The water samples were collected into Winkler bottles of about 50 ml in volume. With the help of microsyringes, 0.25 ml of 40% $MnCl_2$, 0.25 ml of 10% KOH, and 0.25 ml of 3% dye solution were added successively into the bottles which were then closed and shaken thouroughly for 1 min, the precipitate was allowed to settle. After 3 min, 1 ml of 40% citric acid was added and the bottles were again shaken for 1 min in order to dissolve the precipitate. After 10 min, a deep blue colour had developed (figure 2); 10 ml of this blue solution were pipetted out into a 100-ml standard flask and made up to the mark with distilled water. The optical density of the blue colour was read against distilled water in a Eppendorf photometer (Netheler and Hinz, Hamburg) at a wavelength of 578 nm, using glass cuvettes of 1 cm light path.

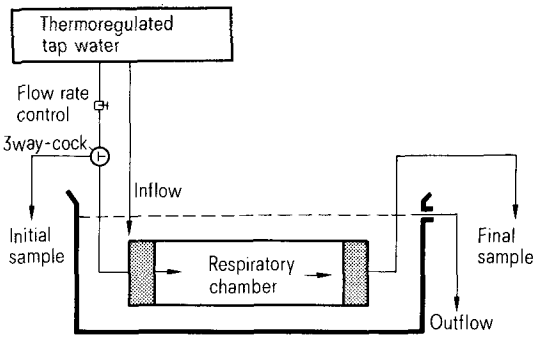

Fig. 1. Continuous flow set up for measuring O_2-consumption of aquatic animals.

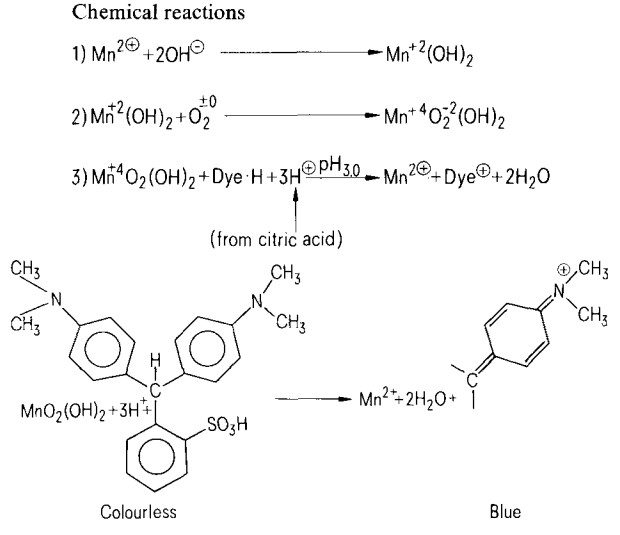

Fig. 2. Chemical reactions involved in the estimation of O_2-concentration with the dye leukoberbelinblue I.

The calibration curve was prepared with the help of original Winkler-method by using water samples of different O_2-concentrations (by bubbling N_2 into tapwater). The equation of the calibration curve reflecting the relation between O_2-concentration and optical density read at 578 nm, was calculated by linear regression. A calibration factor of 6.607 was obtained in this present method. Calculation formula:

$$\frac{\left[\text{O.D.}^{\text{initial}}_{578} - \text{O.D.}^{\text{final}}_{578}\right] \cdot 6.607 \cdot \text{flow rate}}{\text{body weight}} = O_2\text{-consumption}$$

calibration factor	$[\text{ml } O_2 \cdot l^{-1}]$
flow rate	$[l \cdot h^{-1}]$
body weight	[g]
O_2-consumption	$[\text{ml } O_2 \cdot g^{-1} \cdot h^{-1}]$

The reagents were purchased from Merck (Darmstadt) and the dye from Altmann (Berlin). All the solutions were prepared with O_2-free distilled water. In order to prevent decomposition of the dye, an amber-coloured bottle was used and 0.3 ml of 25% NH_4OH per 100 ml solution of the dye was added.

