*J. Exp. Biol.* (1970), 53, 75–92
*With* 10 *text-figures*
*Printed in Great Britain*



# CHLORIDE REGULATION AT LOW SALINITIES BY *NEREIS DIVERSICOLOR* (ANNELIDA, POLYCHAETA)

## I. UPTAKE AND EXCHANGES OF CHLORIDE

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(*Received* 5 *December* 1969)

The ability of the common brackish water polychaete worm *Nereis diversicolor* O. F. Müller to regulate osmotically and ionically has been well established (Schlieper, 1929; Beadle, 1937; Smith, 1955; Hohendorf, 1963; Oglesby, 1970; and others). Jørgensen & Dales (1957) demonstrated an uptake of chloride against the concentration gradient at low salinities, with a maximum rate of exchange under steady-state conditions at external chloride concentrations of 65–120 mM/l, and a drop in uptake rate to about one-third of that maximum in fresh water (Cl = 1·4 mM/l). Fretter (1955) demonstrated a comparable uptake of sodium against the concentration gradient in this species, but without the maximum shown in the chloride exchanges.

Evidence that *N. diversicolor* has a considerably lower permeability to chloride than has the more marine *Nereis virens* was given by Jørgensen & Dales (loc. cit.) while Fretter (loc cit.) demonstrated that *N. diversicolor* is comparably lower in permeability to sodium than the marine *Perinereis cultrifera*. It therefore appears that a lowering of integumental permeability to ions is part of the adaptation of *N. diversicolor* for life in low salinities.

However, whether permeability to water is similarly lowered is a matter of less certainty. Smith (1963) showed that the tendency to lose salts in an acute short exposure to distilled water is markedly less in *N. diversicolor* than in the more marine *Nereis succinea*. *Nereis limnicola*, a species perhaps more tolerant of low salinities than *N. diversicolor*, showed even less tendency to lose salts in the above acute test. But a study of the uptake of $D_2O$ (Smith, 1964) showed that *N. succinea* and *N. limnicola* had essentially the same permeability to inward diffusion of water, indicating that permeability to ions may vary independently of that to water. Jørgensen & Dales (1957) presented evidence that *N. diversicolor* in fresh water decreases its permeability to water to about one-third (< 40%) of that characteristic of higher salinities, and they considered it 'likely' that a comparable decrease in permeability to chloride occurred. On the basis of the observed decrease in permeability to water and the assumed decrease in permeability to chloride, these authors considered that the observed reduction of chloride exchanges in fresh water was consistent with the view that *N. diversicolor* produced a urine isotonic to the body fluid. However, Potts & Parry (1964, pp. 145–155), reviewing the above paper, considered that the evidence

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for changes in permeability to water and chloride was not sufficiently strong and suggested that the reduced chloride exchange in fresh water was a consequence of hypotonicity of the urine. It may be pointed out that these views are not mutually exclusive. The present paper and those that follow (Smith, 1970*a*, *b*) report the results of studies on exchanges of chloride and water and on urine concentration in *N. diversicolor*, in an effort to provide a clearer picture of the mechanisms of its adaptation to very low salinities.

## MATERIAL AND METHODS

*Nereis diversicolor* of medium size (100–500 mg) were collected from muddy intertidal banks of the Wansbeck estuary on the Northumberland coast, where the salinity was less than 50% of SW, and experiments were conducted in the Department of Zoology, University of Newcastle upon Tyne. In the laboratory worms were maintained in plastic food containers, 4–6 worms/vessel in 200–300 ml of water, at a room temperature which usually fluctuated between 14 and 18° C. Containers were provided with short lengths of glass tubing in which the worms took up residence. Media ranged from offshore sea water (SW) of chloride concentration 562 mM/l to local pond water (PW): $Cl^-$, *c.* 0·90 mM/l; $Ca^{2+}$, *c.* 1·14 mM/l; $Na^+$, *c.* 0·81 mM/l; $K^+$, *c.* 0·11 mM/l; $Mg^{2+}$, *c.* 0·39 mM/l). All intermediate salinities were made by diluting SW with PW. Since *N. diversicolor* could be kept for over 4 weeks in PW without feeding, this medium was considered tolerable, and its use in mixtures assured that exposure of worms to low concentrations of chloride and sodium did not confuse the issue by subjecting them to intolerable deficiencies of $Ca^{2+}$ and other ions. The use of deionized water (DW) in making dilutions of SW was given up after it was noted that, when SW was diluted with DW to chloride concentrations of 10 mM/l and less, worms emitted excessive mucus and appeared distressed or not fully normal. The use of glass tubes as dwelling places for *N. diversicolor* is more satisfactory than the use of sand or mud when large numbers of worms are maintained in the laboratory. Like its relatives, *N. diversicolor* secretes a stringy mucus, and an important precaution in handling nereids experimentally, especially when removed from their tubes and isolated in experimental vessels for any length of time, is to remove accumulated mucus. Otherwise, many worms entangle themselves, causing circulatory arrest, damage to parapodia, or even amputation of the posterior part of the body. Before any experimental procedure worms were removed from their glass tubes and placed in a fresh 25 ml of the same ('adaptational') medium for several hours or overnight. Mucus was removed, the worms were gently blotted on filter paper, and then weighed to the nearest milligram on a Sartorius balance.

Net chloride uptake was determined by the decrease in chloride concentration of measured 10 ml volumes of low concentrations of medium (Cl = 3–20 mM/l) in which individual worms were placed following prior adaptation to still lower chloride concentrations. The greatest change in chloride concentration acutely applied was 19 mM/l (representing the shift from PW to 3·6% SW); this, in terms of difference in water concentration, is but a slight osmotic change. Chloride was determined by an Aminco-Cotlove electrometric chloride titrator, using NaCl standards.

In experiments to determine chloride fluxes, *N. diversicolor* was studied in the steady state; osmotic changes were avoided or minimized. The radioisotope $^{36}Cl$ was

obtained as 2 N-HCl and neutralized with NaOH after being added to experimental media. For counting $^{36}Cl$ an Isotopes Developments Ltd. scaler 1·700, read-out unit 2007 and automatic sample changer were used. Samples of media and coelomic fluid containing $^{36}Cl$ were taken in 5 and 10 $\mu$l disposable capillary pipettes (Drummond 'Microcaps'), spread on nickel-plated 25 mm planchets in two drops of DW followed by two drops of 1 M glucose plus 10% liquid detergent, and dried at 110° C before counting.

Other details of method are given in the descriptions of the experiments.

## OBSERVATIONS AND RESULTS

### (1) *General observations*

*Nereis diversicolor* can be adapted in the laboratory to chloride concentrations at least as low as the 0·9 mM/l found in pond water of the Newcastle upon Tyne area. This contains calcium at 1·14 mM/l and other ions as noted above. In this PW worms survived without feeding for over a month, generally remaining motionless in their glass tubes, occasionally undulating for respiratory irrigation, or leaving the tubes to creep about the container. They were not swollen or otherwise abnormal in appearance, and could be presumed to be in a viable steady state. But that they were under significant stress was evident from the fact that the chloride concentration of the coelomic fluid (Fig. 1) is below the 'plateau' of regulation achieved at an external chloride concentration of 10 mM/l or higher. Further evidence of this stress was accidentally provided when the laboratory heating was turned off during Christmas vacation, and the temperature fell to some unrecorded level below 8° C; two-thirds of the worms in PW died, but no deaths occurred among worms adapted to chloride concentrations of 5 mM/l and higher. Worms adapted to 100% sea water (Cl = 562 mM/l) generally appeared somewhat shrunken and gave the appearance of being less active than normal, although survival was not impaired in the period of observation.

