adequate micrographs. Electrodes may be viewed uncoated at low accelerating voltages (less than 2 kV) but the resolution obtained is not sufficient to examine fine electrode tips.

The problems associated with secondary emission can be eliminated by using transmission microscopy. Samples do not need a metal coating and require much less spot current (about 1 pA). The transmission mode has the added advantage of greater attainable resolution than the secondary emission mode. The silhouette image obtained in the transmission mode can be improved by using dark field which allows determination of tip lumen diameter.

In summary, glass microelectrodes were examined with dark field scanning transmission electron microscopy without the necessity of heavy metal coating. The electrodes were not damaged and could be used for physiological recording after examination[9].

Zusammenfassung. Mikroglaselektroden wurden im rasterelektronenmikroskopischen Dunkelfeld transmissionsoptisch ohne Metallbeschichtung untersucht, wobei die Elektroden nicht beschädigt werden und für physiologische Ableitungen wieder benützt werden können.

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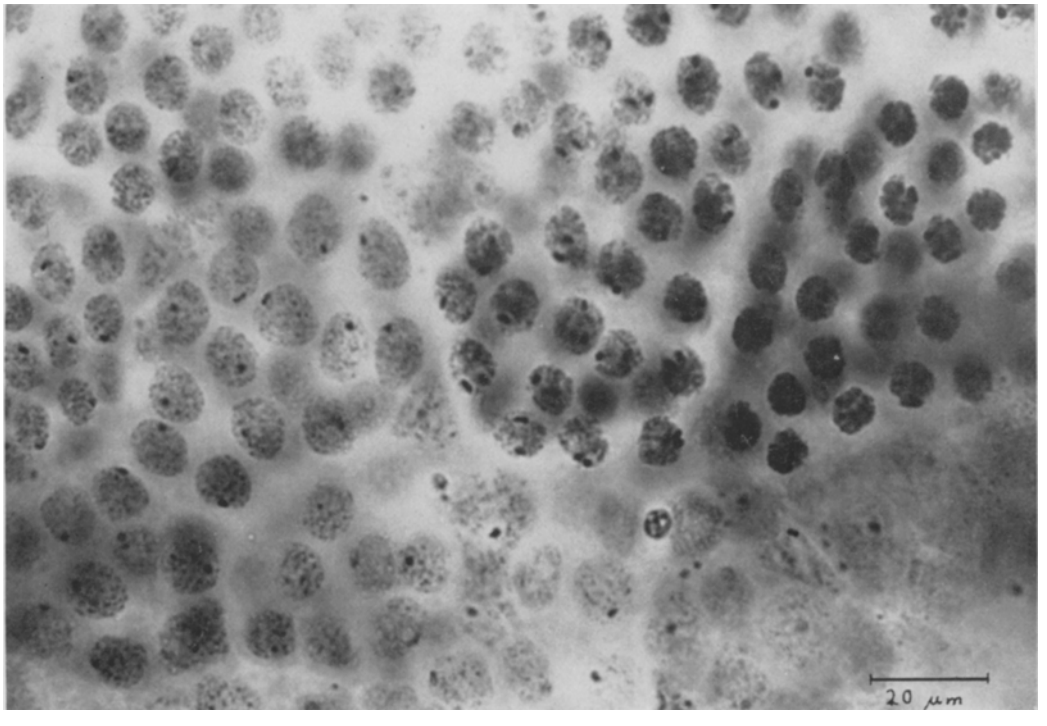
A Simple Method for the Microscopic Examination of Unsquashed, Stained Organs of Insects

In cytologic-microscopic examination of unsquashed objects of insects, with the dyes acetic-lacticorcein[1], carbolfuchsin (Fa. Merck), which are used for the staining of the nucleus, the difficulty arises, that either the object can only be examined in the upper or lower level based on the strong colouring, or the dye is not able to percolate completely by shorter duration of staining. To make possible an equal consideration of the nuclei at all levels, a method was evolved which proved useful for testes follicles of insects, and which is also suitable for discovering the metaphase-I-stages in oocytes.

The testicle is divided in Ringer's solution in separate follicles. The follicles are transported to a small dish with acetic-lacticorcein[1] and are left covered for at least 2 h at room temperature until the object is totally stained. Subsequently the follicle is transported to a slide, on which broken fragments of cover-glass are placed. The cover-glass fragments must be higher than the object to avoid squashing. After covering with a cover-glass, 60% acetic acid is added on one side, and on the other side the dye is sucked off with filter paper, until the liquid sucked off is nearly colourless. The cover-glass is sealed with cover-glass cement. According to the thickness of the object, the slide is kept on for 15 min to 2 h after the addition of the acetic acid. During this time the dye, which still remained in the cytoplasma of the follicle, becomes transparent and shows in all parts a uniform colouring.

[1] L. F. La Cour, Stain Techn. *16*, 169 (1941).



Part of a testicle follicle of *Oncopeltus fasciatus* (Heteroptera), 5th larval stage, from an unsquashed preparation. Acetic-lacticorcein. Note the nuclei situated in cysts.

Then the follicle can be examined without difficulty under the microscope at all levels.

