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Kinetic Study of Gill Epithelium Permeability to Water Diffusion in the Fresh Water Trout, *Salmo Gairdneri*: Effect of Adrenaline

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Summary. Using isolated head perfused at constant flow rates, close to those occurring *in vivo*, the movement of tritiated water through the gill epithelium of the trout, *Salmo gairdneri* was studied.

The analysis of the curves of loading and unloading of tritiated water between the gill epithelium and the external and internal media shows two exponentials with different slopes in each medium. As the rapid exponentials have identical slopes, the external medium, the gill epithelium, and the perfusion medium constitute a system of three compartments in series for water exchanges. The kinetic analysis of rapid exponentials allowed us to calculate the characteristics of water movement through the apical and basal membrane and the size of the pool of water participating in the exchange mechanism.

When the trout head is perfused without adrenaline, the permeability of the apical membrane to water is about 8 times higher than that of the basal membrane, the latter constituting the limiting factor for water diffusion.

When the trout head is perfused with a perfusion medium containing 10^{-5} M adrenaline this hormone produces a double action: it leads to a comparable increase in the permeability of both the apical and basal membranes and also increases the size of the water transport pool by a factor of four.

The study of teleost gills *in vivo* has shown that this epithelium is the site of considerable diffusional fluxes of water (Evans, 1969; Motais, Isaia, Rankin & Maetz, 1969). Pic, Mayer-Gostan and Maetz (1974) observed an increase of diffusional fluxes of water following intraperitoneal administration of adrenaline in *Mugil*. This study, however, did not permit the determination of the nature of this catecholamine's action on gill permeability to water. Indeed, numerous authors have shown that adrenaline provokes a decrease in the resistance of gill vessels to blood flow (Keys & Bateman, 1932; Richards & Fromm, 1970; Wood, 1974).

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Two hypotheses have been advanced to explain this action. Steen and Kruysse (1964) suggested that adrenaline causes a vasodilation of the secondary gill lamellae, while Hughes (1972) invoked the existence of a mechanism of recruitment which would increase the number of lamellae perfused in the presence of adrenaline. These two hypotheses describe the effect of adrenaline in terms of an increase in the functional exchange surface between the gill and the external medium (Bergman, Olson & Fromm, 1974). The development of the technique for perfusing the isolated trout head (Payan & Matty, 1975) led to the demonstration that adrenaline increased the gill permeability towards water diffusion by stimulating β receptors (Haywood, Isaia & Maetz, 1977). Furthermore, Girard and Payan (1977a), by adapting the isolated trout head preparation to experiments involving loading and unloading of radioactive tracers which were developed for other epithelial systems (Schoffeniels, 1957; Morel, 1958; Whittam & Guinebault, 1960; Istin, 1970), showed that the lamellar epithelium, the perfusion medium, and the external medium constitute an ionic exchange system of three compartments in series. These authors have shown that the intermediate compartment (lamellar) is limited by two barriers, the apical and basal membranes, which have different permeability characteristics for Na^+ and Cl^-.

The purpose of the present work was to determine the characteristics of these two membranes in relation to water exchanges and also to study the action of adrenaline on the permeability of these two barriers.

Materials and Methods

The trout used, *Salmo gairdneri*, weighed 158 ± 4.6 g on the average ($n = 14$) and were obtained from a breeding facility near Nice, France. The fish were kept in the laboratory in running aerated fresh water. Water exchange studies were performed on the isolated trout head preparation (Payan & Matty, 1975), using a constant perfusion flow-rate ($180\ ml \cdot h^{-1} \cdot 100\ g^{-1}$; Girard, 1976).

Triated water loading and unloading of the gill epithelium was performed as previously described by Girard and Payan (1977*a–b*). Briefly, the external medium (1), the lamellar compartment (2) and the internal medium (3) are considered as a system of three compartments in series (*see* Fig. 1). The transfer coefficient K_{nm} for water expressed in $\% \cdot min^{-1}$ concerns transfer from compartment n towards compartment m.

