le rôle d'un transporteur d'électrons. Cette hypothèse est étayée par les constatations récentes de KERTESZ[9]; cet auteur a pu montrer que le cuivre de la polyphénoloxydase se trouve sous la forme cupreux, même en présence de substrat et d'oxygène. Des expériences sont en cours pour vérifier cette hypothèse.

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Service de Chimie, Faculté de Médecine, Paris, le 5 août 1958.

Summary

The authors studied the kinetics of the inhibition of the copper-catalysed oxydation of ascorbic acid by versene and some proteins. The versene-inhibition attains slowly the theoretical value, when added after the Cu^{++} to ascorbic acid. Proteins inhibit non-competitively and completely, when added to the vitamine either after or before the copper. This result may be interpreted as indicating an interaction between protein and the complex (AH^- Cu^{++}).

[9] D. KERTESZ, Nature *180*, 506 (1957).
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Mast Cell Destruction Caused by Distilled Water

It is well known that histamin liberators[1,2], anaphylactic shock[3], and intraperitoneal injection of distilled water[2,4] produce disruption of mast cells. The explanation for this latter phenomenon is not yet at hand. For this reason we wanted to discover whether the primary cause of mast cell destruction is the shock caused by intraperitoneal injection of distilled water or the osmotic effect of the water.

Albino rats weighing 220–260 g were used in this study; 10–10 animals in each series. In the first experimental series, 10 ml distilled water/100 g of weight were injected intraperitoneally and afterwards the animals were killed 1, 2, 3, or 4 h later. In the second series the animals were killed 10 min after the injection. Their mesenteries were kept in distilled water for 4 h stretched on a cork plate. In the third series, the rats were killed without intraperitoneal injection and their mesenteries were also kept in distilled water. Finally the mesenteries were stretched on glass slides, fixed in methanol, and stained with alcoholic toluidin blue.

In the first and second series, we have experienced that the destruction of mast cells occurred similary as FAWCETT[2] and HILL[4] had described, nevertheless we have to point out that some of the animals showed no signs of mast cell disruption, but minor granula expulsion only. On the other hand, in the third series, minor granula expulsion and swelling was the only morphological symptom.

These findings indicate that the shock caused by intraperitoneal injection of distilled water plays an extraordinary great role in the mast cell destruction. The osmotic effect without shock produces a much slower mast cell destruction than with it.

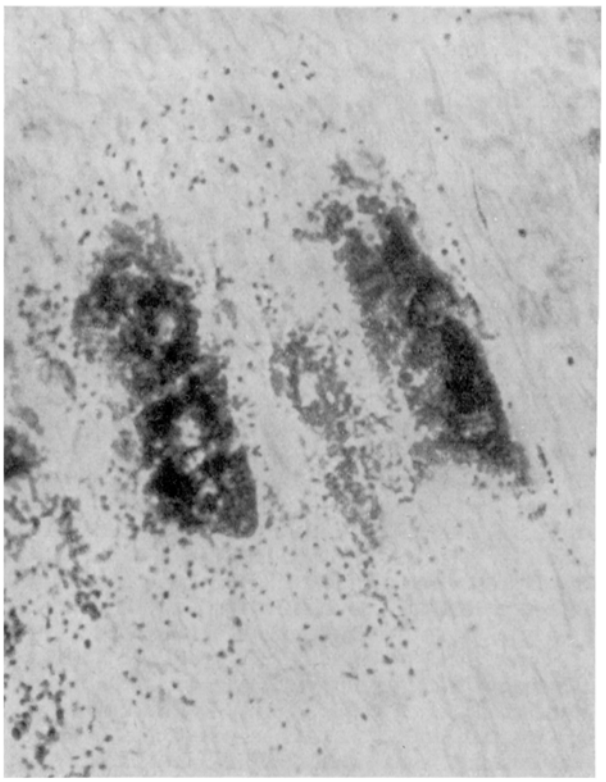

Fig. 1.—Mesentery mast cells of rat killed after 4 h after distilled water injection. (× 900.)

