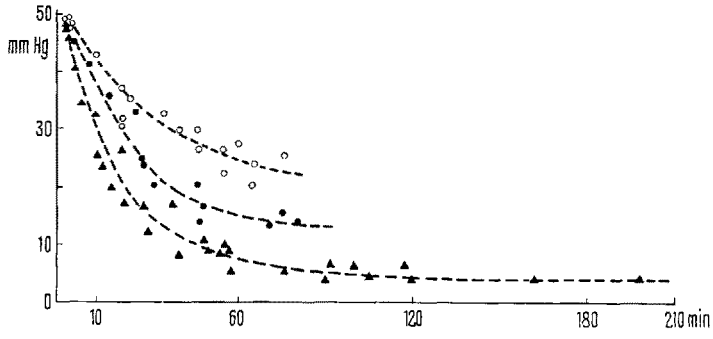

Chambre respiratoire 150 cm³. Seuils limites de la pression partielle d'oxygène mesurés chez différentes espèces, o *X. helleri*, • *C. auratus* and ▲ *A. latifrons*.

	Poids g	Expérimentation normale[5] O_2 mmHg	Durée	Poids g	O_2 minima mmHg	ppm	Durée h	Comportement
A. latifrons	4–6 6–11	36 24	24 h 24 h	2–8	4–8	0,21–0,30	3	Calme. Mouvements operculaires amplifiés
C. auratus	4–6 6–11	34 24	24 h 24 h	2–10	13–15	0,70–0,80	0,40	Phase d'excitation. Montée en surface.
X. helleri	0,7–5	48	24 h	0,9–1,5	25	1,5	0,30	Phase d'excitation. Montée en surface.

est réoxygénée par un courant d'oxygène qui pénètre par une ouverture latérale dans la chambre respiratoire. Ceci permet de répéter l'expérience un certain nombre de fois sur le même animal, sans perturber les conditions expérimentales, ni exciter le poisson.

Résultat et discussion. Sur le graphique, sont représentés les seuils limites de la pression partielle d'oxygène des espèces étudiées.

Le Tableau présente les valeurs de la pression partielle d'oxygène au cours de l'expérimentation normale[4], ainsi que les tensions d'oxygène minima nécessaire à la survie des poissons.

Il est à souligner que les *A. latifrons* tolèrent des pressions d'oxygène très basses, de l'ordre de 4–8 mmHg. En outre, ils se distinguent des autres espèces par leur comportement particulier. Ils restent calmes au fond de la chambre respiratoire, amplifient leurs mouvements de ventilation, mais ne présentent jamais des phases d'excitation.

Ces résultats concordent avec ceux obtenus par WHITWORTH[5] qui a étudié les besoins en oxygène de 30 espèces différentes. En soumettant les poissons à une activité forcée, la majorité des espèces succombe à des taux d'oxygène inférieurs à 2 ppm (38 mmHg). Seuls se distinguent, des *Campostoma anomalum* (Cyprinidés) de grande taille et le groupe des Cichlidés, *Tilapia nilotica* qui survivent à des teneurs en oxygène égales et même inférieures à 1 ppm (19 mmHg).

Il apparaît ainsi que les poissons Cichlidés présentent des particularités dans leurs échanges respiratoires qui semblent être liées à des caractères écologiques et physiologiques.

Summary. The lowest levels of oxygen tension tolerated by 3 Teleost fishes were determined. *Aequidens latifrons* is able to survive at very low O_2 tensions which are lethal for the other species. This particularity can be correlated with ecological and physiological characters of this species.

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Investigation of the Electrical Potential of the Isolated Midgut of the Freshwater Teleost, *Ameiurus nebulosus*

Mechanisms of ion regulation in marine teleosts, originally postulated by SMITH[1], have been confirmed by the in vivo ion transport studies of KEYS[2], MULLINS[3], and HOUSE[4]. These workers have emphasized the role of the gut in water, sodium, potassium and chloride absorption. HOUSE and GREEN[5] have reported active transport of sodium, chloride and water in the isolated midgut of the marine teleost, *Cottus scorpius*. In vitro studies of ion regulation in freshwater fish have not been reported. In the present study, the potential across the midgut wall is examined as an indicator of ion regulatory processes in the fresh water fish.

Ameiurus nebulosus catfish were kept in an aquarium at 21°C from October to late April and May, at which time the experiments were carried out. They were fed with one part ground beef liver to one part trout fry feed. Individ-

uals weighing between 50 and 70 g were decapitated, the midgut was excised, and a 1 cm section was mounted in a perfusion chamber designed by HARVEY and NEDERGAARD[6] for use with the Cecropia midgut. The procedures for adding the bathing solutions and the methods for measuring the potential differences with calomel electrodes and a Radiometer PHM4 potentiometer are also those of HARVEY and NEDERGAARD[6]. Three bathing solutions were used. All were buffered to a pH of 7.2 with phosphate buffer, one part to ten. The basic solution was the physiological saline solution of EKBERG[7] as modified by ROBERTS[8]. The constituents are reported in the Table, and henceforth will be referred to as salines A, B, C. Experiments were carried out, bathing both sides of the gut with saline A. As required, either of the sides or both sides were changed to saline B or C. Potentiometer readings were taken immediately before and after each change and at 10–15 min intervals between changes. In a few experiments, ouabain and 2,4-dinitrophenol were added in concentrations of 10^{-5} and $10^{-4}M$.