The chloride concentration of the coelomic fluid (Fig. 1) shows a 'plateau' of regulation from about 210 mM/l in 35% SW (Cl *c.* 200 mM/l) to 135 mM/l in 1·8% SW (Cl *c.* 10 mM/l). However, at environmental chloride concentrations of 1 and 5 mM/l the chloride concentration of the coelomic fluid dropped below the regulatory plateau to *c.* 110 mM/l. Accordingly, attention was focused on the environmental chloride range of 1–10 mM/l in attempts to demonstrate net chloride uptake.

### (2) *Net chloride loss with activity under steady-state conditions*

In the study of net chloride uptake worms were routinely removed from their tubes and isolated overnight in 25 ml of the adaptational media. They were then weighed, and dropped at 'time zero' into measured 10 ml volumes of the same concentration for 2 h. Early in the study it was noticed that worms thus transferred to fresh volumes of the identical adaptational media showed, on the average, both a weight loss and a chloride loss, as represented at the foot of each curve in Fig. 3. Since there was no osmotic change, it is suggested that the weight loss and chloride loss result from increased urinary output. Characteristically, worms placed in experimental vessels after weighing become intermittently active, swimming or creeping about for varying

periods before settling down. As was suggested by Beadle (1937), it seems very probable that increased body turgor during activity increases urinary output through the open metanephridia. There is a good correlation ($c$ = 0·80) between weight loss and chloride loss to support this conclusion, as shown in Fig. 2A, representing worms adapted in and transferred to a chloride concentration of 10 mM/l, as would be expected if the worms voided urine containing chloride at a higher concentration than that of the dilute medium. That the correlation is not exact could be accounted for by the premise that urinary chloride loss is only part of the total loss, the rest coming from

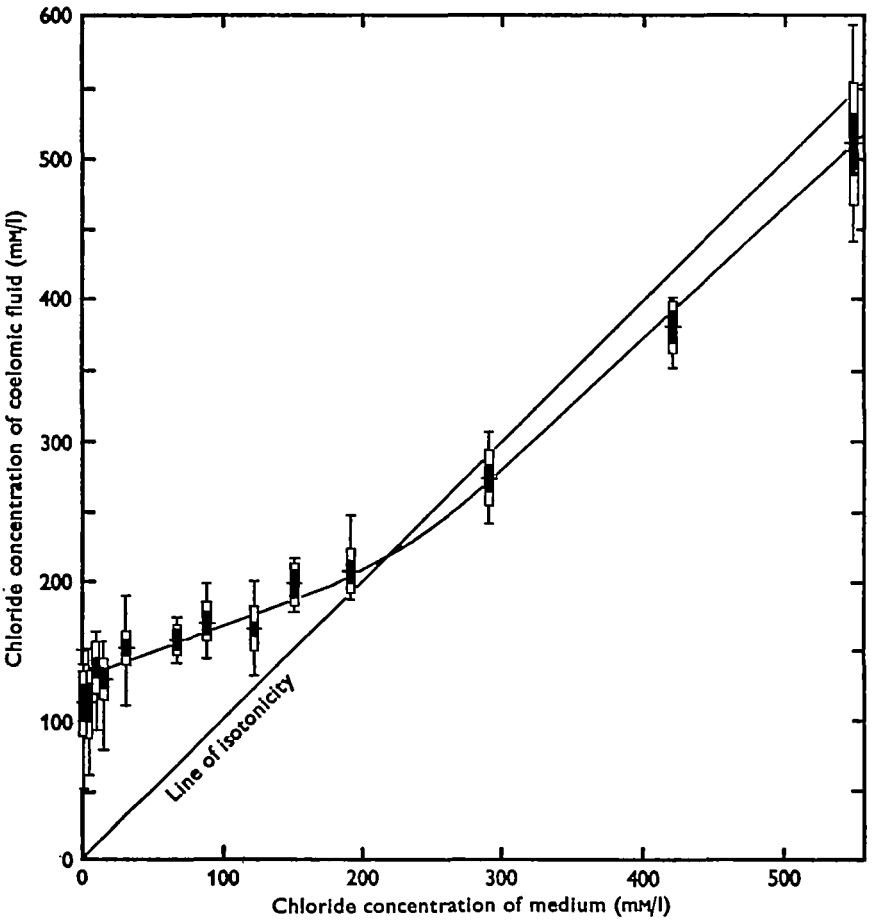


Fig. 1. Regulation of coelomic chloride in *N. diversicolor*, in part from data of Oglesby (1970). In this and following figures, variation about means shown by vertical line spanning the range, open block ± one standard deviation, black block ±2 standard errors, cross-line the mean (method of Dice & Leraas, 1936, in which non-overlap of the ±2 standard error blocks indicates significant difference).

the integument and possibly the gut. In contrast, data from worms adapted in and transferred to PW show a poor correlation ($c$ = 0·23), the chloride loss being roughly the same at different weight losses (Fig. 2B). It is considered possible that the urine produced in PW might be very hypotonic to the coelomic fluid and so might con-

tribute significantly to weight loss but not to chloride loss. This possibility is consistent with the ability of *N. diversicolor* to recover chloride from very low external concentrations, as will be shown below.

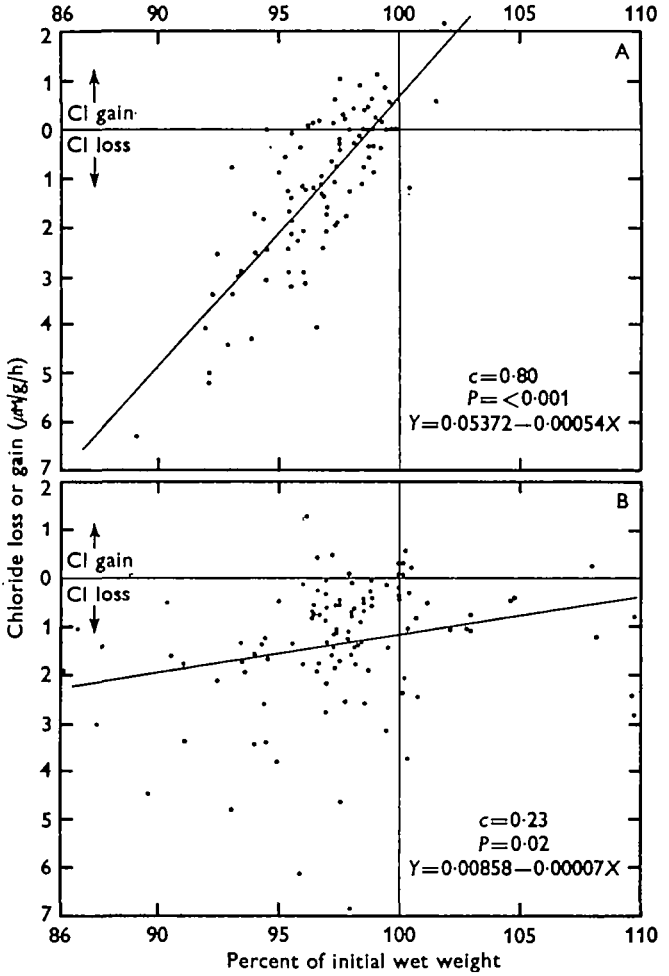


Fig. 2. (A) Loss or gain of chloride by *N. diversicolor* plotted against change in weight (as % of original weight) after a 2 h exposure to a PW/SW mixture having a chloride concentration of 10 mM/l. (B) Similar plot for *N. diversicolor* after a 2 h exposure to PW. In both A and B, worms had been pre-adapted to media identical to those used in test exposures.

