frequenz wird reduziert (STRANZINGER[7]). Die Objektträger sollen flach auf einen Färberost gelegt und mit dest. Wasser überschichtet werden. Sofort anschliessend werden mit einer 0,025%igen Trypsinlösung (37 °C) im pH 7,0 die Objektträger vorsichtig überschichtet und nach 20 Sek. (Wahlweise bis zu 2 Min. zu behandeln) wiederum mit dest. H_2O abgespült.

Die Wahl des Trypsins ist von ausschlaggebender Bedeutung. Wir konnten z. B. mit dem Trypsin der Fa. Merck (Kat.-Nr. Art. 9204) keine Bänderung erzielen und verwenden ausschliesslich das 0,25%ige Trypsin von Gibco, Kat.-Nr. 505, welches wir nach erstmaligem Auftauen in kleinen 10 ml Portionen nochmals einfrieren. Kurz vor dem Färbevorgang wird die entsprechend benötigte Menge Trypsin aufgetaut und aufgelöst und mit auf 37 °C vorgewärmtem Sörensen-Puffer 1:10 verdünnt. Die Verdünnung nimmt eine gelbe Farbe (sauer) an und wird mit 10%iger Bikarbonatlösung auf Färbeumschlag (von gelb auf rot) titriert. Trypsin (1:250) der Firma DIFCO (Art.-Nr. 0152–13) wird in Pulverform geliefert. Verdünnt mit (Mg^{+2} und Ca^{+2} frei) Pucks BSS-Lösung bringt dieses Trypsin ebenfalls gute Bänderung (WANG et al.[8]). Die notwendige Auflösung des Trypsins ist jedoch ein zusätzlicher Aufwand gegenüber der bereits gebrauchsfertigen Gibco-Trypsinlösung. Nachdem das Trypsin, nach 20 Sek. bis 2 Min. Einwirkungszeit, mit dest. H_2O abgespült wurde, wird eine auf 60 °C vorgewärmte Giemsa-Borax-Puffer-Lösung auf die Objektträger gegeben und nach 2 Min. eine neue in Zimmertemperatur aufbewahrte Giemsa-Borax-Puffer-Lösung überschichtet. Nach weiteren 2 Min. können die Objektträger mit Leitungswasser abgespült, getrocknet und unter dem Mikroskop betrachtet werden. Sollte die Bänderung zu schwach, d.h. die Chromosomen noch durchgehend normal angefärbt sein, so kann der Farbstoff mit Fixierlösung entfernt, die Objektträger mit dest. H_2O abgespült und die Trypsinbehandlung mit zeitlichen Modifikationen wiederholt werden. Es kann ausserdem empfohlen werden, dass besonders gute Metaphasen (kreisförmige Lage der Chromosomen ohne Überlagerungen und Berührungen und in der Prometaphase) in den Koordinaten registriert und nach jeder Neubehandlung verglichen werden.

Metaphasen auf einem Objektträger sind sehr unterschiedlich stark gebändert und man kann mit nur 10% der auf dem Objektträger befindlichen Metaphasen für eine Aufnahme rechnen. Die Darstellung einiger Bandkaryotypen bei Rind und Kaninchen soll die Differenzierungsmöglichkeiten spezieller Chromosomenanomalien aufzeigen.

Zusammensetzung des Borax-Puffers.
Stammlösung A: Borsäure H_3BO_3 MG 61,83, 0,2 M = 12,3 g/l 50 Teile
Stammlösung B: Natriumborat $NaBO_2 - 4\ H_2O$, MG 137,86 0,2 M = 27,57 g/l 59 Teile
mit dest. H_2O auffüllen auf 200 Teile.

Zusammensetzung des Sörensen-Puffers.
Sörensen-Puffer (nach SCHNEDL für Bandfärbung M/15 KH_2PO_4/Na_2HPO_4 mit einem pH von 6,8).
KH_2PO_4 MG = 136,09 – 1/15 M = 20,41 g/l
$Na_2HPO_4 \cdot 2H_2O$ MG = 177,99 – 1/15 M = 26,69 g/l.
Der Gehalt an Methylen Violet Bernthsen in Giemsalösung ist von Bedeutung und kann durch leichtes Aufkochen aktiviert werden.

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Construction of a PO_2 Microelectrode for Use in Small Blood Vessels

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Summary. The construction of a Clark-type PO^2 electrode is described. The electrode has a tip diameter of less than 0.4 mm and a 95% response time of about 2 sec.

