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THE TRANSPORT OF ORTHOVANADATE AND SIMILAR OXYANIONS IN RELATION TO SALT AND WATER TRANSPORT ACROSS THE ISOLATED INTESTINE OF THE COMMON EEL, *ANGUILLA ANGUILLA*

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SUMMARY

An intestinal preparation from the common eel (*Anguilla anguilla*) was characterized for both mucosal to serosal and serosal to mucosal transport of water, alanine, sodium and chloride. The preparation showed a net outflux of water and a net influx of alanine, and no net fluxes of sodium or chloride. The influxes of all four substances and the outflux of water were inhibited by ouabain, while the outfluxes of alanine, sodium and chloride were increased by ouabain. Influxes and outfluxes of the oxyanions orthovanadate, phosphate, arsenate, sulphate, selenate and chromate were measured over a range of concentrations from 10^{-8} M to 10^{-4} M. The entry of each anion was dependent on the mucosal concentration, not saturable within the concentration range used or competitive with chloride. There were no net fluxes of these anions and pertechnecate from solutions containing equal mucosal and serosal concentrations of the anion.

INTRODUCTION

The present study was started as part of an investigation into the uptake of orthovanadate into fish. The work was then developed to include the intestinal transport of other naturally occurring trace elements which exist as oxyanions, and relating their transport to the transport of the main anions present in the environment such as chloride, phosphate and sulphate.

Vanadium is an essential trace element in rats (Schwarz & Milne, 1971) and in chicks (Hopkins & Mohr, 1974), and is present in most tissues at concentrations between 10^{-8} and 10^{-6} g-atom kg^{-1} (Byrne & Kosta, 1978; Post *et al.* 1979). $(Na^+ + K^+)$-dependent ATPases from all sources so far studied are inhibited by orthovanadate (e.g. Cantley *et al.* 1977; Quist & Hokin, 1978; Bell & Sargent, 1979). Cantley *et al.* (1977) and Cantley, Cantley & Josephson (1978) suggested a role for the metal in regulating this enzyme based on the inhibitory properties of vanadate and its level in tissues. Pharmacological effects of vanadate, possibly relating to its effects

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on the sodium pump, include an inotropic action on papillary muscle (Hackbarth *et al.* 1978), a natriuretic action in rats (Balfour, Grantham & Glynn, 1978), an inhibition of the sodium current in frog skin (De Sousa & Grosso, 1979), and a potent vasoconstrictor action in fish gills (Bell, Kelly & Sargent, 1979; Kelly, Pirie, Bell & Sargent, 1979). Vanadium is a natural component of fresh and sea waters and we have shown that orthovanadate can enter fish in an apparently uncontrolled, concentration-dependent manner (Bell *et al.* 1980, 1981).

The other anions in the study were chosen for a variety of reasons. Chloride and sulphate are major anions in sea water while chloride and phosphate are found in high concentrations in physiological fluids generally. Technetium is a man-made radioactive element produced in the nuclear industry and exists in the environment as the pertechnecate ion TcO_4^-, which is also used clinically as a chloride marker in scintigraphy studies. The other elements studied here are either in the same chemical group as phosphorus and vanadium (group V, arsenic) or as sulphate (group VI, chromate and selenate). All of them can exist in the natural environment as oxyanions and they are also trace elements in animal nutrition (Underwood, 1981) that are toxic at higher concentrations. A further consideration in studying these anions in the present work was that the elements in question all have convenient radioisotopes for tracer studies.

MATERIALS AND METHODS

Radioisotopes

Tritiated water, ^3H_2O (5 mCi/ml), L-[U-^{14}C] alanine (170 mCi/mmol), [^{24}Na] NaCl (340 μCi/mg Na), [^{32}P] orthophosphate (carrier free, 1 mCi/ml), [^{35}S] sulphate (25–40 Ci/mg S), [^{36}Cl] chloride (> 3 mCi/g Cl), [^{48}V] vanadyl chloride (carrier free, 200 mCi/mg V), [^{51}Cr] chromate (250–500 mCi/mg Cr), [^{74}As] arsenate (1–20 mCi/μg As), [^{75}Se] selenate (2–20 mCi/mg Se) and [^{99}Tc] pertechnecate (17 mCi/g Tc) were obtained from Amersham International Ltd., Amersham, Bucks., U.K. The [^{48}V] vanadyl chloride, supplied in 1 M-HCl, was converted to [^{48}V] orthovanadate by treatment with a 10% (v/v) excess of 1 M-NaOH for at least 24 h before use (Cantley *et al.* 1978).

