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BULK FLOW OF THE MEDIUM AND CUTANEOUS SODIUM UPTAKE IN FROGS: POTENTIAL SIGNIFICANCE OF SODIUM AND OXYGEN BOUNDARY LAYERS

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Summary

To examine the potential impact of fluid dynamic boundary layers on cutaneous ion exchange, we investigated how bulk flow of dilute Na^+ solutions ($\leq$1.0mmol l^{-1}) over the skin of intact frogs (*Rana catesbeiana* and *Rana pipiens*) affects cutaneous Na^+ uptake (J_{in}^{Na}) and transepithelial potential (TEP). Cessation of stirring resulted in a 14–35% decrease in TEP and a 14–65% decrease in J_{in}^{Na}. Two weeks' acclimation to an unstirred bath increased J_{in}^{Na} to levels 70% greater than in frogs acclimated to a continuously stirred bath and to levels comparable to those of frogs acclimated to deionized water. These effects are consistent with depletion of Na^+ in the boundary layer, but are also consistent with depletion of O_2 in the boundary layer, which might limit generation of ATP consumed by ATPases responsible for cutaneous Na^+ uptake. To investigate this latter possibility, we measured TEP and J_{in}^{Na} while manipulating the P_{O_2} of well-stirred external media at constant [Na^+]. Hyperoxia ($P_{O_2}\geq$97kPa) increased J_{in}^{Na} by 28% and had little or no effect on TEP. Hypoxia ($P_{O_2}\leq$1.5kPa) reduced J_{in}^{Na} by 48 % and decreased TEP by 22%. These results suggest that ionic and gaseous boundary layers may interact to affect cutaneous ion transport.

Introduction

Although the unstirred layer or boundary layer is a ubiquitous component of transmembrane processes, many membrane physiologists have deemed such layers of dubious physiological significance other than as a nuisance to the unambiguous determination of membrane properties (Barry and Diamond, 1984). During the past decade, however, boundary layers have been recognized as having a physiologically significant impact on transmembrane processes in diverse circumstances. For example, in the gut the unstirred layer around the villi is the principal rate-limiting step to the absorption of nutrients (Levitt *et al.* 1990); in blood vessels the concentration of various

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agonists in the unstirred layer may be an important means by which endothelial cells sense and respond to shear stress (Dull and Davies, 1991); and unstirred layers are directly responsible for severe local hypoxia at the skin surface in whole animals undergoing cutaneous gas exchange (Pinder and Feder, 1990; Booth and Feder, 1991; Feder and Booth, 1992). The present study investigates whether unstirred layers have a similar impact on transmembrane ion flux in a major model system for membrane studies, the frog skin.

In dilute external media, frogs balance a considerable urinary Na^+ loss with direct cutaneous uptake of Na^+ from the medium. Epithelial Na^+ uptake occurs through amiloride-blockable Na^+ channels (Lindemann and Van Driessche, 1977). In dilute external media, extrusion of H^+ by an apical H^+-ATPase is partially responsible for creating the electrochemical gradient that drives Na^+ inward; a basolateral Na^+/K^+-ATPase also contributes to this gradient and removes Na^+ from the epithelial cell (Ehrenfeld *et al.* 1985; Harvey and Ehrenfeld, 1988). As a result of these processes, a transepithelial potential (TEP) develops, which can be highly correlated with Na^+ influx under physiological conditions (Aceves and Erlij, 1971; Christensen, 1974; Salako and Smith, 1970).