For investigations on oxygen transport in fish, a PO_2 transducer was desired that was small and sturdy enough for use in blood vessels (tip diameter less than 0.5 mm), and fast enough to monitor blood PO_2 changes (95% response time at most 2 sec). The membrane-covered polarographic (Clark type) PO_2 electrode is widely used for in vivo measurements of oxygen tension, and many such electrodes have been described in the literature. Some of them, having very small tips (less than 10 µm)[1,2], were considered to be too fragile. Others have tip diameters over 0.8 mm[3–5], or are too slow[6]. This paper describes the construction of an electrode that meets the requirements mentioned above.

Construction of the electrode. Basically the electrode consists of a platinum wire (the cathode) sealed in glass. The glass is coated with silver paint which results in a silver ring at the tip (the anode). The tip is covered by an electrolyte-absorbing medium over which an oxygen-permeable membrane is fitted.

Platinum wire is coated with glass by a modification of the method described by BALLINTIJN[7] (Figure 1, a, b and c). Then, electrical connections to the electrode are made with varnish-insulated copper wire (Figure 1, d). Contact with the cathode (upper knot) is established by silver paint (DAG S56 colloid silver in solvent, Acheson Colloiden B. V., Scheemda, The Netherlands). Also, a layer of silver paint (the future anode) is applied from the lower knot down to the tip of the electrode (Figure 1, e). After allowing the paint to dry, the entire electrode is insulated by dipping it into chloroform-diluted epoxy resin (resin AW 106, hardener 953U, CIBA AG, Basel, Switzerland). The resin is baked for half-an-hour at 80 °C. 3 to 5 of such layers are applied. A piece of glass tube fitting around the knots is fixed to the electrode, using undiluted epoxy resin (Figure 1, f). This protects the connections and facilitates handling of the electrode.

The tip of the electrode, which has become covered with paint and resin, is ground to a slightly convex shape on a

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[6] H. I. BICHER, J. W. RUBIN and R. J. ADAMS, in *Advances in Experimental Medicine and Biology* (Eds. H. I. BICHER and D. F. BRULEY; Plenum Press, New York, London 1973), vol. 37 A, p. 107.
[7] C. M. BALLINTIJN, Experientia *17*, 523 (1961).

small grinding disc (a metal disc covered with carborundum No. 800 in epoxy resin). As a result, the Pt cathode (diameter 20 µm) is exposed, surrounded by the annular Ag anode (diameter about 300 µm; width some 20 µm). After cleaning the tip with acetone, the silver is chloridized in 0.1 *N* HCl for 30 sec by connecting a 1.5 V battery between the anode and a Ag/AgCl electrode (anode positive).

The electrolyte around the tip and the oxygen-permeable membrane are applied according to the method described by SILVER[2]. The extreme tip of the electrode is coated with collodion by dipping it into a solution of collodion in a 1:1 ether-alcohol mixture. To reduce the response time of the electrode, the collodion layer should be made as thin as possible by choosing empirically the appropriate dilution. After drying, the collodion is soaked in 0.5 *M* KCl. Finally, the tip of the electrode is dipped into the histological mounting agent D.P.X. dissolved in xylol. This layer too should be as thin as possible, the appropriate dilution being determined empirically. Drying is allowed for 1 day, with the tip of the electrode up.

The current meter. The electrode current is measured by a current amplifier according to the principle described by HAHN[8] (Figure 2). With R_1 the polarizing voltage can either be varied in order to measure the polarogram of the electrode or adjusted to 0.8 V for normal use. With R_2 the input current of the operational amplifier and the zero current of the electrode can be compensated for in the range of −1 to +10 nA.

Characteristics and testing of the electrode. With respect to linearity, zero current, and flow dependency, the electrode is comparable with other microelectrodes[9].

The sensitivity of the electrode is of the order of 10 pA/Torr. As sensitivity is temperature-dependent, calibration of the electrode has to be done at the temperature at which the physiological experiments are performed.

When the tip coating of the electrode has been applied properly, the 95% response time is about 2 sec. Response time is measured in a water current fed alternately from 2 reservoirs, one with air-saturated and one with nitrogen-saturated water.