Radioactive loading experiments: THO (Centre d'Etudes Nucléaires Saclay, France) was added (15 μCi) to the external medium (volume: 130 ml) at time 0. The appearance of radioactivity in the perfusion liquid was then kinetically followed for 15 min. Analysis of the curves of cumulative appearance of THO in the internal compartment allows for the calculation of θ (min) according to the equation (Girard & Payan, 1977*a*):

$$\theta = \frac{1}{K_{21} + K_{23}}.$$

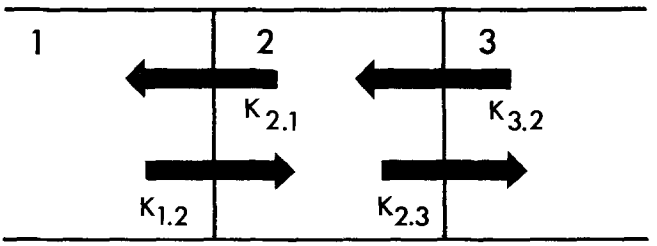

Fig. 1. Model of three compartment system used in this article. Example of transfer coefficients: K_{21}, fraction of water in compartment 2 renewed per min across the membrane from 2 to 1

Thus, the sum of the transfer coefficients which represents the turnover rate of water in the intermediate compartment can be obtained from this equation.

Radioactive unloading experiments: Isotopic equilibrium was first achieved after 20 min of contact with external medium containing the same isotopic concentration as above. The appearance of radioactivity was then followed kinetically in fresh external medium and in the efferent perfusion liquid. Analysis of the curves of unloading of THO appearing in the external and internal media allows calculation of the sum of the transfer coefficients, λ (min^{-1}) according to the equation (Girard & Payan, 1977*a*):

$$\lambda = K_{21} + K_{23}$$

and the ratio of these parameters, r, according to the equation (Girard & Payan, 1977*a*):

$$r = \frac{K_{21}}{K_{23}}.$$

A semilogarithmic plot of the THO appearance in the external and internal media shows two exponentials characterized by different slopes (Fig. 3). The fast exponentials possess an identical half life, $T1/2$ (min), which characterize the intermediate compartment (*see Discussion*). The sum of the transfer coefficients, λ, is obtained from the equation:

$$\lambda = 0.693/T\,1/2.$$

The ratio of the transfer coefficients, r, was calculated for water from the quantities of radioactivity simultaneously appearing in the external and internal compartments during the first minute of the radioactive unloading experiment. The unidirectional diffusion fluxes across these two barriers at equilibrium (J_{21} and J_{23}), as well as overall unidirectional transepithelial flux (J), were also measured and are expressed as $\text{ml} \cdot \text{min}^{-1} \cdot 100\,\text{g}^{-1}$ (*see* Girard & Payan, 1977*a*). The size of the pool ($\text{ml} \cdot 100\,\text{g}^{-1}$) participating in transepithelial water flux is given by the equation (Girard & Payan, 1977*a*)

$$\text{pool} = \frac{\text{transflux}}{K_{21} + K_{23}}.$$

Results

Figure 2 shows the kinetics of appearance of THO in the gill vascular compartment (3) in the presence and absence of adrenaline in the