Unstirred layers may be physiologically significant to ion uptake in frog skin in at least two non-exclusive ways. First, Na^+ uptake is strongly dependent upon $[Na^+]$ in the external medium at $[Na^+]$ less than $1 mmol l^{-1}$ (Greenwald, 1971; Kirschner, 1988). If rates of Na^+ removal from the unstirred layer (e.g. by cutaneous uptake) exceed rates of Na^+ replenishment in the unstirred layer from the bulk medium or the skin itself, then $[Na^+]$ will fall in the boundary layer. If the steady-state $[Na^+]$ in the unstirred layer falls to less than approximately $1 mmol l^{-1}$, Na^+ uptake may be depressed acutely. Second, if rates of cutaneous O_2 uptake from the unstirred layer exceed rates of O_2 replenishment from the bulk medium, the P_{O_2} will fall in the unstirred layer. This decline in P_{O_2} can reduce the oxygen consumption of the entire organism and the skin itself (Vitalis, 1990; Burggren and Feder, 1986; Booth and Feder, 1991). Insofar as the uptake of Na^+ is driven by epithelial ATPases whose activity is limited by ATP supply, hypoxic depression of ATP synthesis could be expected to depress Na^+ uptake. In theory, many other diffusable species could also affect Na^+ flux through accumulation or depletion in the unstirred layer. In any event, stirring of the medium or ventilation of the skin should ameliorate or abolish these effects by dissipating the unstirred layer. The present study reports diverse data that are consistent with these expectations for frogs of two species (*Rana catesbeiana* and *Rana pipiens*) exposed to different convective regimes and external $[Na^+]$. A novel aspect of these findings is the suggestion that oxygen boundary layers have a significant effect on cutaneous Na^+ transport.

Materials and methods

General

Adult *Rana catesbeiana* and *Rana pipiens* were obtained from a commercial supplier (Amphibians of North America, Nashville, TN, USA). While awaiting experimentation, frogs were maintained in tap water derived from Lake Michigan, at room temperature.

Except where otherwise specified, artificial pondwater (Alvarado and Dietz, 1970) was used as a diluent. Because of substantial inter-individual variation in variables under study, whenever possible we examined differences within individual animals exposed to stirred and unstirred conditions and tested for significance with the *t*-test for paired samples. In other cases we employed the two-sample *t*-test. We present mean ± S.E. and sample size for measurements.

Transepithelial potential (TEP)

The transepithelial potential difference between the bathing medium and the extracellular fluid was recorded with calomel half-cells and a Datacan data acquisition system (Sable Systems, Los Angeles, CA). Frogs were initially anaesthetized in MS-222 (1:1000, adjusted to pH7.0) and then partially immersed (up to the sternum) in a 800–1000ml bath of specified [Na^+] in artificial pondwater (Alvarado and Dietz, 1970). MS-222 (1:10000) was added to maintain anaesthesia and the pH of the bath was adjusted to 7.0–8.0. Ringer–agarose salt bridges, made from PE50 tubing, were used to connect the half-cells to the animal and bath. Before experimentation, any asymmetry in salt bridges was recorded and, if necessary, corrected for in subsequent measurements. One bridge was inserted in the ventral lymph sac through a small incision in the ventral skin above the bath level. The other bridge was shielded from flow by inserting it within an open-ended, long but loose-fitting piece of tubing immersed in the bath. Preliminary experimentation established that stirring of the medium produced no streaming potential when the bath electrode was shielded in this way. TEPs typically stabilized within 15–30min of salt bridge positioning. The entire bath assembly rested on an air-driven magnetic stirring apparatus that, when activated, vigorously stirred the bath.

Na^+ uptake (J_{in}^{Na})

Because the TEP may also reflect processes other than Na^+ uptake, we measured Na^+ uptake directly to complement determinations of TEP. Unidirectional flux rates of Na^+ were determined from the disappearance of $^{22}Na^+$ from the bathing solution (Jorgensen *et al.* 1954; Kirschner, 1970). Frogs were caused to void their bladders by suprapubic compression and placed in individual plastic cylinders (11cm diameter×12 cm height) containing 800ml of the specified bath. A plastic screen cone at the water's surface ensured that all skin except the tip of the snout was immersed in the bath, yet allowed the frog access to air. A second screen separated frogs from a magnetic stirring bar on the bottom of the container, which rested on a magnetic stirring apparatus. Stirring was begun and frogs were left for 1h, after which 1.5 μCi l^{-1} of $^{22}Na^+$ was added to the bath. Beginning 5min thereafter, the bath was sampled at recorded intervals. Bath samples were assayed for gamma radiation with a Packard Auto-Gamma 500 gamma counter. [Na^+] concentration of these and other bath samples was determined with a Coleman model 51 Ca flame photometer.

