

IONIC PERMEABILITIES OF THE ISOLATED GILL CUTICLE OF THE SHORE CRAB *CARCINUS MAENAS*

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SUMMARY

Electrical parameters of the cuticle of the posterior gill lamella of the shore crab *Carcinus maenas*, were measured. When the cuticle was perfused with crab saline (CS) inside and sea water (SW) outside, ionic replacements or dilutions of the bathing solutions produced changes in potential that showed a selective permeability to cations with respect to anions. Similar measurements made with the cuticle bathed in single salt solutions gave the following permeability sequence: $\text{NH}_4^+ > \text{Rb}^+ > \text{Cs}^+ > \text{K}^+ > \text{Na}^+ \approx \text{Ca}^{2+} > \text{Li}^+ > \text{Tris}^+ > \text{Mg}^{2+} > \text{HCO}_3^- > \text{CH}_3\text{COO}^- > \text{Cl}^- > \text{SO}_4^{2-}$.

From conductance measurements, the permeability of the cuticle to Ca^{2+} and Na^+ was about $10^{-3} \text{ cm s}^{-1}$. K^+ and NH_4^+ were about five times more permeant, while Mg^{2+} permeability was $5 \times 10^{-5} \text{ cm s}^{-1}$. The permeability of the cuticle to anions lay between 10^{-6} and $10^{-5} \text{ cm s}^{-1}$. In addition, the cuticle showed an asymmetrical behaviour. These results suggest that the low anionic cuticular permeability can interfere with ionic exchanges across the whole gill.

INTRODUCTION

Osmoregulation and ionoregulation in decapod crustaceans have been related to water and salt exchanges across the surface of the gut, the antennal gland and the gills. The latter is the main site of passive loss and active uptake of salts in hyperregulators (Mantel & Farmer, 1983). Isolated decapod gills have therefore been used with the aim of analysing Na^+ , NH_4^+ , H^+ , Cl^- and HCO_3^- transport mechanisms. In relation to these studies, diffusional transgill potentials have been measured in the crayfish *Austropotamobius* (Croghan, Curra & Lockwood, 1965) and in several crab species: *Maja* (King & Schoffeniels, 1969), *Eriocheir* (Pequeux & Gilles, 1981; Gilles & Pequeux, 1985), *Callinectes* (Mantel, 1967; Smith & Linton, 1971) and *Carcinus* (King & Schoffeniels, 1969; Siebers *et al.* 1985; Pequeux, Wanson & Gilles, 1984; Lucu & Siebers, 1986). From these measurements it has

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been suggested that the gills of crayfish and shore crabs are more permeable to Na^+ than to Cl^- .

Any possible action of the cuticle as a barrier (Mantel & Farmer, 1983) has generally been ignored in the studies quoted above on decapod gills. At least two factors have to be considered to relate the transbranchial ion transport to the cuticle properties: first, the cuticular ionic selectivity or relative ionic permeability across the cuticle, and second, the ionic cuticular permeability relative to that of the underlying epithelium. These two factors should be considered in conjunction with the properties of the subcuticular spaces that are limited by the apical infoldings of the gill epithelial membrane. Such spaces were first described by Copeland & Fitzjarrell (1968) in *Callinectes*, and can be observed in most hyperregulators, including *Carcinus* (Gilles & Pequeux, 1985; Towle & Kays, 1986).

Avenet & Lignon (1985) observed a high ionic permeability of the gill cuticle to all ionic species (10^{-3}cm s^{-1}) in the osmoconforming lobsters, *Homarus* and *Nephrops*. Such a high permeability of the cuticle is unlikely to affect the salt exchanges across the gills. However, in hyperregulating freshwater crayfish, Avenet & Lignon (1985) and Lignon & Lenoir (1985) showed a high ionic selectivity of the isolated gill cuticle. Besides, the permeability of the crayfish gill cuticle computed from conductance measurements was found to range from 10^{-7} to 10^{-3}cm s^{-1} depending upon the ionic species. These authors pointed out that, in crayfish, the cuticle could impede the transbranchial flux of some ionic species while allowing almost free passage of other ionic species. In their investigations on *Callinectes* gills, Smith & Linton (1971) also measured the diffusional transcucular potential and stated that the preferential permeability of the gills to Na^+ 'is primarily a function of the cuticle'. Unfortunately, these authors did not make any estimate of the cuticular permeability so their statement is partly speculative. In contrast, Webb (1940) claimed that the permeability of *Carcinus* gill cuticle 'is such that it does not affect the salt and water exchanges of the animal'. However, this claim was based on indirect evidence since Webb measured the salt permeabilities of the cuticle of the lobster foregut and presumed that the gill cuticle of *Carcinus* is more permeable since it is thinner.

In the present investigation, it is shown that the gill cuticle of *Carcinus maenas* has a marked selectivity for cations over anions. The cationic permeability computed from conductance measurements is relatively high while the anionic permeability is moderate. These results are discussed in relation to the overall permeability of the crab gill.

MATERIALS AND METHODS

Experimental set-up

The experiments were performed on large specimens of *Carcinus maenas* at intermoult stage at the Marine Biological Station in Roscoff (Brittany, France). The animals were kept in large tanks of running sea water (SW) prior to the experiment.

The isolated posterior gills were perfused with crab saline (CS) containing Evans Blue and, after a wash with pure CS, a lamella was isolated and cut along its edge

with fine scissors. The two sides of the lamella cuticle were gently pulled apart from each other. Any patch of epithelium (selectively absorbing the dye) still adhering to the cuticle was peeled off mechanically.

The cuticle was sandwiched vertically between two identical half-chambers as described by Avenet & Lignon (1985). The volume of the half-chamber facing the exposed area of the cuticle (3.14 mm^2) was 20 mm^3 . The cuticle was superfused on both sides at a rate of $10\text{--}20 \text{ ml min}^{-1}$ at room temperature ($19\text{--}20^\circ\text{C}$). Salines flowing by gravity from storage vessels could be changed using multiway taps connected to the bottom of the half-chambers. Excess saline was removed by suction at the top of the chambers.

