THE FERTILIZATION REACTION IN THE SEA-URCHIN EGG. THE EFFECT OF NICOTINE

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(With Plate 12 and One Text-Figure)

INTRODUCTION

After insemination of unfertilized eggs of *Psammechinus miliaris*, a change in cortical structure is propagated over the egg surface. A quantitative examination of this phenomenon (Rothschild & Swann, 1949) raised the following questions, requiring further experiments. First, is the observed cortical change the block to polyspermy, or is there a more rapid and so far undetected block to polyspermy as suggested by Gray (1931)? Secondly, is the cortical change directly or indirectly caused by the diffusion of some substance from the sperm head, round the egg surface or through the cytoplasm? The second question is not considered here, though some calculations relating to it have been published elsewhere (Rothschild, 1949).* The first question is the subject of this paper.

Hertwig & Hertwig (1887) discovered that nicotine, among other substances, induced polyspermy in sea-urchin eggs. An investigation of the action of nicotine involves consideration of the following possibilities. If nicotine increases the mean speed of a sperm suspension, the number of sperm-egg collisions will be greater than under normal conditions, thus increasing the incidence of polyspermy. Secondly, if nicotine retards or suppresses the block to polyspermy, and the observed change is the block to polyspermy, it should be possible to detect this action of nicotine. Finally, the suggestion has been made that not every sperm-egg collision is successful in the sense that fertilization follows. Nicotine may have a chemical effect on the egg surface such that the probability of a successful sperm-egg collision is increased, with concomitant changes in the incidence of polyspermy.

The experiments described in this paper were designed to investigate these questions. Eggs, with and without jelly, and spermatozoa of *P. miliaris* were used.

The fertilization reaction and the movements of spermatozoa were recorded by the dark-ground photography methods described elsewhere (Rothschild & Swann, 1949), while sperm densities were measured with the photoelectric absorptiometer (Rothschild, 1950).

* There is a misprint in this paper on p. 179, where the approximate figure 10^3 for the molecular weight should be 2.10^4.

RESULTS

Incidence of polyspermy at different sperm densities. Nicotine-treated and control eggs were placed in different sperm suspensions of the same density. At the time of the first cleavage the numbers of unfertilized, monospermic, polyspermic and cytolysed eggs were counted. The results of an experiment of this type are given in Table 1.

Table 1. *Effect of nicotine on incidence of polyspermy in eggs of* Psammechinus miliaris

Eggs treated with nicotine, N. Eggs not treated with nicotine, C. *Nicotine treatment:* unfertilized eggs placed for 5 min. before fertilization in 1/2000 (v/v) nicotine in sea water. Figures in brackets are percentages. *T*° C., 17. 7 July 1949.

No. of sperm/ml.		Un-fertilized eggs	Mono-spermic eggs	Poly-spermic eggs	Cytolysed eggs	Doubtful
9.07×10^4	C	36 (9·3)	340 (87·9)	1 (0·3)	9	1
9.07×10^4	N	30 (6·4)	326 (69·8)	109 (23·3)	2	0
1.36×10^6	C	5 (1·4)	322 (92·8)	5 (1·4)	15	0
1.36×10^6	N	2 (0·4)	149 (32·6)	289 (63·2)	17	0
2.18×10^7	C	12 (2·9)	351 (85·8)	23 (5·6)	22	1
2.18×10^7	N	1 (0·2)	45 (10·9)	350 (85·0)	16	0
3.48×10^8	C	0 (0)	253 (63·4)	118 (30·0)	28	0
3.48×10^8	N	1 (0·2)	3 (0·7)	437 (98)	5	0

The expected number of successful collisions while the block to polyspermy is being propagated can be calculated from the ratios of monospermic to polyspermic eggs (Text-fig. 1).*