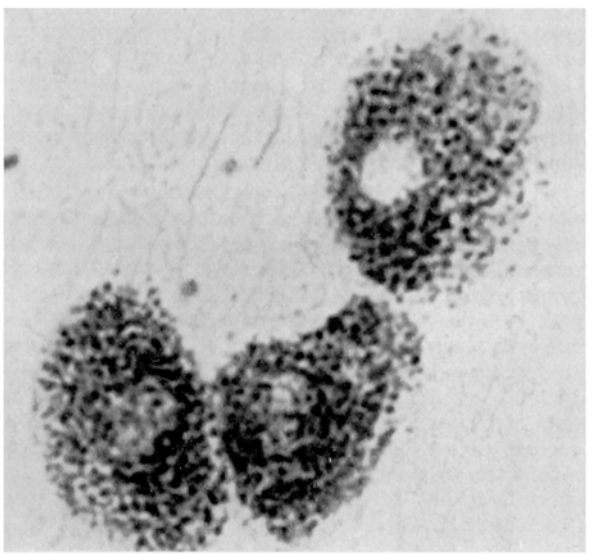

Fig. 2.—Mesentery mast cells of rat killed without intraperitoneal distilled water injection and afterwards keeping 4 h in distilled water.

It is possible that the lack of disruption of mast cells, which sometimes occurred in the first and second series, can be attributed to diminished disposition of these rats to get a shock reaction.

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Department of Pathology, Laszlo-Hospital for Infectious Diseases and Department of Industrial Hygiene, State Institute of Occupational Medicine, Budapest, June 23, 1958.

[1] J. F. RILEY, J. Path. Bact. *65*, 471 (1953).
[2] D. W. FAWCETT, J. exp. Med. *100*, 217 (1954).
[3] I. MOTA, Brit. J. Pharmacol. *12*, 453 (1957).
[4] M. HILL, Exper. *13*, 395 (1957).

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 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).

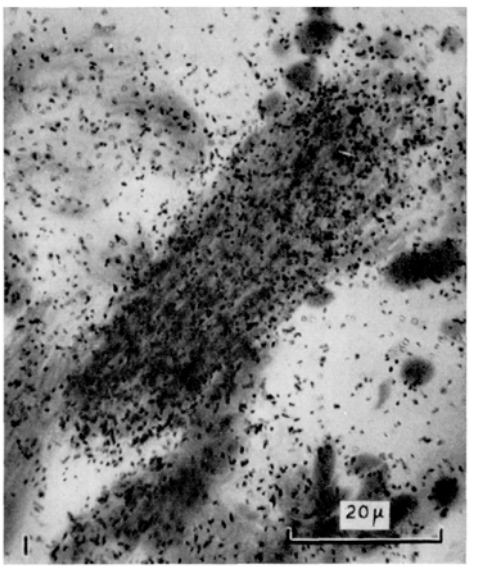

Fig. 1.—Autoradiograph of sectioned testis of *P. hirtellus* showing a sperm mass heavily labelled.

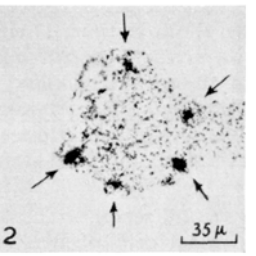

Fig. 2.—Autoradiograph of sectioned testis of *D. melanogaster* after treatment with ribonuclease, showing labelled sperm bundles.

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

[1] B. P. MOORE, Proc. R. ent. Soc. Lond. [B] *26*, 199 (1957).
[2] A. HOWARD and S. R. PELC, Brit. J. Rad. *23*, 634 (1950).
[3] A. GLUCKSMANN, A. HOWARD, and S. R. PELC, J. Anat. *89*, 13 (1955).
[4] J. L. SIRLIN and R. G. EDWARDS, J. exp. Zool. *137*, in press (1958).
[5] J. L. SIRLIN and R. G. EDWARDS, Exp. Cell Res. *9*, 596 (1955).
[6] J. L. SIRLIN, Exp. Cell Res. *15*, 250 (1958).
[7] P. OFTEDAL and J. C. MOSSIGE, *Advances in Radiobiology* (Oliver and Boyd, Edinburgh 1956), p. 457.
[8] B. P. MOORE, G. E. WOODROFFE, and A. R. SANDERSON, Nature *177*, 847 (1956).
[9] T. ALDERSON, Nature *179*, 974 (1957).
[10] J. DLUGOSZ and J. W. HARROLD, Proc. R. Soc. Edinb. [B] *64*, 353 (1952).