Perfusion fluid

Experimental fluids were filtered sea water (SW) from the Station, artificial sea water (ASW) and crab saline (CS) made up with distilled water and *pro analysis* grade salts. ASW composition was close to that of SW, consisting of (in mmol l^{-1}), Na^+ , 479; K^+ , 10; Ca^{2+} , 10; Mg^{2+} , 55.5; Cl^- , 559; SO_4^{2-} , 29; HCO_3^- , 2.5; pH 8.1. CS had the following composition (in mmol l^{-1}): Na^+ , 465; K^+ , 10; Ca^{2+} , 10; Mg^{2+} , 8; Cl^- , 503; SO_4^{2-} , 4. The pH was adjusted to 7.5 with Hepes (2 mmol l^{-1}) and Tris.

In another experimental series, the cuticle was superfused with single salt solutions. The reference solution was usually a 500 mmol l^{-1} NaCl solution, having an ionic strength close to that of SW and CS. The reference concentrations for CaCl_2 , MgCl_2 and Na_2SO_4 were 200 mmol l^{-1} and 100 mmol l^{-1} for MgSO_4 . CaSO_4 was used at 10 mmol l^{-1} (imposed by the solubility product). For comparative purposes, MgSO_4 was also used at 10 mmol l^{-1} . All these solutions were buffered at pH 7.0 with the above-quoted buffer. The partial pressure of CO_2 in the gas mixture used to equilibrate the NaHCO_3 solutions was adjusted so as to hold the pH at 8.0. At this pH, 97% of the total CO_2 would be in the form of HCO_3^- so that Na^+ and HCO_3^- concentrations can be considered as virtually equal.

Single ion activity coefficients were calculated from the salt activity coefficient given in the tables of Parson (1959) according to the convention of Bates (Meier *et al.* 1982).

Potential and conductance measurements

Electrical measurements were performed as described by Avenet & Lignon (1985). The potential (V_{oi}) was measured with KCl (3 mol l^{-1})–agar bridges whose tips were held close to the cuticle. V_{oi} was measured with respect to the inside. The junctional potentials were calculated using the Henderson equation.

Current–voltage (I/V) curves were obtained using constant-current pulses of varying amplitude fed from a floating current source to the cuticle through two silver plates. The current I_{oi} was taken as positive when flowing inwards. The series resistances (R_s : resistance of the fluid layers separating the tips of the bridges from the cuticle) were also calculated from I/V curves established at the end of the

experiment after the cuticle had been disrupted. Means are given with their standard errors.

Potential, conductance and permeability relationships

Ionic flux across a membrane is proportional to the concentration, to the electrochemical gradient and to a permeability coefficient related to the transport properties of the membrane. Simple explicit solutions of the Poisson–Nernst–Planck equations are obtained if the Poisson equation is approximated either by the electroneutrality equation or by the constant-field equation and if boundary conditions are correctly chosen. At zero total transmembrane current ($I = 0$), the potential (V_{oi}) is related to the permeability ratio between different ionic species ($\alpha_{ij} = P_i/P_j$) and to the ionic activities (a_j). Flux rate constants (k_j) and conductances (G_j) from which absolute values of the permeabilities (P_j) can be derived are generally functions of P_j , a_j and V_{oi} .

The method most commonly used to analyse V_{oi} at $I = 0$ is to perform ionic replacements in solutions of constant ionic strength in which all ions have the same absolute valency (z_+). V_{oi} then takes the form of the Goldman, Hodgkin & Katz (GHK) equation:

$$V_{oi} = -\frac{RT}{z_+ F} \ln \frac{\sum P_{Ca} a_C^o + \sum P_{Aa} a_A^i}{\sum P_{Ca} a_C^i + \sum P_{Aa} a_A^o}, \quad (1)$$

in which F , R and T have their usual meaning, C and A stand for cations and anions and a_X is the activity of the ion X inside (i) or outside (o). Equation 1 is also referred to as the generalized null potential equation since the constant-field condition (Goldman, 1943) and the independence principle are not necessary to derive it (Schultz, 1980; Barry & Gage, 1984). It follows from equation 1 that V_{oi} will be close to the Nernst potential for a cation, $C1$, if $C1$ is replaced by an impermeant cation, $C2$, and if P_A is small, but V_{oi} will be small if P_A is large and/or if $C2$ is also permeant.

In the latter case, the selectivity of a membrane for cations over anions will be best shown by diluting a single salt (CA) on one side of the membrane. If the constant-field condition holds, equation 1 becomes:

$$V_{oi} = -\frac{RT}{z_+ F} \ln \frac{a_C^o + \alpha_{AC} \cdot a_C^i}{a_C^i + \alpha_{AC} \cdot a_C^o}, \quad (2a)$$

with

$$\alpha_{AC} = P_A/P_C$$

and the $V_{oi}/\log(a_C)$ relationship is either curvilinear ($\alpha_{AC} \neq 0$) or linear ($\alpha_{AC} = 0$). If, instead, the electroneutrality condition is used, another equation is obtained:

$$V_{oi} = -\frac{RT}{z_+ F} \frac{1 - \alpha_{AC}}{1 + \alpha_{AC}} \ln \frac{a_C^o}{a_C^i}, \quad (2b)$$

and the curve $V_{oi}/\log(a_C)$ is always linear. In both cases, the permeability ratio α_{AC} can then be deduced from the fitting of the curve $V_{oi}/\log(a_C)$.

For a membrane highly selective to cations, the permeability ratio between two cations C1 and C2 is obtained in the bi-ionic case in which C1 is perfused on one side of the membrane and C2 on the other side with a common anion A. If both ions have the same valency (z_+) and if the anion flux is neglected, equation 1 simplifies to:

$$\frac{P_{C1}}{P_{C2}} = \frac{a_{C2}^i}{a_{C1}^o} \exp\left(-\frac{z_+ F V_{oi}}{RT}\right). \quad (3a)$$

If the two cations have different valencies (+2 for C1 and +1 for C2) and if the constant-field condition holds, the following equation (in which the anion flux is neglected) derived by Fatt & Ginsborg (1958) should be used:

$$\frac{P_{C1}}{P_{C2}} = -\frac{a_{C2}^i}{4a_{C1}^o} \exp\left(-\frac{F V_{oi}}{RT}\right) \left[1 + \exp\left(-\frac{F V_{oi}}{RT}\right)\right]. \quad (3b)$$

The absolute values of P_j can be deduced from the area-specific conductances (G_j). For a membrane perfused with identical solutions on both sides, simple relationships can be derived. The relationship between P_j and G_j depends upon the model used. The slope conductance (derivative dI/dV_{oi}) and the chord conductance (ratio I/V_{oi}) are usually equal if dI is small. If, in addition, the independence principle holds and simple models are used, G_j depends linearly upon a_j (Avenet & Lignon, 1985):

$$G_j = P_j \frac{z_j^2 F^2}{RT} a_j. \quad (4)$$

It should, however, be noted that G_j generally exhibits saturation at high concentrations (usually above the physiological level). In that case permeability ratios can still be independent of the concentrations (Eisenman & Horn, 1983; Hille, 1984).