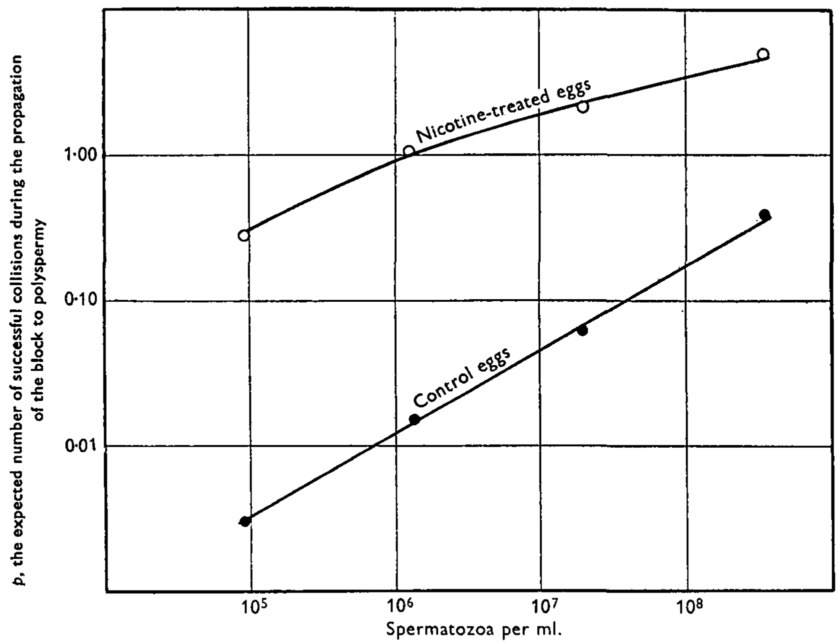
The expected number of successful collisions during the propagation of the block is evidently related to sperm density in an orderly but not a linear way. If the sperm density is increased by a factor of 1000, the increase in the expected number of successful collisions is about 12-fold for nicotine-treated eggs and about 60-fold for untreated eggs. The effect of nicotine is to increase the expected number of successful collisions at all sperm densities, the increase varying from about 100-fold at 10^5 sperm/ml. to about 13-fold at 10^8 sperm/ml.

The existence of an orderly relationship between the expected number of successful collisions and sperm density means that nicotine does not completely suppress the propagation of the block to polyspermy. But the fact that the relationship is not linear suggests that, as predicted, the assumptions involved in treating the collisions between spermatozoa and eggs in terms of the bombardment of a particle of a known radius by gas molecules are unlikely to be justified over wide ranges of sperm densities. One reason concerns the possibility that spermatozoa do not swim at the same speeds at different densities (Gray, 1928). Sperm tracks in the neighbourhood of a drop of undiluted semen, Pl. 12*a*, can be compared with those in a sperm

* The treatment involves the assumption of a Poisson distribution. If it were shown that the expected number of successful collisions and the conduction rate of the block to polyspermy varied from egg to egg, a different distribution would be applicable. Deviations from the Poisson distribution would also suggest this possibility.

suspension of density 10^5/ml., Pl. 12*b*. The evidence in support of a difference in sperm speeds in dense as opposed to dilute suspensions is not strong. The presence of jelly round the egg has little effect on the speeds of spermatozoa moving in it, though there is a tendency for less spermatozoa to be found inside the jelly than on or near its outer surface. Before the problem of the non-linearity of the sperm density-probability relationship can be explained, more detailed observations of the behaviour of spermatozoa near an egg are needed.

The effect of nicotine in increasing the expected number of successful collisions during the period of propagation of the block to polyspermy means that the block itself is slowed down, or that the chance of a collision being successful is raised.



Text-fig. 1. Relationship between *p*, the expected number of successful collisions during the propagation of the block to polyspermy, and sperm density. Using the Poisson distribution, the proportions of monospermic and polyspermic eggs will be e^{-p} and $1-e^{-p}$.

The effect of nicotine on the speeds of spermatozoa and on the rate of propagation of the cortical change. Pretreatment of unfertilized eggs with nicotine has no effect on the mean speeds of spermatozoa. Furthermore, neither the conduction velocity nor the appearance of the propagated change are significantly altered by nicotine. The effect of nicotine in inducing polyspermy can therefore have one of two explanations. Either the block to polyspermy has a much higher conduction velocity than that of the observed cortical change, and *this* block to polyspermy is slowed up by nicotine; or, if the observed cortical change is the block to polyspermy, there must normally be a low probability of a successful sperm-egg collision, which is greatly increased by nicotine. The experiments in the next section constitute an attempt to decide between these alternatives.

The effect of nicotine on the time between insemination and fertilization. If there is a low probability of a successful collision we should expect considerable scatter or dispersion in the 'fertilization' times within a sample of eggs which have been inseminated at $t=0$. If nicotine increases the probability of a successful collision, the dispersion and the mean time at which 'fertilization' occur should be reduced. A series of experiments were carried out in which eggs from the same female were added to sperm suspensions of known densities. The experiments were done in pairs, using nicotine-treated and untreated eggs. Immediately after insemination the eggs were transferred to the microscope slide and dark ground cinematography was begun. The times at which eggs first showed any cortical brightening (i.e. 'fertilization') were determined. The results of the experiments are shown in Table 2.

