administered as ND (fig.). The AUC $\int_0^{60\ min}$ in the serum between ED and ND were almost of a similar order of magnitude (fig.).

C_{max} of P in the serum was maximal following NS (fig.). C_{max} of P was significantly higher ($p < 0.05$) as compared with i.v., ND and ED and also when compared with i.m. ($p < 0.01$).

C_{max} of P in the CSF (fig.) was apparently higher following NS but this level was not significantly different from the C_{max} achieved by i.v. However, C_{max} following NS was significantly higher ($p < 0.05$) as compared with i.m., ND and ED.

C_{max} of P both in the serum and CSF was not statistically different when compared between i.v., i.m., ND and ED.

The best correlation coefficient (r) between serum and CSF levels of P was observed by administering P as ED ($r = 0.827$) followed by NS ($r = 0.676$), ND ($r = 0.671$) and i.v. ($r = 0.613$). Administration of P by i.m. showed the poorest correlation coefficient ($r = 0.202$).

Discussion. The present studies clearly indicate that the bioavailability of P is considerably higher in both the serum and CSF following its being sprayed intranasally and that the enhanced bioavailability is dependent on the method by which P is administered into the nostrils. Previous studies[12] have shown that the bulk of the material sprayed into the nostrils with a glass atomizer is deposited on the olfactory mucosa from where it could enter the subepithelial blood capillaries as well as pass into the CSF.

Transport of the steroid into the blood and CSF across the olfactory mucosa would be a slow process resulting in the steroid also being cleared from the circulation at a slower rate. A slow rate of entry of the steroid into the body fluids accompanied by a slow rate of its clearance could enhance its bioavailability, as indicated in the present studies.

Thus, the present studies not only indicate a novel method of drug delivery which enhances the bioavailability of P but also offer an explanation for the effects observed in terms of impaired ovarian and testicular functions with the intranasal administration of low doses of P[4,5,7].

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PRO EXPERIMENTIS

A simple method enabling standardized handling of small biological objects for light and electron microscopic preparation

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Summary. A glass filtration-apparatus provided with an exchangeable nylon-tissue filter permits collection, fixation, dehydration, staining and preembedding of small biological objects in a simple and rapid way.

Problems arising during the handling of small biological objects for microscopy such as eggs, embryos and small isolated organs of different origins are frequently solved with methods which cannot easily be reproduced[2] for several reasons, such as loss of material due to difficulties in finding the objects after preparation[3], or a high risk of drying[4] or the sticking of the objects during their transport in highly volatile solvents. With the method described here, collection, fixation, dehydration, staining and embedding procedures for light and electron microscopy can be carried out with only minor modifications, whereby optimal conditions for reproducibility are also guaranteed.

Methods and discussion. All procedures take place in a standard vacuum glass filtration apparatus (as used in microbiological methods, Schleicher & Schüll AG, CH-8714 Feldbach, Switzerland) connected by suitable length of tubing to a vacuum membrane pump (Balzers Union, FL-9496 Balzers, Liechtenstein). For biological objects larger than 30 µm, filters consisting of a nylon-tissue disk with a diameter fitting the glass filtration-apparatus and with opening sizes starting from 10 µm (Swiss Silk Bolting Cloth Mfg. Co. Ltd, CH-8027 Zürich, Switzerland) can be used for nearly all of the fixation, staining and dehydration treatments.

After collecting the objects in an appropriate medium, usually a Ringer's solution, the suspension (or isolated larger objects) is given into the cylinder of the glass filtration-apparatus (figure 1). With an appropriate vacuum, the Ringer's solution is sucked away and immediately replaced with the fixative. The objects collected on the filter disk are fixed for an adequate period. The operation is repeated for each subsequent solution according to the chosen treatment. Care must be taken not to overload the apparatus: obstruction of the filter complicates the control of the suction forces, thereby increasing the risk of drying or damaging the objects.

For whole mounts, without embedding for sectioning, the filter disk containing the stained material is carefully taken out of the apparatus with fine forceps after treatment of the objects in the solvent of the balsam (ethanol, xylol). The filter disk is then placed in a little dish previously filled with the balsam (euparal, caedax, eukitt). From there, the objects are transferred to a glass slide by means of a capillary pipette and then covered with a cover slip. The great viscosity of the balsam reduces the capillarity forces in the pipette, so that the transport of the objects can be held under control during the whole process. This largely eliminates drying or sticking of the objects to the glass.

If sectioning of the objects is desired, treatment in the apparatus ends after infiltration in the solvent of the embedding medium (xylol, toluol) or in an antemedium.