Results. The accuracy of the method was tested with 20 estimations by the help of variation coefficient (VC), and we got a VC of 3.5%. The O_2-consumption of *Idus idus* was estimated at different flow rates and a flow rate of 5 l/h was maintained in our experiments. A clear dependence of O_2-consumption on adaptation-temperature was noticed which indicates an adaptation in the sense of a compensation (table)[7,8]. By altering the flow rate and the size of the respiratory chamber, the O_2-consumption of any aquatic animal could be measured.

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Direct visualization of the haemal system in starfish by a staining procedure

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Summary. After perforating the madreporite, an infusion of trypan blue in sea-water was administered to the axial sinus of a starfish. By making the animal subsequently transparent, the haemal system is directly visible by a blue colour.

Part of our research on reproduction physiology of the starfish *Asterias rubens* (L.) is concerned with possible transport routes between storage organs (pyloric caeca) and gonads. The role of the perivisceral fluid in this transport has been examined and discussed by Ferguson[2-5]. Besides the haemal system may be involved in the transport of metabolites. Therefore, it is necessary that this system interconnects the digestive organs and the gonads.

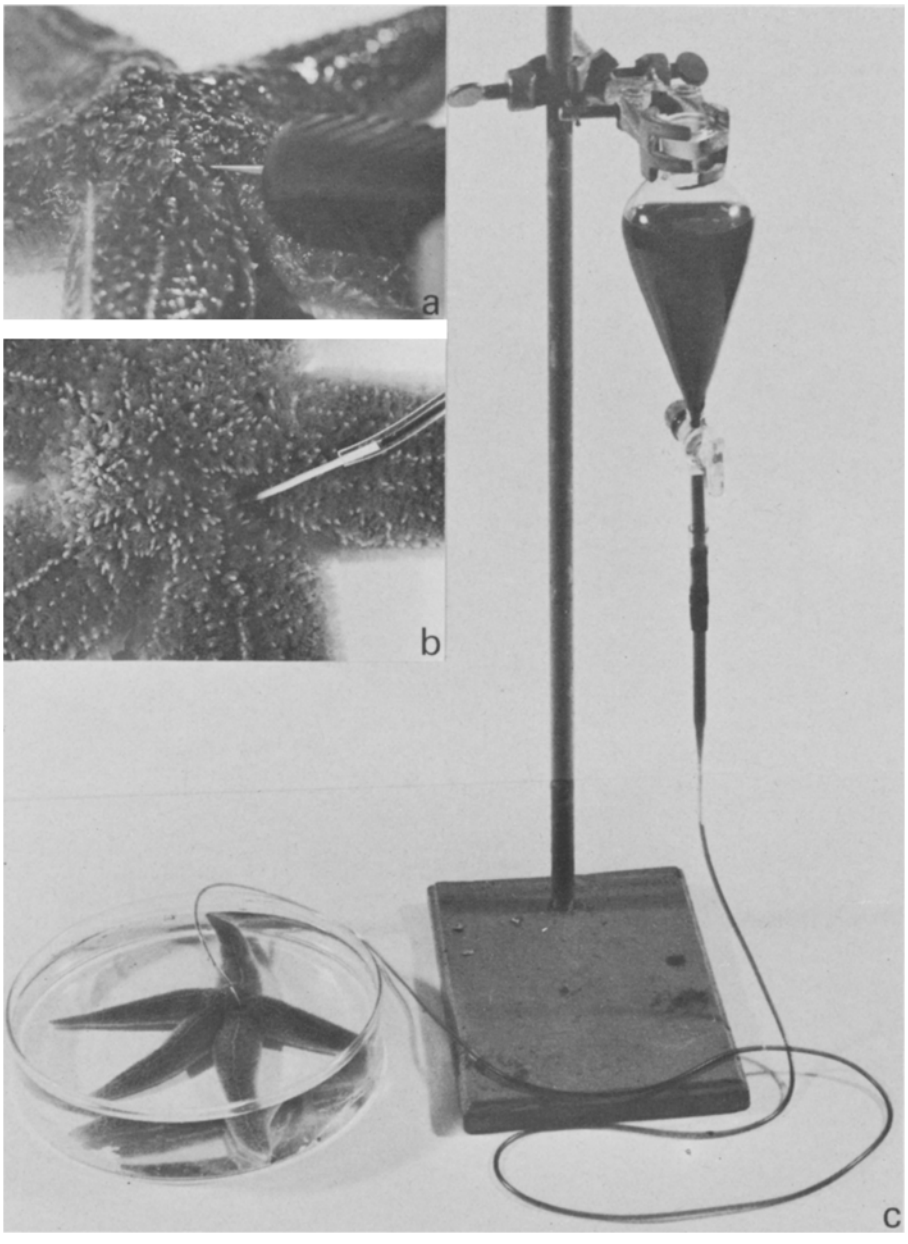
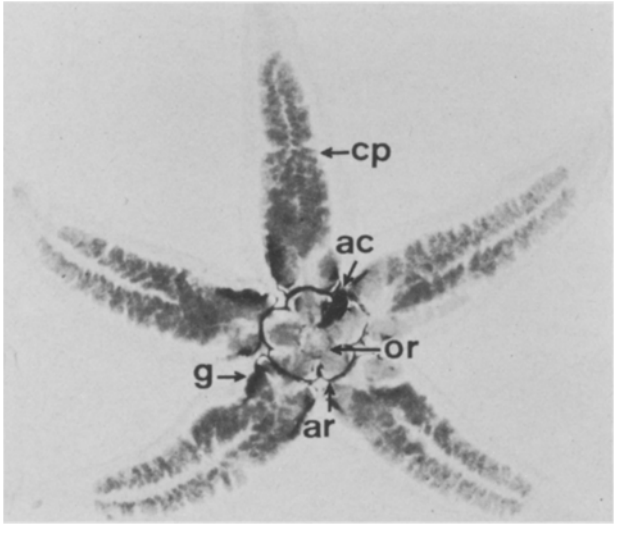