Influx (J_{in}^{Na}) was calculated as:

$$\ln Q^*_{out(t)} = -\frac{J_{in}^{Na}}{Q_{out}} t + \ln Q^*_{out(0)}$$

where Q^*_{out} is the amount of isotope in the bath at the time of sampling (t); $Q^*_{out(0)}$ is the initial amount of isotope at time zero, and Q_{out} is the average amount of Na^+ in the bath during flux determinations (equation 5 of Kirschner, 1970). We assume that the specific activity of the animal is negligible relative to that of the bath.

Experiment A

Rana catesbeiana and *Rana pipiens* were anaesthetized and prepared for TEP determinations as outlined above. The bath was aerated continuously, but the experimental animal was shielded from convection due to the aeration. When TEP had reached steady-state, the bath was alternately stirred or not stirred with the magnetic stirring apparatus. Unstirred periods were ended when the TEP attained an apparent steady state, typically 10–20min after cessation of stirring. Likewise, stirred periods were ended when the TEP reached an apparent steady state, typically 10–15min after initiation of stirring. Bath [Na^+] was 0.75–1.0mmol l^{-1}.

Experiment B

Rana pipiens (N=10; mass 35.6±1.1g) were prepared for Na^+ influx determinations as outlined above. The initial bath concentration was 1mmol l^{-1} Na^+ in artificial pondwater. Beginning 5min after addition of $^{22}Na^+$ to each stirred bath container, animals were exposed to one of two treatments: 12h without stirring or 12h with stirring. In the unstirred treatment, the bath was stirred for the final 5min of the experimental period. Thereafter, a bath sample was taken and each animal was exposed to the other experimental treatment for an additional 12h period. The order of treatments was reversed in half of the experimental animals.

Experiment C

This experiment resembled experiment B except that animals were anaesthetized before (MS-222, 1:1000) and during (MS-222; 1:10000) experimentation. Flux periods were 6h instead of 12h. The initial bath concentration was 0.4mmol l^{-1} Na^+ in artificial pondwater. This concentration was chosen to increase specific activity of $^{22}Na^+$ during the relatively brief flux periods. Subjects were *Rana pipiens* (N=9; mass 33.4±1.7g)

Experiment D

Rana pipiens were housed individually in screened containers through which water could circulate. These containers prevented frogs from emerging from the bath but did not hinder pulmonary ventilation or restrict movement within the container. Containers with frogs were assigned to three experimental treatments: (1) deionized water; (2) 0.75mmol l^{-1} Na^+ in unstirred artificial pondwater; (3) 0.75mmol l^{-1} Na^+ in artificial pondwater continuously stirred through vigorous aeration. Bath volume was 1.25l per frog. The [Na^+] of the bath was determined daily. After 14 days, frogs were removed from their containers and Na^+ influx was determined as described above. At that time, their mass was 37.7±1.0g and did not vary significantly among experimental groups.

Initial bath [Na^+] for flux determinations was 1.8–2.0mmol l^{-1} and the bath was stirred continuously; these conditions are likely to yield near-maximum rates of Na^+ influx. Otherwise, conditions for flux determinations resembled those in experiment B.

Experiment E

This experiment resembled experiment A except that the bath was stirred continuously and the treatments alternated among gassing the bath with air, 100% O_2 or 100% N_2. Within 10min of gassing the bath with O_2, the P_{O_2} of the bath was greater than 97kPa. Within 10min of gassing the bath with N_2, the P_{O_2} of the bath was less than 1.5kPa. Bath [Na^+] was 1.0mmol l^{-1}.

Experiment F

This experiment resembled experiment B except that the bath was stirred continuously and animals were exposed to various bath P_{O_2} values. In the first group, frogs (N=6, mass 29.9±1.9g) were exposed to 12h of either hyperoxia (bath P_{O_2}>97kPa) or normoxia while J_{in}^{Na} was determined; during the subsequent 12h, the frogs were exposed to the alternative treatment. In a second group, frogs (N=6, mass 32.7±1.6g) were exposed to 12h of either hypoxia (bath P_{O_2}<1.5kPa) or normoxia while J_{in}^{Na} was determined; during the subsequent 12h, the frogs were exposed to the alternative treatment. Bath P_{O_2} was established by gassing the bath with air, 100% O_2 or 100% N_2. The initial bath concentration was 1.0mmol l^{-1} Na^+ in artificial pondwater.