(3) *Net chloride uptake from low external concentrations*

Continuing the experimental procedure indicated in the preceding section, at the end of the 2 h period in 'adaptational medium' each worm was weighed a second time and transferred to a 10 ml volume of a 'recovery medium' of a higher chloride concentration (up to 20 mM/l) for a further 2 h period, at the end of which it was removed. All experimental vessels were grouped in covered containers lined with medium-soaked filter paper to check evaporation, and duplicate control vessels without worms were used for each set of 'adaptational' and 'recovery' media. Chloride determinations on experimental and control solutions were made, and the loss or gain of chloride

was calculated for each experimental animal and expressed in m-moles/g of animal (wet weight)/h. The weight at the second weighing was used in this calculation.

Following adaptation in PW, *N. diversicolor* shows some net uptake of chloride from a concentration as low as 3 mM/l, a marked net uptake from 5 to 10 mM/l, and a

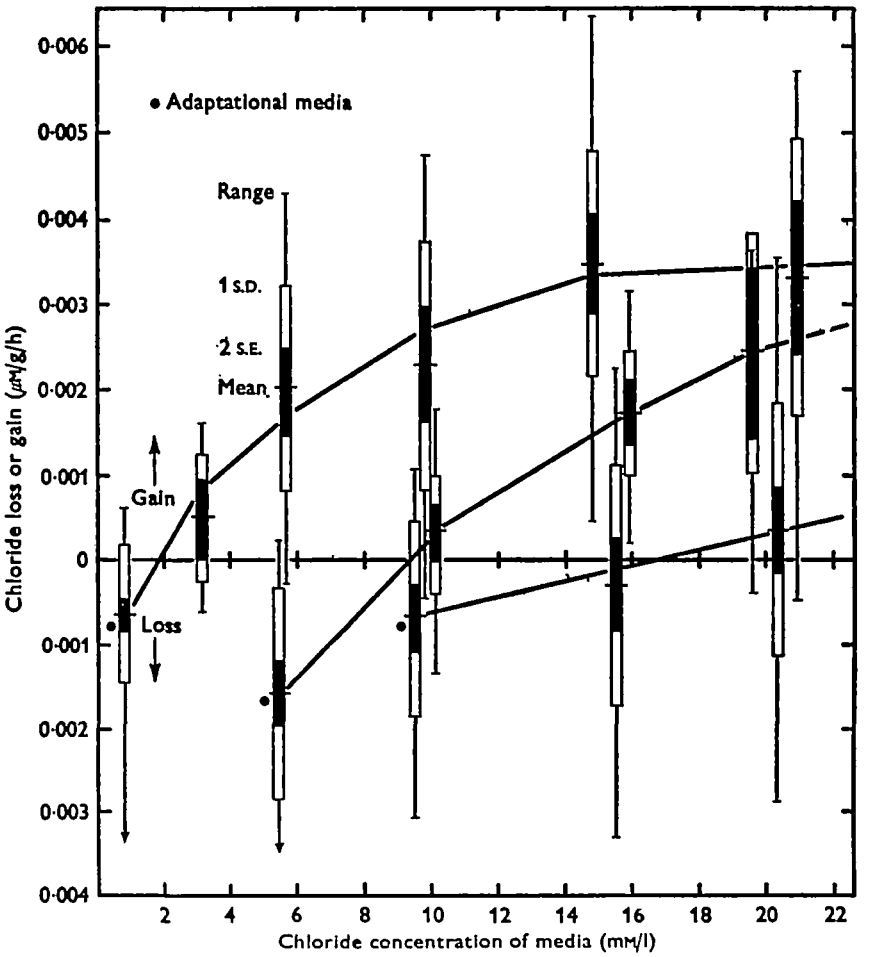


Fig. 3. Net gain or loss of chloride by *N. diversicolor* adapted in media of chloride concentrations 1, 5 or 10 mM/l, exposed 2 h to adaptational media to establish initial point of each curve (●), and then transferred for 2 h to various media of higher chloride concentrations. Details in text.

maximum in 15–20 mM/l (Fig. 3). This net uptake reaches *c.* 3·5 μM/g (wet weight)/h. Worms adapted at a chloride concentration of 5 mM/l show net uptake in 10 mM/l and approach the maximum at some level above 20 mM/l. It can be said that an increase of external chloride concentration by 10 mM/l over an adaptational level of 1 or 5 mM/l results in a significant net uptake. But, on the contrary, worms adapted at a chloride concentration of 10 mM/l show no significant net uptake when placed in 20 mM/l. Experiments at chloride concentrations higher than 20 mM/l did not appear profitable, since the resolution of the chloridometer is inadequate to detect the small percentage differences in concentration produced by net uptake from more concen-

trated recovery media. The form of the chloride regulation curve (Fig. 1) makes it probable that an animal transferred from a medium of 10 mM/l to one of 20 mM/l need make only a minute adjustment of its coelomic chloride concentration, and hence shows no measurable net uptake. But when worms adapted to *c.* 1 mM/l are exposed to recovery media of 10 mM/l, there is an immediate need to raise the coelomic chloride concentration from about 110 to about 130 mM/l, an increase of *c.* 18%, and in consequence a net uptake of chloride can be observed. In a 1 g worm preadapted to PW, and assuming a water content of 90%, this would require about 18 $\mu$M of chloride to complete the adjustment and, at a rate of 2·5 $\mu$M/h, a period of 7 h for the process. The net uptake of 2·5 $\mu$M/g/h is part of an elevated chloride influx which would come to balance efflux as the new steady state is attained, and the net uptake could result simply from the provision of additional external chloride ions to an unsaturated active uptake system. It might be argued that there is some 'activation' of a chloride uptake (active transport) system in worms adapted to less than 10 mM/l, but since worms adapted to 10 mM/l and higher have to make only a slight adjustment of their internal chloride concentration, this process may be completed well before the end of the 2 h test period, thus obscuring an initially high rate of active uptake. Another type of experiment, designed to measure the initial rate of chloride uptake, would be necessary in order to resolve the question of the possible activation of a chloride transport mechanism at low salinities. Furthermore, none of the experiments reported in this paper differentiate between the active transport of the chloride ion and movement of chloride resulting from active transport of other ions. All that is clearly shown is that *N. diversicolor* can recover chloride from the medium against concentration ratios of at least 35 to 1.