RESULTS

Potential in crab saline and artificial sea water

When the cuticle was superfused with CS on both sides, the transcuticular potential (V_{oi}) was equal to zero, as expected from a cell-free system. The potential was negligibly small when SW bathed the outside and CS the inside. The effect of some ionic replacements performed in CS at constant ionic strength are illustrated in Fig. 1. Total replacement of Na^+ by K^+ or Li^+ resulted in changes of V_{oi} by up to 30 mV and indicate the following permeability sequence: $K^+ > Na^+ > Li^+$. Ca^{2+} replacement by Na^+ was almost ineffective. Cl^- replacement by CH_3COO^- induced a 4 mV change in V_{oi} , showing a larger acetate permeability. The effect of the total replacement of Cl^- by SO_4^{2-} would support a larger permeability to SO_4^{2-} but in this case $[Na^+]_i$ was also reduced so that the negative value of V_{oi} could also indicate a Na^+ selectivity of the cuticle. Ionic substitutions performed in SW (ASW) had similar effects (Fig. 1), but the changes in V_{oi} were then of the opposite sign and slightly larger. These results show that the crab gill cuticle is permeable to monovalent cations.

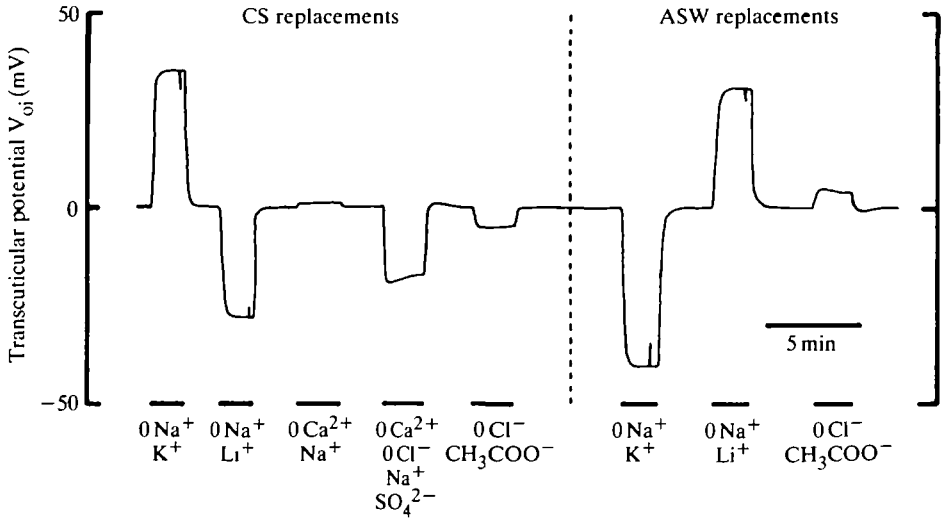


Fig. 1. Effects of ionic replacements on the transcuticular potential V_{oi} . The cuticle was perfused with crab saline (CS) inside and artificial sea water (ASW) outside. Solution changes at constant ionic strength are marked by bars. From the lefthand side to the righthand side solution changes in CS are: Na^+ replaced by K^+ ; Na^+ by Li^+ ; Ca^{2+} by Na^+ ; Ca^{2+} by Na^+ and Cl^- by SO_4^{2-} ; Cl^- by CH_3COO^- . Solution changes in ASW are: Na^+ replaced by K^+ ; Na^+ by Li^+ ; Cl^- by CH_3COO^- .

The small changes in V_{oi} observed could result either from a low selectivity among monovalent cations or from a substantial permeability to anions. To distinguish between these two possibilities, saline solutions were diluted either inside or outside. When CS was diluted, V_{oi} was negative (e.g. -15 mV for a dilution to half concentration; Fig. 2). Conversely, when SW was diluted, V_{oi} was positive. As shown in Fig. 2, V_{oi} increased, respectively, to $+16$, $+49$ and $+86$ mV upon SW dilutions by a factor of 2, 10 or 100. These values of V_{oi} are close to the monovalent cation equilibrium potentials, showing a high selectivity of the cuticle to cations over anions.

Potential in single salt solutions

Single salt solutions were used to distinguish the effects of each ionic species. Replacing either CS or SW by a pure NaCl solution (500 mmol l^{-1}) of similar ionic strength did not change V_{oi} by more than 0.5 mV. Upon dilution of this pure NaCl solution either inside or outside, changes in V_{oi} were recorded (Fig. 3) that were similar to those obtained with CS and SW dilutions (Fig. 2), clearly showing a selectivity of the cuticle for Na^+ over Cl^- .

During dilution of NaCl upon either side, V_{oi} was close to the equilibrium potential for Na^+ (E_{Na^+}). The initial slopes of the experimental curves (fitted by eye) were 57.4 ± 0.6 and 57.2 ± 0.9 mV/decade, respectively, for an external and an internal dilution ($N=5$). These curves were virtually linear over the first two decades but the slopes decreased at higher dilutions so that V_{oi} tended to a maximum value (140 ± 15 mV for an outside dilution). This reduction of the slope of the curve