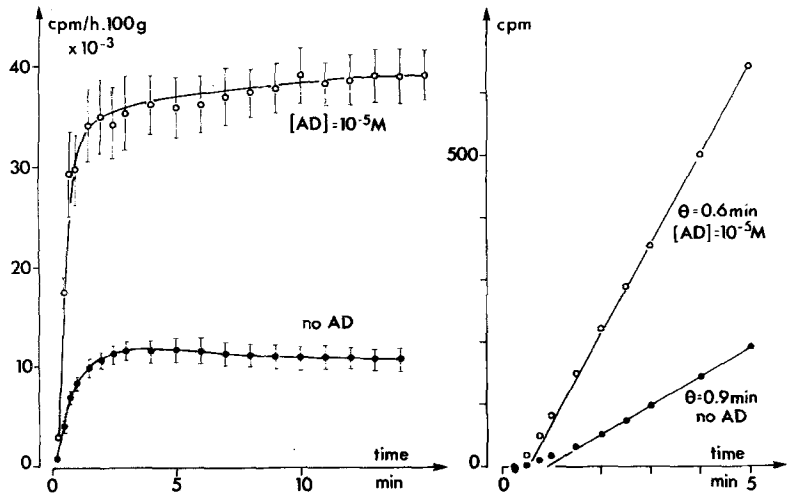

Fig. 2. Loading experiments, *left hand side:* appearance of THO in the internal lamellar compartment without (●) and with (○) adrenaline in the perfusing fluid. The values are normalized to a specific radioactivity of THO in the external medium equal to 1000 cpm/ml. Each point represents the mean ±SE of 8 experiments (no AD) and 5 experiments in the presence of adrenaline. *Right hand side:* experimental curves of the cumulative appearance of THO in the internal lamellar compartment allowing for the graphical determination of θ. θ is determined by the point of intersection of the asymptote of the curve with the t axis (*see* Girard & Payan, 1977*a*). (●) no AD; (○) 10^{-5} M adrenaline

Table 1. Sum of the transfer coefficient $K_{21}+K_{23}$ calculated from radioactive loading and unloading experiments concerning the fast exchangeable compartment[a]

	Loading experiment to: internal compartment	Unloading experiment to: external compartment	Unloading experiment to: internal compartment
No Ad ($n=8$)	113±15	115±19	110±11
[Ad]$=10^{-5}$ M ($n=5$)	192±16	147±12	137±18

[a] Adrenaline effect.

Means values ±SE. $K_{21}+K_{23}$ expressed in min^{-1}. Radioactive loading experiments: $K_{21}+K_{23}$ obtained from the graphical determination of $\theta=\frac{1}{K_{21}+K_{23}}$. Radioactive unloading experiments: $K_{21}+K_{23}$ was calculated from the $T1/2$ (min) of radioactive unloading of the internal and external compartment (*see Materials and Methods*).

perfusion liquid (radioactive charge experiments). The values of the sum of transfer coefficients ($K_{21}+K_{23}$), calculated in the absence and presence of adrenaline in the perfusion liquid, are shown in Table 1.

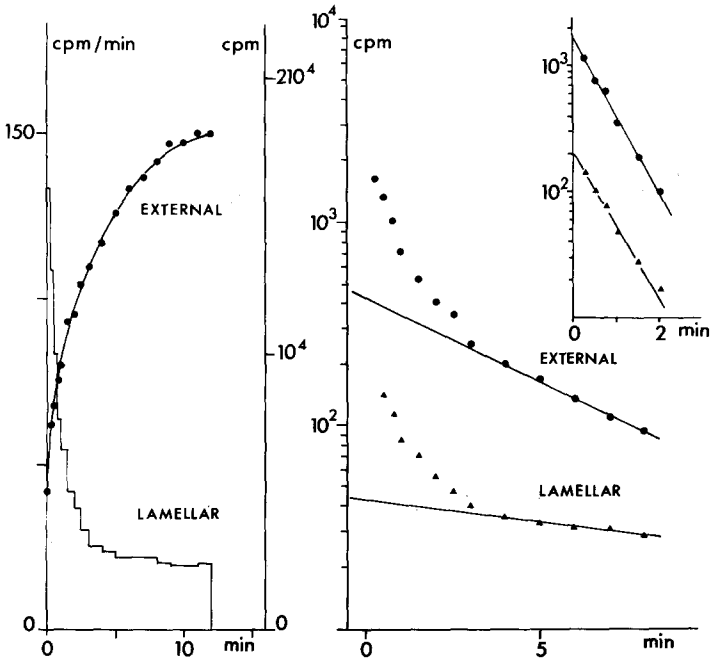

Fig. 3. THO radioactive unloading experiment and its mathematical treatment without adrenaline in the perfusion Ringer's. *Left hand side:* appearance of THO in the external medium in cpm, and in the internal lamellar compartment in cpm/min. *Right hand side:* graphical analysis. In ordinate logarithm of the quantity of radioactivity appearing per unit of time in the external (●) and in the internal (▲) lamellar compartment. Insert represents the graphical determination of the fast exponentials (Girard & Payan, 1977*a*)