Chemicals

L-alanine, ouabain, phenol red and PVP-40 (polyvinylpyrrolidone) were purchased from Sigma (London) Chemical Co., Poole, Dorset, U.K. 4,4'-Diisothiocyanostilbene 2,2 disulphonic acid disodium salt (DIDS) was obtained from Pierce Chemical Co., Rockford, Illinois, U.S.A. Furosemide was a gift from Hoechst Pharmaceuticals, Hounslow, Middlesex, U.K. Amiloride hydrochloride was a gift from Merck, Sharp and Dohme Ltd., Hoddesdon, Herts., U.K. All other chemicals were of AnalaR grade where available, from BDH Chemicals, Poole, Dorset, U.K. Insta-gel was obtained from Packard Instruments, Caversham, Berks., U.K.

Animals

Common eels (*Anguilla anguilla*), which had been reared in fresh water were obtained from Easgan Fisheries Ltd., 12 Seedhill Road, Paisley, Glasgow. They we

kept in a running fresh water aquarium at 5–10 °C without food and used within three months of receipt.

Ringer solutions

Intestinal preparations were bathed serosally in a solution of composition 112 mM-NaCl, 4 mM-KCl, 26 mM-$NaHCO_3$, 2 mM-Na_2HPO_4, 0·4 mM-KH_2PO_4, 0·63 mM-$CaCl_2$, 2 mM-$MgSO_4$, 5 mM-alanine, 5·6 mM-glucose and 20 g/l PVP-40. The solution was passed through a 0·22 μm Millipore filter before use, and gassed with O_2/CO_2 (98/2) to maintain a pH of 7·6 at 15 °C. This Ringer was developed for use with freshwater eels (Bornancin, de Renzis & Maetz, 1977). The mucosal surface of the preparation was bathed with a solution containing 132 mM-NaCl, 4 mM-KCl, 5 mM-Na_2HPO_4, 2·5 mM-$CaCl_2$, 5 mM-alanine and 5·6 mM-glucose. The pH was adjusted to 7·6 with HCl. The tonicity of the two saline solutions is identical. A low chloride mucosal saline was also used. This contained 5 mM-Na_2HPO_4, 2·5 mM-$CaCl_2$, 5·6 mM-glucose and 5 mM-alanine, the pH being adjusted to 7·6 with HCl.

Intestinal preparation

Fish were killed by decapitation and the body cavity opened from the liver to the anus. The entire viscera were removed and the gut dissected free from other tissue, washed out with cold mucosal saline and chilled on ice as quickly as possible. The outer layer of longitudinal muscle was then stripped off the gut as described by Ando & Kobayashi (1978). During stripping, the intestine breaks at the ileorectal valve about 15 mm before the anus. Often there was a sharp change in gross structure between the upper, rather muscular part of the intestine, and the middle regions. Only the mid gut corresponding to mammalian small intestine was used for experiments. The uptake of ^3H_2O, [^{14}C] alanine and [^{32}P] phosphate was not found to vary in this region (unpublished results). The stripped intestine was cut into 15 mm lengths, placed serosal side down on nylon gauze of rectangular mesh 106 μm square (Henry Simon, Stockport, U.K.) then mounted between two Perspex Ussing chambers with a capacity of 2·5 ml on each side (Degnan & Zadunaisky, 1977). The chambers were placed in a water bath at 15 °C. The serosal and mucosal salines were continuously gassed and circulated by an air lift pump which gave a mixing time of 10–15 s. The serosal Ringer was gassed with O_2/CO_2 (98/2) and the mucosal saline was gassed with air. Between 1×10^6 and 15×10^6 c.p.m./ml of isotope were added to one side of the chambers, and phenol red (0·0067 %, w/v) was added to the saline on the side from which transport was being measured so that any leaks could be detected immediately. Eight chambers were used simultaneously so that two influxes and two outfluxes from adjacent pieces of gut from two fishes were measured. Samples were taken at 20 min intervals from the side at which radioactivity appeared. Experiments usually lasted 6 h but the preparations continued to give linear fluxes for at least 10 h. Inhibitors were added in a minimum volume of mucosal saline.

