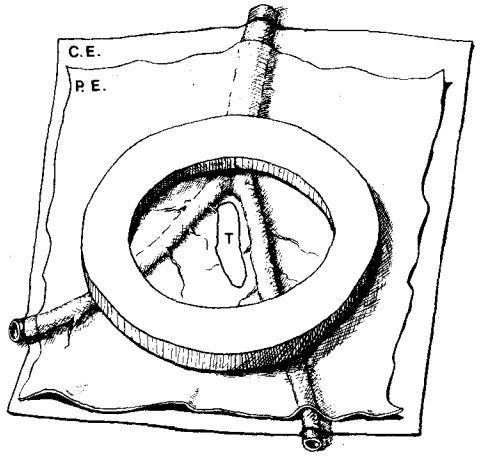
Millipore filter often adhers tightly to the transplant and may become embedded in the CAM. Deformation of the transplant cannot always be avoided. The inclusion of the piece of filter material into the graft may interfere with histological sectioning. The disadvantages of O'Hare's modification can be avoided by the use of a thin film of polyethylene instead of cellulose ester filter material. Square films (side approximately: 10–15 mm, weight: 1,5–2 mg) of polyethylene (trade-mark: Glad, Union Carbide) are pre-sterilized by immersion in 70% ethanol for several h. After drying in a sterile Petri dish, they are ready for use. The transplant or association of transplants is placed in the freshly prepared artificial air space at the surface of a vascularized area of the CAM, and then covered by such a sterilized film of polyethylene (figure). To assure a better contact of the transplant with the underlying CAM, a small ring cut from a silicone rubber tube is placed on the polyethylene film with the graft in a central position. The ring (weight: 30–40 mg) has an external diameter of approximately 8 mm and a lumen width of 4–5 mm. With this accommodation of the CAM grafting, the number of successful transplantations is much higher than with the classical method. For this there are several reasons. Since the polyethylene film is waterproof, the exsiccation of the graft and graft site is avoided. The weight of the rubber ring on the polyethylene sheet usually prevents slipping of the graft on the CAM and assures optimal contact of the transplant with the chorionic ectoderm. The evolution of the transplant can easily be followed, since the polyethylene film is perfectly transparent. During the transplantation period, the polyethylene film always remains stretched at the surface of the CAM and never becomes enclosed in it. So the localization of the graft remains easily detectable, even after a prolonged sejourn on the CAM, and nearly always the polyethylene film can easily be separated from the underlying graft and CAM.



The polyethylene-ring technique of CAM transplantation: view of the graft site after excision. T, transplant; P.E., polyethylene film; C.E., chorionic ectoderm.

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2 J.B. Murphy and P. Rous, J. Am. med. Ass. *58*, 741 (1911).
3 E.R. Clark, Science *51*, 371 (1920).
4 B.H. Willier, Am. J. Anat. *33*, 67 (1924).
5 F.M. Burnet, J. Path. Bact. *37*, 107 (1933).
6 D.A.T. New, The Culture of vertebrate embryos. Academic Press, New York 1966.
7 M.J. O'Hare, J. Embryol. exp. Morph. *27*, 215 (1972).
8 J.J. Harris, Ann. N.Y. Acad. Sci. *76*, 764 (1958).
9 D.H. Ausprunk, D.R. Knighton and J. Folkman, Devl. Biol. *38*, 237 (1974).

A quick and modified Winkler-method for measuring O_2-concumption of aquatic animals[1]

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Summary. A modified procedure for measuring O_2-consumption, based on Winkler-method, is described. Instead of KI and HCl (or H_2SO_4) triphenylmethane-dye leukoberbelinblue I and citric acid are used.

Besides the Winkler-method, the so-called O_2-electrode is applied for the estimation of oxygen consumption of aquatic animals[2]. Although the chemical method is simple in practice, some interferences occur if the sample is taken from lakes or rivers as well as marine habitats. Generally in Winkler-method, the measuring of optical density is preferred to titration with $Na_2S_2O_3$. However, the vapours of strong acids like HCl or H_2SO_4 destroy the sensitive parts of the photometer, even when the cuvettes are carefully covered. The advantage of the polarographic method is undoubtedly a continuous estimation of O_2-concentration as a function of time, but suitable equipment like amplifier and recorder are required for the purpose.

