Zusammenfassung

Intraperitoneal injiziertes *aqua destillata*, welches Schock hervorruft, führt zu einem starken Mastzellenzerfall. Derselbe bleibt bei osmotischer Wirkung ohne Schock wesentlich geringer.

Labelling of Insect Spermatozoa by Adenine-8-^{14}C

Experiments on the behaviour of the male pronucleus in the egg may be facilitated by the use of radioactively labelled sperm. The procedure for labelling spermatozoa of the spider beetle *Ptinus hirtellus* Sturm (recently changed to *P. clavipes* Panzer[1]) and *Drosophila melanogaster* is presented here.

Of the various tracers used to label spermatozoa of vertebrates (phosphorus-32[2], methionine-^{35}S[3,4], adenine-8-^{14}C[5], formate-^{14}C[4]), adenine-8-^{14}C is most suitable because its half-life is sufficient for the long time interval between the administration of tracer and the preparation of autoradiographs. During spermatogenesis, adenine is incorporated into DNA at spermatogonial or pre-leptotene stages[4]. Later work has shown that labelling of the DNA of spermatozoa with thymidine-^{3}H is also feasible[6]. Recently, after feeding newly eclosed *Drosophila* with phosphorus-32, OFTEDAL and MOSSIGE[7] have detected by counter measurements what presumably is DNA-^{32}P in spermatozoa; the interval before the ejaculation of labelled spermatozoa occurs points to incorporation during premeiotic stages.

After examination of the larval testes of *P. hirtellus* in aceto-orcein squashes, larvae which were more than half-grown, and therefore possessed mainly spermatogonia, were chosen for the work. About 0·01 cm^3 of a solution of adenine-8-^{14}C (10 μc/cm^3; 9·6 μc/*mM*) was injected into each larva, using a microneedle. Feeding tracer (as with *Drosophila*, see later) was not possible in this species owing to the dry culture medium[8] used.

The larvae became pupae about 45 days later and the males emerged about 100 days after injection. Testes were fixed in alcohol-acetic acid (3:1), and sections, squashes, or smears were made. Autoradiographs of these were prepared with Kodak AR.10 film and exposed for up to 24 days. The preparations were then stained with methyl green-pyronin and examined with phase microscopy. Only about 20–25% of the sperm masses were labelled. As a higher percentage was required, a second batch of larvae were given one injection, as before, and a second identical dose 30 days later. Pupation and emergence were delayed, and there was a 35% mortality. Autoradiography of the testes showed almost all sperm masses heavily labelled (Fig. 1).

Labelling of the first-produced spermatozoa in *Drosophila* imagos was obtained by feeding larvae from hatching on a dead yeast medium[9] with added adenine-8-^{14}C (10 μc/cm^3 of medium). Testes of just eclosed to 2-day old flies were fixed in alcohol-acetic acid, sectioned at 5 μ, prepared for autoradiography, and exposed for up to 12 days. DNA of sperm bundles was heavily labelled, as seen in Figure 2, which shows the autoradiograph of a

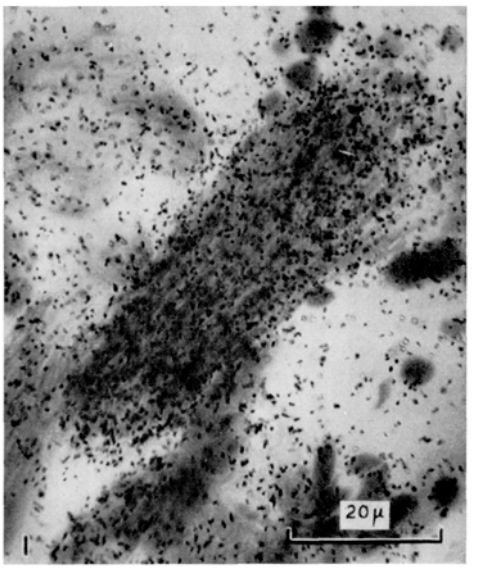

Fig. 1.—Autoradiograph of sectioned testis of *P. hirtellus* showing a sperm mass heavily labelled.

section of the testis after previous treatment with ribonuclease. Tracer is also present in the DNA of other cells, and perhaps also in other materials not removed by the enzyme. The testis of adult *Drosophila* is unfavourable material for the study of individual spermatozoa even in squashes or smears. However, positive autoradiographs were obtained on dispersed bunches of spermatozoa in the vagina and seminal receptacle of females paired with adenine-fed males (Fig. 3).

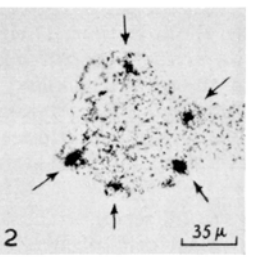

Fig. 2.—Autoradiograph of sectioned testis of *D. melanogaster* after treatment with ribonuclease, showing labelled sperm bundles.