Assay of radioactive material

^3H_2O,L-[^{14}C] alanine, [^{32}P] phosphate, [^{35}S] sulphate, [^{36}Cl] chloride and [^{99}Tc] pertechnecate were assayed in a Packard Tricarb model 3385 using Insta-gel as scintillant. Where possible paired isotopes were used e.g. ^{3}H and ^{36}Cl, ^{3}H and ^{14}C, ^{32}P

and ^{35}S. [^{24}Na] sodium, [^{48}V] vanadate, [^{51}Cr] chromate, [^{74}As] arsenate and [^{75}Se] selenate were assayed in a Packard Autogamma 500C. Again isotopes were paired if possible e.g. ^{51}Cr and ^{74}As, while ^{24}Na was used with ^{3}H and ^{36}Cl, the latter isotopes being counted after the ^{24}Na had decayed (half-life = 15 h). A small sample of saline was taken at the beginning of each experiment from the side from which transport was being measured and counted under the same conditions as the other samples. Short half-life isotopes were corrected for decay over the period of the experiment.

Analysis of data

Samples were taken at 20 min intervals and plotted as c.p.m. versus time for each experiment. When an inhibitor was added best-fit lines were fitted by the least mean squares method to each period pre- and post-treatment with inhibitor. The slopes of the lines were calculated and expressed as a ratio of post-treatment to pre-treatment. Control fluxes were analysed similarly and a slope ratio obtained. A percentage inhibition was obtained from the test lines after correcting for any deviation in the controls. The means of experiments were compared using unpaired students' *t*-test for a difference between means for a two-tailed test. All results were expressed as mean ± S.D.

RESULTS

The intestinal preparation was first characterized for mucosal to serosal and serosal to mucosal transport of $^{3}H_2O$, ^{14}C-alanine, $^{24}Na^{+}$ and $^{36}Cl^{-}$. The results are shown in Table 1. There is a significant difference between the influxes and outfluxes of $^{3}H_2O$ and ^{14}C-alanine, water giving a net outflux and alanine a net influx. The influxes and outfluxes for $^{24}Na^{+}$ and $^{36}Cl^{-}$ are not significantly different. The influxes of all four substances were inhibited by 10^{-4} M ouabain added serosally and mucosally (Table 2). The outflux of $^{3}H_2O$ was also inhibited by ouabain, but the outfluxes of ^{14}C-alanine, $^{24}Na^{+}$ and $^{36}Cl^{-}$ were all increased by ouabain treatment (Table 2). However, the changes in $^{3}H_2O$ and ^{14}C-alanine outfluxes were not significant. The effect of several other possible inhibitors of anion transport were also tested on the $^{3}H_2O$ and ^{36}Cl-fluxes, test compounds being added to both sides of the intestinal preparation. However, 0·2 mM-DIDS, 0·2 mM-furosemide, 0·1 mM-amiloride and 0·1 mM-vanadate all had no effect on the fluxes of $^{3}H_2O$ and $^{36}Cl^{-}$.

