J. Exp. Biol. (1970), 53, 93–100
With 3 text-figures
Printed in Great Britain

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

II. WATER FLUXES AND APPARENT PERMEABILITY TO WATER

BY RALPH I. SMITH*

Department of Zoology and Dove Marine Laboratory (Cullercoats), University of Newcastle upon Tyne

(*Received* 5 *December* 1969)

Evaluation of the chloride exchanges of *Nereis diversicolor* in the steady state has shown that this worm 'cuts its losses', i.e. reduces the fluxes, of chloride at very low salinities (Smith, 1970*a*), and that this reduction of chloride loss takes place mainly in the urinary component of the efflux, as has also been suggested by previous authors. Jørgensen & Dales (1957) measured the rates of osmotic gain or loss of water, and concluded that at low salinities *N. diversicolor* could reduce its permeability to water by over 60%. Assuming a comparable reduction in permeability to chloride, they calculated that the observed reduction of chloride efflux could be accounted for by reduction of urine *volume*, without the necessity of postulating the production of a urine rendered hypotonic to the body fluids by recovery of solutes by the nephridia. Potts & Parry (1964), on the other hand, in their review of the previous authors' work, felt unable to accept the evidence for a reduction of permeabillty to water and, on the assumption of no permeability changes, drew up a balance sheet in support of the hypothesis that reduction of chloride loss was achieved in *N. diversicolor* by recovery of ions from a consequently hypotonic urine. These views are not mutually exclusive, and the problem is not to choose between them but to obtain evidence to indicate the extent to which each postulated mechanism may be operative. In the previous paper (Smith, 1970*a*), evidence was presented compatible with the concept that the urine of *N. diversicolor* may be hypotonic in chloride to the coelomic fluid at low salinities, although this evidence was indirect and not conclusive. In a subsequent paper (Smith, 1970*b*) direct evidence will be presented that the urine of *N. diversicolor* is hypo-osmotic. In order fully to evaluate the hypothesis of Jørgensen & Dales it would be necessary to measure both permeability to water and urine volume. So far, it has not been possible to measure urine volume, but data on permeability to water have been obtained, and an estimate of water fluxes attempted.

MATERIAL AND METHODS

Nereis diversicolor were collected and maintained as described in the first part of this study (Smith, 1970*a*). For determination of permeability to water D_2O (Bio-Rad Laboratories, Richmond, California, 95 moles %) was employed as a 5% solution in

* Present address: Department of Zoology, University of California, Berkeley, California 94720, U.S.A.

several dilutions of sea water (SW) made up with local pond water (PW). The use of D_2O followed closely the method employed in previous studies on nereid polychaetes (Smith, 1964) and on the crabs *Rhithropanopeus* (Smith, 1967) and *Carcinus* (Smith, 1970*c*).

Worms used in this study were selected to give a range in weight from *c.* 100 mg to *c.* 500 mg, in an effort to permit calculation of the relationship of uptake to body weight. Only worms of normal body form were used; worms with extensive caudal amputations, even though fully healed, were discarded. *N. diversicolor* from two sources were used. One group of worms, adapted and tested in salinities ranging from PW to 50% SW, came from the Wansbeck estuary, representing a habitat of salinity below 50% SW and the same population that furnished material for the studies reported in the previous paper and by Oglesby (1970). A second group of worms, adapted and tested at salinities from 25 to 94% SW, was collected from the Black Middens, a fairly clean marine-dominated rocky and sandy area at the mouth of the River Tyne. Tests were carried out at 18–19 °C, except for two groups of Wansbeck estuary worms that were tested at 22–23 °C, and yielded slightly elevated water fluxes.