Results

Experiment A

This experiment was performed to assess whether flow regime affected TEP. In each trial, cessation of stirring decreased TEP and resumption of stirring increased TEP (e.g. Fig. 1). The decrease in TEP by 10min after cessation of stirring averaged 6.9mV (±1.1mV S.E.; N=9 trials) in *Rana catesbeiana*; at the TEP of frogs in stirred water (23.9±4.2mV), this decrease was 34.8±7.0%. The decrease in TEP by 10min after cessation of stirring averaged 5.8mV (±0.5mV; N=10 trials) in *Rana pipiens*; at the TEP of frogs in stirred water (47.9±3.9mV), this decrease was 13.5±2.1%. Upon resumption of stirring, TEP typically increased rapidly at first and slowly thereafter.

Experiments B and C

These experiments were performed to assess whether changes in actual J_{in}^{Na} paralleled the changes in TEP. Experiment B examined awake animals, which could breathe air and move (i.e. ventilate the skin) *ad libitum*. Experiment C examined anaesthetized apnoeic animals, which were incapable of ventilating their skins voluntarily and were treated similarly to the animals for which TEP was determined. In both experiments, animals in the stirred bath had significantly greater J_{in}^{Na} values than animals in the unstirred bath. In experiment B (initial [Na^+]=1mmol l^{-1}), J_{in}^{Na} decreased from 200.4±33.9 μmol kg^{-1} h^{-1} to 144.3±23.1 μmol kg^{-1} h^{-1} when stirring was halted. For the nine animals for which both stirred and unstirred values of J_{in}^{Na} were available, the unstirred rate averaged 85.9%

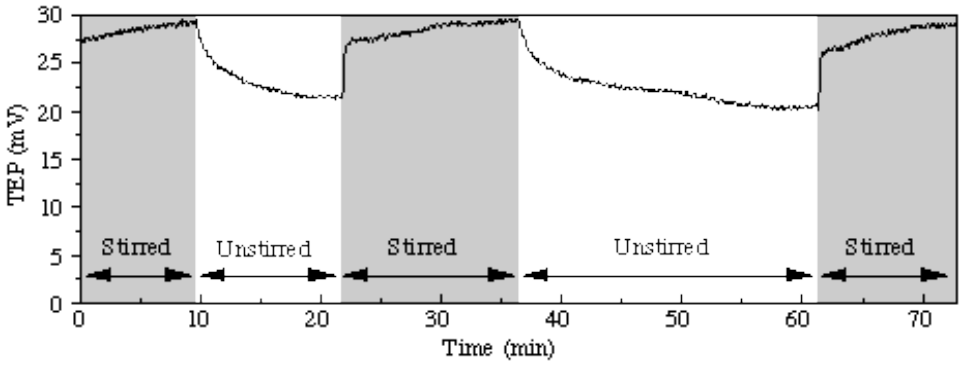

Fig. 1. Typical effect of bulk flow of the external medium on transepithelial potential (TEP) of a bullfrog (*Rana catesbeiana*) in a 0.75mmol l^{-1} Na^+ solution. At indicated intervals, a magnetic stirring apparatus was either activated or inactivated.

of stirred values, a significant (P<0.05, *t*-test for paired comparisons) decrease. In experiment C (initial [Na^+]=0.4mmol l^{-1}), J_{in}^{Na} decreased from 85.0±13.9 μmol kg^{-1} h^{-1} to 40.5±11.2 μmol kg^{-1} h^{-1} when stirring was ceased. For the eight animals for which both stirred and unstirred values of J_{in}^{Na} were available, the unstirred rate averaged 44.5% of stirred values, a significant (P<0.01, *t*-test for paired comparisons) decrease.