Certain conclusions may be drawn from these experiments:

(1) No egg shows a cortical change in less than about 20 sec. after insemination.

(2) Though the mean times of treated samples are somewhat less than those of untreated samples, the difference is not statistically significant.

(3) There is a significant reduction in dispersion as a result of nicotine treatment.

If there is a high-speed block to polyspermy and a high probability of a successful collision, the alternative to a low-speed (20 sec.) block to polyspermy and a low probability of a successful collision, the delay of 20 sec. before any egg shows the cortical change must be attributed to a latent period during which no visible morphological changes occur, even though a spermatozoon has activated the egg. If this hypothesis is correct, all eggs will be fertilized in the first few seconds after insemination, and the considerable dispersion of the times at which cortical changes occur must be attributed to variations in the lengths of this latent period. The reduction of this dispersion in nicotine-treated eggs would have to be explained by the somewhat improbable postulate that this treatment produces some reduction in the variability at which the rates of the chemical and physical reactions responsible for the latent period take place.

If, alternatively, the observed cortical change is the block to polyspermy and there is a low probability of a successful sperm-egg collision, the 20 sec. delay in appearance of the cortical change and the lack of reduction in the mean time at which the cortical change appears are difficult to explain without making further assumptions that have no experimental justification. The reduction in dispersion is, however, consistent with this hypothesis.

Direct observations. Rothschild & Swann (1949) briefly discussed the direct evidence that spermatozoa may collide with an egg without fertilizing it. Moser (1939, p. 428) refers to spermatozoa bombarding the surfaces of eggs of *Arbacia punctulata* 'to the extent that momentary indentations may be seen in the periphery of the eggs'. We have made similar observations using the eggs of *P. miliaris*. This evidence tends to support the hypothesis that the conduction velocity of the block to polyspermy is low and that the probability of a successful sperm-egg collision is low; but there is a possibility that the observed unsuccessful collisions in fact take place after a spermatozoon, which has not been seen, has activated the egg.

Table 2. *Effect of nicotine on time at which cortical change develops after insemination*

C, controls; N, nicotine-treated eggs. The figures in columns C and N refer to the film frame numbers, the number of frames per second being 1·5. The experiments are tabulated in ascending order of sperm densities. T ° C., 17.

Spermatozoa per ml.	Exp. no.	C		N	
		Frames after insemination	Mean	Frames after insemination	Mean
$1·3 \times 10^5$	1	43 86 101	76·7	46 48 67	53·7
5×10^5	9	47 94 113 140	98·5	72 84 85 107	87·0
10^6	8	61 73 75	69·7	39 44	41·5
2×10^6	2	47 52 54	48·5	40 45 59	49·4
	3	44 44 45 45 53		57 61 62	
	7	37 38 52 71		38 40 42 50	
4×10^6	4	29 39 40 45 55 62	45·0	33 43 48	40·0
	5			31 45	
5×10^6	10	—	—	48 54	47·7
	11	—	—	42 43 44 45	
	12	—	—	43 50 51	
	13	—	—	48 49 54 56	
	14	—	—	43 44 49	
8×10^6	15	38 44 45 49	44·0	—	—
10^7	6	38 42 43	41·0	44 51	47·5
5×10^7	16	52 60 61	56·9	—	—
	17	49 51 54 71			

In certain eggs treated with nicotine, two bright regions appear at different points on the cortex (Pl. 12*c*). The times at which these two cortical changes appear strongly suggest that they are each caused by spermatozoa entering the egg at the points in question. The start of two cortical changes in the same egg has been observed and photographed five times, the second change appearing 6, 8·5, 9, 12 and 18 sec. after the first. This suggests that the block to polyspermy had not travelled completely round the egg within these times, and that it might therefore have the same velocity as the cortical change. On the other hand, if the block to polyspermy were a high-speed phenomenon, these effects could be explained if both spermatozoa activated the egg within a fraction of a second of each other, and if the latent period were different in the two cases.

CONCLUSION

The incidence of polyspermy when nicotine-treated eggs are inseminated at different sperm densities shows that this treatment increases the chance of an effective sperm-egg collision, by a factor which may be as much as 100, during the propagation of the block to polyspermy. This is not due to any effect of nicotine on the spermatozoa. If the observed cortical change is the block to polyspermy, the effect of nicotine is not to retard its conduction velocity but to increase the probability of a sperm-egg collision being successful. The experiments cannot be harmonized entirely with this hypothesis, or for that matter with the alternative hypothesis involving a high-speed block to polyspermy.

