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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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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levels. The critical moment for the animal is the extraction of the microchamber. At the 8th day the microchamber is covered by a coat formed by cells and peritoneal secretions; occasionally there can also exist fibrine adherences to the peritoneum or intestine. If microchambers are carelessly taken off the animal recovers from the anaesthesia to die shortly after in shock. This problem can be prevented by careful handling of microchambers or by injecting the animal with 1 mg of hydrocortisone after the second operation.

In some cases it may be advisable not to inject the rodent with colchicine. In these cases, after removing the microchambers are incubated for 3 h at 37 °C in Hanks solution containing 0.2 µg/ml of colchicine. Afterwards the procedure is followed as described above[6].

Resumen. Se describe una técnica que hace posible el estudio cromosómico de pequeños roedores sin sacrificar el animal. El método consiste en: a) se introduce una microcámara de difusión cargada con un antígeno en la cavidad abdominal del animal a estudiar, b) se extrae la microcámara cuando está rodeada por una población de células inmunocompetentes en división activa (el animal se colchiniza 3 h antes de sacar la microcámara), c) se colectan las células adheridas a la cámara mediante tratamiento enzimático con Pronasa, d) se preparan las metafases para estudio cromosómico, mediante shock hipotónico, fijación y preparación de extendidos por secado al aire.

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Simple Method for a Medium-Cell Separation

A rapid progress in the biochemistry of eucaryotic cells depends to a great extent on the development of new techniques. Tissue culture is the most useful model for cytological and biochemical studies of eucaryotic cells[1].

For separation of cells from culture medium, 2 methods have been used up to now. The first one employs centrifugation of cell suspensions followed by washing. This method is not suitable for taking samples at very short intervals, as well as the fact that fragile cells can be damaged during the procedure. The second one, a rapid sampling technique, used in special cases uses the ability of neutrophils to adhere to glass[2]. Neutrophil cells are allowed to adhere to a coverslip and then are easily washed.

In the present paper, another simple method for medium-cell separation with broad spectrum of application is described.

Material and methods. 1. Whatman 3MM paper strips, size shown in Figure 1. 2. Phosphate buffered saline (PBS) or any physiological medium. 3. Vessel varying in length according to the number of processed samples.

Aliquots of cell suspension (in our case 0.1 ml samples were withdrawn) are dropped in the middle of Whatman 3MM paper, and one end of the strip is immediately dipped into the vessel filled with cold PBS. A fixation of the opposite end of paper should assure an ascending flow of the fluid. All extracellular soluble material (including radioactivity) moves rapidly with the front of soaking solution, while cells remain at the same place (Figure 2). By this procedure the cells are washed within 2–3 min, during which time further samples can be taken. The zone with the spotted sample can be cut out of the wet or dried strip and radioactivity measured in dioxane or toluene scintilation liquid, respectively.

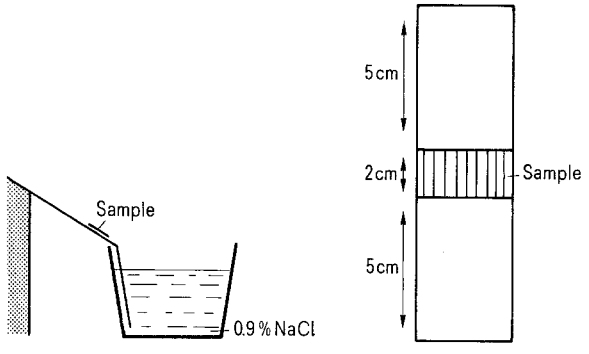

Fig. 1. Schematic representation of the method described.

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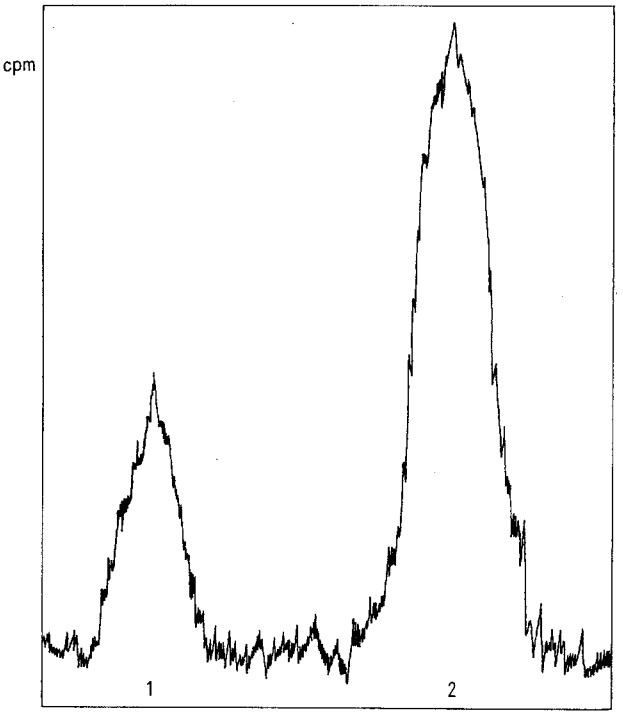

Fig. 2. Radioactivity tracing on paper strip after processing. 1. Zone where suspension of mouse thymus cell labelled by ^{14}C uridine (spec. act. 0.1 mCi/0.46 mg) was spotted. 2. Radioactivity separated from the cell sample by soaking PBS.