Lowering the concentration of chloride from 141 mM (normal) to 2·5 mM (low chloride saline) had very little effect on the fluxes of $^{3}H_2O$, ^{14}C-alanine or $^{36}Cl^{-}$

Table 1. *Control influxes and outfluxes of $^{3}H_2O$, $^{24}Na^{+}$, $^{36}Cl^{-}$ and ^{14}C-alanine*

	Influx	Outflux
$^{3}H_2O$ mmol cm^{-2} h^{-1}	2·34 ± 1·26 (51)	3·98 ± 2·46 (52)*
$^{24}Na^{+}$ μmol cm^{-2} h^{-1}	2·05 ± 1·34 (28)	1·87 ± 0·95 (28)
$^{36}Cl^{-}$ μmol cm^{-2} h^{-1}	2·61 ± 1·34 (51)	2·01 ± 1·77 (52)
^{14}C-alanine μmol cm^{-2} h^{-1}	0·323 ± 0·100 (8)	0·077 ± 0·028 (8)*

All values are means ± S.D. (no. of determinations).
* Significant difference between influx and outflux at $P < 0·001$.

Table 2. *The effect of 10^{-4} M-ouabain added serosally and mucosally on fluxes of ^3H_2O, ^{14}C-alanine, $^{24}Na^+$ and $^{36}Cl^-$*

	Influx	Outflux
^3H_2O	−19·5 ± 12·4 (25)*	−20·2 ± 15·3 (21)
^{14}C-alanine	−32·8 ± 9·1–(5)*	+39·8 ± 33·2 (5)
$^{24}Na^+$	−20·4 ± 12·3 (12)†	+27·5 ± 30·0 (10)†
$^{36}Cl^-$	−16·6 ± 15·4 (20)†	+76·3 ± 96·3 (19)†

− represents a percentage inhibition and + represents a percentage increase. All values are means ± S.D. (no. of determinations).
*$P < 0·001$.
†$P < 0·01$.

Table 3. *The effect of the mucosal chloride concentration on the influxes and outfluxes of ^3H_2O, ^{14}C-alanine and $^{36}Cl^-$*

	Mucosal [Cl] mM	Flux units	Influx	Outflux
^3H_2O	141	mmol cm^{-2} h^{-1}	8·29 ± 2·02 (20)	8·42 ± 2·39 (20)
^3H_2O	2·5	mmol cm^{-2} h^{-1}	5·16 ± 2·02 (20)	7·25 ± 2·64 (22)
^{14}C-alanine	141	μmol cm^{-2} h^{-1}	0·323 ± 0·100 (8)	0·077 ± 0·028 (8)
^{14}C-alanine	2·5	μmol cm^{-2} h^{-1}	0·247 ± 0·109 (7)	0·096 ± 0·038 (8)
$^{36}Cl^-$	141	μmol cm^{-2} h^{-1}	7·75 ± 1·41 (14)	2·51 ± 0·86 (12)
$^{36}Cl^-$	2·5	μmol cm^{-2} h^{-1}	0·087 ± 0·043 (14)	3·34 ± 0·93 (13)

All values are means ± S.D. (no. of determinations). Significantly different values at $P < 0·001$ are the influx of ^3H_2O in the two salines, the influx and outflux of $^{36}Cl^-$ in the two salines and the influx and outflux of ^{14}C-alanine in the normal saline; at $P < 0·01$ the influx and outflux of ^{14}C-alanine in the 2·5 mM-Cl^- saline.

(Table 3). Though the decrease in the influx of ^3H_2O is significant the change is much less than expected after reducing the mucosal tonicity from 305 mOsM to 28 mOsM to give a markedly hypotonic mucosal medium. This result confirms that decreasing the mucosal tonicity does not result in any enhanced osmotic inflow of water in this tissue (Schultz, 1979).

The effects of the mucosal concentration on the rates of influx of phosphate, vanadate, arsenate, sulphate, chromate and selenate were determined (Fig. 1). The influxes of all the oxyanions studied were linear with concentration over the concentration range studied, with slopes of about one, showing that the rate of influx is directly proportional to the mucosal concentration of the anion (Fig. 1).

