# Some Metabolic Properties of the Rabbit Oviduct.* (26543)

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The human endosalpinx displays some unusual metabolic characteristics *in vitro*(1). It resembles certain malignant tissues in that there is a substantial accumulation of lactic acid when it is incubated aerobically with glucose. In the present experiment some *in vitro* metabolic properties of the rabbit oviduct are explored. The rabbit offers the advantage that hormonal conditions can be controlled, and an accurate correlation between *in vitro* metabolic activity and the endocrinological status of the animal is possible.

*Materials and methods.* New Zealand White rabbits weighing 3-5 kg were used throughout. Animals were housed in separate cages and were given a diet of Purina chow and water *ad libitum*. Oviducts were removed under sodium pentobarbital anesthesia and placed immediately in cold Krebs-Ringer phosphate solution (K.R.p.). Specimens were freed of surrounding tissues by careful dissection, supported with pith and sliced transversely into thin sections. These were studied at 37°C in the Warburg apparatus in K.R.p. alone and with added substrates. After 2 hours' incubation, hexose disappearance and lactic acid accumulation were determined. Nelson's modification of the Somogyi method(2) was used for sugar determinations and the method of Barker and Summerson(3) for lactate determinations. The tissue in each flask was dried overnight at 106°C and weighed. Results were expressed as μg/mg dry weight. Intact non-pregnant animals, castrates and mated animals 16-72 hours following coitus were selected for study. In the intact non-pregnant group, no attempt was made to distinguish between anestrous and estrous rabbits. Those whose ovaries contained obvious corpora lutea were excluded. The castrates were ovariectomized 23-49 days prior to study. In mated animals, coitus was confirmed by recovery of spermatazoa from the vagina. Animals whose ovaries failed to show evidence of recent ovulation were excluded.

*Results.* Specimens from intact non-pregnant animals were studied under aerobic conditions in K.R.p. alone and with added glucose, fructose or succinate (Table I). Oxygen uptake in these media was approximately equal in the first and second hours, suggesting that tissue survival was not materially affected by the conditions of study. Oxygen uptake was unchanged by addition of glucose or fructose. It was increased 2-fold in presence of succinate. The mean lactic acid production in K.R.p. with glucose was nearly 10 times that in K.R.p. alone. This high lactic acid production was not observed with fructose or succinate. Uptake of glucose was approximately twice that of fructose.

The oviducts of 6 intact non-pregnant rabbits were studied anaerobically (Table I). Although mean glucose uptake was higher in $N_2$ then in $O_2$, the difference was not statistically significant. In K.R.p. with glucose and K.R.p. with succinate, lactic acid production was significantly higher in $N_2$ than it was in these media in $O_2$. Virtually all of the glucose taken up could be accounted for as lactic acid.

In the castrate group, oxygen uptake in the various media was not significantly different from that observed in intact animals (Table I). Glucose uptake was not significantly different in the 2 groups. Lactic acid production in glucose was significantly greater in the castrate group ($p<0.01$).

Animals studied at 16-25 hours, 43-49 hours and 64-72 hours after mating were so grouped (Table I). Oxygen uptake was not significantly different from that of intact non-pregnant rabbits. It remained essentially unchanged with time followig mating.

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TABLE I. Oxygen Uptake, Hexose Uptake and Lactic Acid Production of Oviducts Removed from Intact Animals, 23-49 Day Castrates, and Mated Animals 16 to 98 Hours after Coitus.

| | Oxygen uptake, $\mu$l/mg dry wt/2 hr ± S.E. | | | | Hexose uptake, $\mu$g/mg dry wt/2 hr ± S.E. | | Lactic acid production, $\mu$g/mg dry wt/2 hr ± S.E. | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | KRp | KRp + Glucose | KRp + Fructose | KRp + Succinate | Glucose | Fructose | KRp | KRp + Glucose | KRp + Fructose | KRp + Succinate |
| Intact animals* | 7.50 ± .63 (18) | 7.74 ± .54 (18) | 8.13 ± .67 (14) | 16.06 ± 1.10 (20) | 50.7 ± 4.3 (16) | 25.3 ± 3.5 (13) | 2.6 ± .5 (18) | 24.9 ± 1.4 (18) | 3.7 ± .4 (14) | 4.3 ± .6 (20) |
| *Idem** (in $N_2$) | — | — | — | — | 77.8 ± 12.8 (6) | — | 9.0 ± 1.3 (6) | 57.6 ± 4.6 (6) | — | 9.7 ± 1.0 (6) |
| 23-49 day castrates | 5.77 ± .66 (8) | 7.12 ± .22 (7) | — | 16.03 ± .57 (7) | 61.6 ± 3.9 (7) | — | 1.4 ± .2 (7) | 31.8 ± 1.3 (8) | — | 3.3 ± .4 (7) |
| 16-25 hr after mating† | 7.32 ± 1.09 (4) | 7.43 ± .40 (4) | — | 22.38 ± 2.97 (4) | 63.8 ± 14.8 (4) | — | 2.5 ± .2 (4) | 26.6 ± 1.8 (4) | — | 7.8 ± 2.0 (4) |
| 43-49 hr " † | 6.57 ± .50 (6) | 7.57 ± .52 (7) | — | 17.64 ± 1.10 (7) | 54.9 ± 5.8 (6) | — | 1.6 ± .4 (5) | 27.9 ± 5.5 (6) | — | 3.9 ± .8 (6) |
| 64-72 hr " † | 7.42 ± .77 (4) | 8.27 ± .65 (4) | — | 19.33 ± 1.34 (3) | 60.0 ± 12.8 (4) | — | 3.4 ± .9 (4) | 32.9 ± 1.8 (4) | — | 6.1 ± 2.1 (4) |

* Those with ovaries containing recent corpora lutea excluded.
( ) = No. of animals for which final values were obtained.
† Ovaries of all contained recent corpora lutea.