Figure 3 shows the curves of radioactive discharge from the intermediate compartment into the external medium and into the perfusion liquid in the absence of adrenaline. Analysis of these curves demonstrates that for water two exponentials are obtained having different slopes for the external and internal compartments. The fast exponentials have comparable slopes and allow for the calculation of the sum of the transfer coefficients (*see* Materials and Methods) which are identical whether they are calculated from the external or internal compartments (Table 1). On the other hand, the slow exponentials possess slopes which are significantly different (no Ad ($n=8$), external compartment; $\lambda = 24.0 \pm 2.2$ min^{-1}; internal compartment, $\lambda = 4.0 \pm 0.2$ ($P < 0.01$); [Ad] $= 10^{-5}$ M($n=5$); external compartment, $\lambda = 25.0 \pm 5.4$; internal compartment, $\lambda = 1.0 \pm 0.1$ ($P < 0.001$)). The ratio of the transfer coefficients, r, calculated from the radioactive unloading experiments are 7.6 ± 1.2 ($n = 8$) in absence of adrenaline and 6.6 ± 0.7 ($n=5$) in presence of 10^{-5} M adrenaline.

Table 2. Transfer coefficients (K) and unidirectional diffusional water fluxes (J) across the apical (1.3) and basal (2.3) barriers of the lamellar epithelium[a]

[adrenaline] in perfusing solution	$\frac{1}{\theta}=K_{21}+K_{23}$	K_{21}	K_{23}	J_{21}	J_{23}	Transflux	Lamellar pool
	(min^{-1})			($ml \cdot min^{-1} \cdot 100\,g^{-1}$)			(ml 100 g)
No Ad ($n=8$)	112 ± 7	98 ± 7	14 ± 2	1.40 ± 37	0.18 ± 0.03	0.17 ± 0.03	0.13 ± 0.02
10^{-5} M ($n=5$)	158 ± 11[c]	136 ± 8[c]	22 ± 3[b]	4.93 ± 0.50[d]	0.77 ± 0.05[d]	0.65 ± 0.03[d]	0.42 ± 0.03[d]

Means $\pm$SE. In brackets, the number of experiments. Statistical comparison between no Ad and Ad $=10^{-5}$ M. $K_{21}+K_{23}$ values were the average of the values obtained from radioactive loading and unloading experiments (*see* Table 1).

[a] Effect of adrenaline.

[b] $P<0.05$.

[c] $P<0.01$.

[d] $P<0.001$.

Individual values of K_{21} and K_{23} have been calculated from the sum $K_{21}+K_{23}$ (average of the values obtained from radioactive loading and unloading experiments) and the ratio r. Results are pooled in Table 2. In the absence of the catecholamine, the turnover rate of water in the intermediate compartment is seven times more rapid across the apical than across the basal membrane. Although maintaining an identical ratio between K_{21} and K_{23}, adrenaline significantly increases the values of these two parameters.

The unidirectional fluxes J_{21} and J_{23} across the apical and basal barriers are shown in Table 2. In the absence of adrenaline, J_{21} is more intense than J_{23}, the intensity of the latter being comparable to that of the transepithelial flux (Table 2). It can be seen from this table that the catecholamine leads to a greater increase of the unidirectional fluxes J_{21} and J_{23} than of the transfer coefficients K_{21} and K_{23}. It appears that the action of adrenaline on the transfer coefficients is related to an increase in the size of the water pools, whose values are presented in Table 2.

Discussion

Validity of the Technique

The results obtained with the constant flow-rate perfusion technique in the presence and absence of adrenaline (10^{-5} M) (Table 2) are con-

sistent with those published by Haywood *et al.* (1977), which were obtained with the constant pressure perfusion technique. These authors reported unidirectional water fluxes equal to $0.18\,ml \cdot min^{-1} \cdot 100\,g^{-1}$ in the absence of adrenaline and $0.39\,ml \cdot min^{-1} \cdot 100\,g^{-1}$ in the presence of 10^{-6} M of the catecholamine.