Some observations on spermateleosis in *P. hirtellus* warrant mention. The specialized nature of the spermatozoa in *P. tectus* has been reported earlier[10]. Although the mature spermatozoa in *P. hirtellus* is also of this type, e.g. 'all head', it appeared that the mode of transformation of the spermatid into the spermatozoon differs from the other species. In *P. hirtellus* the entire spermatid nucleus progressively elongates and is directly transformed into

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the mature spermatozoon, while all the cytoplasm of the spermatid is discarded except that which will later form the delicate membrane of the spermatozoon and the small

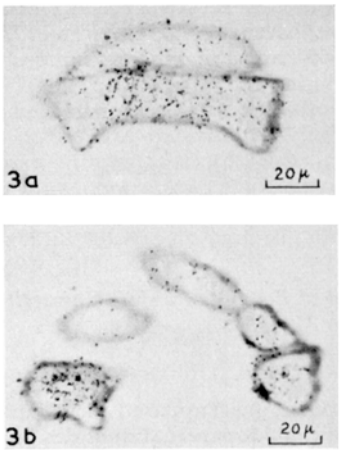

Fig. 3 *a* and *b*.—Autoradiograph of sectioned seminal receptacle of a female *D. melanogaster* showing labelled spermatozoa.

(3–5 μ) persisting tail remnant[11]. An early spermatid after nuclear elongation has started is shown in Figure 4, and on average 10–15 grains associated with the nucleus were

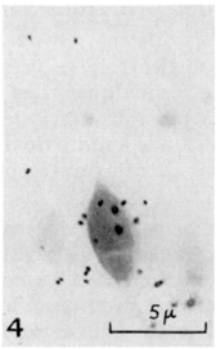

Fig. 4.—Autoradiograph of an early spermatid of *P. hirtellus* (smear).

observed. In more fully developed spermatozoa, in which the nucleus has greatly elongated, 20–25 grains were observed (Fig. 5 and 6). Though there has been an increase

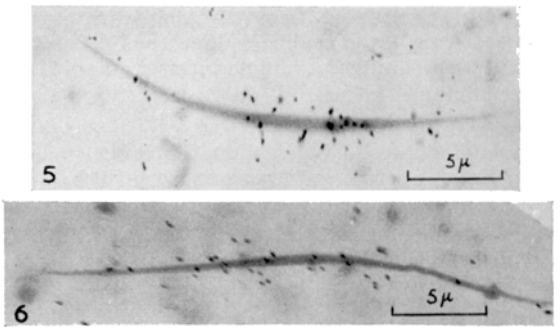

Fig. 5 and 6.—Autoradiographs of two stages of immature spermatozoa of *P. hirtellus* (smear).

[11] J. Jacob, Cytologia, in press (1958).

in nuclear content the amount of DNA has presumably not increased[4] (the intensity of staining decreasing with maturation) and the RNA investment of the nucleus has become much thinner[11]. The increase in tracer content may therefore indicate that the two generations of cells which gave rise to the less developed and more fully developed spermatozoa have incorporated different amounts of tracer in DNA, which could have been caused by the timing of the two injections.

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Institute of Animal Genetics, Edinburgh, June 18, 1958.

Résumé

La technique de marquage avec de l'adénine-8-^{14}C des spermatozoïdes mûres du coléoptère *Ptinus hirtellus* et de *Drosophila melanogaster* est décrite. L'isotope était administré par injection dans les larves de *Ptinus* et par bouche dans celles de *Drosophila*.

* Research Worker of the British Empire Cancer Campaign.

What Constitutes a Network in the Acinar Cells of Amphibian and Mammalian Pancreas?

Bowen[1], Beams[2], Duthie[3], Subramaniam[4], Chodnik[5], and Nassonov[6], all of whom have worked on vertebrate pancreas, described the so-called 'Golgi apparatus' as a reticulum consisting of solid strands. According to Lacy[7], however, it is in the form of canals. On the other hand, some modern cytologists like Baker[8, 9], Nath[10], and Thomas[11] have denied categorically the existence of such net-like and canalicular 'Golgi apparatus' in both the somatic and germ cells. Hirsch[12] and Morgan[13] have likewise stated that such structures are non-existent in mammalian pancreatic cells. The reasons put forward by the antagonists of the 'Golgi networks' for the appearance of such structures in pancreas can be summarized as follows:

(1) Silver and osmium, used in the classical 'Golgi' techniques, over-impregnate the lipid bodies or the neutral red granules and also fill up the spaces in between them[8, 12, 13].

(2) Golgi network may be formed simply by the deposition of silver or osmium between crowded secretion granules[8].

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