There was no significant difference in glucose uptake between mated and unmated animals. Among the mated animals, analysis of variance revealed no difference in glucose uptake with time following mating.

Lactic acid production in K.R.p. alone and K.R.p. with succinate was essentially the same for mated and unmated animals and it did not vary significantly in the interval following mating. In the presece of glucose, lactic acid production increased in the third 24-hour period after mating. This change was statistically significant by analysis of variance ($p<0.01$).

*Discussion.* When fresh specimens of human endosalpinx were incubated with glucose under aerobic conditions, 60% of the glucose disappearing from the medium at the end of 2 hours could be accounted for as lactic acid (1). Oxygen uptake was depressed significantly by addition of glucose to the medium. The human oviduct was stripped of serosa and muscularis, whereas the rabbit specimens in the present study included the whole oviduct.

The oviduct of the rabbit, like that of man, utilizes the anaerobic route of metabolism preferentially when incubated aerobically with glucose. Unlike the human endosalpinx, the rabbit oviduct takes up oxygen equally well in K.R.p. and K.R.p. with added glucose. When studied in nitrogen, the ability of the rabbit oviduct to utilize the anaerobic route is illustrated further.

The rate of anaerobic glycolysis becomes greater in the third day after successful mating. In this interval oxygen uptake and glucose uptake remain unchanged, while lactic acid production is increased.

The secretions of the rabbit oviduct contain rather large amounts of lactic acid(4). A significant increase in lactic acid concentration occurs during the first 3 days of pregnancy(5). Diffusion of lactic acid from the tissues of the oviduct into the tubal lumen could account for its presence in tubal fluid. The increased lactic acid production *in vitro* coincides in time with the increased concentration in tubal fluid collected continuously *in vivo*. Lactic acid is essential to the devel-

opment of the 2-cell mouse ovum when cultured *in vitro*(6), and certain mammalian spermatazoa are capable of utilizing lactate for energy under aerobic conditions(7).

*Summary.* The oviducts of non-pregnant intact rabbits, castrates and mated rabbits 16-72 hours after coitus were studied in the Warburg apparatus. In the intact, non-pregnant group, $O_2$ uptake was essentially the same in Krebs-Ringer phosphate solution (K.R.p.) alone, K.R.p. with glucose and K.R.p. with fructose. It was increased 2-fold by addition of succinate. Lactic acid production was substantially higher in glucose than in fructose or in K.R.p. alone. At the end of 2 hours, glucose uptake was 50.7 ± S.E. 4.3 $\mu$g/mg dry weight of tissue and lactic acid production was 24.9 ± S.E. 1.4 $\mu$g mg dry weight of tissue. Under anaerobic conditions virtually all of the glucose taken up could be accounted for as lactic acid. In castrates, $O_2$ uptake in various media and glucose uptake were not significantly different from those observed in intact, non-pregnant animals, but lactic acid output in glucose was higher. Among mated animals $O_2$ uptake was similar, and it did not vary significantly among specimens studied from 16-72 hours after mating. Lactic acid production in glucose was increased in the third 24-hour period following mating ($p<0.01$), indicating an increased utilization of anaerobic pathways during this interval.

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1. Mastroianni, L., Winternitz, W. W., Lowi, N. P., *Fertil. and Steril.*, 1958, v9, 500.
2. Nelson, N., *J. Biol. Chem.*, 1944, v153, 375.
3. Barker, S. B., Summerson, W. M., *ibid.*, 1941, v138, 535.
4. Bishop, D. W., *Internat. J. Fertil.*, 1957, v2, 11.
5. Mastroianni, L., Wallach, R. C., *Am. J. Physiol.*, 1961, in press.
6. Whitten, W. K., *Nature*, 1957, v179, 1081.
7. Mann, T., *Biochemistry of Semen.* London Methuen and Co., London, 1957, 53.

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## Inoculation of Chicken Sarcoma Virus into Chicken Thymus. An Electron Microscopy. (26544)

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It has been reported that in the chicken sarcoma, when the tumor is transplanted subcutaneously(1,2,3,4) or intraperitoneally(5), the virus particles identifiable by electron microscope are about 50 or less per single cell section. This number has been somewhat increased by X-ray irradiation(6). Bernhard's recent report deals chiefly with extracellular viruses of Rous sarcoma(7).

We found recently that a significantly large number of virus particles was attached to the tumor cell when virus suspension was injected into the thymus.

The sarcoma of Chiba strain(8), which is 100% transplantable, was used in this experiment. A 10 g piece of tissue taken from the tumor, implanted subcutaneously 15 days before, was homogenized with a glass homogenizer in 50 cc of physiological saline and centrifuged at 4,500 rpm for 20 minutes. One ml of the supernatant virus suspension was injected into the 2nd or the 3rd thymus of 120- to 150-day-old male chickens. A tumor of myxosarcomatous pattern developed in the interlobular stroma of the thymus at site of injection, reached a macroscopic size 4 days after injection, and increased in size subsequently.

Virus particles were first detected in the tumor 4 days after injection. They were round or ovoid, 70 to 85 m$\mu$ in diameter, and consisted of a central nucleoid body, 30 to 40 m$\mu$ in diameter, surrounded with a single or double membrane (Fig. 2).