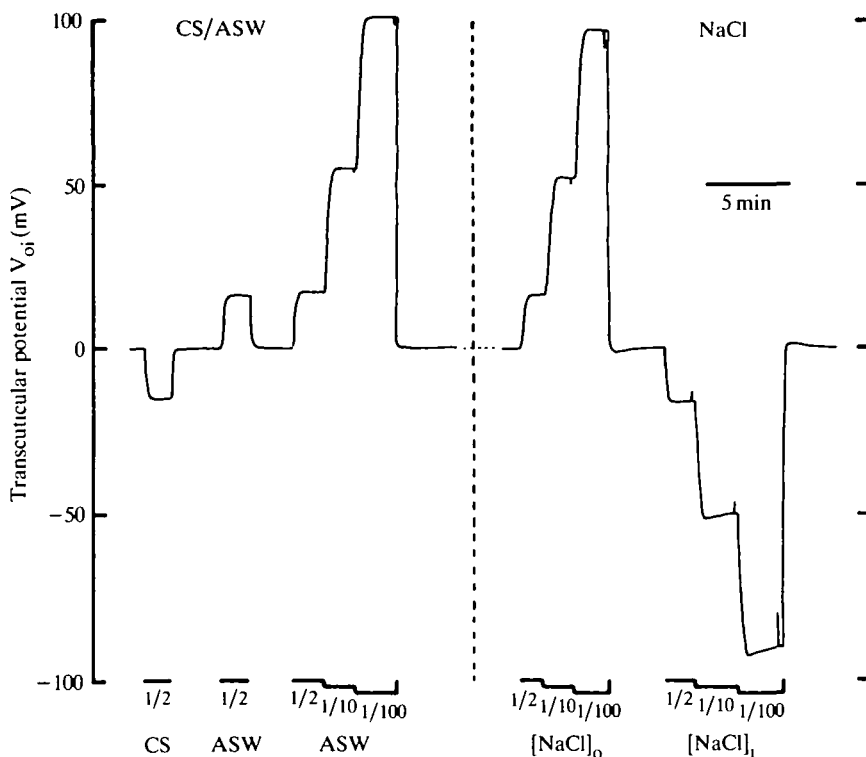


Fig. 2. Effects of the dilution of the perfusing solutions on the transcuticular potential V_{oi} . The cuticle was initially perfused with crab saline (CS) inside and artificial sea water (ASW) outside (left) and then with pure NaCl on both sides (right). Solution changes are marked by bars. From the lefthand side to the righthand side the changes are: CS reduced to 1/2; ASW to 1/2; ASW to 1/2, 1/10 and 1/100; $[\text{NaCl}]_o$ to 1/2, 1/10 and 1/100 and $[\text{NaCl}]_i$ to 1/2, 1/10 and 1/100. Same cuticle as in Fig. 1.

$V_{oi}/\log(a_{\text{NaCl}})$ always occurred at higher NaCl activity when the dilution was performed inside. The maximum absolute value of V_{oi} was also found to be 20–50 mV less than for an outside dilution. Similar results were obtained upon dilution of a 500 mmol l⁻¹ KCl or LiCl solution. Absolute values of V_{oi} were slightly larger with KCl than with NaCl and slightly smaller with LiCl. It can thus be concluded that Na⁺, K⁺ and Li⁺ are much more permeant than Cl⁻ across the crab gill cuticle.

Similar dilution experiments were performed with 500 mmol l⁻¹ solutions of Na⁺ salts containing Cl⁻, CH₃COO⁻ or HCO₃⁻. NaCl and NaCH₃COO dilution performed at pH 7.0 and 8.0 did not show any significant differences. NaHCO₃ dilutions were performed at pH 8.0. In every case the cuticle showed a marked Na⁺ selectivity. However, the slopes of the curves $V_{oi}/\log(a_{\text{Na}^+})$ increased with the following salt sequence: NaHCO₃, NaCH₃COO, NaCl, indicating a larger impermeability of the cuticle to Cl⁻ than to CH₃COO⁻ and HCO₃⁻. For an outside dilution, typical values of the initial slope and V_{oi} maximum for NaCl, NaCH₃COO

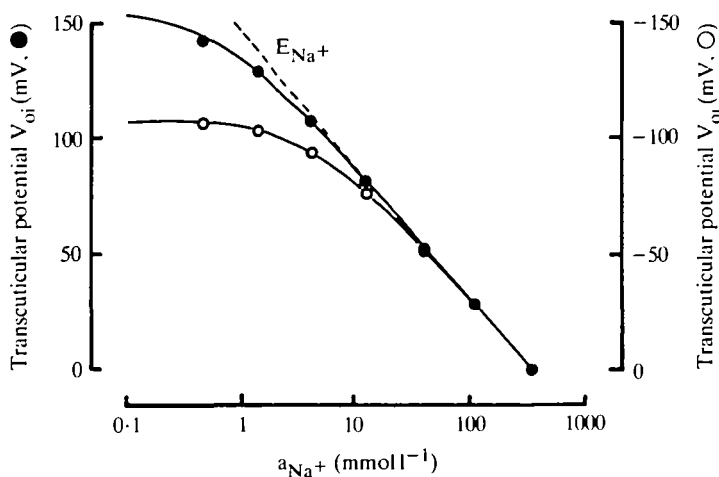


Fig. 3. Transcuticular potential V_{oi} versus activity of Na^+ (a_{Na^+}) of a cuticle perfused with pure NaCl at pH 7.0. Left ordinate: dilution of NaCl (●) outside; the internal NaCl concentration was 500 mmol l^{-1} . Right ordinate: dilution of NaCl (○) inside; the external NaCl concentration was 500 mmol l^{-1} . Symbols: experimental values. Dashed line: equilibrium potential for Na^+ (E_{Na^+}). Continuous lines: theoretical prediction of the Goldman equation with apparent permeabilities as defined in the Discussion for the two-layer model of the cuticle with $P_{\text{Na}^+} = 9.28 \times 10^{-4} \text{ cm s}^{-1}$, $P_{\text{Cl}^-} = 1.9 \times 10^{-6} \text{ cm s}^{-1}$ for the external layer and $P_d = 7.03 \times 10^{-4} \text{ cm s}^{-1}$ for the internal layer. Note the smaller absolute value of V_{oi} when the dilution is performed inside.

and NaHCO_3 were 57.5, 57.0 and 55.5 mV/decade and 150, 120 and 115 mV, respectively.