Results from radioactive loading and unloading experiments show that the rapid exponentials of the internal and external compartments have identical slopes. This is expected from a compartment which is losing radioactivity exponentially across two different surfaces even if at different rates. In addition, the calculation of the sum of the transfer coefficients from loading experiments leads to identical values. Consequently, as Girard and Payan (1977*a*) showed for Na^+ and Cl^-, the external medium, the lamellar epithelium, and the vascular compartment can also be considered as a system of three compartments in series for water movement. The rapid exponentials correspond to the fraction of water in the intermediate compartment which is rapidly exchanged.

The observed difference in λ values for the slow exponentials for water (*see Results*) does not allow an identical mathematical exploitation and we do not, at this stage, attempt to explain this difference.

It is possible to criticize the demonstration of a clearance kinetics from the intermediate compartment which is more rapid across the apical than the basal membrane. Indeed, certain factors inherent in the particular experimental conditions could interfere with the measurements of water exchanges. The only factors one could imagine are the perfusion flow-rate and/or the unstirred layers of water on the internal and external sides of the lamellar epithelium. In the framework of cardiac flow-rate and utilizing the hemodynamic model of Vogel, Vogel and Kremers (1973) and Vogel, Vogel and Pfautsch (1976), confirmed by Girard and Payan (1976), the totality of blood leaving the heart crosses the secondary gill lamellae. Since the perfusion flow-rate was $3\,ml \cdot min^{-1} \cdot 100\,g^{-1}$, i.e., 17 times (without adrenaline) and 4 times (with adrenaline) greater than the measured diffusion flux, perfusion flow-rate flux thus cannot represent a limitation of the measurement technique.

It is not necessary to consider the limiting action of any eventual unstirred water layers (Dainty & House, 1962*a*, *b*) on water flux measurements in this type of preparation. Indeed, the greatest portion of the exchange surface ($400\,cm^2/100\,g$; Hughes & Morgan, 1973) is composed of respiratory epithelium having a thickness less than 5 μm. The diffusion coefficient for water across this epithelium can be calculated according to the Fick's equation:

$$J = \frac{A \cdot D \cdot C}{dx}$$

in which J represents the diffusional water flux ($mM \cdot sec^{-1} \cdot 100\,g^{-1}$), A: the gill area ($cm^2 \cdot 100\,g^{-1}$); D: the transepithelial diffusional water coefficient ($cm^2 \cdot sec^{-1}$); C: the water concentration ($55.2\,mM \cdot cm^{-3}$); and dx: the thickness of the epithelium (cm). Thus, the diffusion coefficient for water across this epithelium is about $3.10^{-9}\,cm^2 \cdot sec^{-1}$ in the absence of adrenaline in the perfusion liquid. Since the coefficient of self-diffusion of water at 15 °C is $1.5 \times 10^{-5}\,cm^2 \cdot sec^{-1}$ (Wang, Robinson & Edelman, 1953), it then becomes evident that if the unstirred water layers were the limiting factor, their thickness would have to be about 3 cm. This is physically and physiologically impossible, considering the internal irrigation and the external ventilation of the gills in the present experimental conditions.

Characteristics of the Lamellar Epithelium in Relation to Diffusional Water Exchanges

Two points are clearly shown from the radioactive loading and unloading experiments:

1) An intermediate compartment which probably consists of intracellular water exists for water exchanges. This compartment is limited by two barriers, the apical and basal membranes. Indeed, in the absence of an intermediate compartment, the curve of appearance of THO in the vascular compartment would give a value of θ equal to 0, contrary to that which is observed in Figure 2. The fact that the flux across the basal membrane (J_{23}) is comparable to the transflux measured by this technique or during *in vivo* experiments (Haywood *et al.*, 1977) demonstrates that the intermediary compartment can be equated to the secondary lamellar epithelium. Indeed, the internal medium collected *via* the dorsal aorta bathed solely the secondary lamellae of the gill.