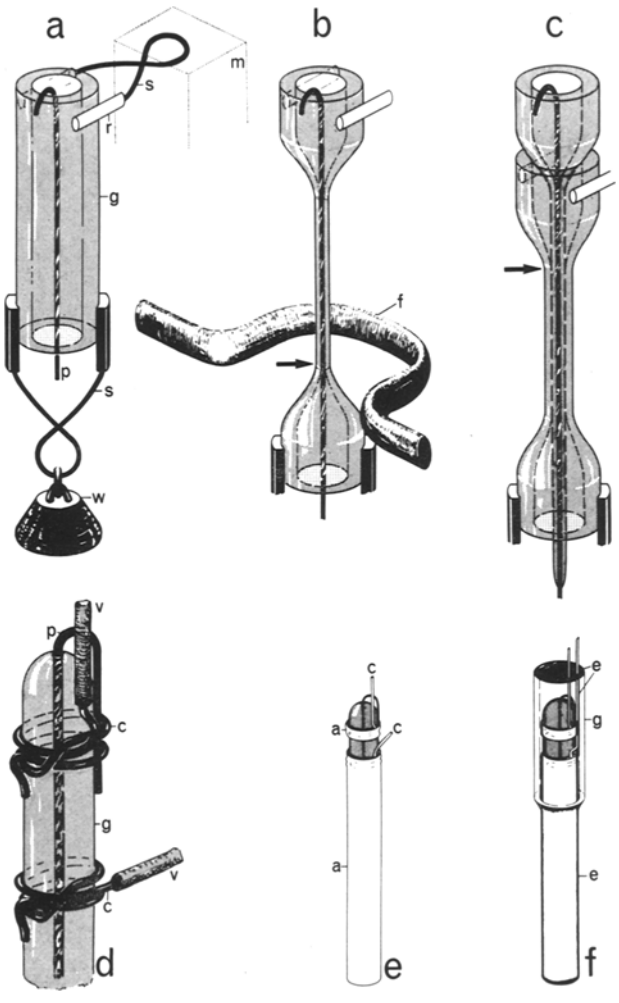

Fig. 1. Construction of PO_2 elctrode. a) Platinum wire (length 4 cm) is inserted into glass tube (length 2 cm, o.d. about 0.5 mm). b) First layer of glass coating is applied. Lower end of glass tube is broken off at arrow and removed from Pt wire. c) More layers of glass are applied, until diameter of electrode is some 0.3 mm. Upper ends of glass tubes, together with Pt wire, are broken off at arrow. This end will become tip of electrode. d) Position of electrical connections (electrode turned upside down with respect to c)). Knots are shaped beforehand around a pin. e) Silver paint is applied to upper knot and from lower knot down to tip. f) Electrode is coated with epoxy resin and provided with protective glass tube. Meaning of symbols: a, silver paint; c, Cu wire 50 µm; e, epoxy resin; f, heating filament (0.6 mm chromium-nickel wire); g, pyrex glass; m, micromanipulator; p, Pt wire 20 µm, r, heat resistant silicone rubber tubing; s, "serre fine" spring clamp; v, varnish insulation; w, weight (about 1 g).

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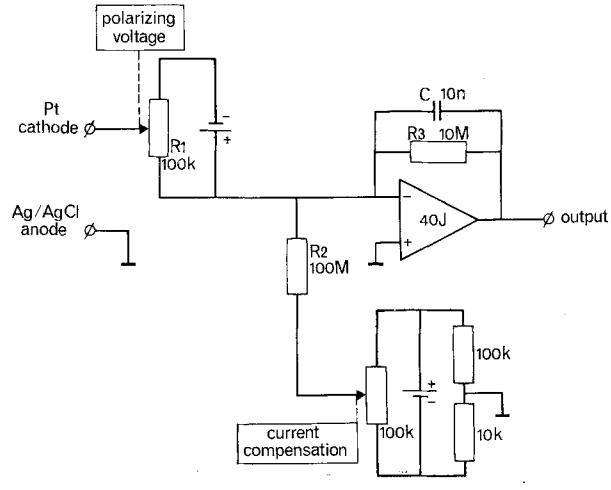

Fig. 2. Current amplifier circuit. FET-input operational amplifier (type 40 J, Analog Devices) has input bias current of less than 50 pA. Batteries (1.35 V, 1000 mAh, type RM1H, Mallory) are soldered into the circuit without switches to avoid contact noise.

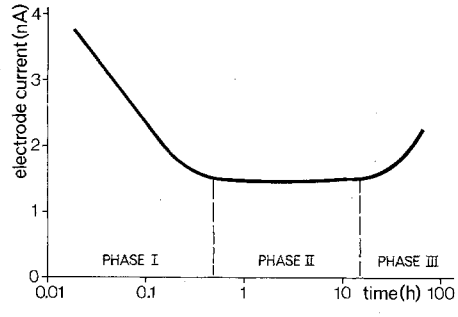

Fig. 3. Typical time course of electrode current (redrawn from long duration recording).

The continuity of the membrane is tested by connecting one of the input terminals of the current amplifier to the cathode of the electrode and the other to a separate Ag/AgCl electrode, both electrodes being dipped into 0.1 *M* KCl.

The electrode current changes throughout its useful life-time as shown in Figure 3. Calibrated measurements without the need of frequent recalibration are possible in phase II. Therefore the electrodes are stabilized prior to use in air-saturated water until this phase is reached. Phase II is normally long enough for one day of experimentation. When the electrode has reached phase III, the tip cover can be removed with acetone and the electrode can be recycled, starting with grinding the tip.