Table 4 compares the influxes and outfluxes of the various oxyanions measured under normal conditions of 141 mM-mucosal and 117 mM-serosal chloride. The concentrations of the test oxyanions were usually the same for both sides of the tissue allowing a direct comparison of the influxes and outfluxes from adjacent pieces of intestine. However, in the case of phosphate the mucosal concentration was 5 mM and the serosal concentration was 2·4 mM, and for pertechnecate no unlabelled carrier is available. In this latter case it should be noted that, to give sufficient counts in the system to allow determination of accurate flux rates, the amount of isotope added gave a concentration of 2·4 mM. Fluxes of pertechnecate were therefore measured down

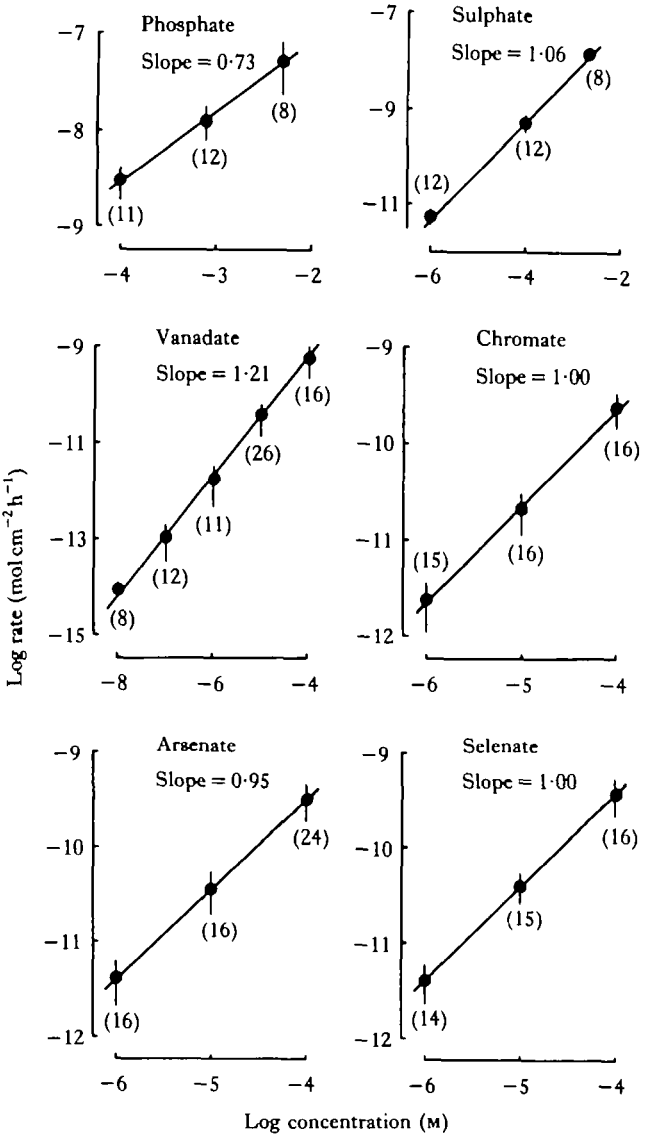

Fig. 1. Influxes determined for phosphate, vanadate, arsenate, sulphate, chromate and selenate at different mucosal concentrations of each anion, and plotted as log rate (mol cm^{-2} h^{-1}) against log concentration (M). Each point is the mean ± S.D. of the number of determinations in brackets. The slopes were all determined from a line-fitting programme.

gradients of 2·4 mM to zero. Table 4 also shows the effect on the influxes and outfluxes of the oxyanions of lowering the mucosal chloride concentration to 2·5 mM. This caused slight increases in both the influxes and outfluxes of most of the anions but none of the differences were significant (Table 4). This result shows that there is no competition between the oxyanions and chloride. The rates of influx and outflux are very similar in most cases and any net fluxes are small and insignificant. Only in the case of pertechnecate under conditions of low mucosal chloride are the influxes and

Table 4. *The influxes and outfluxes of seven oxyanions determined in the presence of two mucosal chloride concentrations, 141 mM and 2.5 mM*

