BRIEF COMMUNICATION

THE EFFECT OF X-IRRADIATION OF RABBIT SPERM-
ATOZOA *IN VITRO* IN DIFFERENT GASES ON EGG
DEVELOPMENT AND SPERM DEHYDROGENASES

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The insemination of mammalian spermatozoa which have been treated *in vitro* with X-rays at a dose level of 400 to 800 r results in failure of a large number of eggs to develop beyond the blastocyst stage in rabbits (Amoroso & Parkes, 1947; Chang, Hunt & Romanoff, 1957); mice (Edwards, 1957) and cattle (Rowson & Dott, 1964). Higher doses of X-rays caused development of the eggs to fail at earlier stages (Amoroso & Parkes, 1947; Chang *et al.*, 1957; Edwards, 1957). Yet the metabolism and motility of irradiated spermatozoa appear to be unaffected even by much higher doses of X-rays, at least in those species which have been examined (cf. Mann, 1964). It seems likely that it is the deoxyribonucleic acid (DNA) in the spermatozoa which is affected by X-irradiation rather than their metabolism, motility or fertilizing capacity.

X-rays can, presumably, act on DNA either directly by virtue of their ionizing activity on DNA molecules, or indirectly by producing free radicles. Two of the methods which have been used to protect biological material from the indirect action of ionizing radiation are: (i) performing the irradiation experiment at a low temperature; and (ii) irradiating under anaerobic conditions. The first of these methods was used by Rowson & Dott (1964) in their experiments with bull semen; the results indicated that X-irradiation with 500 r caused less damage if carried out at -79°C than at $+4^{\circ}\text{C}$. However, additional experiments (Rowson & Dott, unpublished) failed to confirm this preliminary result and cast some doubt on the value of the low temperature method as a means of protecting spermatozoa effectively against ionizing radiation. In the present study the second method was used.

Rabbit semen was collected by means of an artificial vagina and diluted with 0.9% NaCl so as to contain 100×10^6 spermatozoa/ml. The diluted semen was then divided into six samples and put into six glass tubes which were filled in pairs with nitrogen, air or oxygen for 10 to 12 min. The oxygen tension was checked with a platinum electrode. The six tubes were sealed and one tube from each of the three pairs was exposed to 800 r at 100 r/min, the other served

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as a control. After irradiation, 0.5 ml (50×10^6 spermatozoa) samples were inseminated into does which were then intravenously injected with 90 i.u. HCG to induce ovulation. The does were killed either 2 or 6 days after insemination and the eggs recovered and examined as described by Chang *et al.* (1957).

The results of inseminations are given in Table 1. They agree closely with those previously reported by Chang *et al.* (1957), namely that the insemination of spermatozoa after X-irradiation *in vitro* with 800 r in the presence of air did not affect the proportion of eggs fertilized, but the number of normal blastocysts produced was distinctly reduced. Furthermore, neither the proportion of eggs fertilized nor the proportion of normal blastocysts produced was altered by replacing the air with either oxygen or nitrogen. This might be taken as an

TABLE 1

RESULTS OF INSEMINATIONS WITH RABBIT SPERMATOZOA WHICH HAD BEEN IRRADIATED *in vitro* IN THE PRESENCE OF NITROGEN, AIR OR OXYGEN

Gas phase at irradiation	Nitrogen		Air		Oxygen		Total (all gases)	
Irradiation dosage (r)	0	800	0	800	0	800	0	800
Time between insemination and examination (days)	2 6	2 6	2 6	2 6	2 6	2 6	2 6	2 6
No. of animals inseminated	5 6	6 5	6 5	6 7	6 5	6 5	17 16	18 17
No. of corpora lutea	65 78	61 60	84 50	62 82	65 59	62 54	214 187	185 196
No. of eggs recovered	61 73	52 45	69 43	46 62	55 46	54 50	185 162	152 157
% of eggs that had cleaved	84 95	83 89	87 86	93 60	88 87	72 94	86 90	82 79
% of eggs which developed into normal blastocysts	55	15	68	24	48	17	56	19

indication that the damage suffered by spermatozoa at irradiation results from a direct ionizing action of X-rays on DNA.

On three occasions the diluted semen was incubated at 37° C with nitro-blue tetrazolium (Borden Chemical Co.) and appropriate substrates to demonstrate the dehydrogenase activity against succinate, ethanol, lactate, α -glycerophosphate and glutamate (Hess, Scarpelli & Pearce, 1958a, b). After incubation the colour intensity of the reaction produced in the midpiece of the spermatozoa was estimated by examining the spermatozoa under an oil immersion objective ($\times 1000$). No difference was noted between spermatozoa from the irradiated and control samples. These results are in accordance with the fact that even much higher doses of irradiation do not affect the metabolism of spermatozoa (cf. Mann, 1964).

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