BRIEF COMMUNICATION

ABNORMAL FERTILIZATION OF RAT EGGS AFTER
INJECTION OF SUBSTANCES INTO THE AMPULLAE
OF THE FALLOPIAN TUBES

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Summary. The fertilization of rat eggs after various treatments of, or the injection of various substances into, the Fallopian tubes just before fertilization was studied. After all treatments, an increase was observed in the number of eggs which contained supplementary spermatozoa and in the numbers of supplementary spermatozoa per egg. Most treatments produced a small number of eggs in which a second spermatozoon entered the vitellus later than the first, and these eggs were said to be 'refertilized'. Several other abnormalities were produced by the treatments.

After the injection of 1 μ l of M/500 versene into the ampullae of Sherman rats 2½ to 3 hr after mating, Piko (1961) found that 46·2% of the eggs recovered 20 to 50 hr later had supplementary spermatozoa in the perivitelline space, compared with 22·6% of control eggs. Moreover, in 9% of these eggs 'refertilization' had occurred.

In the present paper 'refertilization' is taken to mean that appearances indicated a second and later sperm entry. The work of Piko was repeated and experiments were carried out to test the specificity of versene in the refertilization phenomenon. The effects of various solutions were tested and observations were also made on eggs collected from untouched ampullae (the untouched control series), from ampullae into which a needle had been inserted without injection and from ampullae which had been withdrawn through the opening in the body wall and replaced. Hooded rats were used, fed on Oxoid 41B rat pellets and kept under 14 hr of light by controlled lighting. They were mated between 08.30 and 09.30 hours; this results in 'delayed mating' as described by Austin & Braden (1954a), who showed that sperm penetration occurred 2 to 6 hr later. Immediately after mating various experiments (see Table 1) were carried out under ether anaesthesia which lasted less than 5 min and therefore precluded the danger of parthenogenic effects from the anaesthesia (Austin & Braden, 1954b). The ovarian capsule was

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TABLE 1
OBSERVATIONS MADE 24 HR AFTER THE INJECTION OF 2 μ l OF DIFFERENT SOLUTIONS INTO THE AMPULLAE OF HOODED RATS IMMEDIATELY AFTER MATING COMPARED WITH VARIOUS CONTROL EXPERIMENTS

	<i>Versene in physiological saline</i> M/500	<i>Isosmotic CaCl₂.6H₂O</i> (2.6% w/v)	<i>Isosmotic NaCl</i> (0.8% w/v)	<i>Tyrosine solution</i>	<i>Isosmotic glucose</i> (11.1% w/v)	<i>Distilled water</i>	<i>Uterine fluid</i>	<i>Control experiments</i>		
								<i>Untouched ampullae</i>	<i>Needle inserted into Fallopian tubes</i>	<i>Fallopian tubes withdrawn and replaced</i>
Total rats	45	26	24	23	23	17	13	51	28	28
Total eggs	236	148	127	108	102	91	40	220	81	63
Unpenetrated eggs	53 (22.5%)	41 (28.0%)	59 (46.5%)	31 (28.7%)	16 (15.7%)	26 (28.6%)	15 (37.5%)	25 (11.4%)	28 (34.6%)	13 (20.6%)
Eggs with supplementary spermatozoa	53 (22.5%)	42 (28.4%)	36 (28.3%)	38 (35.2%)	32 (31.4%)	31 (34.1%)	11 (27.5%)	21 (9.5%)	26 (32.1%)	26 (41.3%)
'Normal' dispermic eggs	13 (5.6%)	10 (6.7%)	7 (5.5%)	8 (7.4%)	10 (9.8%)	6 (6.6%)	1 (2.5%)	7 (3.2%)	6 (7.4%)	2 (3.2%)
Refertilized eggs	7 (3.0%)	14 (9.5%)	3 (2.4%)	3 (2.8%)	1 (1.0%)	3 (4.4%)	None	None	1 (1.3%)	None
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punctured with a $\frac{1}{2}$ in. 17 gauge needle to allow injection into the ampulla. Eggs were recovered 24 hr later and their manipulation was as described by Austin (1961).

From Table 1 it can be seen that injection of all substances (except uterine fluid, which was only tried in a small series) induced between 1.0 and 9.5% refertilization. There was a marked increase in numbers of eggs with supplementary spermatozoa in the perivitelline space and in the numbers of supplementary spermatozoa compared with the untouched controls (Table 2). A general increase in the incidence of 'normal' dispermic eggs was also found which probably resulted from excess spermatozoa in the perivitelline space at the time of penetration. Both male pronuclei in these eggs appeared to be at the same stage of their development, thus the term 'normal' dispermic is used to describe these eggs. Mechanical disturbance of the environment probably caused the increase of unpenetrated eggs after various treatments.

TABLE 2
SAMPLE DISTRIBUTION OF SUPPLEMENTARY SPERMATOZOA IN EGGS FROM
UNTOUCHED AND TREATED AMPULLAE OF THE RAT

	Total eggs	No. of supplementary spermatozoa					
		1	2	3	4	5	6 to 10
Eggs from untouched ampullae	30	25	4	1	0	0	0
Eggs from treated ampullae	30	13	6	5	3	1	2

Commonly a refertilized egg contained two or more sperm tails, and toluidine blue staining showed a normal first cleavage metaphase group of chromosomes and one or more dark oval masses, as described by Piko. The dark masses were also found in stained eggs with normal male and female pronuclei, but with an extra sperm tail in the vitellus. After staining, eggs became detached from the glass and irregular surfaces prevented satisfactory photography. The darkly staining masses were presumably chromatin material from the degenerating heads of refertilizing spermatozoa. Occasionally the additional sperm head was enclosed in a curious vacuole (Pl. 1, Fig. 1).

Other abnormalities were observed after various treatments. Several eggs were unpenetrated although there were spermatozoa in the perivitelline space. In five of forty eggs observed after uterine fluid treatment, the tail of the fertilizing spermatozoon was only partly incorporated into the vitellus, although normal pronuclear development was proceeding (Pl. 1, Fig. 2). Subnuclei were found in about 3% of fertilized eggs after all the treatments except versene. The formation of subnuclei is an indication of abnormal development and was reported by Austin & Braden (1954b) after many different treatments of rodent eggs.

Both mock treatments caused an increase in eggs with supplementary spermatozoa compared with controls, and one refertilized egg was found in this small series. Alteration of temperature, pH or oxygen tension had probably occurred.

These results suggest that a delicate balance of factors is essential for normal fertilization reactions to proceed. The refertilization phenomenon is influenced by various factors, although these different factors may all operate by producing the same effect, such as an alteration in calcium balance in the plasma membrane.

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PLATE 1

FIG. 1. Part of an egg refertilized after treatment with distilled water. This egg contained three sperm tails, indicating the entry of three spermatozoa. This figure shows one of two male pronuclei of equal size which were present in the vitellus, two of the three sperm tails and, on the right-hand side, an abnormal vacuole-like 'pronucleus'. There was also a small female pronucleus.

FIG. 2. Abnormal development of the head of a spermatozoon which was only partially incorporated into the vitellus of an egg after treatment with uterine fluid.