Dilutions of salts containing divalent ions were also performed to assess the selectivity of the cuticle to Ca^{2+} , Mg^{2+} and SO_4^{2-} . The slope of $V_{oi}/\log(a_{\text{Na}^+})$ was close to 58 mV/decade upon diluting Na_2SO_4 and indicates a much higher permeability of the cuticle to Na^+ than to SO_4^{2-} . V_{oi} was positive when CaCl_2 was diluted outside. The curve relating V_{oi} to $\log(a_{\text{Ca}^{2+}})$ was linear over two decades and had a slope of $28 \pm 1 \text{ mV/decade}$ ($N = 3$). V_{oi} was slightly negative (-10 mV at most) when MgCl_2 was similarly diluted outside. This indicates that Mg^{2+} and Cl^- have similar permeabilities while the cuticle permeability to Ca^{2+} is much higher. The dilution of both CaSO_4 and MgSO_4 (from 10 mmol l^{-1} down to 0.1 mmol l^{-1}) outside induced the development of a positive potential, indicating a higher permeability of the cuticle to cations. The slopes of the curves $V_{oi}/\log(a_{\text{M}^{2+}})$ were $28 \pm 1 \text{ mV/decade}$ for CaSO_4 ($N = 4$) and $18 \pm 4 \text{ mV/decade}$ for MgSO_4 ($N = 5$), respectively. Dilutions down to 1/100 were performed from solutions containing either 10 or 100 mmol l^{-1} MgSO_4 and gave similar initial slopes. However, the slope of the $V_{oi}/\log(a_{\text{MgSO}_4})$ curves was reduced when a 100 mmol l^{-1} MgSO_4 solution was further diluted to 0.1 mmol l^{-1} . V_{oi} values of similar amplitude but of opposite sign were obtained when the dilutions were performed inside. These results indicate that SO_4^{2-} is the less permeant ion across the crab cuticle and that Mg^{2+} is the less permeant cation while Ca^{2+} has a much higher permeability.

Ionic replacements

Permeability ratios between cations were assessed from V_{oi} measurements in bi-ionic conditions (total replacement of one cation by another one) (Table 1). When 500 mmol l^{-1} NaCl was perfused inside and replaced outside, the potential sequence varied according to the sequence NH_4Cl , RbCl , CsCl , KCl , NaCl , LiCl , CaCl_2 , TrisCl , MgCl_2 for each experiment (as given for the mean in Table 1). The same monovalent cation sequence was also obtained using SO_4^{2-} instead of Cl^- as a common ion. The values of V_{oi} were then slightly larger. Perfusing the cuticle at 10 mmol l^{-1} with $\text{CaSO}_4/\text{MgSO}_4$ (outside/inside) showed that Ca^{2+} is more permeant than Mg^{2+} . Similar information was obtained when MgCl_2 and CaCl_2 were used (200 mmol l^{-1}).

A similar ionic sequence was obtained when replacements of NaCl were performed inside while maintaining a 500 mmol l^{-1} NaCl solution outside. The polarity of V_{oi} was reversed and the absolute values of V_{oi} were usually slightly smaller. Exchanging MgCl_2 and CaCl_2 also resulted in a small drop (3–4 mV) in the absolute value of V_{oi} . In every case the time course of the change in V_{oi} was slower when the ionic replacements were performed inside as compared with the effect of an external replacement.

Substitutions of Cl^- in a 500 mmol l^{-1} NaCl solution were also performed. In agreement with the much larger permeability of the cuticle to Na^+ , the changes in V_{oi} thus observed were small. These substitutions nevertheless confirm the following permeability sequence: $\text{HCO}_3^- > \text{CH}_3\text{COO}^- > \text{Cl}^-$.

Conductance measurement

The area-specific conductance (G) of the cuticle to various salts was estimated from the slope of the I/V curve obtained when both sides of the cuticle were perfused

Table 1. *Bi-ionic potentials in Carcinus maenas gill cuticle*

External medium (mmol l^{-1})	Internal medium (mmol l^{-1})	V_{oi} [mV, mean \pm S.E. (N)]	Permeability ratio*
500 NH_4Cl	500 NaCl	-47.5 ± 4.2 ($N = 5$)	$P_{\text{NH}_4^+}/P_{\text{Na}^+} = 6.7$ (1)
500 RbCl	500 NaCl	-40 ± 1 ($N = 2$)	$P_{\text{Rb}^+}/P_{\text{Na}^+} = 4.9$ (1)
500 CsCl	500 NaCl	-38 ± 1 ($N = 2$)	$P_{\text{Cs}^+}/P_{\text{Na}^+} = 4.5$ (1)
500 KCl	500 NaCl	-33.2 ± 2.7 ($N = 5$)	$P_{\text{K}^+}/P_{\text{Na}^+} = 3.8$ (1)
500 NaCl	500 NaCl	0 ($N = 5$)	1 (1)
500 LiCl	500 NaCl	$+29 \pm 1$ ($N = 4$)	$P_{\text{Li}^+}/P_{\text{Na}^+} = 0.32$ (1)
500 TrisCl	500 NaCl	$+86.5 \pm 7$ ($N = 4$)	$P_{\text{Tris}^+}/P_{\text{Na}^+} = 0.032$ (1)
200 CaCl_2	500 NaCl	$+41 \pm 3$ ($N = 4$)	$P_{\text{Ca}^{2+}}/P_{\text{Na}^+} = 0.45$ (2)
200 MgCl_2	500 NaCl	$+95 \pm 4$ ($N = 3$)	$P_{\text{Mg}^{2+}}/P_{\text{Na}^+} = 0.043$ (2)
200 MgCl_2	200 CaCl_2	$+25.1 \pm 1.4$ ($N = 4$)	$P_{\text{Ca}^{2+}}/P_{\text{Mg}^{2+}} > 7$ (2)
10 CaSO_4	10 MgSO_4	-31 ± 3 ($N = 4$)	$P_{\text{Ca}^{2+}}/P_{\text{Mg}^{2+}} = 12$ (1)

* Permeability ratios were calculated using equations 3a and 3b as described in the Discussion with the following conditions: negligible P_{anion} (1) or constant-field theory and negligible Cl^- flux (2).