(4) *Chloride influx as a function of environmental salinity*

*N. diversicolor* adapted for a week or more at various adaptational salinities were selected at random in respect to size, isolated in adaptational media, weighed, dropped at recorded times into media enriched with $^{36}Cl$, and sampled at either 4 or 18 h. Provided the radioactive media were of the same chloride concentration as the adaptational media, the chloride influx was nearly constant with time (Fig. 4). Test media contained $^{36}Cl$ at a strength yielding 1000–8000 counts/1000 sec from a 10 $\mu$l sample. This resulted in comparable count rates in coelomic fluid at salinities above the regulatory range (above 35% SW) but as isotopic equilibrium was approached in very low salinities the count rate of coelomic fluid was, as expected, many times greater than that in the medium. Counting was continued to a maximum of 10,000 counts or 1000 sec, mostly on duplicate 5 or 10 $\mu$l samples of medium and of coelomic fluid centrifuged free of cells after being drawn by puncture of the muscular anterior part of the body of the worm. At no point in the preparation and counting procedure was material digested in the acid chloridometer reagent, since counts made on material exposed to acid digestion gave noticeably, although irregularly, lower counts, presumably resulting from loss of $^{36}Cl$ as gaseous $H^{36}Cl$. The determinations of chloride flux were carried out at 16–18° C.

Chloride determinations on media containing $^{36}Cl$ were made on suitable subsamples; agreement was so close that these values were averaged, except at concentrations below 10 mM/l, where net loss or gain of chloride might significantly alter

individual media. Chloride determinations on coelomic fluid were made on single 5 or 10 $\mu$l subsamples from each worm, taken from the same centrifuged sample that supplied subsamples for the counting of $^{36}Cl$. Since the variation between worms was vastly greater than the variability of repeated chloride determinations on the same lot of fluid, the coelomic chloride concentration of each individual worm was considered separately in calculation of the specific activity of $^{36}Cl$ expected in its coelomic fluid

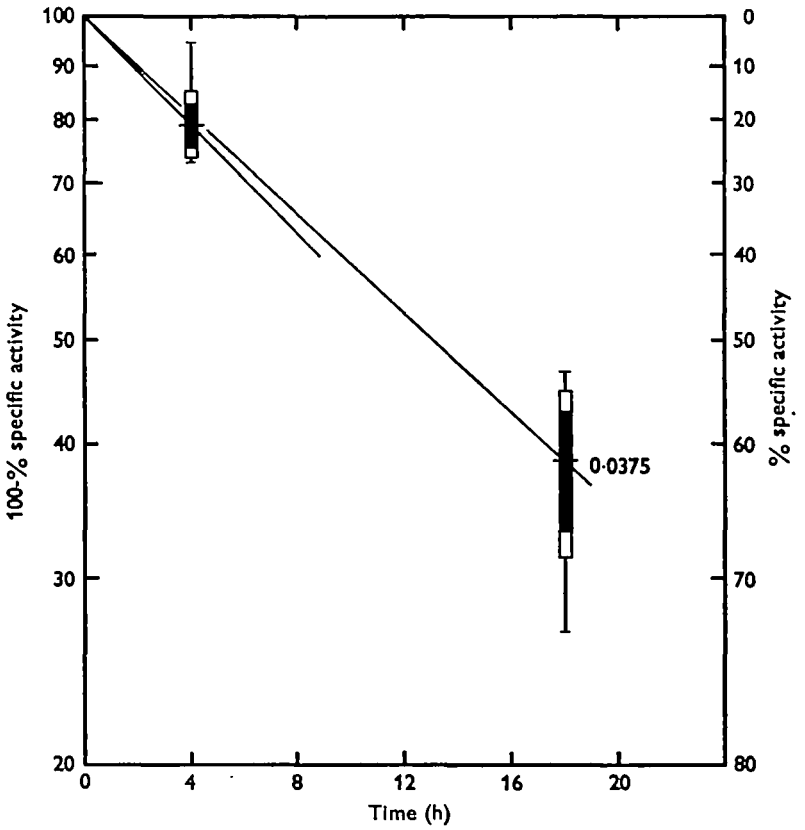


Fig. 4. Concentration of $^{36}Cl$ in coelomic fluid (expressed as % specific activity at right and as 100-% specific activity at left) plotted semilogarithmically against time, showing that influx is essentially constant with time. Hourly exchange fraction ($K$) at 18 h is 0·0375. Details in text.

when in isotopic equilibrium with the medium. The specific activity of $^{36}Cl$ in the coelomic fluid was expressed as a percentage of that of the medium and plotted as (100-% specific activity) against time on semilogarithmic paper (Figs. 4, 7). The 'hourly exchange fraction', $K$ (the fraction of body chloride exchanged/h), was calculated for the means at 4 and 18 h by the equation:

$$K = \frac{2{\cdot}3}{t} \log_{10}\left(\frac{100}{100-x}\right),$$

in which $K$ = fraction of total body chloride exchanged/h, $t$ = time in hours, $x$ = specific activity of $^{36}Cl$ in coelomic fluid at time $t$, expressed as percentage of the specific activity of $^{36}Cl$ in the medium.

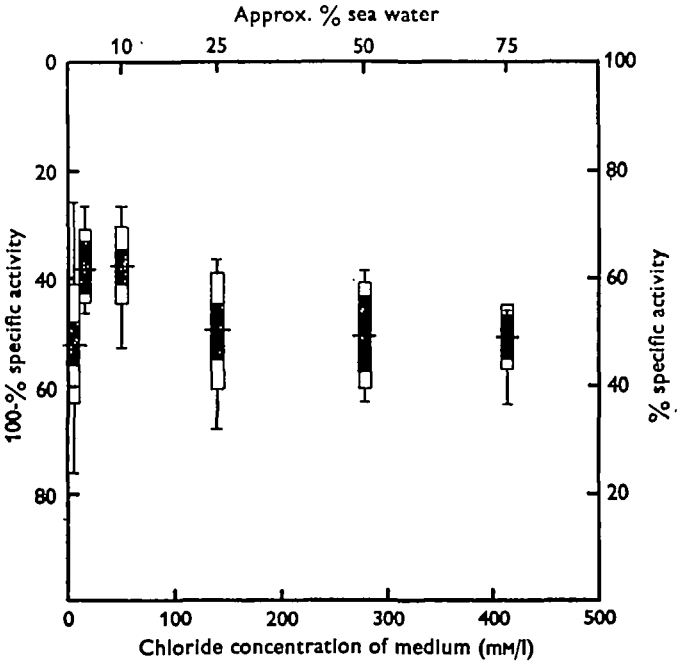


Fig. 5. Percentage specific activity of $^{36}Cl$ attained in 18 h as a function of the chloride concentration of the medium. Note that the percentages at 10 and 60 mM/l are significantly elevated above those at higher salinities or in 2·5 mM/l.