Half the worms selected for a given day's experiment had been adapted for a week or more at a high salinity, half at a low salinity. This procedure made for a more effective use of the density-gradient columns (see below), and helped to randomize the tests. Prior to testing, worms were isolated overnight in 20 ml of their respective adaptational media. Each worm was weighed after blotting on filter paper and returned to its vessel. Exposure of each individual to medium containing 5% D_2O was for 5 min, following which the worm was blotted dry on filter paper, and a sample of coelomic fluid was taken by puncture of the anterior third of the body with a short glass tube drawn to a capillary tip. This tip was quickly sealed in a flame, the tube was centrifuged, and the cell-free supernatant was picked up in a fresh capillary. This subsample was discharged into the large end of a numbered Aloe disposable pipette, this end was tightly plugged with a small cork, and the tip was sealed in a flame. A sample of the medium containing D_2O was placed in another such pipette and in other pipettes several samples of centrifuged coelomic fluid from worms not exposed to D_2O were used as controls. The pipettes containing samples from about 20 worms thus exposed to media containing D_2O, together with samples of media and controls, were laid on a slide-warmer set at 50 °C overnight. Water and D_2O distilled from the samples and condensed in the tips of the pipettes, which projected 3 in from the slide-warmer and were covered by a strip of facial tissue paper kept wet by its ends dipping into vessels of water. Evaporative cooling gave effective condensation. The following morning, the drops of condensate in the pipette tips were discharged into a pair of kerosene/bromobenzene density-gradient columns, together with standard D_2O solutions of 0–5% D_2O, and the relative positions of standards and unknowns were plotted. By the procedure detailed in Welsh, Smith & Kammer (1968) the D_2O concentrations of media and of samples of coelomic fluid (corrected for controls) were determined graphically, and the coelomic concentrations attained in 5 min were expressed as percentages of the D_2O concentration of the medium. For reasons to be explained below, the % concentration values in this particular study were not referred to unit weight, but simply averaged for each test group. From these mean % con-

centration values the hourly water-exchange fraction (K) was calculated by the equation:

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

in which K = fraction of body water exchanged/h, t = time of exposure to D_2O in hours, x = concentration of D_2O in coelomic fluid at time t, expressed as a percentage of the concentration of D_2O in the medium.

RESULTS

(1) *Influx of D_2O as a function of weight*

In several studies previously carried out on nereid polychaetes and crabs (Smith, 1964, 1967, 1970*c*), influx of D_2O had been found to be weight-specific. However, in the present study, despite the use of worms of a wide weight range (100–500 mg) the scatter did not permit very consistent values of $(b-1)$ to be obtained when the data were treated by the usual equation: Uptake = a weight$^{(b-1)}$, as is evident from the points in Fig. 1. Since the mean weights of worms in the several groups were similar and the values for $(b-1)$ quite low, it seemed better not to extrapolate to unit weight, but simply to use the % concentration values directly.

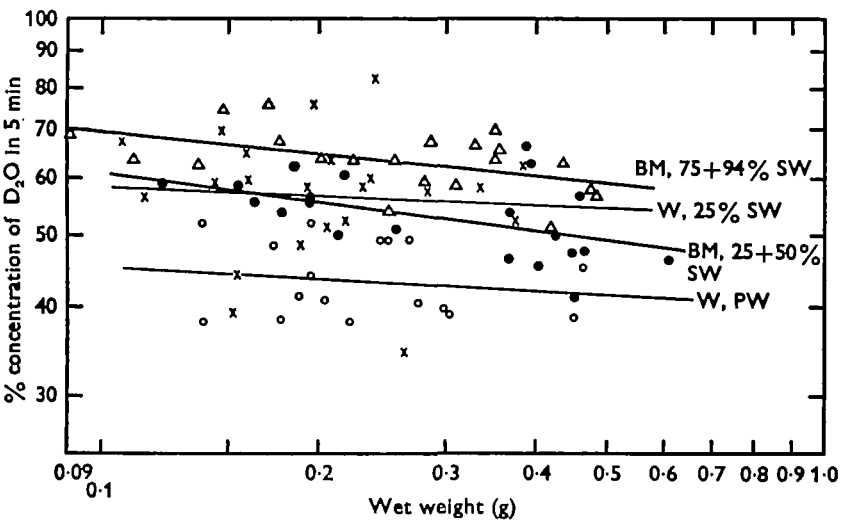

Fig. 1. Double logarithmic plots of D_2O uptake (expressed as percentage of the concentration of D_2O in the medium) by *N. diversicolor* in 5 min exposure, as a function of wet weight. Data are for four groups, totalling 81 individuals; curves calculated by method of least squares. Triangles: Black Middens worms in 75 and 94% SW; dots, Black Middens worms in 25% and 50% SW; X's, Wansbeck worms in 25% SW; circles, Wansbeck worms in PW.