2) There exist two barriers which water molecules must successively pass in order to cross the epithelium, apical and basal. In the absence of adrenaline, the basal barrier is about eight times less permeable and thus constitutes the limiting factor for water diffusion. These results were confirmed by recent experiments (Isaia & Isaia, 1978), showing that, although the intracellular water of the gills is partially bound to the macromolecules forming these cells, it is not a limiting factor for the

transepithelial diffusion of water. The basal membrane also limits Na^+ and Cl^- exchanges (Girard & Payan, 1977*a*, *b*) as well as exchanges of hydrophilic molecules such as mannitol (Masoni & Isaia, 1973). In this respect, the gill epithelium differs from the toad urinary bladder, where identical studies have shown that the apical membrane is the limiting factor for water diffusion (Parisi & Piccini, 1973).

The present results show that the size of the pool which participates in transepithelial diffusional flux is $140\,\mu l \cdot 100\,g^{-1}$ of body weight in the absence of adrenaline. The gill of a 100 g trout weighs about 2.9 g and the gill filaments weigh 1.6 g. Since 80% of the weight of gill filaments is water, the total quantity of water in the gills would then be $1300\,\mu l \cdot 100\,g^{-1}$ of body weight. This calculation shows that only 13% of the total intracellular water would participate in diffusional exchanges. A comparable result was obtained by Girard and Payan (1977*b*) for Na^+ and Cl^- in the gill of the fresh water trout: the transport pool represents only a small percentage (0.3 to 2% of the intracellular ion content).

Mechanism of Action of Adrenaline

The action of adrenaline is manifest by an increase of the transfer coefficients (about 1.4 times) and of fluxes (about 4 times). The exchangeable pool is increased in parallel by a factor of 3 (Table 1). The increase in the size of the rapidly exchangeable pool may be secondary to an increase in the number of cells which participate in the exchanges. This is the lamellar recruitment model proposed by Hughes (1972). Nonetheless, Haywood *et al.* (1977) demonstrated that the effect of adrenaline on water permeability could not be explained by this hypothesis. The increase in the size of the rapidly exchangeable pool could result from an increased participation of intracellular water in the rapid exchanges of water.

Adrenaline action on gills differs from that of neurohypophyseal hormones on other tissues. Thus, oxytocin increases the permeability of the luminal barrier of the toad urinary bladder and leads to the accumulation of tritiated water in the tissue. This suggests the existence of a second barrier to water movement which is located beyond the luminal barrier (Parisi & Piccinni, 1973).

In conclusion, adrenaline increases transepithelial water exchanges both by increasing the fraction of intracellular water participating in the exchanges and by increasing the permeabilities of the apical and basal

membranes. Further study is required to elucidate the intimate mechanism of action of adrenaline on membrane permeability.

References

Bergman, H.L., Olson, K.R., Fromm, P.O. 1974. The effects of vasoactive agents on the functional surface area of isolated-perfused gills of rainbow trout. *J. Comp. Physiol.* **94**:267

Dainty, J., House, C.R. 1966*a*. 'Unstirred layers' in frog skin. *J. Physiol. (London)* **182**:66

Dainty, J., House, C.R. 1966*b*. An examination of the evidence for membrane pores in frog skin. *J. Physiol. (London)* **185**:172

Evans, D.H. 1969. Studies on the permeability to water of selected marine freshwater and euryhaline teleost. *J. Exp. Biol.* **50**:689

Girard, J.P. 1976. Salt excretion by the perfused head of trout adapted to sea water and its inhibition by adrenaline. *J. Comp. Physiol.* **111**:77

Girard, J.P., Payan, P. 1976. Effect of epinephrine on vascular space of gills and head of rainbow trout. *Am. J. Physiol.* **230**:1555

Girard, J.P., Payan, P. 1977*a*. Kinetic analysis and partitioning of sodium and chloride influxes across the gills of sea water adapted trout. *J. Physiol. (London)* **267**:519

