Respiratory Metabolism of Mammalian Eggs.* (22407)

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Little is known of the metabolic mechanisms of mammalian eggs although biochemical investigation of this tissue would seem to be of fundamental importance in the study of development and differentiation. Extensive investigations have been carried out on the eggs of several other species mainly marine animals. During the course of mammalian evolution, egg production has been reduced in number and confined to a specific phase of the sexual cycle. Consequently, metabolic studies on mammalian eggs have been very much restricted. Smith and Kleiber(1) reported that the oxygen consumption of rabbit eggs is greater than that of other eggs of similar size. Fridhandler *et al.*(2) have shown that there were little or no differences between the O_2 uptake of fertilized, unfertilized and activated (capable of subsequent cleavage) unfertilized rabbit eggs and the O_2 consumption of unfertilized ova was not depressed after remaining *in vivo* up to 57 hr *in situ*.

This paper deals with the changes in respiratory rate of rabbit eggs during development. Oxygen uptake of the early stages was measured in the Cartesian diver while that of the later stages was measured in the Warburg apparatus.

Materials and methods. Virgin rabbits weighing from 7 to 8 lbs were purchased from Rockland Farms. They were superovulated using sheep pituitary extract(3). The animals were sacrificed 0.5, 1.5, 2.5, 3.5, 5.5 and 6.5 days post-coitum. The tubes or uteri were flushed with Ca^{++}-free Krebs-Ringer phosphate pH 7.4 containing 0.1% glucose (4) "RPG." Bovine hyaluronidase (Wyeth) was used to separate the ova from any attached cumulus cells. In some cases the blastocysts were punctured and the blastocyst fluid washed out and in other cases the blastocyst was punctured and the cellular mass dissected from the zona pellucida. Only healthy ova, morulae and blastocysts were transferred to freshly prepared RPG. The respiratory rates of intact ova, morulae and 4-day-old blastocysts and punctured, dissected 5- and 6-day-old blastocysts were measured in the Cartesian diver. The Warburg respirometer was used to measure respiration in intact and punctured 6-day-old blastocysts. The Cartesian diver has been described by Linderstrøm-Lang(5,6) and Boell *et al.*(7) and Holter(8). It is capable of measuring gas exchange of less than one mμl. The ova, morulae or punctured blastocysts were pipetted into the divers using a braking pipette(8). The volume of the medium ranged from 0.7 to 1.0 μl whilst the volume of the gas space ranged from 5 to 15 μl and each diver contained 7 to 15 ova or one dissected blastocyst. Thirty to fifty 6-day-old intact or punctured blastocysts were transferred to Warburg flasks (fluid volume 1 ml) using freshly prepared RPG as medium. In all experiments, air was the gas phase and the temperature of the water bath was 35°C.

Results. The average O_2 uptake of the fertilized rabbit ova 16 hours post-coitum was 0.61 mμl/ovum/hr. Detaching the cumulus cells from the freshly shed ova was found to be necessary, since they respire at a high rate. A mass of cumulus cells roughly equivalent in volume to one ovum consumes O_2 at the rate of 2.5 mμl/hr. Smith and Kleiber (1) reported that the O_2 uptake of the fertilized one-cell rabbit ovum was 26 mμl/ovum/hr as measured in the Cartesian diver. The difference between their value and that reported here is too great to be attributed to

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strain, live weight or nutritional differences in the experimental material. It should be pointed out that their conditions (incubating medium, temperature and gas phase) were different from the present experiment. Dragoiu *et al.*(9) reported that the respiratory rate of cow's follicular eggs (with germinal vesicle) was 5000/mμl/egg/hr. This value probably represents the respiration of follicular cells of corona radiata predominantly, for, in the experiments cited, a single egg plus its halo of follicle cells had an average dry weight of more than 8 μg, a figure which is some 10 times larger than the estimated dry weight of a single bovine egg. Moreover in the cow's egg cumulus cells are present in the large follicles(10). In the rat, the O_2 consumption of follicular eggs and fertilized ova was 1.11 and 0.72 mμl/egg/hr respectively(11).

The average O_2 uptake of the fertilized ova 40, 65, 90 and 160 hr post-coitum was 0.51, 0.48, 2.56 and 200 mμl/hr/ovum. There was no increase in the O_2 uptake during the first 65 hr post-coitum *i.e.* up to the morula stage. There was a sudden rise in the respiratory rate at the early blastocyst stage (Fig. 1). This was definitely associated with the morphological changes which generally supervene by the 80th hr post-ovulation. If the morulae, however, failed to develop into blastocysts by the 80th hr post-ovulation, the respiratory rate remained essentially unchanged as compared with that of the one-cell stage.