(2) *Rate of exchange of D_2O as a function of salinity*

Percentage concentration values are given in Fig. 2 and Table 1, together with standard deviations, standard errors, hourly water-exchange fractions, and the probabilities (by t-test) that adjacent mean values differ by chance. The overall picture is that permeability to water (as D_2O) is significantly less at low salinities, especially in

Table 1. *Results of D_2O uptake tests (expressed as percentage of the concentration of D_2O in the medium attained in a 5 min exposure), and the resulting hourly water-exchange fractions* (K)

Populations and remarks	Chloride concentrations of media in mM/l: Adaptation	D_2O test	*n*	Mean uptake % in 5 min	S.D.	S.E.	Hourly water exchange fraction (*K*)	P
Wansbeck, tested at 18–19 °C	0·9–1·0 (PW)	0·8–0·9	*17	43·8	5·2	1·3	6·93	
	9·6	9·4	9	38·7	4·8	1·6	5·88	0·05
	63	58	6	54·1	4·0	1·6	9·38	0·001
	143	140	*22	58·1	10·8	2·3	10·47	0·02
	276	277	14	62·8	3·3	0·89	11·90	<0·1
Wansbeck, tested at 22–23 °C	57	32	12	51·6	5·6	1·6	8·73	0·001
	98	98	12	60·1	3·3	0·95	11·06	
Black Middens, tested at 18–19 °C	139	139	*11	53·6	6·6	2·0	9·24	N.S.
	276	276	*10	53·8	7·1	2·2	9·29	0·01
	426	412	*10	62·7	4·7	1·5	11·87	N.S.
	510	515	*11	64·7	7·2	2·2	12·53	

Total 134 (*) curves plotted in Fig. 1.

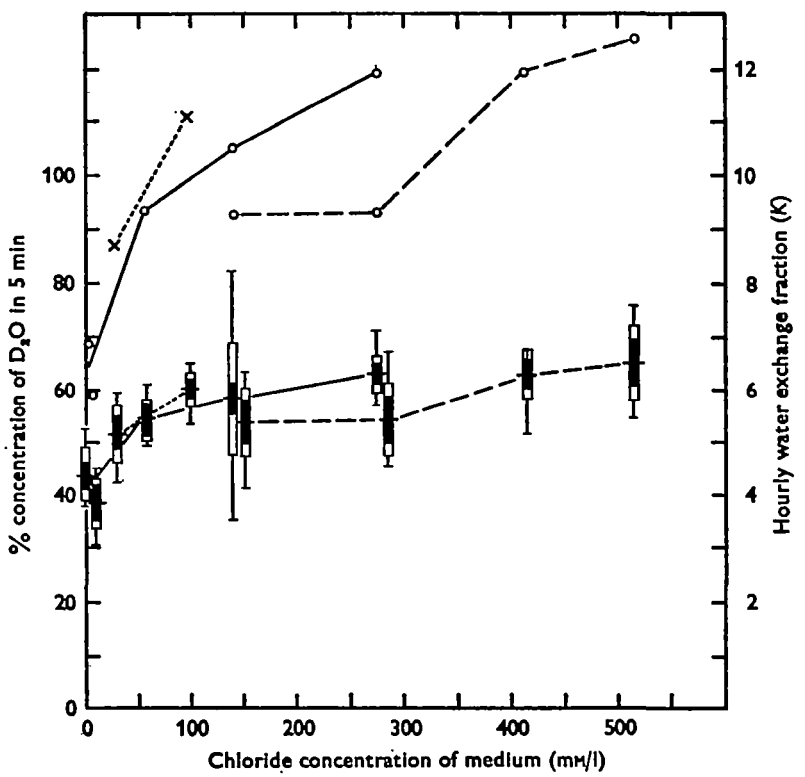

Fig. 2. Lower curves (left ordinate), % concentration of D_2O attained in 5 min as a function of the chloride concentration of medium. Upper curves (right ordinate), hourly water-exchange fractions (*K*) derived from means of % concentration.

PW and 1·8% SW. The trend is evident when worms from Wansbeck estuary and from Black Middens are considered separately, although no explanation can be offered for the apparent tendency of the latter group of worms to show lower permeability than their Wansbeck counterparts in 50% and 25% SW. The hourly water-exchange fraction (K) drops from 12·53 in 95·5% SW to 5·88–6·93 as PW is approached, a decrease of nearly 50%.

(3) *The effect of temperature on the rate of exchange of* D_2O

Two groups of Wansbeck worms had to be tested on a hot day when laboratory temperature rose to 22–23 °C. There is a noticeable elevation of the % concentration attained in these groups of worms, indicating the need for caution when comparing water-exchange rates of animals tested under uncontrolled conditions.