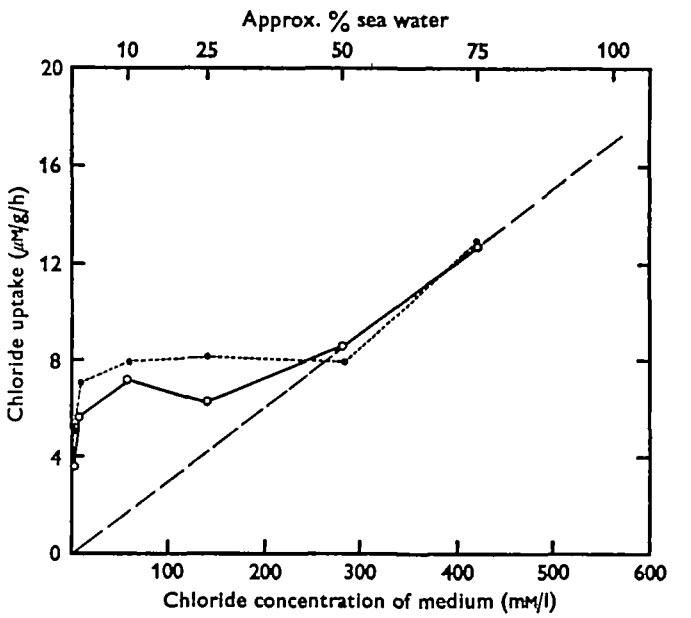


Fig. 6. Chloride influx (μM/g/h) under steady-state conditions as a function of the chloride concentration of the medium. Note proportionality of influx to external concentration in 50 and 75 % SW. ●, calculated at 4 h; ○ and solid line, calculated at 18 h.

Calculations of chloride influx in $\mu$M/g (wet weight)/h were made by using the mean values for coelomic chloride concentration, the mean $K$ for each group, and the water content from data of Oglesby (1970), on the assumption that the chloride was distributed throughout all the water of the body. The results are summarized in Table 1 and plotted in Fig. 6.

The chloride influx is in general rather slow, having a half time of up to 18 h. The time-constants of chloride influx, $1/K$ (the time required to reach a % specific activity of $(1-1/e)$, or 63·2% in the presence of back diffusion, or 100% in the absence of back diffusion) are included in Table 1. Chloride influx is clearly proportional to the external chloride concentration in salinities of 50% SW and higher (Fig. 6), suggesting a process of simple diffusion. However, in the osmoregulatory range the influx is higher than can be accounted for by simple diffusion and indicates active uptake or 'exchange diffusion'. There is a marked elevation of the influx in the concentration range of 10–60 mM/l (Figs. 5, 6), and an indication of a lowering of the influx at an external concentration of 2·5 mM/l (Fig. 6), in which the % specific activity at 18 h is close to that in high salinities (Fig. 5); likewise the hourly exchange fraction ($K$) at 2·5 mM/l is close to that in high salinities (Table 1). These facts do not seem to suggest that permeability to chloride is lowered in fresh or nearly fresh water.

Table 1. *Hourly exchange fractions* (K) *and time constants* (1/K) *for chloride influx (measured by use of* $^{36}Cl$) *at various salinities*

| Approximate % SW | | 0·4 | 1·8 | 10 | 25 | 50 | 75 |
|---|---|---|---|---|---|---|---|
| Chloride concentration of media, mM/l | | 2·5 | 10 | 62 | 143 | 283 | 415 |
| Chloride concentration of coelomic fluid, mM/l | | 110 | 135 | 155 | 188 | 268 | 382 |
| Water-content (Oglesby, 1970) | | 0·90 | 0·88 | 0·88 | 0·87 | 0·86 | 0·85 |
| Hourly chloride exchange fraction ($K$) | 4 h | 0·050 | 0·058 | 0·058 | 0·057* | 0·034 | 0·039 |
| | 18 h | 0·036 | 0·047 | 0·054 | 0·036 | 0·036 | 0·038 |
| Chloride influx, $\mu$M/g/h | 4 h | 6·81 | 6·96 | 7·90 | 9·37* | 7·91 | 12·73 |
| | 18 h | 3·88 | 5·64 | 7·29 | 5·82 | 8·69 | 12·69 |
| Time constant (1/$K$) (h) | 4 h | 20·0 | 17·2 | 17·2 | 17·5* | 29·4 | 25·6 |
| | 18 h | 27·8 | 21·3 | 18·5 | 27·8 | 27·8 | 26·3 |

* Net uptake possibly occurring; chloride concentration of adaptational medium was not checked at time of transfer.

(5) *Variation in the chloride influx with time*

As mentioned above, the chloride influx was nearly constant with time in most experiments, e.g. in Fig. 4 (10% SW). However, in all but one salinity there was a slight change in influx with time, such as to indicate a faster uptake in the first 4 h than would be calculated on the basis of 18 h exposures (Table 1, Fig. 6). Such an initially higher influx could be accounted for in two ways.

One of these is that, after transfer to labelled media, the worms commonly became active and swam about a great deal in the first hour or two. This undoubtedly provided a better irrigation of body surfaces and an increased internal circulation, and so reasonably accounts for the fact that chloride influx might be higher in the first few

hours than would be indicated by a measurement at 18 h, after the worms had rested undisturbed overnight.

At very low concentrations a second complication is that non-steady-state conditions and consequent net movements of chloride may occur. Net loss of chloride may result from activity (Fig. 2), even when experimental solutions are carefully adjusted. Further, since $^{36}Cl$ is supplied in a high concentration (2N-HCl) of low specific activity, its addition to PW raises the chloride concentration very considerably—in the experiments reported here, from 0·9 to 2·5 mM/l—thus providing conditions for net uptake. Thus if one adds $^{36}Cl$ to PW the result is a nearly threefold increase in chloride concentration, which, if not taken into account, could lead to apparently very high uptake values from what was supposed to be fresh water. As shown in Fig. 7,

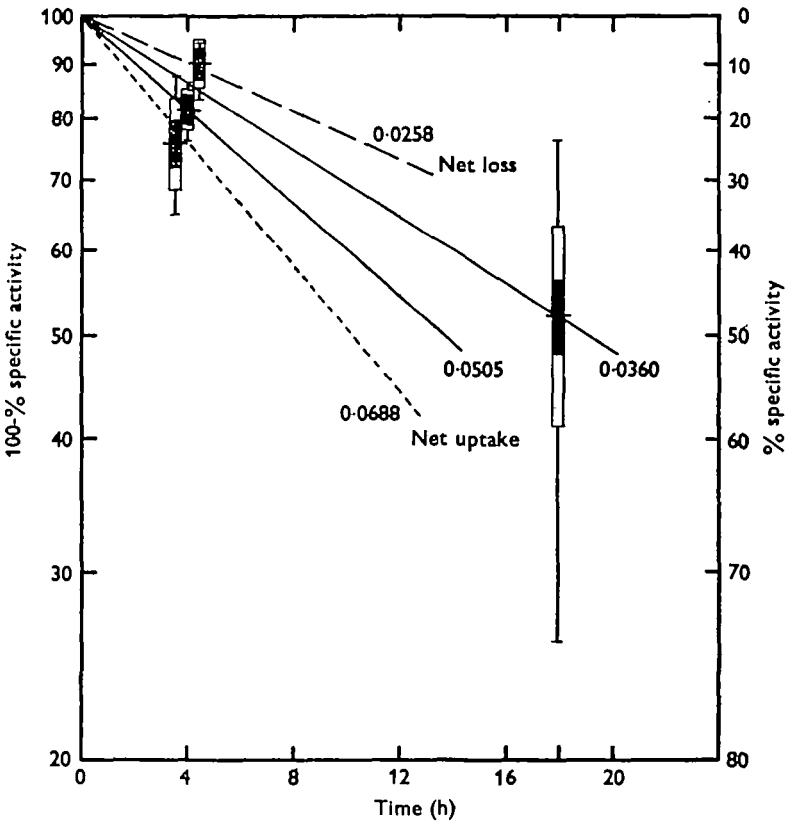


Fig. 7. Concentration of $^{36}Cl$ in coelomic fluid (expressed as % specific activity and plotted as in Fig. 4) showing variation of influx over 4 h. Lower dashed line, exposed to 2·5 mM/l after adaptation in 0·9 mM/l; upper broken line, exposed to 2·5 mM/l after adaptation in 10 mM/l; solid lines, exposed to 2·5 mM/l after adaptation in identical concentration. 18 h values, although diverging in same sense as 4 h values, were not significantly different among themselves, and are shown as a single pooled sample. $K$ values (hourly exchange fractions) are given for each line.

