16141 P

Relation between Time of Fertilization and Follicle Cell Dispersal in Rat Ova.*

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Following the discovery that the enzyme hyaluronidase from sperm disperses the follicle cells of recently ovulated mammalian ova, a concept has arisen that the action of this enzyme facilitates fertilization by denuding the ova prior to or simultaneously with sperm entry.[1-4] The complete cell dispersal seen when mammalian ova are denuded by hyaluronidase *in vitro*[1,3] and the observation of Gilchrist and Pincus[5] who state "the freeing of adherent follicle cells is an inevitable accompaniment of sperm penetration..." have undoubtedly promulgated this concept. Observations to be reported in this note indicate no mass removal of the follicle cells prior to fertilization and that sperm penetration precedes the gross denudation of the ovum in the rat.

Methods. Female rats were bred and 12-26 hours later the ova were removed from the oviducts. The ova were examined in a depression slide for the disposition of the follicle cells and then transferred to a microscope slide to determine if fertilization had occurred. Hyaluronidase (0.1% solution) was drawn under the coverglass to remove the adhering follicle cells when it was necessary to observe the ovum proper.

In another experiment, 0.2 cc of hyaluronidase from bull or rat testes, in concentrations of 30-60 turbidity reducing units per cc, was introduced into each horn of the uterus of a rat in heat and the horns ligated near the cervix to prevent leakage. The enzyme was dissolved in either Ringer's solution or in the uterine fluid which was removed before administering the enzyme.

Experimental. In the first experiment, the ova of 9 rats were observed to be covered with follicle cells and remained in a compact mass when removed from the oviduct. They were similar in appearance to ova recovered from non-bred rats (100 cases). The ova proper could not be dissected free of their follicle cells with dissecting needles. After adding hyaluronidase, which removed the adhering follicle cells from the 65 ova recovered, spermatozoa were identified within the perivitelline space and polar bodies were present in every instance. These fertilized ova were obtained 12-16 hours post-coitus.

From 10 other rats, 31 fertilized ova were recovered, and they were either partially or completely denuded of their follicle cells. A jelly-like matrix surrounded the ova in several instances, but the follicle cells were few and scattered. Dead and motile sperm also were observed in this jelly. The denuded fertilized ova were recovered in two instances 13 hours post-coitus; the remainder at 16-26 hours.

Following the introduction of hyaluronidase into both uterine horns of 12 rats in heat, masses of ova were recovered in every instance 18-24 hours later. In not one case were the ova denuded. Addition of the uterine fluid of these rats to their own ova, *in vitro*, induced complete denudation in 10-20 minutes in all trials.

The results indicate that sperm make their way between the follicle cells and fertilize the ovum before any visible dispersal of the follicle cells occurs. Somewhat later the follicle cells are dispersed, freeing the ova. Furthermore, our observations indicate that

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2 Joël, C. A., and Eichenberger, E., *Schweiz. Med. Wchnschr.*, 1945, Nr. **27**, 601.

3 Leonard, S. L., and Kurzrok, R., *Endocrinology*, 1945, **37**, 171.

4 Swyer, G. I. M., *Biochem. J.*, 1947, **41**, 409.

5 Gilchrist, F., and Pincus, G., *Anat. Rec.*, 1932, **54**, 275.

the bulk of the sperm and hyaluronidase remain in the uterus following copulation and no mass movement of the enzyme from the uterus into the tubes seems to play a part in the process of denudation and fertilization. We are of the opinion, however, that hyaluronidase is produced by those sperm which gain access to the tubes, and with the aid of this enzyme, the sperm traverse the follicle cell mass to fertilize the ovum. Later the concentration of enzyme is sufficient to denude the ova as was observed 24 hours post-coitus.

Conclusion. Fertilization of the rat ovum occurs before mass displacement of the surrounding follicle cells; denudation of the ova occurs subsequently. Since hyaluronidase, introduced into the uterus, does not pass into the tubes to denude the ova, it seems that only the enzyme associated with the sperm which reach the oviduct disperses the follicle cells.

16142

Influence of *M. varians* on Oral Infectivity of Mouse-Hamster (M-H) Virus.*

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During the course of experiments on the susceptibility of white Swiss mice (Webster strain) to orally-administered M-H (Mouse-Hamster) virus, an unexplained reduction in the usual mortality of the mice occurred. During the period of January 5 to June 15, 1946 eight experiments involving 214 mice were conducted. The mortality to an orally-administered, highly concentrated mouse-brain suspension of the M-H virus in these 8 experiments varied between 48 and 80%, or an average mortality of 60%. Quite unexpectedly on July 26, the administration of the same dose of the M-H virus that had been used in the previous experiments now produced a mortality of only 20%. No explanation for this reduced mortality was available at that time as the virus end-point by intracerebral or foot-pad inoculation had not altered as compared with the previous 8 experiments. This reduced mortality to the M-H virus occurred again on December 21, 1946 (Experiments 12, 13, Table I). Thus, in the 13 experiments conducted during this year, there were only 2 in which the mortality to the usual dose of the virus was less than 44%-80%, and these mortalities were only one-half to one-fourth of the lowest mortality previously achieved in the other 11 experiments. A review of the protocols of the 13 experiments and the method of preparation of the virus used in the oral feeding experiments shed some light on the problem.

Up to May 15th, consecutive passages of the M-H virus by intracerebral and foot-pad inoculation into the mouse had been performed until a seventh-passage virus was obtained on March 20, 1946. Contemplating a large number of experiments, subsequent to April, 1946, 200 mice were obtained and were inoculated subcutaneously into the foot-pad with a fatal dose of the seventh passage M-H virus. The brains were harvested when paralysis or death occurred, and a 25% emulsion in Ringer's solution was prepared. This total volume of the brain emulsion was then divided into 10 equal portions and stored in sterile lusterloid tubes in the CO_2 icebox at -40° C until needed in the future. Thus, the first experiment conducted with one of the samples of this new batch of virus was done in May, 1946, approximately 4 days after

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