The Figure shows a part of a testicle follicle from *Oncopeltus fasciatus* (Heteroptera) treated in the way described. The nuclei (secondary spermatogonia) are seen to be arranged in cysts of different size. In the nuclei the sex chromosomes are recognizable as heterochromatic bodies. With the help of the described method it was possible to show that in *Oncopeltus* ♂ the transformation of the second sex chromosome into the heterochromatic state takes place following the 6th (= last) mitotic division of the secondary spermatogonia, i.e. during the formation of the 64-cell-cyst (Publication in preparation). After that, the primary spermatocytes develop from the spermatogonia.

This method has the considerable advantage, compared with the examination by aid of series sections[2], that no time-consuming preparation must be done and that no summary of the interpretation of single sections is necessary to obtain the result. In addition, if compared with the squash-preparations[3], this method is superior where the problem is that the nuclei are to be counted or the succession of the single mitotic or meiotic stages in a testicle follicle is to be examined.

Zusammenfassung. Am Beispiel von Hodenfollikeln von *Oncopeltus fasciatus* (Heteroptera) wird eine Methode beschrieben, die bei gefärbten, ungequetschten Objekten eine lichtmikroskopische Untersuchung in allen Ebenen zulässt.

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17 December 1974.

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[3] C. D. DARLINGTON, L. F. LA COUR, *Methoden der Chromosomenuntersuchung* (Franckhsche Verlagshandlung, Stuttgart 1963).
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A Technique for Chromosome Analysis of Small Rodents Without Sacrificing the Animal

In many experimental designs it would be greatly convenient to have information on the chromosomal constitution of small rodents without killing the specimen. Thus far, leukocyte or skin[1-3] cultures have been the most common methods employed to study the chromosomes of living animals. However, both type of cultures are difficult or even impossible to obtain in rodents.

Leukocyte or skin cultures from various species of vole mice of the genus Akodon (Rodentia Cricetidae) constantly fail to yield suitable amounts of mitosis for cytogenetic analysis. Thus, we decided to employ a modification of the in vivo chamber technique used to study the kinetics of antibody response or to grow tumour cells[4,5]. The method basically consists in: a) to introduce a microchamber charged with an antigen into the abdominal cavity of the animal to study, b) to extract the microchamber when it is surrounded by an actively dividing population of immunocompetent cells (the animal is colchicinized 3 h before extracting the microchamber), c) to harvest the immunocompetent cells by means of the enzyme pronase, d) to prepare metaphase spreads by hypotonic shock and air drying procedures.

Materials and method. Diffussion chambers (0.18 ml capacity) were constructed by cementing a circular Sartorius filter (0.2 µm pore size) to each side of an acrylic ring with an external and internal diameter of 14 and 12 mm respectively (the cement employed is MF Cement Millipore Co). Chambers are placed in open Petri dishes and sterilized at 80 °C for 48 h. Petri dishes are covered and the sterilization is completed by placing the dishes at 37 °C for 24 h and at 80 °C for another 48 h.

The sterilized chambers are filled with total blood from an heterologous species half diluted with Hanks or Earle saline (for Akodon we usually employ mice or rat blood). The chamber filling is performed with a needle and a syringe through a hole performed in the wall of the acrylic ring. Then the hole is sealed with a drop of sterile paraffin.

Receptor animals are anaesthetized with 1 µg/g body weight of Na pentobarbital (the Na pentobarbital solution is prepared by dissolving 550 mg of the drug in a mixture of propylene glycol 20 ml, absolute ethanol 10 ml, distilled water 80 ml). Afterwards the abdomen is opened, 1 or 2 microchambers are placed into the peritoneal cavity and the abdominal wall is sutured. While the animal is being operated, the blood-charged microchambers are maintained in sterile Hanks solution.

We have tried various time-lapses to draw microchambers. However we have found that the peak of mitotic activity in the immunocompetent cells which surround the microchamber appears at about 8 days after their introduction in the recipient animal. Accordingly, at this moment the microchambers are taken from the peritoneal cavity of animals. 3 h before removing the microchambers the animals are injected i.p. with 1 µg/g body weight of colchicine (0.04% solution in distilled water).

Immediately after being removed the microchambers are placed in a 0.5% solution of pronase (Calbiochem) in normal saline and gently shaked for 10 to 15 min to free the cell coat which covers them. The pronase solution is centrifuged at 600 rpm for 7 min and the cell pellet obtained resuspended in a 0.7 *M* solution of KCl at 37 °C for 15 min. Then cells are centrifuged again and fixed in 3/1 absolute ethanol/glacial acetic acid. After one washing in fresh fixative, chromosome spreads are prepared by air drying and stained with carbol-fuchsin, giemsa, acetic orcein or any other chromosomal stain.

Results and discussion. We have studied with the above procedure a large number of rats and mices and more than 50 specimens of Akodon belonging to 3 different species. In all cases we were able to obtain a considerable amount of good metaphases suitable for chromosome analysis or banding procedures. During the first trials 30% of animals died after the removal of microchambers (no animal died during or after the first operation). However, as we gained experience the mortality decreased to zero

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