In the Warburg apparatus, there was a

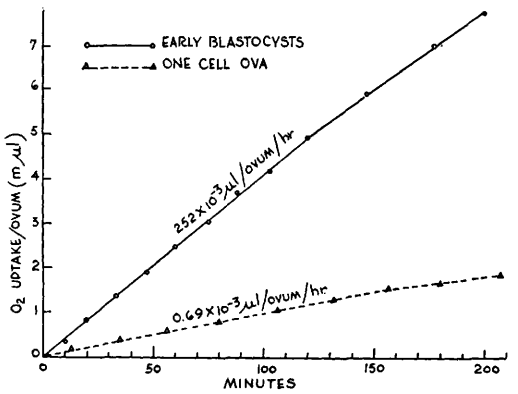

FIG. 1. O_2 consumption of one-cell ova and early blastocysts as measured in the Cartesian diver.

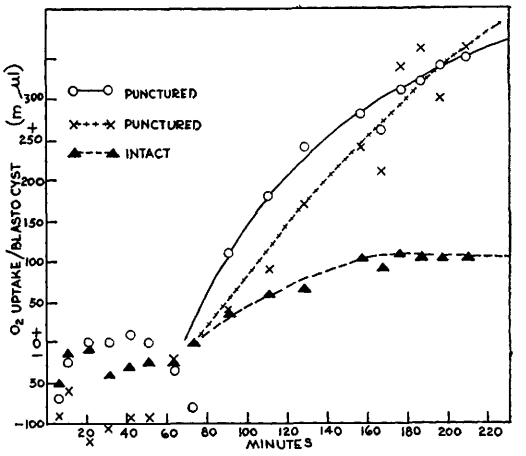

FIG. 2. O_2 consumption of rabbit blastocysts 160 hr post-coitum as measured in Warburg respirometer.

period of no apparent O_2 uptake in the first 30 minutes after the closure of all the vessels. This was followed by a simultaneous initiation of O_2 uptake in all the vessels. The rate was uniform but tended to decline with time. The initial rate was lower in the intact as compared with the punctured blastocysts (Fig. 2). In fact, in the intact blastocyst the respiration stopped entirely after 30 minutes. This is probably due to the high bicarbonate content of the blastocyst fluid which caused, in the presence of the CO_2 free atmosphere in the vessels, a rise in the pH to 9.5.

Five- and 6-day-old blastocysts were punctured, dissected from the zona pellucida, washed free of blastocyst fluid and split into 2 segments. The average O_2 uptake for the 5- and 6-day-old blastocyst was 400 and 800 mμl/blastocyst/hr respectively. The zona pellucida did not show any respiratory activity. It must be noted that the O_2 uptake was observed with no lag period in the divers in contrast with the apparent absence of O_2 uptake in the first 30 minutes in the Warburg vessels. As yet there is no apparent explanation for this difference in result.

The cellular mass of a dissected blastocyst was pipetted into a calibrated capillary tube to measure its approximate volume. Judging from this and from the diameter of the ovum the marked increase in the respiratory rate

(about 1600-fold) is not accompanied by a corresponding increase in the tissue mass. It is therefore suggested that a change in metabolism takes place at the early blastocyst stage; either an increase in the overall rate per unit of tissue mass or a shift of emphasis from one pathway to another. Boell and Nicholas(11) have suggested that the increase in respiration is intimately correlated with the increase in the volume of the egg. It must be remembered here that they worked in a narrow range of stages of development (one- to 16-cell stage). Moreover, the increase in egg volume does not necessarily represent an actual increase in dry weight. It may be mentioned here that water uptake occurs in eggs of other species during the transformation of yolk into active protoplasm(12-14) and that this process is associated with an increase in respiration.

Microscopic examination of the eggs after the respiratory measurements revealed that the morphological appearance was essentially normal. The critical test for viability by transplanting the eggs into the uteri of pseudopregnant rabbits was not performed. It has been found that the rabbit eggs do not cleave in RPG *in vitro* nor do they cleave in several synthetic media tried by Hafez and Pincus(15). Therefore the egg in RPG is not a developing organism, but a resting one and interpretation of results must be made with this in mind.

Summary. Respiration of fertilized rabbit eggs (intact, punctured or dissected) in different pre-implantation stages was measured in the Cartesian diver and Warburg apparatus. Average O_2 uptake of eggs 16, 40, 65, 90, 120, 140 and 160 hr post-coitum was 0.61, 0.51, 0.48, 2.56, 200, 400 and 800 mμl/ovum/hr respectively. The high respiratory rate of blastocysts was associated with the morphological changes yet the rate of increase was not directly proportional to cell mass increase. It is suggested that these pacts reflect a change in metabolic pathways. Intact blastocysts (in Warburg) respire at a lower rate than punctured ones due to the bicarbonate content of blastocyst fluid which raised the pH in the CO_2-free atmosphere. Cumulus cells respire at a high rate while the zona pellucida does not respire at all.

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