SUMMARY

1. Unfertilized eggs of *Psammechinus miliaris* which have been allowed to stand in sea water containing nicotine and are then inseminated in normal sea water are polyspermic.

2. Polyspermy is not due to an increase in the speeds at which spermatozoa swim in these circumstances; nor to a decrease in the rate at which the change in cortical structure of the egg, the first sign of fertilization, is propagated over the egg surface.

3. Experiments to test alternative explanations of the effect of nicotine have been carried out.

4. The experiments do not enable a firm decision to be made between (*a*) a high-speed block to polyspermy (which has not been observed) with a high probability of a successful collision, and (*b*) a low-speed (20 sec.) block to polyspermy with a low probability of a successful collision.

When discussing the morphology of inseminated oocytes in a previous paper (Rothschild & Swann, 1949), we referred to Seifriz's work on this subject (1926), but not to that of E. B. Harvey (1938) and in particular to her remarkable photographs of protoplasmic papillae on the surfaces of inseminated oocytes. We welcome the opportunity of rectifying this omission.

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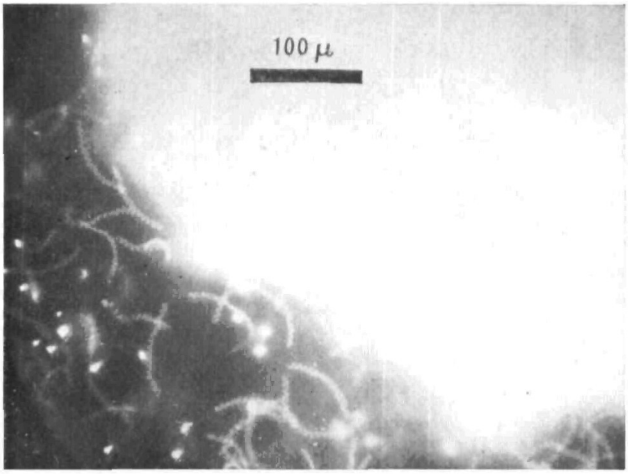
EXPLANATION OF PLATE 12

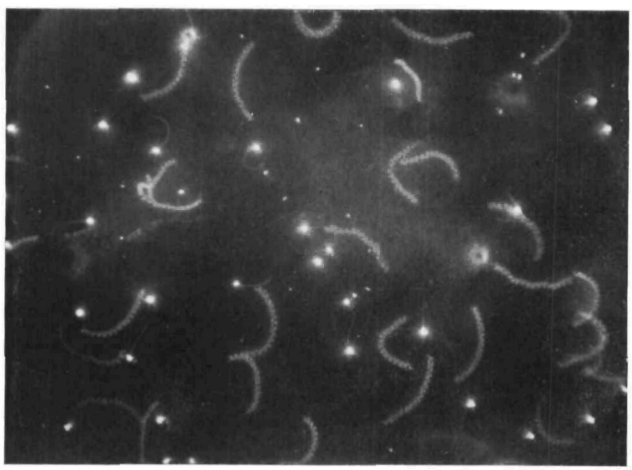
a. Tracks of sperm heads (*P. miliaris*) in the neighbourhood of undiluted semen, under dark ground illumination. Exposure, 0·5 sec. $T°$ C., 17.

b. Tracks of sperm heads (*P. miliaris*) in a suspension of density 10^5/ml., under dark ground illumination. Exposure, 0·5 sec. $T°$ C., 17. Magnification as in Pl. 12*a*.

c. Propagated cortical changes in dispermic egg of *P. miliaris*, pre-treated with nicotine. Dark ground illumination. Exposure, 0·38 sec. Interval between photographs, 0·75 sec. $T°$ C., 17. Diameter of egg, 100μ.



a.



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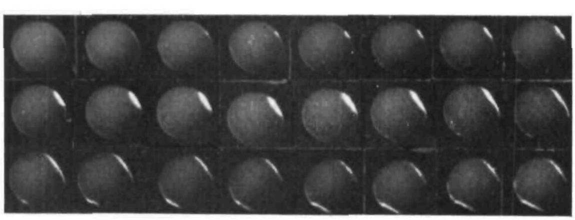

c.

ROTHSCHILD AND SWANN—FERTILIZATION IN SEA-URCHIN EGG