ANOMALIES IN RAT, MOUSE, AND RABBIT EGGS

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[*Manuscript received June* 30, 1954]

Summary

Records were kept of 7284 rat eggs, 1120 mouse eggs, and 221 rabbit eggs. Amongst these were 22 rat eggs, 21 mouse eggs, and 1 rabbit egg that were unusual: "giant" eggs of about twice the normal volume, primary oocytes recovered from the fallopian tubes, probable instances of gynogenesis and parthenogenesis, eggs showing abnormalities of fertilization such as the presence of a single pronucleus or of a male and two female pronuclei, and 2-cell eggs with a binucleate blastomere. These anomalies are discussed and attempts are made to explain their occurrence.

I. Introduction

During the study of fertilization in rat, mouse, and rabbit eggs, a variety of spontaneously occurring anomalies were encountered. The commonest was polyspermy, and this has been made the subject of two previous communications (Austin and Braden 1953*a*, 1953*b*). The purpose of the present paper is to describe some of the rarer anomalies that have been seen. In all instances the eggs appeared viable and showed no sign of imminent degeneration.

II. Methods

Both rat and mouse eggs were recovered from the fallopian tubes, and then examined by phase-contrast microscopy, in the manner described by Austin and Smiles (1948). Ovulation was induced in immature rats by the method of Rowlands (1944), except that B.D.H. "Serogan" and "Gonan" were used as sources of gonadotrophins.

Rats were mated by two different procedures, referred to as "normal" mating and "delayed" mating. For normal mating, the females were caged with males overnight from the early afternoon and examined the following morning for copulation plugs. For delayed mating, oestrous females were placed with males for ½-1 hr at 10 p.m., 3 a.m., 7 a.m., or 11 a.m. and examined for copulation plugs. It has been shown that ovulation occurs in this colony between midnight and 4 a.m. (Austin and Braden 1954*b*).

III. Observations

(*a*) Rat Eggs

Eggs were obtained from the fallopian tubes of a total of 717 rats, of which 241 were normally mated adult rats, 370 were delay-mated adults, and 106 were

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TABLE 1

DISTRIBUTION OF ANOMALOUS EGGS OBSERVED IN RATS, MICE, AND RABBITS

	Mating	Number of Animals	Total Eggs	Anomalous Eggs						
				"Giant" Eggs	Primary Oocytes	Gynogenetic Eggs	Parthenogenetic Eggs	Abnormal Fertilization	Binucleate Blastomere	Total
Rats	Normal	241	2064	3	2	1	0	1	0	7
	Delayed	370	3481	5	3	1	2	1	1	13
	Normal (immature rats)	106	1739	1	1	0	0	0	0	2
	Totals	717	7284	9	6	2	2	2	1	22
Mice	Normal and delayed	147	1120	1*	15	0	0	4	1	21
Rabbits	Normal and delayed	27	221	1	0	0	0	0	0	1

*This egg was also a primary oocyte.