The average potential across the midgut bathed in saline A was about 2.5 mV, the mucosal side being negative. The potential always increased by an average of 3.4 mV (Figure 1) when the mucosal side was bathed in saline B (sulfate substituted for chloride), and the serosal side was bathed in saline A. No effect on the initial potential was observed by reversing the bathing solutions (Figure 1). Substitution of choline for sodium (saline C) on the mucosal side caused appreciable change in the potential. The direction of change was in the positive side (Figure 2). Saline C on the serosal side increased the negativity of the potential. Ouabain, an inhibitor specific for active sodium transport (KOEFOED-JOHNSEN[9]), as well as 2,4-dinitrophenol, had no effect on the potential at 10^{-5} and $10^{-4}M$.

A slight potential difference across the isolated midgut wall, bathed in saline A, is in agreement with the findings of HOUSE and GREEN[5]. The results indicate a very negligible amount of ion movement, due to potential gradient occurring across the gut wall, when both sides are bathed in a physiological saline solution. HOUSE and GREEN[5] have shown that, in *C. scorpius*, sodium and chloride on both sides of the gut move as one neutral molecule.

Sulfate is a non-penetrating ion compared with chloride[10]. The substitution of sulfate for chloride in the bathing solution increases the potential by a few millivolts, suggesting a net flux of positively charged ions, i.e. sodium, from the mucosal to the serosal side. A more affirmative conclusion is not possible without an isotopic study. The change in direction and magnitude of the potential, when sodium on the mucosal side is substituted by choline, definitely demonstrates a sodium diffusion potential. This suggests that independent movement of sodium as an ionic species is possible and does not correlate with the findings of HOUSE and GREEN[5] in the marine teleost. In the silkworm, an animal which has a high blood potassium content, HARVEY and NEDERGAARD[6] have observed a similar rise in initial potential when potassium was substituted by choline on the mucosal side. When sodium is substituted on the serosal side, the mucosal side becomes more negative, indicating diffusion of positively charged sodium from mucosal to serosal side. The magnitude of the potential is not as great as in the previous case. If the potential difference were due to sodium movement alone, a differential permeability of the midgut to sodium ions could be postulated, the direction from serosal to mucosal side being the more favorable to ion movement.

Compositions of bathing solutions (g/l)

	Saline A	Saline B	Saline C
NaCl	6.5	–	–
KCl	0.15	0.15	0.15
$CaCl_2$	0.12	0.12	0.12
$NaHCO_3$	0.20	0.20	0.20
Na_2HPO_4	0.01	0.01	0.01
Na_2SO_4	–	7.88	–
Choline Cl	–	–	16.53
Na succinate	2.0	2.0	–
Glucose	2.5	2.5	3.83

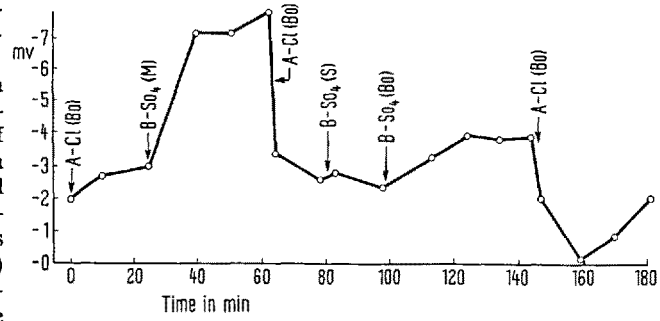

Fig. 1. Effects of sulfate Ringer's solution on the midgut potential. (A) Normal chloride solution, (B) sulfate solution, (Bo) both sides of the gut, (M) mucosal side of the gut, (S) serosal side of the gut.

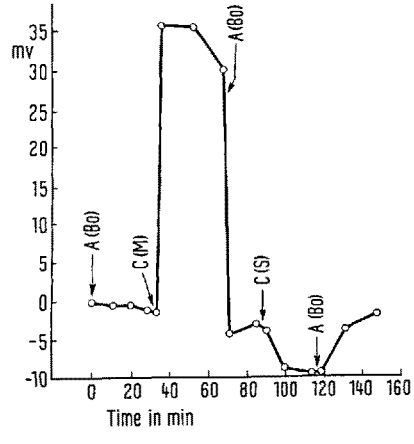

Fig. 2. Effects of substitution of sodium on the midgut potential. (A) Normal sodium solution, (C) choline chloride solution, (Bo) both sides of the gut, (M) mucosal side of the gut, (S) serosal side of the gut.

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The changes in potential observed by substituting sulfate and choline respectively for chloride and sodium indicate that both the ions permeate more freely from serosal to mucosal side than in the opposite direction.