after such a transfer the chloride influx calculated over 4 h is much higher than that calculated over 18 h ($K \approx 0{\cdot}0688$ as opposed to 0·0360 at 18 h). The converse experiment, involving a probable net loss of chloride, was performed by pre-adapting worms

at 10 mM/l and then determining chloride influx at 2·5 mM/l. This gave a very low initial influx ($K$ = 0·0258). Finally, in an experiment where the chloride concentration of the pre-adaptational medium was very carefully adjusted to match that of the radioactive medium, the influx over 4 h, while still greater than that over 18 h, was intermediate in value ($K$ = 0·0505). In this last instance the effect of initial activity is presumed to be seen alone, without effect caused by net loss or gain except for chloride loss in the urine, which might be expected not to affect the influx. Another possible explanation for this set of results is that the transport mechanism for chloride is in a more active state in worms pre-adapted in 0·9 mM/l (PW) than in 2·5 or 10 mM/l. This activation difference, pronounced during the 4 h following transfer, may perhaps be lost during the longer 18 h exposure, since the 18 h influxes although differing, in the same sense as the 4 h influxes, did not differ significantly, and are shown pooled in Fig. 7.

(*b*) *Electrical potentials across the body wall*

As the final part of this study a rather brief series of measurements was made of the electrical potential across the body wall of *N. diversicolor* adapted to PW (Cl = *c.* 0·9 mM/l), 10% SW (Cl = *c.* 56 mM/l) and 50% SW (Cl = *c.* 280 mM/l). A Vibron Electrometer, chart recorder and a pair of calomel half-cells were used, with one half-cell immersed in the external medium, and the other immersed in 'Nereis-Ringer' contained in the puncturing capillary in the form of SW diluted with PW to chloride concentrations of 110, 155 and 280 mM/l respectively. The worms were punctured anterodorsally by the Ringer-filled glass capillary, to which they were tied by a piece of cotton thread, and suspended in the bathing media. Swimming movements occurred during the reading, but level and steady traces were obtained in several instances. In some records the trace showed sharp oscillations; possibly in such cases the impaling capillary tip was embedded in the musculature.

In PW initial deflexions of −12 to −32 mV were obtained (inside-negative). These subsided in three 'steady' worms to an average of −17·3 mV (−14 to −20 mV). In five 'unsteady' examples a mean potential of −8·6 mV was obtained (−6 to −12 mV, all inside-negative).

In 10% SW, three successful 'steady' readings averaged −3·7 mV (−2·5 to −5·5 mV, inside-negative).

In 50% SW, essentially no potential was recorded; the mean of five 'steady' examples was +0·6 mV, which is not considered significantly different from zero.

Since the potentials measured were all inside-negative in the regulatory range of salinities tested, it is clear that the hyperregulation of chloride cannot be the result of passive movement of chloride ions in response to an electrical gradient. Rather, chloride must be moved inward against the electrical as well as the chemical gradient. However, the cause of the observed inside-negative potential is not indicated by these experiments.

## DISCUSSION

For purposes of discussion of chloride exchange *N. diversicolor* may be represented schematically as a surface enclosing coelomic fluid, which drains to the outside in the form of urine via an open nephridium (Fig. 8). In the non-feeding animal the

gut cavity may be ignored, but were it to be significantly open to exchange with the exterior it could be regarded as part of the surface. In the steady-state condition the influx of water, or of chloride or other ions, exactly balances an efflux attributable to two components, urinary and integumental (Fig. 8A). In the case of a worm regulating at salinities below 35% SW, the concentration of ions in the coelom is higher, sometimes much higher, than in the medium, and water is at a correspondingly lower concentration.

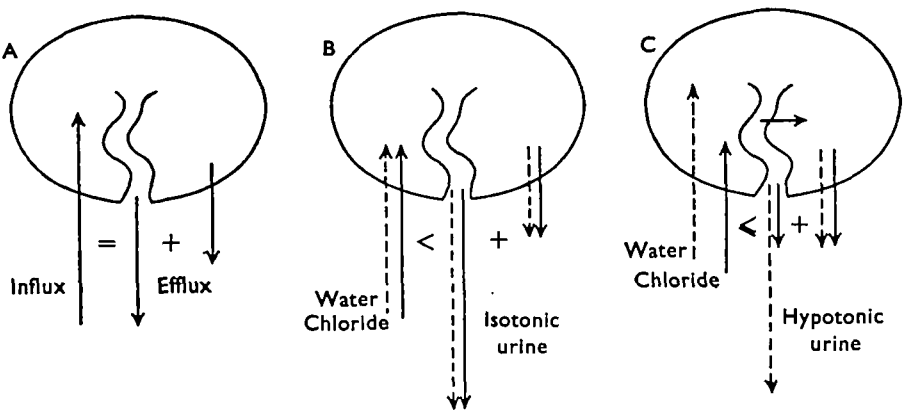


Fig. 8. (A) Schematic nereid in steady state (influx = efflux). (B) Discharging increased volume of isotonic urine as result of activity and consequently elevated hydrostatic pressure. (C) Active as in B, but discharging urine rendered hypotonic by solute resorption in nephridium.

A net loss or gain of chloride in this model can be attributed to change in the influx or in one or both of the effluxes. Thus, the net chloride loss combined with weight loss seen following shift of a worm from a 10 mM/l solution to an identical solution can be most simply explained by an increase in the rate of elimination of a urine having the same composition as the coelomic fluid, or at least of markedly higher concentration than the medium (Figs. 2A, 8B). On the other hand, the loss of weight without a well-correlated loss of chloride when a transfer is made from PW to PW could be explained if the worm in PW were producing a very hypotonic urine, as a result of active resorption of chloride (Figs. 2B, 8C). The loss of water as urine would cause weight loss, but such hypotonic urine would not contribute significantly to the net chloride loss. This is consistent with the view of Potts & Parry (1964) that the urine of *N. diversicolor* may be hypotonic to the body fluid at low salinities, and the correlation of weight loss with chloride loss at an external chloride concentration of 10 mM/l is in agreement with their view (loc. cit., p. 151) that the urine may become hypotonic only at very low salinities. However, the present data do not show whether or not the urine is hypotonic in a medium of 10 mM/l, but only that the urine might have a considerably higher chloride concentration than the medium.