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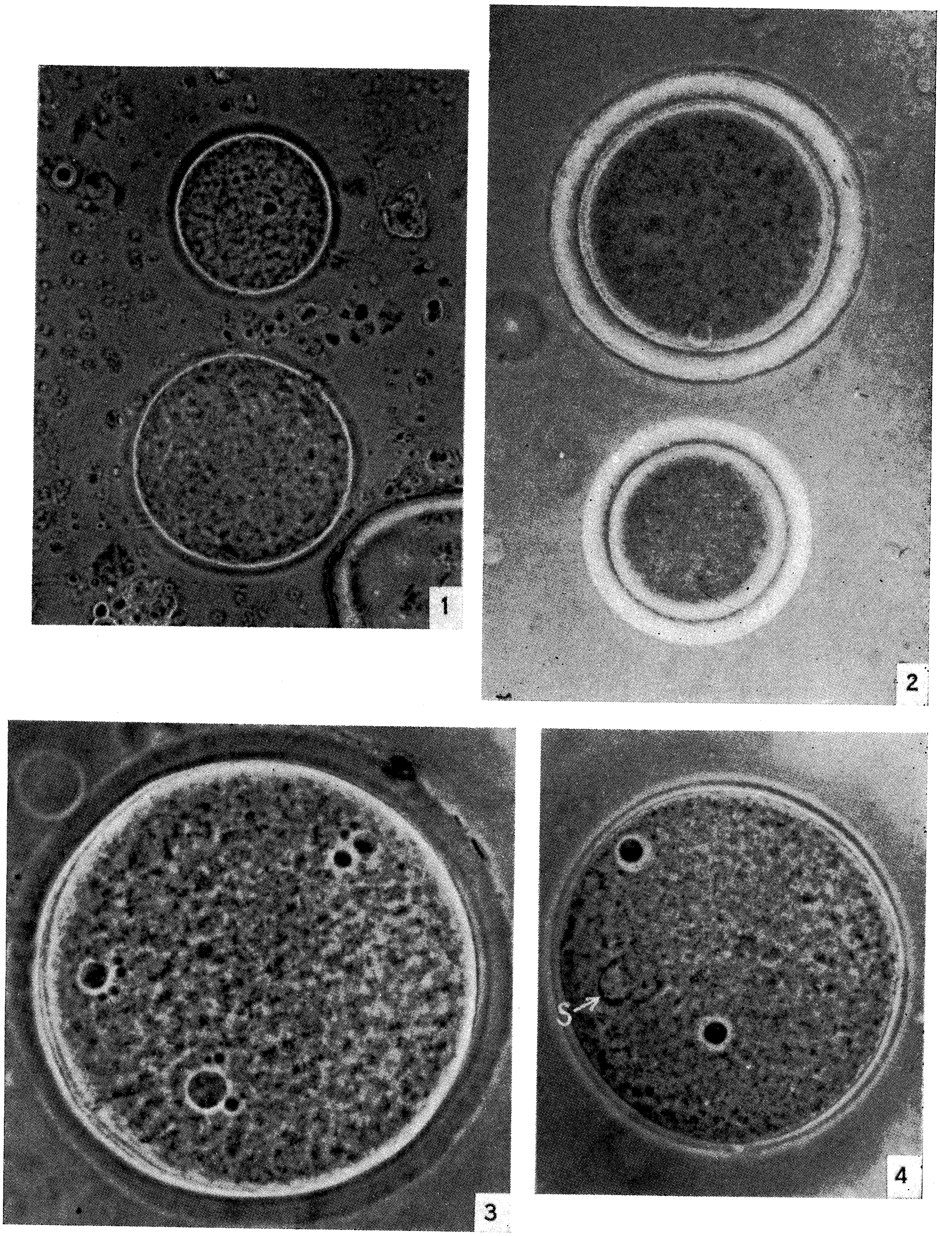

