LETTERS TO THE EDITORS

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X-Irradiation of Rabbit Spermatozoa *in vitro*

THE classic experiments of Hertwig on the X-irradiation of frog spermatozoa were recently extended by Rugh[1], who reported that exposure of the spermatozoa to doses from 15 r. to 10,000 r. caused a progressive increase in embryonic mortality; at the higher dosage only 1·6 per cent of the embryos hatched. Further increase of the dosage, however, increased the number of viable embryos; at 50,000 r., 90·5 per cent hatched. These embryos were morphologically uniform and very similar to haploids produced by other means. Rugh concluded that with the higher doses the spermatozoa, which showed normal motility, were able to penetrate the egg, but were unable to effect syngamy, so that the development initiated was parthenogenetic.

Comparable effects with the small doses have been demonstrated in mammals. Doses of 800 r. to mice[2] and 1,000 r. to rats[3] do not diminish the capacity of the existing spermatozoa to effect fertilization, but early mortality among the embryos reduces litter size. The mammalian work on X-rays, with the solitary exception, so far as we know, of some inconclusive experiments by Asdell and Warren[4], has involved the mating of irradiated males, a technique which has several disadvantages. Pincus and Enzmann[5], however, carried out a few experiments on rabbits by inserting into the Fallopian tube spermatozoa which had been exposed *in vitro* to ultra-violet irradiation, and obtained some rather inconclusive evidence of penetration and activation of the eggs without syngamy. We have now carried out, also on rabbits, experiments involving the *in vitro* X-irradiation of semen, followed by artificial insemination. In addition, the induction of superovulation by the injection of gonadotrophin has proved most useful in augmenting the number of eggs used for the study of the segmentation stages.

The females were killed and the tubal or uterine contents examined at various times up to 40 hr., at 9 days, or at 28 days after ovulation. Results with the various dosages were briefly as follows:

50 *r. and* 100 *r.* The great majority of the eggs segmented normally and normal implantations at 9 days and normal young at term were also obtained.

250 *r. and* 500 *r.* Most of the eggs were segmenting normally at 40 hr., but there was a definite increase in the percentage of those which were retarded or arrested. Normal implantations and apparently normal young at birth were obtained, but in small numbers.

1,000 *r.* The great majority of the eggs had started to divide but had no more than 10 cells at 40 hr.; less than 3 per cent were apparently normal. No implantations at 9 days or young at term were obtained with this or any higher dose.

2,500 *r.*–10,000 *r.* More than 70 per cent of the eggs recovered at 40 hr. had started to segment, but most of these were in the 3- or 4-cell stage. None was segmenting normally.

25,000 *r.*–50,000 *r.* Most of the eggs had made a single division only at 40 hr., and were arrested in the 2-cell stage.

100,000 *r.* Only 8 of the 70 eggs obtained at 40 hr. had divided, and 6 of these were in the 2-cell stage.

Cytological examination was made of many of the eggs fertilized by spermatozoa receiving between 500 r. and 100,000 r. The presence of male nuclei within the cytoplasm of the egg showed that penetration had been effected at all dosages. Spermatozoa were abundant in the perivitelline space and zona pellucida at all dosages up to 50,000 r., but at 100,000 r. very few were seen. Delay in migration and vesiculation of the male nucleus was observed at all doses from 1,000 r. to 10,000 r. and constituted the earliest indication of any organic change caused by irradiation of the spermatozoa. Cytological evidence of damage to the male nucleus appeared only with doses of 10,000 r. and above. At lower doses the nuclei were single and oval or spherical, while with the higher doses the majority were hypertrophied and lobed.

At the stage of fusion of the pronuclei the effects of various dosages may be summarized as follows:

1,000 *r.* Syngamy was complete with no outward sign of irregularity in the male pronucleus.

10,000 *r.* Syngamy took place, but the male pronucleus was very irregular and hypertrophied, and the chromosomes remained dispersed.

25,000 *r.* Syngamy might take place, but in some eggs the occurrence of polyspermy produced derangement in the distribution of the chromosomes. Cytoplasmic division and separation in these cells might be incomplete.

50,000 *r.* Syngamy occurred, but the formation of the male chromosomes was delayed or suppressed, so that zygotic combination was weakened. Because of the delay in polar migration of the chromosomes the cleavage spindles were irregular.

100,000 *r.* Extrusion of the polar bodies and migration of the male nucleus was observed, but not syngamy in any of the eggs studied. However, the recovery of 2-celled eggs with diploid chromosomes showed that the absence of syngamy may not be constant. The occurrence in one egg of a single segmentation spindle with haploid chromosomes suggested that spermatozoa exposed to 100,000 r. can sometimes activate the egg without fusion of the pronuclei. No evidence of continued gynogenetic development was, however, obtained.

A full account of the experiments outlined above is being prepared, but it may be noted here that the technique of semen collection, artificial insemination and, where appropriate, induction of superovulation, offers exceptional facilities for the type of work described above, and is being used by us for the study of the effects of chemical and serological as well as of physical agents on the physiological and genetic properties of mammalian sperm.

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