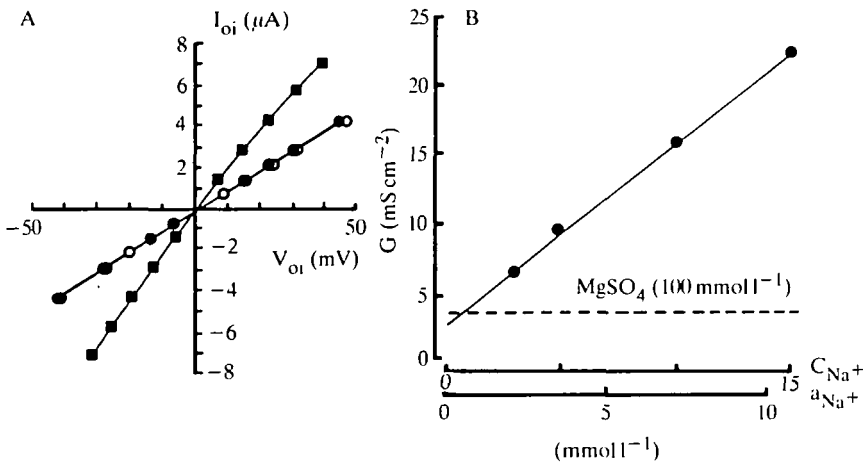


Fig. 4. Effects of Cl^- and Na^+ on the I/V curves and on the conductance of a gill cuticle initially perfused with pure $MgSO_4$ ($100 mmol l^{-1}$) on both sides. (A) I/V curves obtained when the cuticle was perfused with identical saline solutions on both sides. Solutions are: $MgSO_4$, $100 mmol l^{-1}$ (○); $MgSO_4$, $98.5 mmol l^{-1}$ + $MgCl_2$, $1.5 mmol l^{-1}$ (●); $MgSO_4$, $98.5 mmol l^{-1}$ + Na_2SO_4 , $1.5 mmol l^{-1}$ (■). Current pulses were delivered in alternate directions. (B) Area-specific conductance (G) of the cuticle used in A and perfused with the same solution on both sides as a function of Na^+ concentration (C_{Na^+}) or activity (a_{Na^+}). The conductance was first measured in pure $MgSO_4$ ($100 mmol l^{-1}$, dashed line). $MgSO_4$ was then progressively replaced by Na_2SO_4 . The measurements were performed at low current intensities and G was calculated from the slope of the I/V curves as illustrated in A.

with identical solutions. Both the total resistance (R_t) and the series resistance (R_s) were measured for each salt and for each concentration. As illustrated on Fig. 4A, the I/V curves were linear showing the ohmic behaviour of the cuticle in these conditions. G was deduced from the difference between R_t and R_s .

The conductance of the cuticle perfused with $500 mmol l^{-1}$ $NaCl$ was $G_{NaCl} = 3.4 \pm 0.7 S cm^{-2}$ ($N = 5$) and was not significantly different from the conductance obtained with CS and SW perfusions. Under these conditions, R_t and R_s were found to be of the same order of magnitude, thus increasing the relative error made for G_{NaCl} . More precise measurements were obtained when the cuticle was perfused with the relatively impermeant salt $MgSO_4$. In this case R_t was much larger than R_s . G_{MgSO_4} was $5.2 \pm 2.7 mS cm^{-2}$ ($N = 5$) when the cuticle was perfused with $100 mmol l^{-1}$ $MgSO_4$ (activity = $15 mmol l^{-1}$). G_{MgSO_4} dropped to $3.5 \pm 0.6 mS cm^{-2}$ ($N = 3$) when $[MgSO_4]$ was $10 mmol l^{-1}$ (activity = $4 mmol l^{-1}$). Replacing Mg^{2+} by Ca^{2+} ($CaSO_4 = 10 mmol l^{-1}$) evoked a large increase of the cuticle area-specific conductance to $84.6 \pm 38.5 mS cm^{-2}$ ($N = 3$) in agreement with the fact that Ca^{2+} is much more permeant than Mg^{2+} .

G_{Na^+} and G_{Cl^-} were also obtained by perfusing both sides of the cuticle with $MgSO_4$ ($100 mmol l^{-1}$) and progressively replacing either Mg^{2+} by Na^+ or SO_4^{2-} by Cl^- up to $20 mmol l^{-1}$ (Fig. 4). R_s was then kept constant and the change in G could be directly related to G_{Na^+} and G_{Cl^-} . It can be seen that the slope of the I/V curve was almost unchanged when a small amount of SO_4^{2-} was replaced by Cl^- . In

contrast, the slope of the I/V curve increased markedly when a small amount of Mg^{2+} was replaced by Na^+ , indicating a large cuticular G_{Na^+} (Fig. 4A). The change of the slope was linearly related to $[Na^+]$ (Fig. 4B). This observation allows us to calculate a molar area-specific conductance of the crab gill cuticle to Na^+ (g_{Na^+}). This was found to be $2.05 \pm 0.53 \text{ mS cm}^{-2} (\text{mmol l}^{-1})^{-1}$ ($N = 5$) on the concentration scale and $2.96 \pm 0.76 \text{ mS cm}^{-2} (\text{mmol l}^{-1})^{-1}$ on the activity scale. The increase in G was noticeably smaller when SO_4^{2-} was replaced by Cl^- than when Mg^{2+} was replaced by Na^+ : 50 ± 5 ($N = 3$) times smaller.

DISCUSSION

The experimental results in crab saline or sea water show a high selectivity of the crab gill cuticle for cations over anions normally present in SW and in the haemolymph. This was confirmed in single salt experiments that were performed to obtain precise values of the permeability ratios and of the conductance to each ionic species: monovalent cations and Ca^{2+} are much more permeant than other ionic species. The similarity of the results obtained in both conditions shows that the main properties of the crab gill cuticle are not modified if some ions are excluded from the perfusion medium. This allows for the use of permeability values, determined in single salt experiments, to compute the ionic fluxes in more complex solutions such as SW and CS. These permeability values should then be incorporated into a model that accounts for both the selectivity and the asymmetrical behaviour of the crab gill cuticle.

Cuticular permeabilities

P_{Na^+}/P_{anion} ratios were deduced from the fitting of the $V_{oi}/\log(a_{NaX})$ curves obtained by dilution of Na^+ salts containing Cl^- , SO_4^{2-} , CH_3COO^- or HCO_3^- . The experimental $V_{oi}/\log(a_{NaCl})$ curves cannot be fitted by a model using the electroneutrality condition (equation 2b) that predicts a linear relationship. The curvilinear characteristic of the experimental $V_{oi}/\log(a_X)$ relationships such as those illustrated on Fig. 3 supports the use of the constant-field condition (equation 2a). A P_{Na^+}/P_{Cl^-} ratio of 260 ± 120 ($N = 5$) gave a relatively good fit (within a few mV) of the $V_{oi}/\log(a_{NaCl})_o$ curves. This ratio was reduced by a factor of 2–3 for $NaCH_3COO$ and $NaHCO_3$. The fit of the $V_{oi}/\log(a_{NaCl})_i$ curves was not as good but supported a P_{Na^+}/P_{Cl^-} ratio of about 120. A $P_{Mg^{2+}}/P_{SO_4^{2-}}$ ratio of 7 was computed from the V_{oi} values recorded in diluting $MgSO_4$. $P_{Ca^{2+}}/P_{SO_4^{2-}}$ was found to be greater than 100. The slope of the $V_{oi}/\log(a_{Ca^{2+}})$ curves (28 mV/decade) also indicated a large $P_{Ca^{2+}}/P_{Cl^-}$ value upon dilution of $CaCl_2$.