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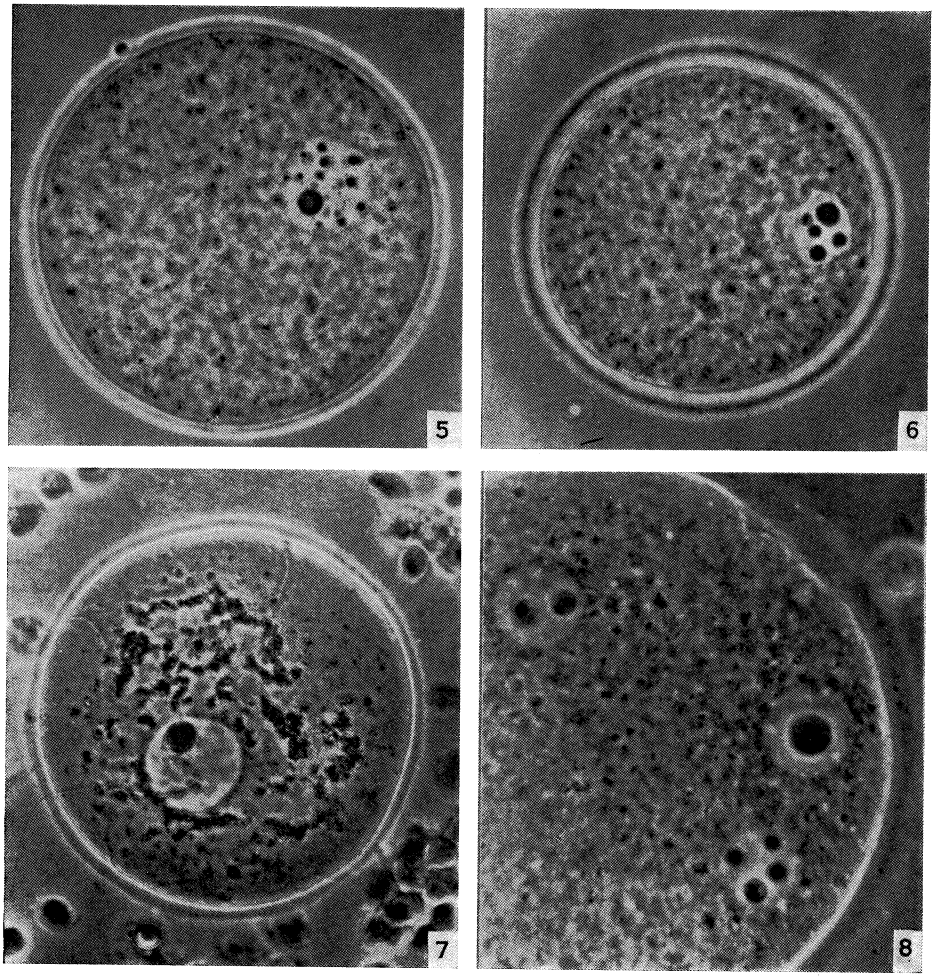

normally mated immature rats. The total number of eggs recovered was 7284 and amongst these there were 22 eggs that presented unusual features (Table 1).

(i) *Giant Eggs*.—Nine "giant" eggs were seen; they were quite distinctive, for their diameter was about 1·3 times that of normal eggs so that their volume was about twice that of normal eggs (Plate 1, Fig. 1). Seven of the "giant" eggs contained sperms and were undergoing fertilization. Two of these were polyspermic (dispermic), and one was monospermic but had two female pronuclei (Plate 1, Fig. 3); in the remaining four eggs the process of fertilization appeared to be quite normal. A single metaphase chromosome group was observed in the two unpenetrated "giant" eggs, but the chromosome number could not be determined.

(ii) *Primary Oocytes*.—The primary oocyte was easily recognized by its large spherical nucleus, usually with only a single large nucleolus, and by the characteristic texture of the cytoplasm. Six oocytes were recorded in the present series, two from the normally mated rats, three from the delay-mated, and one from the immature rats. One oocyte had been penetrated by six sperms which all lay in the perivitelline space, none having entered the vitellus.

(iii) *Early Gynogenesis*.—Two eggs appeared to be undergoing gynogenetic development, or more specifically gyno-merogeny. In each of them, the sperm mid-piece lay, loosely coiled, in a small mass of cytoplasm that was clearly separate from the rest of the vitellus. The sperm head had changed and given rise to a small ill-formed pronucleus, and the acrosome could be seen close by. One of the eggs was undivided; it had a polar body and a single large nucleus, resembling in size and form a male pronucleus. The other egg was in the 2-cell stage; the cytoplasm appeared normal but the nuclei were smaller than usual and had only one nucleolus each (Plate 1, Fig. 4).

(iv) *Rudimentary Parthenogenesis*.—Two eggs were recovered, each of which had a large, well-formed nucleus, but showed no evidence of sperm penetration (Plate 2, Figs. 5 and 6). The nuclei, particularly the one shown in Plate 2, Figure 5, bore a strong resemblance to male pronuclei both in size and structure. Most of the other eggs that were obtained from the same fallopian tubes as these two had been penetrated by sperms and were in the course of apparently normal fertilization.