In view of these complications, we present here a quick and modified Winkler-method using the dye leukoberbelinblue I[3] and citric acid instead of expensive KI and strong acids like HCl or H_2SO_4. In our chemical method, the compound MnO_2 $(OH)_2$ oxidizes the triphenylmethane-dye at pH 3.0 to a deep blue berbelinblue I which has a maximum absoprtion at the wavelength of 620 nm. The optical density of the blue solution of this dye is a measure of O_2-concentration in water.

Material and methods. The O_2-consumption of the fish *Idus idus* L. (Cyprinidae) was measured by the set-up (figure 1) as described[4–6]. A respiratory chamber (made out of transparent plastic material) with a diameter of 7 cm and measuring 20 cm in length was used.

In the present experiments, 3 fishes of similar size (3–4 g b.wt) were introduced into the respiratory chamber covered with a black foil.

O_2-consumption of the fish *Idus idus* L. as a function of adaptation-temperature (experimental temperature 25 °C)

Adaptation-temperature [°C]	O_2-consumption [ml $O_2 \cdot g^{-1} \cdot h^{-1}$]
10	0.32
20	0.22

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.

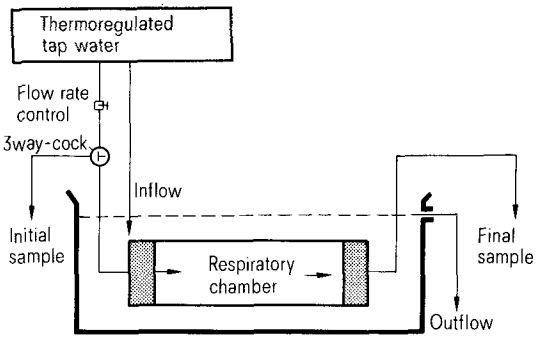

Fig. 1. Continuous flow set up for measuring O_2-consumption of aquatic animals.

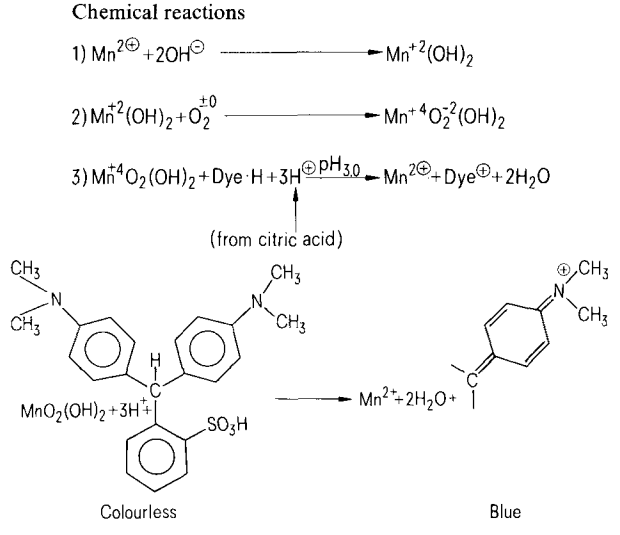

Fig. 2. Chemical reactions involved in the estimation of O_2-concentration with the dye leukoberbelinblue I.

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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2 J. Kanwisher, Limnol. Oceanogr. *4*, 210 (1959).
3 H.J. Altmann, Z. analyt. Chem. *262*, 97 (1972).
4 H. Precht, Z. vergl. Physiol. *44*, 451 (1961).
5 G. Krüger, Z. wiss. Zool. *167*, 87 (1962).
6 D. Schultze, Z. wiss. Zool. *172*, 104 (1965).
7 G. Berkholz, Z. wiss. Zool. *174*, 377 (1966).
8 H. Precht, J. Christophersen, H. Hensel and W. Larcher (eds.), Temperature and Life, p. 334. Springer Verlag, Heidelberg/Berlin/New York 1973.

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.