Experiment D

Previous studies have shown that frogs upregulate J_{in}^{Na} after prolonged exposure to deionized water, which depletes them of salt (e.g. Greenwald, 1971). This experiment was performed to assess whether prolonged exposure to an unstirred 0.75mmol l^{-1} Na^+ bath, in which the skin might contact a salt-depleted boundary layer, has the same effect. During the 14-day exposure to the experimental treatments, actual [Na^+] of the bath was less than 0.1mmol l^{-1} in the deionized water group and fluctuated between 0.69 and 0.99mmol l^{-1} in the other two groups. J_{in}^{Na} in the three experimental groups was as follows (Fig. 2): (1) deionized water, 407.1±35.5 μmol kg^{-1} h^{-1} (N=10); (2) unstirred 0.75mmol l^{-1} Na^+, 417.0±73.3 μmol kg^{-1} h^{-1} (N=5); (3) stirred 0.75mmol l^{-1} Na^+, 244.9±38.1 μmol kg^{-1} h^{-1} (N=8). The mean J_{in}^{Na} of the unstirred 0.75mmol l^{-1} Na^+ group was 70% greater than that of the stirred 0.75mmol l^{-1} Na^+ group (P<0.05, *t*-test) and did not differ significantly from that of the deionized water group (P=0.89, *t*-test).

Experiment E

This experiment was performed to assess whether changes in the P_{O_2} of a well-stirred bath could directly affect TEP and thus to assess whether a hypoxic boundary layer might affect Na^+ influx by depressing ATP-requiring transporters. Bath hyperoxia resulted in a modest (7.4%) increase in TEP in one frog (Fig. 3A), but no change in others (N=4). Bath hypoxia, by contrast, resulted in a considerable (9.0±2.3mV; N=6) decrease in TEP by 10min after the initiation of gassing with N_2 (Fig. 3B); at the TEP of frogs in normoxic water (41.8±3.2mV), this decrease was 21.7±5.0%. This decline continued as long as the

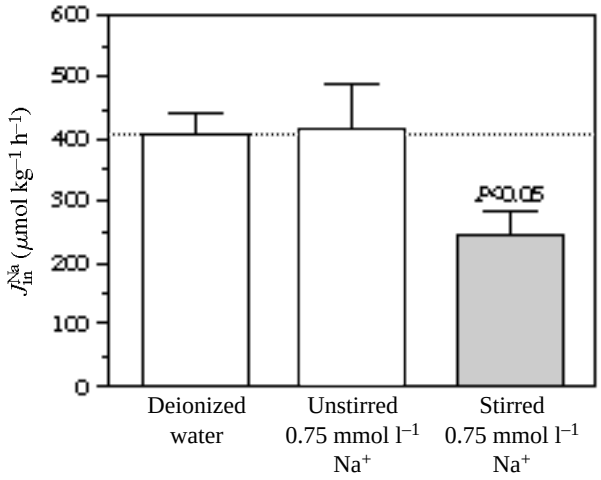

Fig. 2. Effect of acclimation to a stirred or unstirred external medium (nominal $[Na^+]$=0.75mmol l^{-1}; range: 0.69–0.99mmol l^{-1}) on Na^+ influx in frogs, *Rana pipiens*. For comparison, an additional group of frogs was acclimated to deionized water, which induces an upregulation of Na^+ influx (see text). Means are plotted + standard error. The latter two means differed significantly from one another (*t*-test).

bath remained hypoxic. Upon gassing the bath with air, TEP rapidly returned to normoxic levels.

Experiment F

This experiment was performed to assess whether P_{O_2}-induced changes in actual Na^+ uptake paralleled the P_{O_2}-induced changes in TEP in experiment E. In the first experimental group, J_{in}^{Na} was greater in the hyperoxic bath (292.8±22.5 μmol kg^{-1} h^{-1}; N=6) than in the normoxic bath (198.7±29.3 μmol kg^{-1} h^{-1}; N=4). For the four animals for which both hyperoxic and normoxic values of J_{in}^{Na} were available, the hyperoxic rate averaged 128.1% of normoxic values, a near-significant (P=0.052, *t*-test for paired comparisons) change. In the second experimental group, J_{in}^{Na} was lower in the hypoxic bath (123.9±16.8 μmol kg^{-1} h^{-1}; N=6) than in the normoxic bath (250.5±29.5 μmol kg^{-1} h^{-1}; N=6). The hypoxic rate averaged 52.3% of normoxic values, a significant (P<0.01, *t*-test for paired comparisons) decrease.