(v) *Abnormal Fertilization*.—Two eggs were seen that had been penetrated by sperms and were undergoing apparently normal fertilization except that they had two distinct female pronuclei in addition to a male pronucleus. One of these was a "giant" egg and has already been described (Plate 1, Fig. 3). The other was of normal size; it had no polar body. A photograph of this egg was published in an earlier communication (Austin and Braden 1953*b*, Plate 3, Fig. 18). A third penetrated egg had a single nucleus resembling a normal male pronucleus; no egg chromosomes could be distinguished.

(vi) *Two-cell Egg with Binucleate Blastomere*.—One 2-cell egg was seen that contained a single sperm and appeared quite normal, except that there were two well-formed nuclei in one blastomere. A picture of this egg was also published in the paper just referred to (Plate 3, Fig. 20).

(*b*) *Mouse Eggs*

From 147 mated mice, a total of 1120 tubal eggs was obtained, of which 21 were anomalous (see Table 1). The majority (16) of these were primary oocytes (Plate 2, Fig. 7), including one that was of "giant" proportions, and resembled in nuclear structure the primary oocyte of the rat; the egg shown had been penetrated by four sperms, which were all in the perivitelline space. The corona radiata in these eggs was much less dense than that of the normal eggs in the same tube. The other anomalies were: three penetrated eggs that contained only one pronucleus each, another egg with two well-formed female pronuclei in addition to a male pronucleus, and a 2-cell egg that had two nuclei in one of the blastomeres but was otherwise normal (Plate 2, Fig. 8).

(*c*) *Rabbit Eggs*

Among 221 eggs obtained from 27 mated rabbits there was one "giant" egg, which, like those observed among the rat eggs, had about twice the normal volume (Plate 1, Fig. 2).

IV. Discussion

The origin of the "giant" eggs is enigmatic. They may have resulted from overgrowth of a normal oocyte while in the follicle, but the fact that they were regularly about twice the volume of normal eggs suggests an origin early in oogenesis. They may have been initially tetraploid, but this possibility was not investigated. "Giant" eggs appear to be more prone to irregularities of fertilization, for, out of the seven fertilized rat eggs seen, two were polyspermic and another had two female pronuclei. If the "giant" eggs were indeed diploid after meiosis, these irregular forms may have been capable of giving rise to tetraploid and pentaploid embryos.

The presence of primary oocytes in the fallopian tube may result from the accidental ovulation of a normal, but immature, follicle or from the ovulation of a follicle in which maturation of the egg alone has been delayed. There appears to be no earlier report of the occurrence of tubal primary oocytes in rats or mice. Longley (1911), however, described an oocyte that was recovered from the fallopian tube of a cat, and the ovulation of primary oocytes is evidently normal for the dog (Van der Stricht 1923), the fox (Pearson and Enders 1943), and the horse (Hamilton and Day 1945). In the present series, oocytes were seen much more frequently in mice than in rats, but the reason for this is obscure. The fact that one rat oocyte had been penetrated by six sperms and one mouse oocyte by four sperms is of particular interest because there is evidence that the membranes of normally ovulated rat eggs must undergo a form of maturation in the fallopian tube before sperm penetration can occur (Austin and Braden 1954*b*). Evidently the same kind of change can take place in the outer membranes of an oocyte after ovulation even though the vitellus is in an immature state. The presence of all the penetrating sperms in the perivitelline space suggests that the immaturity of the vitellus precluded the entry of sperms into it. Lack of maturity in the vitellus is apparently not a bar to sperm entry in all species, however, for in the dog, penetration occurs before the first meiosis (Van der Stricht 1923).