DISCUSSION

The lessened osmotic uptake of water reported for *Nereis diversicolor* by Jørgensen & Dales (1957) has thus been shown to have a physical basis in a lowered diffusional permeability to water (as D_2O). However, it would be better to use the term 'apparent permeability' in this context, since there is no evidence to differentiate a true permeability change from a change in the exchange rate of D_2O brought about by, e.g. circulatory changes in the animal. With this reservation in mind, the K values presented here should permit a preliminary estimate of the net uptake of water entering by diffusion and available for release as urine, at different salinities, provided that certain assumptions may be accepted for purposes of the calculations. Unfortunately data on the osmotic concentration of coelomic fluid were not obtained in the present study, but an approximation may be made by correcting the data available for chloride concentration of *N. diversicolor* of the Wansbeck estuary population (Oglesby 1970; Smith 1970*a*). Oglesby (1969), in summarizing several studies by different authors, concluded that osmotic concentration of coelomic fluid in *N. diversicolor*, if expressed as equivalent NaCl, is higher at all salinities than the measured molarities of Na^+ and Cl^- would indicate. Hohendorf (1963) found that inorganic ions accounted for all but 7% of the total osmotic concentration of the coelomic fluid of *N. diversicolor*. In a study subsequent to the present one, it was found (Smith, 1970*b*) in a different population of this species, that the chloride molarity of coelomic fluid, reckoned as NaCl, accounts for 93·3% of the osmotic concentration as equivalent NaCl. Thus, for purposes of calculation, the osmotic concentration of the coelomic fluid of *N. diversicolor* is taken as equivalent to the measured chloride molarity × 2 × (100/93). The resultant osmolarity of coelomic fluid is expressed in Table 2 (line 6) as equivalent SW, with 100% SW considered to have an osmolarity of 1·0. Since this arbitrary correction of chloride concentrations of coelomic fluid gives values slightly below those of 75 and 94·5% SW, and since Hohendorf (1963) has shown that the body fluid of *N. diversicolor* is always hyperosmotic to the medium, even in SW, the values of coelomic fluid in 75 and 94·5% SW have been further raised to 0·01 osmole above the medium. Hourly water exchange fractions used in Table 2 (line 7) were obtained in this study, using average values where the curves from Wansbeck worms and Black Middens worms overlap. A calculation, e.g. for a worm in 50% SW, may be made

on the assumptions stated and yields the data for net water uptake as % of body weight/h (Table 2, line 14). The calculation is made as follows: The osmolarity of 50% SW is 0·50, the mole fraction of water is $55{\cdot}56/(55{\cdot}56+0{\cdot}50) = 0{\cdot}9911$ (line 8); for a worm in 50% SW, the osmolarity of coelomic fluid is estimated as 0·52 (see above), the mole fraction of water in coelomic fluid is $55{\cdot}56/(55{\cdot}56+0{\cdot}52) = 0{\cdot}9907$ (line 9); the mole fraction difference is 0·0004 (line 10). This difference accounts for the *net* flux of water (excess of water diffusing in over water diffusing out), and this net flux represents $0{\cdot}0004/0{\cdot}9911 = 0{\cdot}0404\%$ of the influx in any given time (line 13). Taking the water content of *N. diversicolor* in 50% SW as 85·8% (Oglesby, 1970), and assuming that all water is exchangeable, the water influx is $K \times 85{\cdot}8\% = 909{\cdot}5\%$ of body weight/h (line 12). Net flux is 0·0404% of 909·5 = 0·37% of body weight/h (line 14). This is the net amount of water entering by diffusion and available for release as urine. The results of this and similar calculations are given in Table 2 and the potential urine volumes are plotted in Fig. 3.

Table 2. *Calculation of net flux of water as % body weight/h, which is equal to urine volume/h* (line 14)

(Estimate of loss of chloride via the urine if urine is assumed to be isotonic in chloride to coelomic fluid (line 15). In line 16, the urinary chloride efflux as estimated on basis of exchange of ^{36}Cl (Smith, 1970*a*). For explanation of calculations, see text.)