[10] The author is indebted to Dr. C. M. BALLINTIJN for valuable advice during the development of the electrode, to Dr. C. J. DEN OTTER and Mr. W. J. BEUKEMA for comments on the manuscript, and to Mr. G. THOMAS for suggestions regarding the English text.

Wireless Microphone for Studies of Animal Vocalizations

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Summary. A microphone collar for obtaining good quality recordings of animal vocalizations is described. An inexpensive, commercially available wireless microphone was modified and mounted on a collar with a hearing-aidbattery pack. The complete assembly weighs 25 g, and is readily accepted by domestic cats.

In studying the vocalizations of animals, it is often difficult to record the sounds of low intensity. The conventional solution for this problem is to use highly directional microphones (e.g., parabolic reflectors). The disadvantages are that the axis of the microphone must be aimed rather carefully at the particular animal, and that the unwieldy dimensions of the reflector, which become inevitable at lower frequencies, result in certain practical limitations. Further, unsurmountable difficulties appear if faint vocalizations of two individuals in close proximity are to be distinguished, or if the animal which is to be recorded has a noise source situated behind it. Due to these technical difficulties, a serious bias in favor of loud signals is introduced into studies of animal vocalization and communication by means of sound.

One of us (W.M.S.) has experimented several years ago with radio transmitters to record the soft contact calls of turkeys during movement of the flock, but at that time it proved too expensive to come up with a system of sufficient range, frequency response, and reliability. Recently we found a commercially available wireless microphone[1] operating in the FM range (tuneable between 88 and 92 MHz) which can be modified easily to fit a cat's collar, and which allowed us to make high quality recordings of a variety of vocalizations.

Because the microphone in its original form was too bulky, we discarded the housing and arranged the components on a 25 mm wide strip of soft chamois leather fitted with a button fastener (Figure 1). The electret condenser microphone (a cylinder of 11 mm length, 9 mm diameter, and 1.8 g weight) was attached in the middle of the collar, and to either side was attached the circuit board (18 mm × 58 mm, with the components 15 mm thick) and the battery pack respectively. In an earlier version we used the standard nine-volt transistor battery (15 mm × 25 mm × 50 mm, 33.5 g), but now have substituted eight 1.35 V mercury hearing aid batteries (Mallory Duracell RM312) spot-welded in series. Weight

[1] 'Realistic' brand 'Apollo FM-91 Wireless Microphone', marketed by Radio Shack, A Tandy Corporation Company, catalog number 33-1048A selling currently (August 1975) for $ 19.95.

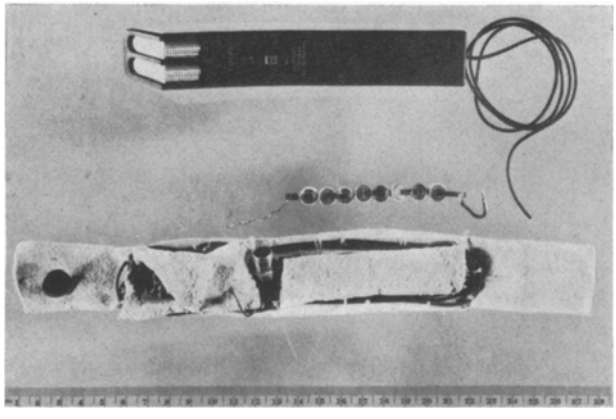

Fig. 1. Wireless microphone in its original, commercially available form (above), and stripped version mounted on collar (below); the collar is displayed with the battery pack pulled out and the circuit board exposed at one corner.

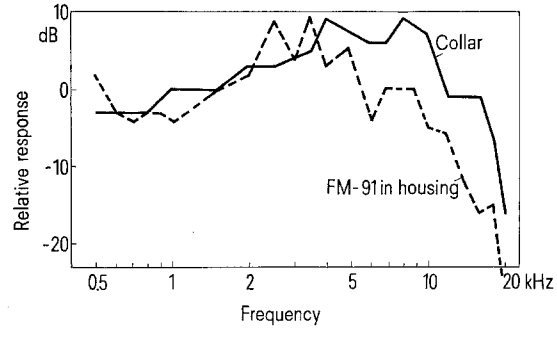

Fig. 2. Combined frequency response of the wireless microphone, receiver (Blaupunkt Frankfurt-Stereo US, Model #7630629), and tape recorder (Uher 4000 Report L). At 1.0 kHz, +64 dB re 2×10^{-3} N/m² gave full saturation on tape recorder (0 dB on meter).