Active transport of sodium and chloride has been demonstrated in the fish gall bladder by DIAMOND[11], and in the rat ileum, in vivo, by CURRAN and SOLOMON[12]. In the present study, $10^{-4}M$ ouabain and 2,4-dinitrophenol had no noticeable effect on the potential differences generated, leading to the conclusion that active transport was not involved in our observations. The sources of sodium and chloride in freshwater fish are food and water. They can actively absorb sodium and chloride against a high concentration gradient[13]. Rapid diffusion of sodium from the serosal side definitely suggests that absorption of the ion is not accomplished through the midgut[14].

Zusammenfassung. Durch Veränderung des innern oder äussern Milieus beim isolierten Mitteldarm eines Teleostiers wird das Potential quer zur Wand verändert. Dies ermöglicht Schlüsse auf die Ionenwanderung und damit Ionenregulation dieses Süsswasserfisches.

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Noradrenalin-Freisetzung aus isolierten Hypothalamus-Vesikeln

Versuche mit isolierten Hypothalamus-Vesikeln haben ergeben, dass bei der Inkubation bei 37 °C Noradrenalin spontan freigesetzt wird und ferner, dass die spontane Aminabgabe durch Kalzium und Acetylcholin gesteigert wird[1]. Beide Substanzen sind in der Lage, auch die spontane Noradrenalin-Freisetzung aus den aminspeichernden Partikeln der sympathischen Ganglien und Nerven zu erhöhen[2], während aus den chromaffinen Granula des Nebennierenmarks die Hormonabgabe durch Kalzium jedoch nicht durch Acetylcholin[2-4] gesteigert wird.

In der vorliegenden Arbeit wurde die Wirkung der monovalenten Ionen Natrium und Kalium sowie von Nikotin auf die Amin-Freisetzung untersucht.

Methoden. Noradrenalinspeichernde Vesikel vom Schweine-Hypothalamus wurden, wie früher beschrieben, durch Differentialzentrifugieren isoliert, in $0{,}3M$ Saccharose-Lösung pH 6,8 suspendiert und 60 min lang bei 37 °C inkubiert[1]. Die Inkubate und die nicht inkubierten Kontrollen wurden mit $0{,}4N$ $HClO_4$ extrahiert[1] und im Überstand die Amine nach der Methode von PALMER[5], im Rückstand das Eiweiss nach der Methode von LOWRY et al.[6] bestimmt. Der Noradrenalin-Gehalt der Ansätze wurde pro mg Eiweiss berechnet. Als Ausgangswert für die Berechnung der prozentualen Noradrenalin-Freisetzung diente der Noradrenalin-Gehalt/mg Eiweiss der nicht inkubierten Kontrollen. Kalzium, Natrium, Kalium, Acetylcholin und Tetracain wurden als Chloride, Nikotin als Base verwendet.

Versuche. Bei der Inkubation isolierter Hypothalamus-Vesikel bei 37 °C werden spontan 24% des vesikulären Noradrenalins freigesetzt (Figur 1). Zugabe von Kaliumchlorid in ansteigenden Konzentrationen führt zu einer dosisabhängigen Steigerung der Aminabgabe, wobei die maximale Noradrenalin-Freisetzung mit $20 \times 10^{-3}M$ Kaliumchlorid erreicht wird. Auch durch Natrium-Ionen wird die spontane Aminabgabe stark erhöht; durch $20 \times 10^{-3}M$ Natriumchlorid werden 57% des Noradrenalins freigesetzt (Figur 1). Höhere Konzentrationen (bis $150 \times 10^{-3}M$ NaCl) sind nicht in der Lage, die Amin-Freisetzung weiter zu steigern. Somit vermögen neben Kalzium- auch Natrium- und Kalium-Ionen in Konzentrationen, die in der Zelle vorkommen, die Abgabe von Noradrenalin dosisabhängig zu erhöhen.

Um festzustellen, ob neben Acetylcholin auch andere depolarisierende Stoffe die Amin-Freisetzung beeinflussen können, wurden Hypothalamus-Vesikel mit Nikotin inkubiert. Als Vergleich wurden Versuche mit ansteigenden

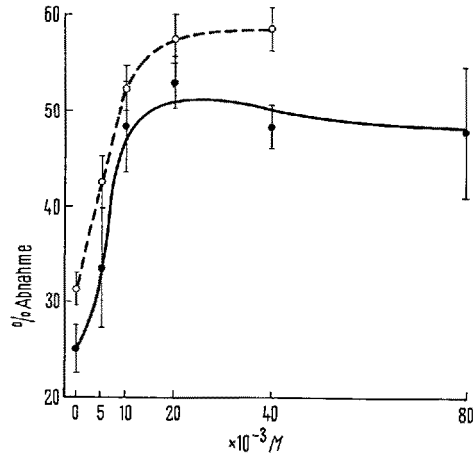

Fig. 1. Freisetzung von Noradrenalin durch Natrium (O----O; $n = 4$) und Kalium (●——●; $n = 5$). Inkubation: 60 min bei 37 °C. Mittelwerte und deren mittlere Fehler.

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