Discussion

Effect of convection on TEP and cutaneous Na^+ uptake

Numerous studies have examined the significance of environmental ion concentrations for organismal ion homeostasis in aquatic organisms, as well as the physiological mechanisms for maintaining ion homeostasis in the face of often unfavourable environmental ion concentrations in fresh and saline water [Boutilier *et al.* (1992) and Shoemaker *et al.* (1992) review the relevant information for amphibians]. The present study suggests that the convective environment of an aquatic organism (i.e. the range of

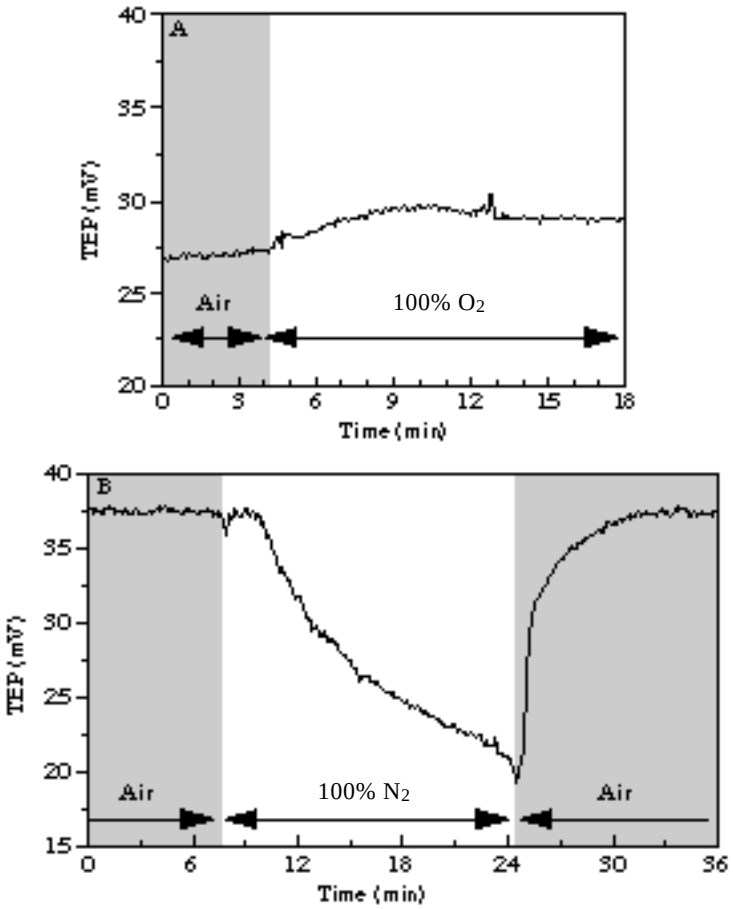

Fig. 3. Effect of (A) hyperoxia and (B) hypoxia of the external medium on transepithelial potential (TEP) of leopard frogs (*Rana pipiens*) in continuously stirred 1.0mmol l^{-1} Na^+ solutions. Within 10min of gassing, bath P_{O_2} values were greater than 97kPa or less than 1.5kPa, respectively.

flow velocities that an organism naturally encounters or induces through its own movements and whether these flows are laminar or turbulent) may be just as significant for ion homeostasis as ion concentrations in the bulk medium. Immersion of frogs in an unstirred bath resulted in a 14–65% decline in TEP and/or J_{in}^{Na}, and stirring of the bath alleviated this diminution (experiments A–C). Such changes have the potential to compromise Na^+ homeostasis. Although the significance of the convective environment for cutaneous gas exchange is well known (Vogel, 1983; Feder and Pinder, 1988; Pinder and Feder, 1990; Booth and Feder, 1991; Feder and Booth, 1992), the present study is the first documentation of parallel phenomena in cutaneous ion exchange.