Fig. 1. *a* Perforation of the dorsolateral place of the madreporite with a dentist's drill. *b* Insertion of the infusion tube into the perforated madreporite. *c* Infusion with trypan blue in sea-water into the starfish.



To visualize the haemal system in situ, a small perforation was made into the most interbrachial site of the madreporite using a dentist's drill with a diameter of 1 mm (figure 1, a). The hole is now located on top of the axial sinus without any damage to the stone canal. Into the perforation was inserted a small cooled PVC tube, with a diameter of 1 mm at room temperature, that fixed itself by expansion (figure 1, b). This tube was connected with an infusion flask filled with a 0.05% (g/v) solution of trypan blue in sea water. Then the starfish was placed in a Petri disk (figure 1, c). After 2 days, the animal was killed by fixation in a 4% formaldehyde solution. From this point, a modification of the method after Spalteholz[6] described for vertebrates was applied.

Fig. 2. Transparent starfish with coloured axial complex, haemal system and gonads due to an infusion with trypan blue in sea-water into the perforated madreporite. cp, pyloric caeca; ac, axial complex; g, gonad; ar, aboral ring of the haemal system; or, oral ring of the haemal system.

After fixation for 3 days (in which time the solution was replaced once), the animal was decalcified in a continuously stirred 5% HNO_3 solution until the total body was soft. Decolourization was performed in 3% H_2O_2 (if necessary 5%) till the skin was completely colourless. In the pyloric caeca and the gonads some colour was left, probably due to lipid components. Formaldehyde and acid residues were washed out by running tap-water for 3 days and then by putting the animal 3 times in aqua dest for 2 days. Then the preparation was dehydrated by bathing it in 50, 70, 90, 96, 100 and 100% ethanol for 12 h in each concentration.

Now the animal was placed for 2 days in benzene, which was replaced 3 times, after which the preparation was placed in a mixture of methylsalicylate and benzylbenzoate (15:5.5, v/v). This liquid shows the same refraction index as that of the tissues of the animal. To facilitate the removal of benzene and air bubbles, the tips of the arms were removed and the preparation was placed in a vacuum desiccator.

