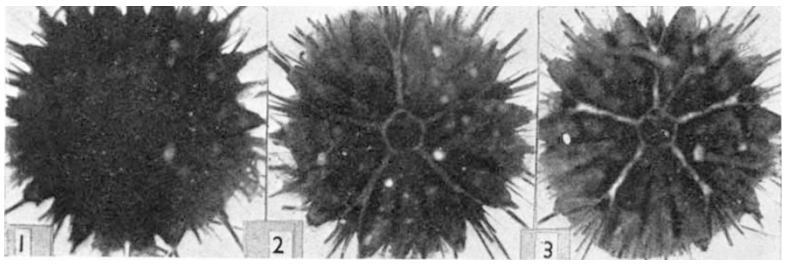

Photographs of young *Diadema* (diameter of test, 18·0 mm.). (1) Dark phase (photographed at 10.0 a.m. after being in sunlight); (2) intermediate phase (photographed at 10.0 a.m. after being in light-proof tank for 23 hr.); (3) pale phase (photographed at 8.0 p.m. after being in darkness for 3 hr.).
These photographs are of the same animal, using the same camera, illumination and plates. They were developed simultaneously in the same tank, printed on the same paper and given equal exposures both for photographing and printing

Since it has already been shown[4] that the whole surface of *Diadema* is remarkably sensitive to changes in light intensity, and moreover that the sensitivity increases markedly in the pale phase and declines in the dark, the dispersed superficial pigment in strong light may well serve as a protective shield for more deeply situated and widely scattered light-sensitive elements.

A full account of the investigation will be published in the near future.

N. Millott

Department of Zoology,
University College of the West Indies,
Jamaica.
Jan. 25.

[1] v. Uexküll, J., *Z. Biol.*, **34**, 315 (1897). Kleinholz, L. H., *Pub. Staz. Zool. Napoli*, **17**, 53 (1938).
[2] Mortensen, Th., "A Monograph of the Echinoidea", **3**, 1 (Copenhagen, 1940).
[3] Millott, N., and Jacobson, F. W., *J. Invest. Dermat.*, **18**, 91 (1952).
[4] Millott, N., *Biol. Bull.*, **99**, 329 (1950).

The 'Capacitation' of the Mammalian Sperm

It was shown recently[1] that sperms injected into the periovarian sac of the rat after ovulation did not begin to enter the eggs until four or five hours later. In the rabbit, too, sperms introduced into the Fallopian tubes shortly *after* ovulation seldom penetrated the eggs; but if sperms were introduced a few hours *before* ovulation, the majority of the eggs were later observed to be fertilized. Chang[2] reported similar findings in the rabbit, and also showed that sperms, injected into the Fallopian tubes after ovulation, were able to penetrate a larger proportion of eggs if they had first spent about five hours in the uterus of another rabbit.

The conclusion drawn by both workers was that, in rats and rabbits, the sperm must undergo some form of physiological change or capacitation before it is capable of penetrating the egg. The need for such a change in the sperm has not been postulated before on direct evidence, and further confirmation is desirable. In the investigations already mentioned, the animals were necessarily subjected to laparotomy and the sperms to manipulation *in vitro*. Both these procedures may render somewhat abnormal the circumstances in which the penetration of the egg is to take place, and the following results are therefore of interest because they have been obtained with a minimum of experimental interference.

Mature female rats were selected for œstrus by examining the vaginal smear between 6.0 and 7.0 a.m. and were placed in cages with males for 10–15 min. between 7.0 and 7.30 a.m. That ovulation is normally complete at this time was shown in a preliminary test by the recovery of eight or more eggs from the Fallopian tubes of sixteen out of twenty rats which were killed between 5.0 and 7.0 a.m. The females which showed evidence of copulation were selected, and individuals were killed at 1, 2, 2½, 3, 4, 5 and 6 hr. later. The Fallopian tubes were separated from the uteri by severing the isthmus of the tube in order to avoid contamination with sperms from the uterus. The distended portion of the ampulla was incised to permit the eggs in cumulus, together with the surrounding fluid, to escape on to a slide. The tube was discarded, and a coverslip with 'Vaseline' on the edges was placed over the preparation. The eggs were inspected to see if sperm penetration had occurred, and the number of sperms in the cumulus and surrounding fluid was counted. Results were obtained from a total of seventy-five rats (see table).

Mean Number of Sperms Observed about the Eggs, and Total Number of Eggs and Number of Penetrated Eggs Recovered from Rats Killed at the Specified Hours after Coitus

Hours after coitus	Number of rats used	Mean number of sperms about eggs	Total eggs	Penetrated eggs	Per cent penetrated eggs
1	10	6·6	74	0	0
2	15	20·6	139	1	0·7
2·5	10	33·5	96	21	21·9
3	10	32·1	88	39	44·3
4	10	49·9	94	65	69·1
5	10	55·4	91	78	85·7
6	10	59·6	82	80	97·6

The number of sperms found in the cumulus and surrounding fluid increased from a mean of 6·6 per tube at 1 hr. after coitus to a mean of 59·6 per tube at 6 hr. In spite of the presence of sperms, none of the eggs examined at 1 hr., and only 1 out of 139 eggs examined at 2 hr., were penetrated. At later hours, increasingly larger numbers of sperms were seen about the eggs and increasingly larger proportions of the eggs were found to contain sperms. At 6 hr. after coitus, eighty of the eighty-two eggs recovered (97·6 per cent) were penetrated.

Clearly, there is a delay of about two hours before sperm penetration of the eggs commences, and this is interpreted as support for the hypothesis that a period for capacitation is required by the sperm before it can penetrate the zona pellucida. Thereafter sperm penetration proceeds readily.

I am grateful to Miss H. N. Turner for advice on the significance of results, and to my assistant, G. S. Thring, for his careful work.

C. R. Austin

Division of Animal Health and Production,
Commonwealth Scientific and
Industrial Research Organization,
McMaster Animal Health Laboratory,
Sydney, N.S.W.
May 1.

[1] Austin, C. R., *Aust. J. Sci. Res.*, B, **4**, 581 (1951).
[2] Chang, M. C., *Nature*, **168**, 697 (1951).