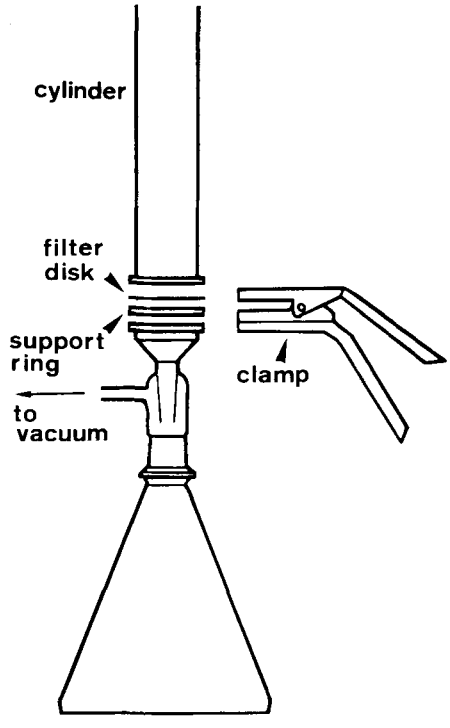

Fig. 1. Diagram of the glass filtration-apparatus.

Embedding is carried out by taking the filter disk containing the objects into a mould filled with the embedding medium. The filter disk can be easily separated from the objects after the hardening of the block.

For electron microscopy, after fixation and block staining as mentioned before, dehydration can be carried out in 2 different ways. Treatment follows in the apparatus till infiltration in 30% plastic is finished. The filter disk is then taken out of the apparatus and placed in a little dish previously filled with 30% plastic. Or, the objects are washed in bidistilled water after the last fixation or block staining step and taken out of the apparatus for dehydration in a desiccator according to the method of Sitte[6]. In this case, the filter disk is placed in a watch glass previously filled with bidistilled water, and the watch glass is brought into the desiccator. After dehydration, 70% plastic is added to the watch glass to obtain a final plastic concentration of about 30%. This 2nd method provides better anatomical preservation of the objects and avoids tedious cleaning of the apparatus after contamination with the plastic. In both cases, embedding is completed by transporting the objects into drops of higher concentrations of plastic by means of a capillary pipette. Final inclusion follows in a mould, in gelatine capsules or similarly. If exact orientation is desired, the objects can be oriented under a binocular microscope in a drop of 100% plastic on a glass-slide. Polymerisation follows overnight. Then, a gelatine capsule filled with fresh plastic is inverted over the hardened drop containing the objects, and is also polymerized. Immersion in liquid nitrogen allows separation of glass-slide and plastic.

For both dehydration procedures, with this method, repeated and uncontrolled transport of the objects (as required by other methods[5]) is avoided. An advantage over the method of Treiblmayr and Pohlhammer[3] is that pipetting or pouring in the highly volatile solvent is eliminated. The reduced size of the apparatus also makes it possible to carry out all the treatments in a refrigerator at 4 °C, after disconnecting the vacuum-pump and closing the cylinder with a rubber stopper. The apparatus should be taken out of the refrigerator only for changing the previously cooled solutions.

To illustrate the appropriateness of the described procedures, preparations of small chorionless eggs and embryos (sized 40–400 µm) of a paedogenetic Dipteran insect, *Heteropeza pygmaea*[7], have been made. 2–3-day-old mother larvae containing eggs and embryos were homogenized through a Cellector tissue sieve (Bellco Glass, Inc., Vine-

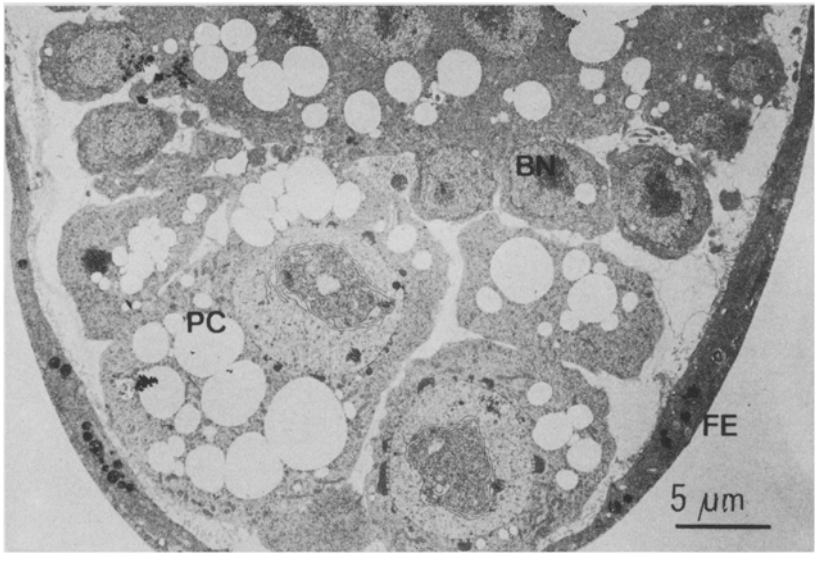

Fig. 2. Ultrathin section through the posterior pole of a syncytial blastoderm stage of a *Heteropeza pygmaea* egg showing follicular epithelium (FE), pole-cells (PC) and syncytial blastoderm nuclei (BN).