Since chloride can be taken up against a concentration difference of over 100 mM/l when *N. diversicolor* is adapted to PW, it would seem reasonable for the worm to utilize this ability to move chloride from its urine against a far smaller concentration gradient back into its body fluid, the more so as the nephridia are considered to be of

ectodermal origin (Goodrich, 1946) and hence should be expected to respond to whatever factors promote active uptake by the epidermis. Thus, chloride regulation in *N. diversicolor* might be analogous to sodium regulation in *Gammarus duebeni* in which Sutcliffe (1967) has suggested 'that increases in sodium uptake in the antennary glands, resulting in a hypotonic urine, are linked with increases in uptake at the body surface. Both uptake systems are possibly activated by a single internal regulator responding to changes in the blood concentration.' In *N. diversicolor*, net chloride uptake is marked in just the salinity range where the coelomic fluid shows a drop in chloride concentration below the regulatory plateau. It has been suggested by Jørgensen & Dales (1957) that the permeability to chloride of *N. diversicolor* is decreased at very low salinities. The present results suggest that in PW the chloride uptake mechanism is unsaturated and the total chloride influx is thereby reduced. This implies a comparable reduction in chloride efflux and, since urine is undoubtedly being produced, it is possible that the reduction in chloride loss is accomplished by rendering the urine hypotonic; but equally possible would be some reduction in loss of chloride via the integument by a lowering of permeability to chloride, or a reduction of urine volume. Until the actual chloride concentration and the volume of the urine can be measured, one cannot with confidence apportion the chloride loss between urine and integument. Until the urine volume is measured, one cannot be certain that a reduction in urine volume at low salinities does occur, although a direct measurement of permeability to water would help to clarify the matter, and will be reported in the following paper (Smith, 1970*a*).

Potts & Parry (1964, pp. 145–152) have made a diagrammatic representation of the fluxes of water and chloride in *N. diversicolor*, based on the data of Jørgensen & Dales (1957), but on the assumptions that permeability to water and chloride does not change and that no potential difference exists across the body wall. A similar balance sheet using the same assumptions has been constructed from the present data (Fig. 9). On their given assumptions, the picture is comparable to that presented by Potts & Parry, but allows for some urinary loss of chloride at all salinities. In Fig. 10 these results are plotted in an attempt to depict the relative chloride losses by the urinary and integumental routes. In higher salinities, where diffusional exchange of chloride predominates in direct proportion to external concentration, the bulk of chloride efflux is integumental. Urinary chloride, although it must be high in concentration, is low in amount because the volume of urine must be low. Urinary chloride loss shows a minimum in the 25–50% SW range, where the coelomic chloride concentration has dropped to near the level of the regulatory plateau, but where hyperregulation is only just beginning (Fig. 1). Urine volume is low and urinary chloride concentration, probably isotonic to that of coelomic fluid, is also close to that of the medium. When the external chloride concentration has dropped to *c.* 60 mM/l (*c.* 10% SW), hyperosmotic regulation is very pronounced, and a large volume of urine is to be expected; if this urine were isotonic to coelomic fluid, the observed increase in urinary loss or efflux of chloride could be accounted for. Chloride influx against the gradient must also be maximal, since the animal is in a steady state (influx = efflux). But when the external chloride concentration has dropped to 10 mM/l or lower, the animal is clearly 'cutting its losses' of chloride, and this reduction of chloride efflux would seem mainly to affect the urinary route (Figs. 9, 10).

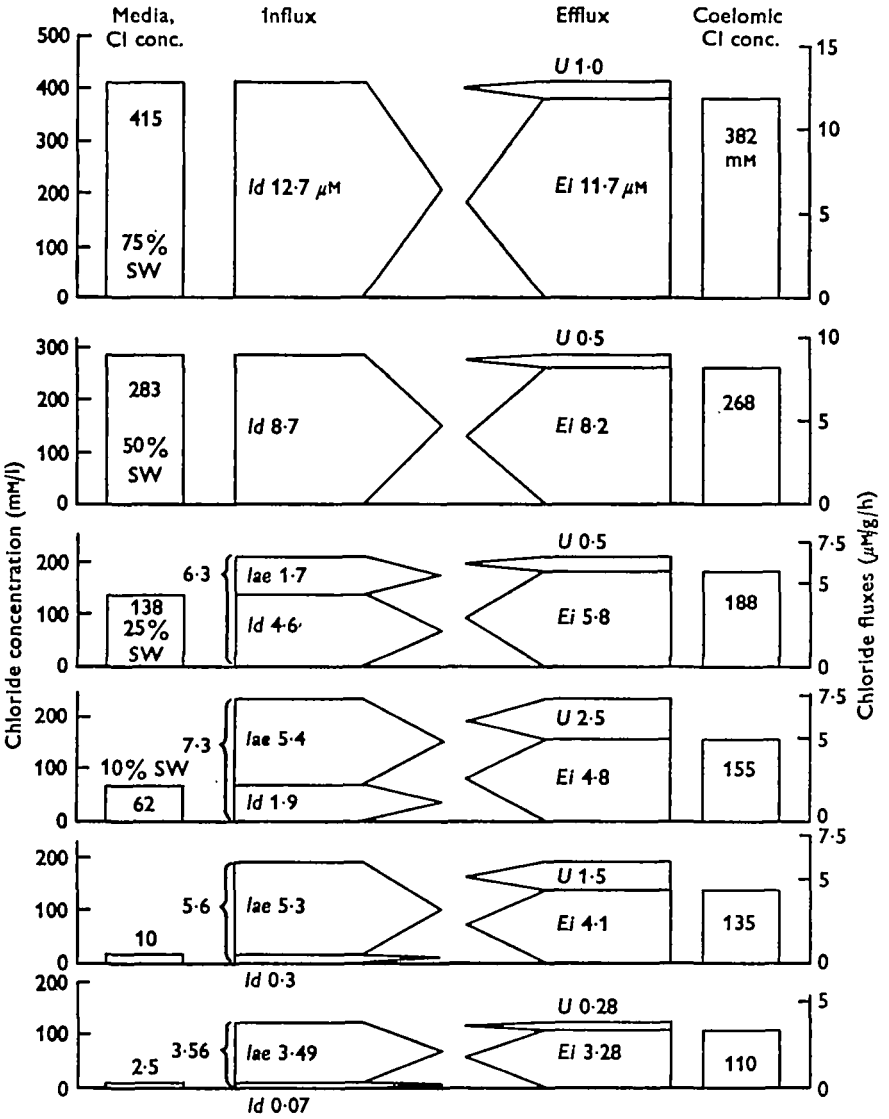


Fig. 9. Representation of chloride fluxes in *N. diversicolor*, based on present data, formulated following the assumptions and diagrammatic method of Potts & Parry (1964). *Id* = diffusion; *Iae* = active uptake + exchange diffusion; *u* = urinary; *Ei* = integumental (diffusion + exchange diffusion).