Since the ratios P_{cation}/P_{Cl^-} and $P_{cation}/P_{SO_4^{2-}}$ are large, equation 3a was used to calculate the permeability ratios between cations of like valencies from the V_{oi} values recorded under bi-ionic conditions. The values of the computed ratios are shown in Table 1 and give the following two sequences: NH_4^+ (6.7) > Rb^+ (4.9) > Cs^+ (4.5) > K^+ (3.8) > Na^+ (1) > Li^+ (0.32) > $Tris^+$ (0.032) and Ca^{2+} (12) > Mg^{2+} (1). The values of the permeability of the divalent cations relative to that of Na^+ were

estimated using equation 3b and gave the following sequence: Na^+ (1) > Ca^{2+} (0.45) > Mg^{2+} (0.043).

The absolute values of the permeabilities were calculated, using equation 4, from the conductance measurements performed at low current intensities when both sides of the cuticle were perfused with identical solutions. The permeability to Na^+ computed in this way was $7.1 \pm 1.8 \times 10^{-4} \text{ cm s}^{-1}$ ($N = 5$; activity scale) using g_{Na^+} values obtained by addition of Na^+ to a MgSO_4 solution. A similar P_{Na^+} value was deduced from the value of G_{NaCl} . P_{Cl^-} was, of course, 50 times lower ($1.5 \times 10^{-5} \text{ cm s}^{-1}$) as g_{Cl^-} was less than g_{Na^+} . P_{Cl^-} can also be calculated in a different way from the individual pairs of g_{Na^+} values and $P_{\text{Na}^+}/P_{\text{Cl}^-}$ ratios (as determined above) giving a lower P_{Cl^-} value of $2.9 \pm 1.9 \times 10^{-6} \text{ cm s}^{-1}$ ($N = 5$). The large change in G when MgSO_4 was replaced by CaSO_4 can be entirely attributed to Ca^{2+} . $P_{\text{Ca}^{2+}}$ was then calculated as $1.3 \pm 0.6 \times 10^{-3} \text{ cm s}^{-1}$ ($N = 3$). G_{MgSO_4} is the sum of $G_{\text{Mg}^{2+}}$ and $G_{\text{SO}_4^{2-}}$. Both permeabilities and conductances were assumed to be proportional to compute $P_{\text{Mg}^{2+}}$ ($5 \times 10^{-5} \text{ cm s}^{-1}$) and $P_{\text{SO}_4^{2-}}$ ($5.7 \times 10^{-6} \text{ cm s}^{-1}$).

Equivalent two-layer model of the cuticle

Permeabilities can be calculated with a limited number of assumptions from conductance measurements performed under symmetrical conditions. However, the permeabilities determined above should be incorporated in a model that accounts for the properties of the cuticle to predict transcuticular fluxes. Apart from its high selectivity to cations, the crab gill cuticle exhibits a functional asymmetry with regard to the time course and absolute values of V_{oi} recorded when the salines are modified on either sides of the cuticle. This is not predicted by the classical model of Goldman (1943) that makes use of a single homogeneous membrane. The cuticle should, therefore, be divided into at least two functional layers. The larger potential obtained when the salts are diluted outside is best explained by a model such as that developed by Avenet & Lignon (1985) for the Cl^- -selective gill lamina cuticle of the crayfish.

In that model, the cuticle is treated as being composed of an outer selective layer (permeability P_X) apposed to an inner non-selective layer (permeability P_d). In the inner layer, all ions have the same permeability and the electroneutrality condition holds. In the outer layer, the constant-field condition is assumed to hold and permeabilities (P_X) vary with the ionic species. The Goldman equations for fluxes, conductances and potential can then be used in their ordinary form provided that they are used with apparent permeabilities (P'_X) related to P_d and P_X by the following equation:

$$P'_X = \frac{P_X}{1 - \frac{P_X}{P_d} \frac{E}{1 - \exp(E)}}, \quad (5)$$

with

$$E = \frac{zF}{RT} V_{oi},$$

in which E is the dimensionless potential and V_{oi} is the total measured transcuticular

potential. For small P_X values (as for Cl^-), P'_X and P_X are almost identical. For large positive values of V_{oi} , P'_{Na^+} approaches P_{Na^+} while it tends to be limited by P_d at negative V_{oi} . This accounts for the difference between the Na^+/Cl^- permeability ratios deduced from the $V_{oi}/\log(a_{\text{NaCl}})_o$ and $V_{oi}/\log(a_{\text{NaCl}})_i$ curves. To find P_d for each preparation, a $P_{\text{Na}^+}, P_{\text{Cl}^-}$ couple was first introduced to fit the $V_{oi}/\log(a_{\text{Na}^+})_o$ curve with an infinite P_d value. P_d was then chosen to fit the $V_{oi}/\log(a_{\text{Na}^+})_i$ curve. The procedure was then continued by iteration to obtain the best fit of the two curves. As exemplified in Fig. 3, both $V_{oi}/\log(a_{\text{NaCl}})$ curves were well fitted with a single set of P_{Na^+} and P_{Cl^-} values when P_d was close in value to P_{Na^+} (about $10^{-3} \text{ cm s}^{-1}$). Besides, the conductance g_{Na^+} measured at low V_{oi} and I_{oi} values is related to P'_{Na^+} as defined by:

$$1/P'_{\text{Na}^+} = 1/P_{\text{Na}^+} + 1/P_d. \quad (6)$$

This partly explains the difference between the Na^+ and Cl^- permeability ratios obtained from the fitting of the $V_{oi}/\log(a_{\text{NaCl}})_o$ curves and from the $g_{\text{Na}^+}/g_{\text{Cl}^-}$ ratio. One obvious effect of the non-selective layer is to reduce the overall selectivity of the cuticle. This conclusion is identical with that given by Eisenman & Horn (1983), who used the rate-theory approach to channel ion selectivity when a non-selective barrier is apposed to a highly selective barrier.