Exposure to natural or artificial environments of extreme ion concentrations can induce acclimatory or evolutionary adjustments of ion fluxes that compensate for environmental conditions. For example, several weeks' exposure to deionized water, which ought to reduce ion concentrations in body fluids of amphibians, results in a substantial

upregulation of J_{in}^{Na} in ranid frogs (Greenwald, 1971). Experiment D of the present study showed that exposure to unstirred water can have the same effect, in this case resulting in a 70% increase above the J_{in}^{Na} for frogs in stirred water of similar [Na^+]. The mechanism of this increase is presently unknown but might involve changes in either the numbers or properties of the components of the Na^+ uptake system or in the ability of epithelial cells to maintain ATP supply to the various ATPases in the face of a hypoxic boundary layer.

Na^+ depletion in the boundary layer as a potential mechanism

A layer of unstirred or poorly stirred medium surrounds all physical objects immersed in a fluid (Vogel, 1983). If Na^+ uptake from this boundary layer exceeds Na^+ replenishment from the bulk medium outside the boundary layer or *via* cutaneous efflux of Na^+, then [Na^+] in the boundary layer will decrease. Given the apparent saturation kinetics of Na^+ uptake in frog skin, a large decrease in [Na^+] in the boundary layer could account for the observed decline in J_{in}^{Na} and TEP in unstirred water, but is not the only possible explanation (see next section). In physiologically relevant conditions (i.e. open-circuit conditions and a dilute external medium), J_{in}^{Na} of frog skin has an apparent K_m of $0.2 mmol\,l^{-1}$ Na^+ and approaches an upper limiting value at approximately $1 mmol\,l^{-1}$ Na^+ (Greenwald, 1971; Kirschner, 1988). These kinetics appear to be due to coupling with outward H^+ transport in open-circuit conditions (Harvey and Ehrenfeld, 1988; Kirschner, 1988) and are demonstrable in both whole frogs and frog skin (Greenwald, 1971; Kirschner, 1988).

At issue in support of the Na^+ depletion hypothesis are the normal range of [Na^+] of the bulk medium, how much [Na^+] declines in the boundary layer in unstirred medium and whether the apparent saturation kinetics of J_{in}^{Na} are such that the steady-state J_{in}^{Na} at the steady-state [Na^+] of the boundary layer will be less than that in well-stirred medium. Average values of [Na^+] are $0.17 mmol\,l^{-1}$ for soft lake water and $0.39 mmol\,l^{-1}$ for river water (Schmidt-Nielsen, 1990). Although natural [Na^+] can clearly be greater, these average values suggest that aquatic amphibians often (if not typically) experience bulk medium [Na^+] sufficiently low for changes in [Na^+] to affect J_{in}^{Na} markedly. How much [Na^+] declines in the boundary layer is presently unclear. P_{O_2} clearly declines in the boundary layer, sometimes as much as 19kPa (142mmHg) below its level in the bulk medium (Feder and Booth, 1992). Na^+, however, has both a greater solubility and a greater diffusion coefficient in water than does oxygen, suggesting that its diminution in the boundary layer should be less than for oxygen. Calculation of the [Na^+] at the mucosal surface from first principles is problematic because flow regime around intact frogs is poorly characterized. We have made a single measurement of [Na^+] just outside the skin of an intact, MS-222-anaesthetized frog with a Na^+-selective microelectrode [ionophore: ETH157 (Fluka); bulk medium: $3 mmol\,l^{-1}$ NaCl at normoxic P_{O_2}; species: *Rana catesbeiana*]. [Na^+] is $3 mmol\,l^{-1}$ at 125 μm from the mucosal surface and declines to $1.33 mmol\,l^{-1}$ at some distance between that point and the mucosal surface. If proportional declines in [Na^+] occurred in experiments A–C, they might well be sufficient to account for the observed declines in J_{in}^{Na} and TEP. Obviously, a definitive evaluation of the Na^+ depletion hypothesis will need to await future studies; none of the present results, however, is inconsistent with this hypothesis.