Ion	Concentration (mM)	141 mM-Cl mucosal saline Influx	141 mM-Cl mucosal saline Outflux	2·5 mM-Cl mucosal saline Influx	2·5 mM-Cl mucosal saline Outflux
PO_4^{3-}	5, 2·4	50·6 ±29·1 (8)	10·9 ±3·7 (8)	50·1 ±13·8 (7)	13·8 ±4·3 (8)
VO_4^{3-}	0·1	1·04 ± 0·40 (8)	0·76 ± 0·33 (8)	1·11 ± 0·51 (15)	—
AsO_4^{3-}	0·1	0·34 ± 0·14 (8)	0·39 ± 0·19 (7)	0·59 ± 0·34 (10)	0·71 ± 0·30 (10)
SO_4^{2-}	2	14·3 ± 3·1 (7)	20·2 ±5·4 (8)	20·9 ± 8·5 (7)	23·0 ±6·7 (8)
SeO_4^{2-}	0·1	0·37 ± 0·18 (16)	0·61 ± 0·23 (8)	0·54 ± 0·33 (7)	0·80 ± 0·26 (6)
CrO_4^{2-}	0·1	0·25 ± 0·10 (8)	0·39 ± 0·26 (7)	0·58 ± 0·50 (10)	0·69 ± 0·41 (10)
TcO_4^-	2·4	41·3 ±13·5 (7)	28·0 ±2·5 (8)	48·5 ±13·5 (8)*	22·1 ±7·4 (8)*

The concentration of each anion mucosally and serosally was the same except for phosphate (5 mM mucosal, 2·4 mM serosal) and pertechnecate which was measured down a gradient of 2·4 mM to zero. All values are means ±S.D. (no. of determinations). Flux units are nmol cm^{-2} h^{-1}. * Significantly different at $P < 0·001$.

outfluxes significantly different. Differences between the influxes and outfluxes of pertechnecate under normal conditions and selenate under conditions of low mucosal chloride have a low significance ($P < 0·05$ and $P < 0·2$ respectively).

Finally the rates of influx and outflux for each anion were compared with the rates for chloride (Table 5). Since flux rates are directly proportional to concentration (Fig. 1) all the rates were corrected to an oxyanion concentration of 1 mM and expressed as a fraction of the projected rate with 1 mM chloride. Note that two batches of fish were used, necessitating two control chloride fluxes. With the exception of pertechnecate the calculated flux rates of all the oxyanions are similar.

DISCUSSION

The 'stripped muscle' intestinal preparation used in the present work was developed by Ando & Kobayashi (1978) to study the potential difference and water fluxes across eel intestine. These authors established that removal of the longitudinal muscle enhanced the potential difference by a factor of four, the potential difference under these circumstances being dependent on both Na^+ and Cl^-, and inhibited by ouabain. Stripping removes the longitudinal muscle layer and some of the circular muscle of the intestine thus decreasing serosal barriers to diffusion.

The intestinal preparation used here was characterized with respect to ^3H_2O, ^{14}C-alanine, $^{24}Na^+$ and $^{36}Cl^-$ transport. It should be noted that several different batches of fish were used during this work and each gave slightly different characteristics. ^3H_2O and $^{36}Cl^-$ fluxes were routinely determined as control values for each batch of fish. The water and chloride fluxes given in Tables 1 and 3 and the chloride fluxes in Table 5 are thus slightly different. It is not known what caused these differences; as far as is known the fish were reared under identical conditions. The large exchange fluxes of water, sodium and chloride are consistent with the view that gut is a leaky epithelium where much of the transport occurs at the loose junctions between cells (Schultz, Frizzell & Nellans, 1974). There is a very significant net outflux of water and net influx of alanine, while the net influxes of sodium and chloride have low significance ($P < 0·2$ and $P < 0·1$ respectively) and are at best very small.

Table 5. *A comparison of the rates of influx and outflux of the various anions relative to chloride*

The rates were corrected for concentration assuming a linear relationship of rate to concentration as found. Note that the experiment was carried out in two parts resulting in different chloride flux control values.

