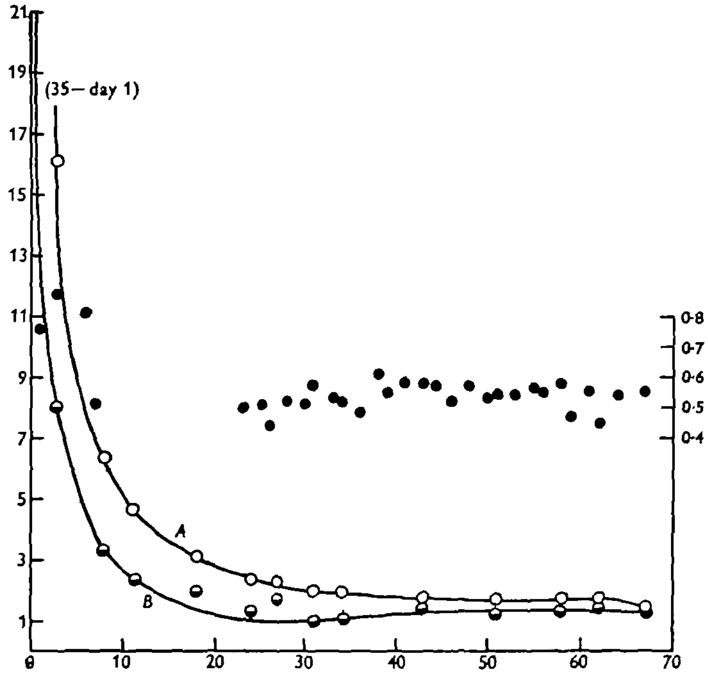

Fig. 2. Metabolic rate, based on embryonic weight (*A*) and combined weights of embryo and yolk-sac (*B*). Ordinate: mm.^{3} O_2/g./hr. in hundreds. Abscissa: age in days after deposition. Solid circles, R.Q. (ordinate on right-hand scale).

Carbon dioxide production. The curve of total CO_2 produced (Fig. 1) is shown in relation to O_2 consumed and embryonic growth. It is apparent that CO_2 production is initially relatively higher than the rate of O_2 consumption, and this is confirmed by the higher R.Q. values of days 1–8 (Fig. 2). This may reflect a utilization of carbohydrate as an energy source during the early part of development as suggested by Needham (1942). If supplemented by protein at this period, when ammonia is the end product, the R.Q. would also remain high.

Of particular interest is the persistently low R.Q. values from the 23rd day onward, having a mean value of 0·53. It was concluded from the study of excreted nitrogen that during the latter part of development protein (degraded to urea and later to uric acid) supplemented fat as an energy source. The maximal R.Q. which might be expected on the basis of combustion therefore would be approximately 0·7. The observed R.Q. may be accounted for by allocation to non-combustive processes of approximately 100 ml. of respiratory gases. The following possibilities suggest themselves: (1) ureotelic and uricotelic metabolism of protein, in which process potential CO_2 is incorporated in the excreted molecules; (2) oxygen used in pigment formation, which since it is consumed, increases the denominator of the fraction but does not lead to excretion of CO_2; (3) conversion of fat to carbohydrate, having a tendency to depress the R.Q. for the reason cited in item (2); and (4) the possible fixation of CO_2 in the form of organic acid or carbohydrate. Quantitative data are available only for the potential CO_2 of excretory products (approximately 5·0 ml.). Colorimetric estimation of liver glycogen of newly-hatched, juvenile and adult snakes by the method of Van der Kleij (1951) indicates that a substantially greater quantity is present in the newly hatched than in either of the two latter. Such data, though only suggestive, support the hypothesis of carbohydrate synthesis. It is hardly conceivable that more than 10% of the unaccounted gas can be concerned with pigment synthesis. The possibility of CO_2 fixation is supported by observations of several workers, of which Wood (1946) and Crane & Ball (1951) may be consulted for further references. Both found pigeon liver most effective, though other tissues also fixed CO_2; pyruvate is known to be involved, though the latter authors point out that there are other, unidentified avenues of incorporation of CO_2 in organic molecules.

These speculations do not constitute a solution to the problem, but possess the merit of amenability to experimental analysis.

SUMMARY

Data on exchange of respiratory gases are presented for the entire post-deposition period of development of the black snake. Of special note among the data are (1) an initial relatively high rate of O_2 consumption and CO_2 production, (2) an initially high R.Q., followed by a persistently low R.Q. (0·53).

Suggestions to account for the peculiarities are offered.

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