1	Approx. chloride concentration of medium, mM/l	0·44	1·8	10	25	50	75	94·5
2	Medium as % SW, approx.	2·5	10	56	140	280	420	530
3	Approx. osmolarity of medium	0·004	0·018	0·10	0·25	0·50	0·75	0·945
4	Chloride concentration of coelomic fluid, mM/l	110	135	155	188	268	382	495
5	Chloride concentration of coelomic fluid + 100/93	118	145	167	202	288	411	532
6	Estimated osmolarity of coelomic fluid	0·21	0·26	0·30	0·36	0·52	(0·76)	(0·96)
7	Hourly water exchange fraction (K)	(6·40)	(6·40)	8·73	(9·85)	(10·60)	11·87	12·53
8	Mole fraction of water in medium	0·9999	0·9997	0·9982	0·9955	0·9911	0·9867	0·9833
9	Mole fraction of water in coelomic fluid	0·9962	0·9953	0·9946	0·9936	0·9907	0·9865	0·9830
10	Mole fraction difference	0·0037	0·0044	0·0036	0·0019	0·0004	0·0002	0·0003
11	Water content, % (Oglesby, 1970)	88·7	88·6	88·2	87·5	85·8	84·3	82·8
12	Influx as % body weight/h	567·7	567·0	770·0	861·9	909·5	1000·6	1037·5
13	Net flux as % of influx	0·370	0·440	0·361	0·191	0·0404	0·0203	0·0289
14	Net flux (equal to urine volume) as % body weight/h	2·10	2·49	2·78	1·65	0·37	0·20	0·30
15	Chloride loss in μM/g/h in *isotonic* urine	2·31	3·36	4·31	3·10	0·99	0·76	1·48
16	Calculated chloride efflux via urine in μM/g/h (Smith, 1970*a*)	0·28	1·5	2·5	0·5	0·5	1·0	—
17	Total chloride efflux in μM/g/h (Smith, 1970*a*)	3·6	5·6	7·3	6·3	8·7	12·7	—

Table 2 indicates that net loss of water as urine has a maximum (2·78% of body weight/h) in 10% SW, and is reduced (2·10% of body weight) in nearly fresh water. As expected, urine volumes are very low in 50% SW and above, in which the worm is nearly iso-osmotic with the medium. The overall results support the conclusion

that urine volume in FW is reduced by about 25% below the peak volume in 10% SW, and that this volume reduction is a consequence of a lowered apparent permeability to water.

Jørgensen & Dales (loc. cit.) give estimates of urine volumes in *N. diversicolor* based on osmotic experiments and weight changes: in 21% SW, 5·4% of body weight/h; in 12% SW, 9·3%/h; in FW, *c.* 4%/h. These estimates are over twice the present estimates based on exchange of D_2O, but may be said to be in general agreement.

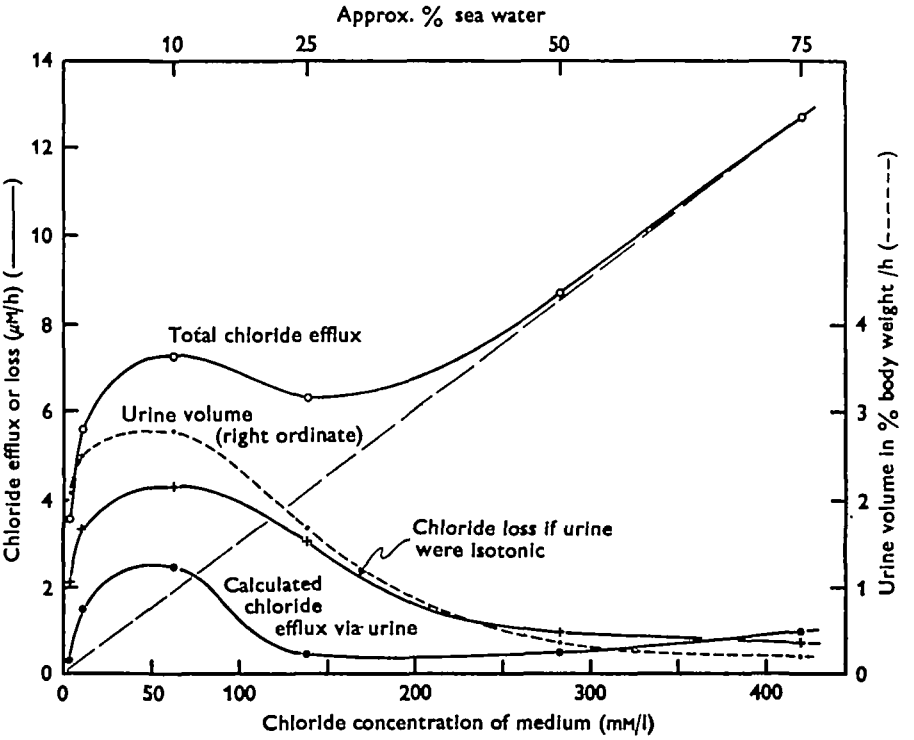

Fig. 3. Total chloride efflux and calculated chloride efflux via the urine μM/g/h (from Smith, 1970*a*); urine volume in % body weight/h (right ordinate); and the chloride efflux calculated on assumption that the urine was isotonic to coelomic fluid.