Anion	Mucosal concentration (mM)	Serosal concentration (mM)	Influx (nmol cm^{-2} h^{-1})	Outflux (nmol cm^{-2} h^{-1})	Ratio In:Out	Influx relative to Cl	Outflux relative to Cl	M–O Å
Cl^-	141	117	4420 ± 1670 (8)	2470 ± 840 (8)	1·48	—	—	1·80†
SO_4^{2-}	2	2	14·3 ± 3·1 (7)	20·2 ± 5·4 (8)	0·71	0·23	0·48	1·44
PO_4^{3-}	5	2·4	50·6 ± 29·1 (8)	10·9 ± 3·7 (8)	2·21	0·32	0·22	1·65
VO_4^{3-}	0·1	0·1	1·04 ± 0·40 (8)	0·76 ± 0·33 (8)	1·35	0·33	0·36	1·56
Cl^-	141	117	2730 ± 1590 (12)	1810 ± 1000 (12)	1·25	—	—	1·80†
CrO_4^{2-}	0·1	0·1	0·25 ± 0·10 (8)	0·39 ± 0·26 (7)	0·63	0·13	0·25	1·6
AsO_4^{3-}	0·1	0·1	0·34 ± 0·14 (8)	0·39 ± 0·19 (7)	0·89	0·18	0·25	1·78
SeO_4^{2-}	0·1	0·1	0·37 ± 0·18 (16)	0·61 ± 0·23 (8)	0·61	0·20	0·39	1·61
TcO_4^-	2·4*	2·4*	41·3 ± 13·5 (7)	28·0 ± 2·5 (8)	1·48	0·89	0·75	1·75

* No carrier, fluxes down gradient 2·4 mM to zero.
† Ionic radius.
M–O is the metal to oxygen bond length.

The influxes of $^{3}H_2O$, $^{24}Na^{+}$ and $^{36}Cl^{-}$ were significantly inhibited when 10^{-4} M-ouabain was added to both sides of the preparation. Though the outflux of $^{3}H_2O$ was inhibited by ouabain the outfluxes of ^{14}C-alanine, $^{24}Na^{+}$ and $^{36}Cl^{-}$ were increased by treatment with ouabain. However, the results of the effects of ouabain on water and alanine outfluxes are of low significance ($P < 0{\cdot}02$ and $P < 0{\cdot}05$ respectively). The data therefore show a trend in this preparation towards small net influxes of sodium and chloride which in the ouabain-inhibited system are changed to substantial net outfluxes ($^{24}Na^{+}$, 0·18 μmol cm^{-2} h^{-1} IN to 0·73 μmol cm^{-2} h^{-1} OUT; $^{36}Cl^{-}$, 0·60 μmol cm^{-2} h^{-1} IN to 1·28 μmol cm^{-2} h^{-1} OUT, for the active non-inhibited and ouabain-inhibited systems respectively). These findings are consistent with a net outward passage of water, sodium and chloride in the ouabain-inhibited system and, under normal circumstances, with an active sodium pump that transports sodium and chloride back across the gut to establish a balance (zero net fluxes) of sodium and chloride. The preparation behaves typically for alanine in that there was a marked asymmetry to the transport with a large net influx from Ringers containing equimolar alanine on each side. The alanine influx was inhibited by ouabain, consistent with an active sodium-coupled uptake of amino acids across intestine (Schultz & Curran, 1970).

The properties of this preparation were similar to those found by other authors for intestine. Thus the value for the water influx here was very close to that given for rabbit (Leng, 1978) and the values for alanine, here measured at 15 °C, were about a quarter of those for rabbit measured at 37 °C (Leng, 1978; Field, Schultz & Curran, 1967). The alanine influx across the intestine of the turtle was 0·59 μmol cm^{-2} h^{-1} from a 5 mM solution at 25 °C (Nassar, Khuri & Hajjar, 1980), only twice the value observed here. The alanine influx across rabbit intestine was inhibited about 70 % by 10^{-4} M-ouabain (Field *et al.* 1967), a larger inhibition than found here.