The model described above thus gives a relatively good account of the properties of the crab gill cuticle as far as Na^+ and Cl^- permeabilities are concerned. The outer selective layer could be tentatively identified as the epicuticle normally facing the SW. Such a conclusion that the impermeability and/or selectivity of the cuticle could be related to the epicuticle has also been stated by Jahn (1936) for the grasshopper egg membrane, by Yonge (1936) for the foregut of the lobster and by Avenet & Lignon (1985) for the crayfish gill lamina cuticle.

Physiological role of the cuticle

Our results on *Carcinus maenas* gill cuticle confirm and extend those of Smith & Linton (1971) on *Callinectes sapidus* in that the gill cuticle shows a marked selectivity for cations over anions. The larger V_{oi} values obtained with *Carcinus* cuticle are likely to result from technical differences: Smith & Linton only diluted the external saline without perfusing the inside, thus creating an artificial unstirred layer that added to the effect of the endocuticle in reducing the cuticle selectivity. Besides, from our conductance measurements, the permeability of *Carcinus* cuticle to Ca^{2+} and Na^+ is about $10^{-3} \text{ cm s}^{-1}$. K^+ and NH_4^+ are about five times more permeant, while Mg^{2+} permeability is $5 \times 10^{-5} \text{ cm s}^{-1}$. The cuticle permeability to anions is noticeably lower and lies between 10^{-6} and $10^{-5} \text{ cm s}^{-1}$.

It is now of interest to know how far the cuticle could interfere with Na^+ and Cl^- losses and uptake that occur when shore crabs are transferred to media of different salinities. Since Na^+ and Cl^- are found in equivalent amounts in the blood, they should be considered as ultimately being cotransported even though this overall cotransport can to some extent be dissociated when $\text{Na}^+/\text{NH}_4^+$ and $\text{Cl}^-/\text{HCO}_3^-$ (Pressley, Graves & Krall, 1981; Henry & Cameron, 1983; Lee & Pritchard, 1985)

exchange mechanisms are switched on. The cuticle should not impede $\text{Na}^+/\text{NH}_4^+$ and Na^+/Na^+ exchanges across the gills since its permeability to NH_4^+ and Na^+ is high. However, this is not so for a $\text{HCO}_3^-/\text{Cl}^-$ exchange, since both Cl^- and HCO_3^- have permeabilities lower than $10^{-5} \text{ cm s}^{-1}$. In this regard the cuticle could create a functional subcuticular compartment that should be taken into account when considering such an exchange across the gills. This conclusion could also hold for a $\text{Na}^+ \cdot \text{Cl}^-$ cotransport (either loose or tight) since the salt flux will be limited by the slowly moving Cl^- flux across the cuticle to satisfy the zero current condition across it.

These observations are, of course, to be considered in relation to the medium in which the gills are bathed. In full strength SW, it is most likely that the subcuticular ionic composition is close to that of SW. The transecticular potential will then be zero and the cuticle as a whole will merely act as a single diffusion layer whose equivalent aqueous thickness will be given by the ratio of the cuticle permeability to the diffusion coefficient in water. This equivalent thickness is about $100 \mu\text{m}$ for cationic exchanges but about 10 mm for exchanges involving anions (either cotransported or counter-transported). For gills perfused with diluted SW, the subcuticular compartment could equilibrate with the external medium. The transepithelial ion leaks would thus be from the haemolymph to the subcuticular compartment. Recycling of these ions by the underlying epithelium could then occur from this compartment, favouring an apparent impermeability of the whole system in the steady state not only to ions but also to water. Alternatively, transbranchial ionic exchanges (either leak or uptake) could occur. In this case, a transecticular electrochemical gradient will be established that will depend upon the relative permeabilities of the cuticle and of the epithelium and upon the pumping activity of the epithelium.

With respect to the latter alternative, the ionic permeabilities of the isolated cuticle, as determined in the present work, can be compared to the permeabilities of the whole gill (cuticle plus epithelium) deduced from the work of Lucu & Siebers (1986). According to these authors, the unidirectional Na^+ flux across the isolated gill of *Carcinus maenas* perfused with 500 mmol l^{-1} NaCl on both sides is $4000 \mu\text{mol l}^{-1} \text{ h}^{-1}$ and V_{oi} is then zero. They also calculated a $P_{\text{Cl}^-}/P_{\text{Na}^+}$ ratio of 0.34 for the whole gill. Taking into account a gill surface area of about $50 \text{ cm}^2 \text{ g}^{-1}$ fresh mass, the overall gill P_{Na^+} and P_{Cl^-} would be 5×10^{-5} and $1.5 \times 10^{-5} \text{ cm s}^{-1}$, respectively. The gill P_{Na^+} is much smaller than the gill cuticle P_{Na^+} . However, the overall gill P_{Cl^-} is close to the gill cuticle P_{Cl^-} . This, therefore, strongly suggests that the effect of the gill cuticle has to be taken into account in the study of transgill ion transport. Along these lines it is worth mentioning that the isolated cuticle and the whole gills both show a Na^+ selectivity in *Callinectes* and that for a similar concentration gradient the transgill and the transecticular potentials are close to each other (Smith & Linton, 1971). Published values of the transbranchial potential in *Carcinus maenas* (King & Schoffeniels, 1969; Pequeux, Wanson & Gilles, 1984; Siebers *et al.* 1985; Lucu & Siebers, 1986) also clearly show a Na^+ selectivity, as we have observed for the isolated cuticle. However, it could be that both the cuticle and

the epithelium show a Na^+ selectivity and more extensive conductance measurements should be made before going further.

Our measurements, therefore, show that the inference made by Webb (1940) should be discarded: the gill cuticle of *Carcinus maenas* can no longer be considered as freely permeable to all ionic species. As mentioned above, a Na^+ - Cl^- cotransport will be limited by the cuticle permeability to the slowest moving ion to satisfy the zero current condition across it. This permeability is $10^{-3} \text{ cm s}^{-1}$ in the lobster gill cuticle. It is less than $10^{-6} \text{ cm s}^{-1}$ in the freshwater crayfish (Avenet & Lignon, 1985). It lies between these values for *Carcinus maenas* gill cuticle. Since the lobster does not osmoregulate while *Carcinus maenas* has a limited power of regulation and the crayfish has an even higher power of regulation, it is tempting to relate the NaCl permeability of the cuticle to the power of regulation of decapods. A similar observation has been made for the carapace permeability of decapod crustaceans to salt and water: the carapace permeability has been related to the habitat of the animals (Gross, 1957; Herreid, 1969).

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