The reduction of chloride loss in PW is attributed by Potts & Parry (loc. cit.) to a recovery of chloride from the consequently hypotonic urine. But the data of Jørgensen & Dales (1957) led them to conclude that, given some reduction in integumental permeability to chloride, the 60% decrease in permeability to water is sufficient to account for the observed reduction of chloride loss by a reduction in urine volume. However, the requisite reduction in permeability to chloride has yet to be unequivocally demonstrated, and it seems probable that hypotonicity of the urine is part of the

mechanism of adaptation to fresh water. In the following paper (Smith, 1970*a*) it will be shown that *N. diversicolor* does in fact exhibit a reduction in permeability to water, such that the hypothesis of Jørgensen & Dales (1957) can also be supported. And in the second following paper (Smith, 1970*b*) the urine of *N. diversicolor* will be shown to be

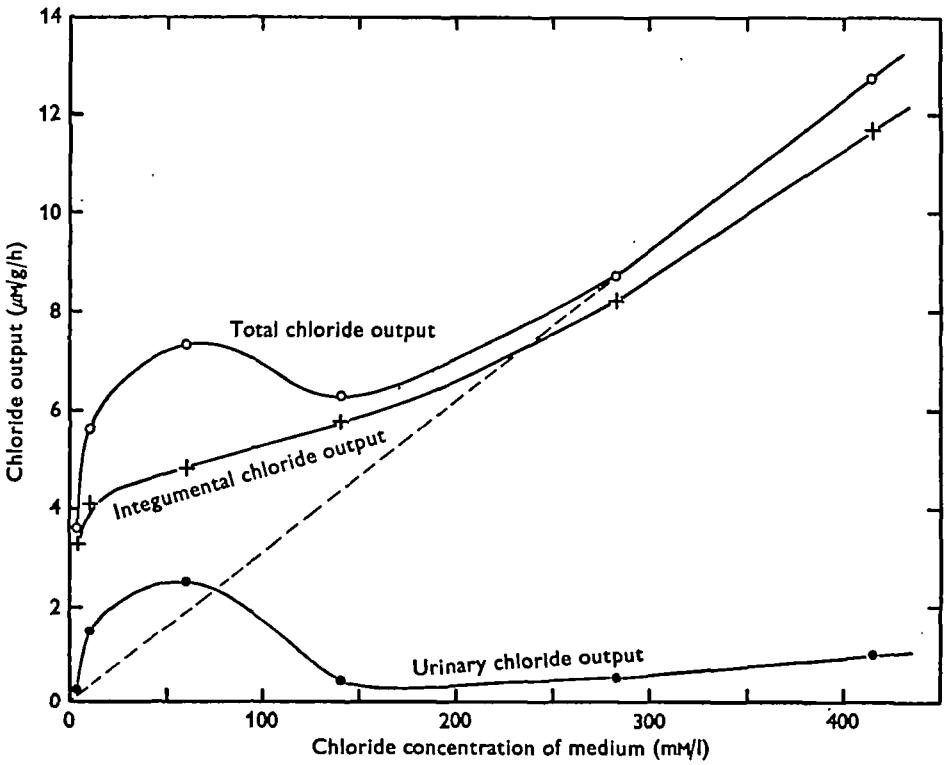


Fig. 10. Relation of urinary and integumental effluxes of chloride in *N. diversicolor* under steady-state conditions as a function of external chloride concentration in dilutions of SW, showing proportionality of total efflux to concentration in > 50 % SW, maximum of urinary chloride loss in range of *c.* 10 % SW, and reduction of urinary chloride loss as FW is approached. Data from Figs. 6 and 9.

hypo-osmotic to coelomic fluid at low salinities, although data on urinary chloride concentration are still lacking. In all probability both hypotonicity of urine and reduction of urine volume are used in the adaptation of *N. diversicolor* to very low salinities.

The demonstration that *N. diversicolor* can move chloride inwards against both electrical and concentration gradients, coupled with the observation that the chloride influx is much above that accountable for by simple diffusion at low salinities (Figs. 9, 10), suggests that an active process is responsible. This is not the same as saying that the chloride ion itself is actively transported but simply that something is being actively transported. Either chloride is itself transported, or it moves passively in response to, or coupled with, the active transport of some other ions; the present study shows that the existing electrical gradient opposes the inward movement of chloride. In the paper of Jørgensen & Dales (1957), as well as in the review of Potts &

Parry (1964), any movement of chloride against the chemical gradient is essentially equated with active transport. This is, perhaps, an over-simplification, and omits mention of one factor which may contribute to the elevated chloride exchange in the concentration range of *c.* 60 mM/l indicated both in the present work and in that of Jørgensen & Dales. In this salinity range, as indicated by the scheme of Potts & Parry (1964, fig. IV. 23) and in the present study (Fig. 9) using the same assumptions, chloride influx can be represented by a significant diffusional component plus what Potts & Parry call 'active uptake'. However, the possibility exists that some fraction of this second component of the exchange does not represent net inward transport, but may be accounted for by what is called 'exchange diffusion'. What is actually referred to is a coupled process by which a chloride ion being transported in is coupled to one being moved out. Such a coupled exchange of chloride would produce no *net* transfer of chloride, but could account for some fraction of the increased chloride influx observed in the regulatory range. Another possibility might be that for several chloride ions pumped in, a 'leaky' or inefficient chloride pump might throw one out. The activity of such an inefficient pump could result in an elevated chloride influx which is greater than the net active transport inwards. Thus in Fig. 9 the component of influx designated as 'active uptake' by Potts & Parry is called 'active uptake plus exchange diffusion', and is considered to represent an exchange of which some undetermined fraction (probably less than half) is attributable to 'exchange diffusion', plus a major fraction of net inward movement against chemical and electrical gradients. Attention is called to the possible presence of some coupled exchange or 'exchange diffusion' of chloride, but with the reminder that the present study has done nothing to evaluate it.

SUMMARY

1. *N. diversicolor* from estuarine conditions in north-eastern England can be adapted to a chloride concentration in a pond water (PW) medium at least as low as 0·9 mM/l, and shows a net uptake of chloride when returned to a medium 3–10 mM/l more concentrated. But in comparable transfers after adaptation at a chloride concentration of 10 mM/l, net uptake is not measurable.

2. Net uptake of chloride is demonstrable in the lowest salinities, where coelomic chloride concentration drops below the regulatory plateau. Net uptake reaches 3·5 μM/g wet weight/h.

3. Chloride loss is well correlated with weight loss after adaptation in 10 mM/l, but poorly so after adaptation in PW, suggesting that the urine is very hypotonic to body fluid in PW, and isotonic (or less hypotonic) at environmental chloride concentrations of 10 mM/l or higher.

4. Uptake of chloride occurs against both electrical and chemical-concentration gradients over the lower third of the environmental salinity range, which is the range in which hyperosmotic and hyperionic regulation are most pronounced.

5. The electrical potential across the body wall is maximal in PW (17 mV, inside-negative), and decreases to zero in 50% SW.

6. Chloride influx (as measured with $^{36}Cl$) is highest in SW, and decreases in proportion to chloride concentration down to 50–25% SW, rises to a secondary maximum in 10% SW or less, and decreases as fresh water is approached.

7. Urinary chloride loss is low, and proportional to external chloride concentration in higher salinities, maximal in the *c.* 10% SW range of salinities, and apparently decreases to a minimum in FW. This may be in part the consequence of recovery of chloride from an hypotonic urine, in part the consequence of a reduction in urine volume. Evidence for these last two possibilities will be given in the papers which follow.

The studies reported in this and the following papers were made possible by a grant of sabbatical leave from the University of California, Berkeley, and the greatly appreciated support of the John Simon Guggenheim Memorial Foundation. I am particularly indebted to Professor John Shaw of the Department of Zoology, University of Newcastle upon Tyne, in whose laboratory this work has been performed, and to whom I owe thanks for many helpful suggestions. I am likewise grateful to Professor and Department Head Robert B. Clark for the hospitality shown me by him and his department, and for the help given by the staff of the Dove Marine Laboratory. Dr L. C. Oglesby kindly permitted me to use his then-unpublished data on chloride regulation and water content.

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