After characterizing the system for the transport of water, alanine, sodium and chloride, the rates of transport of the oxyanions were measured under various conditions. The results given in Fig. 1 show that the uptake of all the oxyanions tested is solely dependent on the mucosal concentration of the anion. The influxes were not saturable and therefore were apparently uncontrolled. These data are consistent with the idea that in a leaky epithelium such as gut most of the exchange flux is occurring across the spaces between cells. Lowering the mucosal concentrations of chloride had no effect on the influxes of any of the oxyanions tested. This shows that there is no competition for entry sites between chloride and the other anions. Lack of carrier-mediated transport was supported by the finding that 0·2 mM-DIDS, known to inhibit anion transport across red blood cells, had no effect on this system. This result also suggests that most of the transport is through the junction between cells. In the present work most of the fluxes were measured under equilibrium conditions with the same concentration of the ion on either side of the tissue. The influxes and outfluxes of the ions under these conditions were similar. However, there did seem to be trends towards net influxes of phosphate and pertechnecate and net outfluxes of sulphate and selenate. The extent of any net fluxes *in vivo* would depend on the removal of the anion from the plasma and this in turn will depend on chemical form and the types of sequestration available.

The finding that the influxes of the oxyanions are solely dependent on concentration and uncontrolled has important implications. Specific mechanisms do not seem to be required for the uptake of any of the elements tested. Uptake from the diet, or for fish from the environment, should be directly proportional to the amount of the element, as an oxyanion, reaching the gastrointestinal tract. All the essential elements tested here, vanadate, arsenate, chromate and selenate, are toxic at higher levels. Chemical speciation is important in overcoming toxicity e.g. vanadate $^{V}VO_4^{3-}$ appears to be reduced to vanadyl $^{IV}VO^{2+}$ intracellularly (Cantley & Aisen, 1979) and this cationic form shows none of the inhibitory properties of vanadate in biological systems.

The influx of vanadate across intestine was found to be identical to the *in vivo* uptake of vanadate into elvers, which was also linear with concentration and apparently uncontrolled up to 10^{-4} M when mortality occurred (Bell *et al.* 1980). The results obtained here also indicate that intestinal absorption accounts for between 20 % and 50 % of the *in vivo* uptake found at the various external concentrations of vanadate. Further experiments have shown that the uptake across gills is very low, so the main route of vanadate entry into fish is probably through the gut (M. V. Bell & K. F. Kelly, unpublished results).

Chromate $^{VI}CrO_4^{2-}$ can also be reduced to ^{III}Cr which is always complexed and therefore less readily available. However, both arsenate and selenate are not reduced to cations biochemically. It is not known how biological systems react to a completely new element like technetium. ^{99}Technetium has a very long half-life of 210 000 years and undoubtedly presents a hazard to the environment in terms of persistence of radioactivity. However, an additional aspect of isotopes with very long half lives is that they have very low specific radioactivities, i.e. to generate sufficient radioactivity a relatively large mass must be present (a practical outcome of this was the relatively high concentration of 2·4 mM pertechnecate required to give measurable radioactivity in the experimental system used here). Nonetheless there is no evidence at present that technetium is a harmful element chemically.

All the anions used in the present work are of similar size. Chloride is a spherical monovalent anion. The other anions are tetrahedral with metal to oxygen bond lengths shown in Table 5. Allowing for an additional radius of the oxygen component of the bond length, all these anions will approximate to spheres slightly larger than chloride. They will, however, have different charge densities. Pertechnecate is the only monovalent ion like chloride and gives higher rates than the other oxyanions, which at physiological pH values exist as a mixture of monovalent and divalent forms ($H_2PO_4^-/HPO_4^{2-}$, $H_2VO_4^-/HVO_4^{2-}$, $H_2AsO_4^-/HAsO_4^{2-}$) or as divalent ions (SO_4^{2-}, CrO_4^{2-}, SeO_4^{2-}). There does not seem to be a pattern between the observed rates and the charge or size of the ion.

The present work therefore shows that the uptake of oxyanions by intestine is uncontrolled and is simply a function of the concentration of the ion at the mucosal surface. The tonicity of the mucosal fluid has no effect on the uptake. This result has important implications in the fields of nutrition and environmental pollution. Potential control sites that remain to be investigated are rate of delivery of anions to the intestine, i.e. rate of ingestion of food and water, and the fate of the anions in the serosal fluid.

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