There seems little doubt that two of the eggs seen were indeed undergoing a form of gynogenesis, for the sperm mid-piece and acrosome, as well as the ill-formed male pronucleus, could be seen in a clearly separated portion of the vitellus. It appears likely that the sperm, having entered the vitellus and thus provided the activating stimulus, became accidentally included in an extrusion of the cytoplasm. As one of these eggs had undergone cleavage, it seems that, in the rat, development may proceed at least to the 2-cell stage without the normal involvement of the male pronucleus. The relatively small size of the nuclei in this egg is more likely due to their recent formation than to a haploid condition, although it has been shown that eggs that are probably haploid have nuclei of slightly less than the normal size (Austin and Braden, unpublished data). The occurrence of gynogenesis in mammals has previously been described only after irradiation of the sperm (Amoroso and Parkes 1947).

Rudimentary parthenogenesis seems an appropriate designation for the process displayed by two other eggs in this series. Neither contained a sperm, yet each had a large, well-formed nucleus. The formation of a single nucleus after artificial stimulation has been reported to occur in some rabbit eggs (Thibault 1949), and recently it has been found possible to produce this result in rat eggs (Austin and Braden, unpublished data). It is interesting that the nuclei, particularly in one egg (Plate 2, Fig. 5), resembled male pronuclei in size and structure. This suggests that there is, in normal fertilization, some competition between the pronuclei for nuclear constituents which plays a part in limiting the size of the female pronucleus. The same explanation may account for the size and form of the nucleus in the undivided gynogenetic egg.

With both rats and mice, the eggs that exhibited abnormal fertilization had either a single pronucleus, or two female pronuclei in addition to the male pronucleus. In the eggs with a single pronucleus, it seems likely that this was the male and that scatter of the chromosomes was the reason for the non-development of the female pronucleus. Such a result has been observed in eggs subjected to artificial stimulation (Austin and Braden 1954*a*), and spontaneous chromosome scatter may be associated with long-delayed sperm penetration. On the other hand, Pesonen (1949) considered that, in mouse eggs, the male and female pronuclei may occasionally unite to form a single nucleus. When two female pronuclei are present in addition to the male pronucleus, the mechanism probably involves inhibition of second polar body formation, such as has been induced by appropriate treatment before sperm penetration in both *Triturus* eggs (Fankhauser and Godwin 1948) and rat eggs (Austin and Braden 1954*a*). Presumably such eggs could give rise to triploid embryos as suggested by Fischberg and Beatty (1952) for mice.

It is difficult to account for the binucleate blastomeres seen in 2-cell eggs. Van der Stricht (1910) noted a similar condition in the egg of a bat. In frog eggs, binucleate blastomeres are seen when polyspermic fertilization has occurred (Wilson 1925) but this explanation does not appear valid for the rat egg (Austin and Braden 1953*b*).

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Explanation of Plates 1 and 2

Photographs were taken with the aid of a phase-contrast microscope.

Plate 1

Fig. 1.—A "giant" rat egg compared with an egg of normal size. Both are undergoing fertilization. × 170.

Fig. 2.—A "giant" rabbit egg compared with an egg of normal size. Neither has been penetrated by sperms. × 170.

Fig. 3.—A "giant" rat egg showing abnormal fertilization: there are two female pronuclei in addition to the male pronucleus. × 350.

Fig. 4.—A 2-cell rat egg showing supposed early gyno-merogeny. The small size of the nuclei is evident and part of the coiled sperm mid-piece(*s*) may also be discerned. Compression of the egg has obscured the individuality of the blastomeres and of the cytoplasmic extrusion containing the sperm mid-piece and male pronucleus. × 350.

Plate 2

Figs. 5 and 6.—Two rat eggs exhibiting rudimentary parthenogenesis. In each there is a single well-formed nucleus; neither egg contains a sperm. × 350.

Fig. 7.—The primary oocyte recovered from the fallopian tube of a mouse. This egg has four sperms in the perivitelline space but none within the vitellus. The scarcity of coronal cells is in contrast to the density of these cells about oocytes recovered from mature follicles. × 350.

Fig. 8.—A 2-cell mouse egg with a single nucleus in one blastomere and two apparently normal nuclei in the other. × 650.