A similar discrepancy exists between direct estimates of the urine volume of the crab *Carcinus* and the net flux of water based on exchange of D_2O (Smith, 1970*c*). It would not be surprising if the true urine volume of *N. diversicolor* proves to be above the present estimates, and by direct observation (Smith, 1970*b*) the urine flow of *N. diversicolor* may be seen to be copious.

When the volumes of urine as estimated in the present study are compared with the calculated losses of chloride via the urine (Smith, 1970*a*), it may reasonably be concluded that the urine is hypotonic at low salinities. Thus, if we calculate the loss of chloride that would occur in urine isotonic to coelomic fluid (Table 2, line 15), and compare these values with the chloride efflux via the urine, calculated from exchange of ^{36}Cl (line 16), we see that chloride loss in isotonic urine agrees well with calculated chloride efflux via the urine in 50% SW and higher, where urine is undoubtedly isotonic. But in the osmoregulatory range, in 25% SW and below, isotonic urine would

account for far more than the calculated chloride efflux, indeed, for over half the total efflux (line 17). Hence, it is concluded that the urine, in salinities below 25% SW, is probably hypotonic in chloride to the coelomic fluid.

Confirmation or refutation of the last hypothesis would require determination at least of the chloride concentration of urine. This has not yet been done, but in the following paper (Smith, 1970*b*) the hypo-osmoticity of the urine will be directly demonstrated. It thus appears evident that reduction of urine volume and hypotonicity of urine are both features of the chloride regulation of *N. diversicolor*.

SUMMARY

1. By studies of D_2O exchange the apparent permeability of *Nereis diversicolor* to water has been shown to vary in response to changes of salinity.

2. The hourly water-exchange fraction drops from over 12 in SW nearly to 6 in fresh water.

3. Calculation of the net flux of water indicates potential urine volumes of 0·3% of body weight/h in SW, a maximum of 2·8% in 10% SW, and a reduction to 2·1% in fresh water.

4. It is calculated that, if such urine volumes are produced, the urine is probably hypotonic in chloride to the coelomic fluid at salinities of 25% SW and lower.

REFERENCES

HOHENDORF, K. (1963). Der Einfluss der Temperatur auf die Salzehaltstoleranz und Osmoregulation von *Nereis diversicolor* O. F. Muell. *Kieler Meeresforsch.* **19**, 196–218.

JØRGENSEN, C. B. & DALES, R. P. (1957). The regulation of volume and osmotic regulation in some nereid polychaetes. *Physiologia comp. Oecol.* **4**, 357–74.

OGLESBY, L. C. (1969). Inorganic components and metabolism; ionic and osmotic regulation: Annelida, Sipuncula and Echiura. In *Chemical Zoology*, Chap. 9, Vol. 4, pp. 211–310. Eds. M. Florkin and B. T. Scheer, New York: Academic Press.

OGLESBY, L. C. (1970). Studies on the salt and water balance of *Nereis diversicolor*. I. Steady-state parameters. *Comp. Biochem. Physiol.* (In the Press.)

POTTS, W. T. W. & PARRY, G. (1964). *Osmotic and Ionic Regulation in Animals*, 423 pp. Pergamon Press.

SMITH, R. I. (1964). D_2O uptake rate in two brackish-water nereid polychaetes. *Biol. Bull. mar. biol. Lab., Woods Hole* **126**, 142–9.

SMITH, R. I. (1967). Osmotic regulation and adaptive reduction of water-permeability in a brackish-water crab, *Rhithropanopeus harrisi* (Brachyura, Xanthidae). *Biol. Bull. mar. biol. Lab., Woods Hole* **153**, 643–58.

SMITH, R. I. (1970*a*). Chloride regulation at low salinities by *Nereis diversicolor* (Annelida, Polychaeta). I. Uptake and exchanges of chloride. *J. exp. Biol.* **53**, 75–92.

SMITH, R. I. (1970*b*). Hypo-osmotic urine in *Nereis diversicolor*. *J. exp. Biol.* **53**, 101–8.

SMITH, R. I. (1970*c*). The apparent water-permeability of *Carcinus maenas* (Crustacea, Brachyura, Portunidae) as a function of salinity. (In the Press.)

WELSH, J. H., SMITH, R. I. & KAMMER, A. E. (1968). The estimation of D_2O in water or blood. In *Laboratory Exercises in Invertebrate Physiology*, pp. 184–188. Minneapolis, Minn.: Burgess Publ. Co.