Girard, J.P., Payan, P. 1977*b*. Kinetic analysis of sodium and chloride influxes across the gill of the trout in fresh water. *J. Physiol. (London)* **273**:195

Haywood, G.P., Isaia, J., Maetz, J. 1977. Epinephrine effects on branchial water and urea flux in rainbow trout. *Am. J. Physiol.* **232**:R110

Hughes, G.M. 1972. Morphometrics of fish gills. *Respir. Physiol.* **14**:1

Hughes, G.M., Morgan, M. 1973. The structure of fish gills in relation to their respiratory function. *Biol. Rev.* **48**:419

Isaia, J., Isaia, A. 1978. Measurements of water exchanges in the eel gill compared by nuclear magnetic resonance and isotopic techniques. *J. Comp. Physiol.* **124**:137

Istin, M. 1970. Rôle du manteau dans le métabolisme du calcium chez les lamellibranches. *Bull. Inf. Sci. Techn. Saclay* **144**:53

Keys, A.B., Bateman, J.B. 1932. Branchial response to adrenaline and to pitressin in the eel. *Biol. Bull.* **63**:327

Masoni, A., Isaia, J. 1973. Influence du mannitol et de la salinité externe sur l'équilibre hydrique et l'aspect morphologique de la branchie d'anguille adaptée à l'eau de mer. *Arch. Anat. Microsc.* **62**:293

Morel, F. 1958. Interprétation de la mesure des flux d'ions à travers une membrane biologique comportant un "compartiment" cellulaire; exemple des mouvements de sodium à travers la peau de grenouille. *In:* Méthode des Indicateurs nucléaires dans l'Étude des Transports Actifs d'Ions. pp. 155–184. Pergamon, Oxford

Motais, R., Isaia, J., Rankin, J.C., Maetz, J. 1969. Adaptative changes of the water permeability of the teleostean gill epithelium in relation to external salinity. *J. Exp. Biol.* **51**:529

Parisi, M., Piccinni, Z.F. 1973. The penetration of water into the epithelium of toad urinary bladder and its modification of oxytocin. *J. Membrane Biol.* **12**:227

Payan, P., Matty, A.J. 1975. The characteristics of ammonia excretion by a perfused isolated head of trout (*Salmo gairdneri*): Effect of temperature and CO_2-free Ringer. *J. Comp. Physiol.* **96**:167

Pic, P., Mayer-Gostan, N., Maetz, J. 1974. Branchial effects of epinephrine in the seawater adapted mullet: I. Water permeability. *Am. J. Physiol.* **226**:698

Richards, B.D., Fromm, P.O. 1970. Sodium uptake by isolated-perfused gills of rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.* **33**:303

Schoffeniels, E. 1957. An isolated single electroplax preparation: II. Improved preparation for studying ion flux. *Biochim. Biophys. Acta* **26**:585

Steen, J.B., Kruysse, A. 1964. The respiratory function of teleostean gills. *Comp. Biochem. Physiol.* **12**:127

Vogel, W., Vogel, V., Kremers, H. 1973. New aspects of the intrafilamental vascular system in gills of a euryhaline teleost, *Tilapia mossambica*. *Z. Zellforsch.* **144**:573

Vogel, W., Vogel, V., Pfautsch, M.1976. Arterio-venous anastomoses in rainbow trout gill filaments. *Cell. Tissue Res.* **167**:373

Wang, J.W., Robinson, C.V., Edelman, I.S. 1953. Self-diffusion and structure of liquid water: III. Measurement of the self-diffusion of liquid water with H^2, H^3, O^{18} as tracers. *J. Am. Chem. Soc.* **85**:466

Whittam, R., Guinnebault, M. 1960. The efflux of potassium from electroplax of electric eels. *J. Gen. Physiol.* **43**:1171

Wood, C.M. 1974. A critical examination of the physical and adrenergic factors affecting blood flow through the gills of the rainbow trout. *J. Exp. Biol.* **60**:241