The result is a transparent starfish with a blue haemal system and slightly coloured pyloric caeca and gonads (figure 2). The picture clearly shows the connection between the axial complex and the gonads. The fact that the gonads are coloured, whereas the digestive system is not, shows that the trypan blue has gone in the direction of the gonads. This transport direction has already been suggested by Cuénot[7]. Research on the interconnection between digestive system and axial complex is in progress.

1 The authors are much indebted to Dr P.A. Voogt and to Dr R.C.H.M. Oudejans for reading critically the manuscript.
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A method to collect cervical smears from small breeds of monkeys

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Summary. A simple method is described to collect cervical smears and to have a clear view of cervix for small breed of monkeys. This method was found useful to collect adequate cytology smears with good preservation of cellular morphology.

There is an increase in the use of primate models for various physiological and pharmacological investigations. In studies in reproduction, cytological examination of cervical mucosa is regularly used for studying different phases of menstrual cycle, for detection of ovulation, for breeding and for research purposes, for screening cervical malignancy during studies with or without contraceptives, etc. The usual way of collecting the smears is by the introduction of a sterile cotton swab through the vagina[1] or by examining the vaginal washings[2]. The disadvantages of these procedures are that a) no information is obtained regarding the origin of cells, b) increase in the number of inadequate smears with lack of cellular material and more of mucus, c) cellular contamination from vulva and vagina, d) fecal contamination. The following simple method has been found to be useful in overcoming the above disadvantages and has been found practicable in our primate colony especially for smaller sized bonnet (*Macaca radiata*) females.

Method. An attempt was made to use the small size Simm's and Cusco's bivalve speculum which was found to be useful only for bigger animals like adult langur monkeys. For smaller bonnet monkeys, this could not be used. The procedure used for collection of cervical smears from the bonnet monkeys is discussed below. The perineal and the external genitalia were cleaned with antiseptic lotions and a sterile nasal speculum of 35 mm (figure 1) was introduced into the vagina. The bivalve speculum which opens sideways was opened after insertion into the vagina and a light source from above was provided to have a clear view of the cervix (figure 2). The cervix could be clearly seen and cervical scrape smears and vaginal pool smears were taken

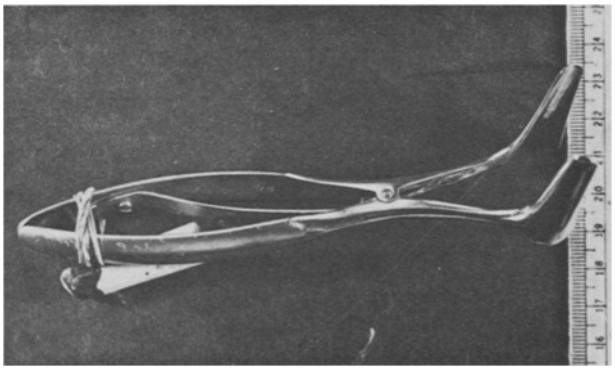

Fig. 1. Nasal speculum, 35 mm.

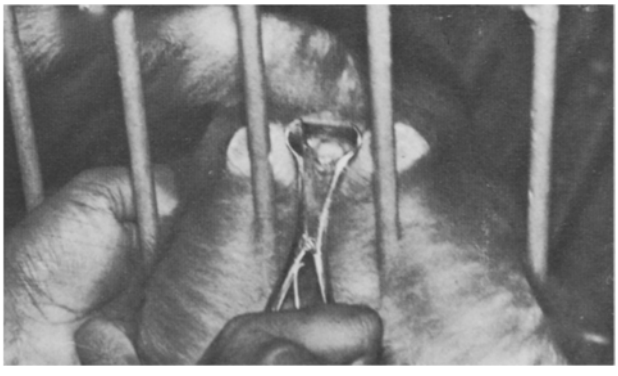

Fig. 2. Speculum in situ, exposing the cervix clearly.