O_2 depletion in the boundary layer as a potential mechanism

As with Na^+, a decline in P_{O_2} in the boundary layer sufficient to compromise the supply of ATP to the ATPases to which Na^+ uptake is linked might account for or contribute to the observed decreases in J_{in}^{Na} and TEP in unstirred medium. The evidence for such an oxygen depletion effect is more substantial at present than for Na^+ depletion, but is not yet definitive.

Previous studies convincingly document that P_{O_2} declines considerably in the boundary layer surrounding the skin of amphibians in normoxic unstirred water (Pinder and Feder, 1990; Booth and Feder, 1991; Feder and Booth, 1992). In such circumstances, the steady-state P_{O_2} is well below the critical P_{O_2} (Booth and Feder, 1991) and, as a result, oxidative metabolism declines unless the affected cells have an alternative source of oxygen. Exposure to unstirred water directly decreases oxygen consumption of intact amphibians (Burggren and Feder, 1986; Pinder and Feder, 1990; Booth and Feder, 1991) and exposure to values of P_{O_2} expected at the skin's surface in unstirred water similarly affects the oxygen consumption of isolated frog skin (Vitalis, 1990). Zerahn (1956) firmly established the link between oxygen consumption and Na^+ uptake in frog skin. Insofar as cutaneous oxygen uptake is affected by the convective environment, so should oxygen-dependent Na^+ uptake.

A variety of experimental data is consistent with this hypothesis. Both mitochondrial inhibitors of oxidative phosphorylation and anoxia block Na^+ uptake in frog skin (Harvey and Ehrenfeld, 1988). Frog skin apparently does not deploy anaerobic metabolism to mitigate affects of limited O_2 supply (Vitalis, 1990). A surprisingly large classical literature, in advance of an adequate mechanistic understanding of Na^+ uptake, showed that hypoxia or anoxia reduce TEP (e.g. Taylor, 1936; reviewed by Vitalis, 1990), as does experiment E of the present study. Although we were unable to demonstrate a consistent or large effect of hyperoxia on TEP, Taylor (1936) reported just such an effect. J_{in}^{Na} increases modestly in a well-stirred hyperoxic bath and decreases considerably in a well-stirred hypoxic bath (experiment F).

A challenge to the oxygen depletion hypothesis is in demonstrating actual inhibition in mitochondrial ATP supply to the relevant ATPases in frog skin. The P_{O_2} required to inhibit mitochondrial oxidative phosphorylation is low and the P_{O_2} actually experienced by the mitochondria of mitochondria-rich cells in frog skin is unknown. On the one hand, the relevant ATPases reside in cells in the most superficial layers of the skin and the mitochondria of these cells may receive sufficient O_2 despite the reduction in P_{O_2} in the boundary layer. Furthermore, the skin is richly vascularized and its cells may be sufficiently oxygenated regardless of the P_{O_2} of the boundary layer. On the other hand, the P_{O_2} outside the skin in the boundary layer is already low and the skin itself is a major resistance to the diffusion of oxygen (Piiper, 1982; Pinder and Feder, 1991). Moreover, the measurements of Vitalis (1990) suggest that the skin itself is primarily responsible for its own oxygen uptake, with little O_2 delivered *via* the circulatory system. This situation could, however, reverse when the skin undergoes severe hypoxia. Direct measurements of intracellular P_{O_2} and ATP concentrations would contribute to the resolution of these issues.

In conclusion, although the coupling of gas exchange and ionoregulation is scarcely a novel concept, the primary focus of interest has been on the interrelationship of CO_2 excretion, acid–base regulation and ion homeostasis, largely within the integument itself and inside the organism (Boutilier *et al.* 1992; but see Gonzalez and McDonald, 1992; Randall *et al.* 1991). The present study, however, suggests that a novel and physiologically relevant form of such coupling may involve oxygen and the convective environment and come about through the common effect of fluid dynamic boundary layers or unstirred layers on solute species outside the skin. Data on both natural convective environments and on several key components of the proposed coupling are still few. If borne out, however, the coupling of cutaneous ion and oxygen uptake in the boundary layer may have significant implications for both adaptation to natural convective environments and general models of transmembrane exchange.

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