land, New Jersey 08360, USA) provided with a 40- or 80-mesh screen (380- or 190-µm opening size) in a dish filled with Ephrussi/Beadle modified Ringer's solution. The homogenate was sieved through 500-, 250-, 140-, 105-, 85-, and 62-µm nylon meshes in different combinations for elimination of contaminants and selection of chosen stages. The final suspension was placed in the cylinder of the glass filtration-apparatus provided with a filter disk (30 mm diameter) of nylon tissue (17- or 27-µm opening size). Whole mounts were obtained with a high yield after different staining methods (Heidenheim's iron-haematoxylin, Mayer's haematoxylin-eosin, Daddi's sudan for lipids, Kunick's methyl green-pyronin, orcein-lactic acid for chromosomes). Paraffin embedding was carried out over cedarwood oil. For electron microscopy (figure 2), after sequential fixation by glutaraldehyde and osmium tetroxide[8], objects were dehydrated in acetone and embedded in epon and araldite.

The above mentioned investigation exemplifies the mode of use of the given method for a specific object. For both light and electron microscopy of these objects, the results were comparable to those previously obtained with much more tedious and less efficient methods[7].

1 This work was supported by grant No 3.218.-077 of the Swiss National Science Foundation.
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Intrasplenic infusion. A simple method for intraportal infusion in rats

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Summary. A simple and time-saving method for intraportal acute or chronic infusion in the rat by the insertion of a catheter into the spleen is described. This method has proved to be especially useful for performing chronic infusions in conscious animals.

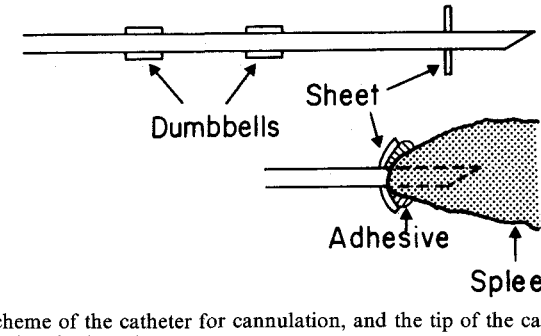
There is a great variety of experiments which require administration of different solutions into the portal system. Cannulation of the portal vein requires extensive surgery, viscera manipulation with loss of fluid and, frequently, great alterations in the portal circulation. Cannulation of a primary mesenteric vein causes great hemodynamic alterations in the splanchnic area, and cannulation of secondary mesenteric veins requires the use of very small catheters, with the problem of small flows and great resistances. The method here presented allows the infusion of any kind of solution even at high infusion rates, without disturbing the portal circulation.

PE 50 tubing (polyethylene tubing, 0.5 mm inner diameter; 1.0 mm outer diameter; Vigon France) was used for this purpose. One end of the catheter was cut with an angle less than 30°, giving a well beveled tip. A small round (1 cm in diameter) piece was cut from flexible polyethylene sheeting, 0.05 mm thick, and a small hole was bored in its center with a sharpened steel tube. The beveled tip of the catheter was then threaded through the sheet and glued to the tube with polyethylene adhesive. 2 small dumbbells made of PE100 tubing were put 3 and 5 cm away from the sheet and used for tying the catheter to the abdominal muscle. All materials were sterilized in a 1:1000 solution of benzalkonium chloride (Armil) for 24 h and rinsed with sterile saline immediately before implantation.

The rats were anesthetized with nembutal (30 mg/kg b.wt i.p.). An abdominal incision about 2 cm in length was made in the linea alba 3 cm below the sternon. The tube was passed under the skin to the back of the neck and filled with heparinized isotonic saline. The spleen was exposed and a loose ligature made in the conjunctive tissue of one of its ends with a double two zero silk. The beveled tip of the catheter was then inserted into the spleen following the direction of its long axis until the sheet touched it, and a drop of cianoacrylate esther adhesive (Eastman 910 adhesive, Eastman Chemical Products, Kingsport, Tenn., USA) was put in the site of puncture (fig.). The ligature was tied to the tube behind the sheet and after 2 min to allow the adhesive to solidify, the spleen was replaced in the abdomen, the catheter tied by the proximal dumbbell to the internal face of the abdominal muscles and the muscular layers closely sutured around the catheter. Then the tube was tied to the external face of the abdominal muscle by the distal dumbbell and the skin was closed.

The external end of the catheter, which during the experiment had been connected to an insulin syringe filled with heparinized saline, was sealed by insertion of a stainless steel rod, and left free in the neck or tied with a suture to the skin.

After the operation the animals were put into individual large cages on top of sterile absorbent pads, and allowed to wake and move freely in the cage. Penicillin was given on the day of surgery and 4 consecutive days thereafter (100,000 U i.m. daily).



Scheme of the catheter for cannulation, and the tip of the catheter in place in the spleen.