J. Physiol. (1974), **240**, *pp.* 535–566
With 12 *text-figures*
Printed in Great Britain

ACTIVE TRANSPORT OF IODIDE AND OTHER ANIONS ACROSS THE CHOROID PLEXUS

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(*Received 13 August 1973*)

SUMMARY

1. An *in vitro* preparation of the frog choroid plexus was used to study mechanisms of anion transport.

2. It was observed that, in the absence of electrochemical potential gradients, there were net fluxes of I^-, SCN^-, TcO_4^-, and Br^- across the plexus, from the ventricular to the serosal surface. The net flux of I^- reached a maximum at a concentration of 250 μM.

3. On the basis of competition effects it was concluded that the affinity of the transport process for anions was:

$$ClO_4 > ReO_4 \sim BF_4 > SCN \sim SeCN > I > NO_3 > Br > Cl.$$

4. Ouabain, oligomycin, phloretin and 2,4-DNP inhibited the net transport of anions, but phlorrhizin, furosemide, 2,4,6-trinitro-*m*-cresolate, reducing agents, and antithyroid agents did not. Ouabain and phloretin were only effective on the ventricular side of the preparation.

5. Anion transport required the presence of both Na and K. The requirement for Na was specific, but Rb, and to a lesser extent Cs, could substitute for K. Na in either the ventricular or the serosal fluids could partially stimulate anion transport, but K was only effective in the ventricular solution.

6. TcO_4^-, SCN^- and I^- were accumulated within the choroidal epithelium from the ventricular fluid, but not from the serosal fluid. Accumulation was inhibited by ouabain and ClO_4^-.

7. The unidirectional influx of I^- across the apical cell membrane was about an order of magnitude greater than the flux across the epithelium. This flux was inhibited by ClO_4^-, ouabain, and Na-free solutions.

8. These experiments suggest the following mechanism for anion transport across the plexus: anions are actively transported into the epithelium, by a ouabain sensitive, Na/K dependent pump located in the brush border

membrane. The anions are accumulated within the epithelium, and, finally, they pass into the serosal fluid down their electrochemical potential gradient. Relations between anion transport, Na/K transport, and Na/K ATPases are discussed.

INTRODUCTION

Over the past few decades, cation transport across cell membranes has been the subject of an ever increasing amount of research. In contrast anion transport has received much less attention, even though a large number of cells actively accumulate anions. Iodide, for example, is actively transported by the thyroid glands, salivary glands, gastric mucosa, mammary glands, choroid plexus, ciliary body and seaweed (see Brown-Grant, 1961; Wolff, 1964). The purpose of this paper is to report on a study of anion transport mechanisms in the choroid plexus.

It is well known that the concentration of anions such as I^-, SCN^- and Br^- in the cerebrospinal fluid (c.s.f.) is much lower than in plasma. *In vivo* experiments have established that these concentration gradients are maintained by the active transport of anions from c.s.f. to blood, and by the low permeability of the 'blood–cerebrospinal fluid barrier' (see Davson, 1967). The choroid plexuses have been implicated in these transport processes ever since it was shown that anions were actively accumulated within the isolated epithelium (Becker, 1961; Welch, 1962*a*, *b*), and this was substantiated by recent observations on SCN^- transport across the isolated choroid plexus of the sheep (Pollay & Kaplan, 1972).

The present study of anion transport across the choroidal epithelium was carried out on the preparation of the frog choroid plexus that was used earlier to study cation transport (Wright, 1972*a*). The results show that I^-, SCN^-, TcO_4^- and perhaps Br^-, are actively transported across the *in vitro* preparation from the ventricular to the serosal surface. Transport across the epithelium occurs in two stages; the first is the active accumulation of anions within the epithelium by a pump located in the apical cell membrane, and the second is the transport of anions down their electrochemical potential gradient from the cell into the blood. Active transport at the apical membrane requires the presence of Na and K, and is inhibited by ouabain and oligomycin. This suggests that active anion pumping is linked to either the Na/K ATPase or the Na/K exchange pump. A preliminary account of some of these results has already been presented (Wright, 1973).

METHODS

These studies were carried out on the posterior choroid plexus of the bull-frog, *Rana catesbeiana*. This choroid plexus consists of a single layer of cuboidal epithelial cells, resting upon a thin supporting layer of connective tissue that is richly endowed with a network of blood vessels. The epithelial cells are joined together at the apical surface by so-called tight junctions. The apical membrane, or brush border, of the epithelium normally faces the c.s.f. in the ventricle, while the baso-lateral membranes face the blood (Wright, 1972*a*; Quinton, Wright & Tormey, 1973). In these *in vitro* studies the fluid compartment bathing the apical surface is referred to as the ventricular compartment, while that bathing the vascular, or baso-lateral, side of the epithelium is referred to as the serosal compartment. Thus J_{vs} and J_{sv} are abbreviations for unidirectional fluxes between the ventricular and serosal compartments.

Transepithelial fluxes. The techniques used for the dissection of this plexus, mounting it between Lucite flux chambers, unidirectional flux measurements, and electrical recordings (transepithelial potential differences and conductances) were identical to those used in a previous study (Wright, 1972*a*).

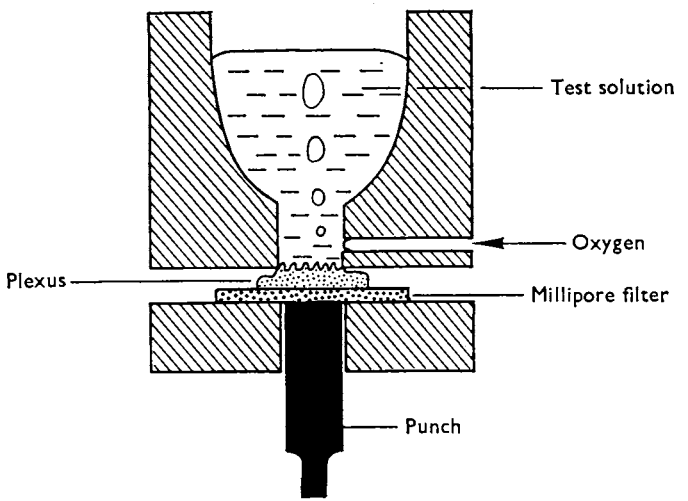

Fig. 1. The chamber used to measure the unidirectional fluxes across the apical membrane of the choroidal epithelium. The area of the exposed membrane was 6·2 mm² (serosal area) and the volume of the solution in the upper compartment varied between 0·25 and 0·5 ml.

Accumulation. The accumulation of anions within the plexus from the ventricular and/or serosal solutions was measured as described for amino acids (Wright, 1972*b*).

Fluxes across the apical cell membrane. Unidirectional influxes across the ventricular surface of the choroid plexus were measured with the chamber illustrated in Fig. 1. The principle involved was to measure the amount of isotope in the plexus after exposure of the ventricular surface of the tissue to a solution containing radioactive isotopes for brief periods of time. The chamber was a modified version of the chamber used to measure transepithelial fluxes, but this one was held in the vertical plane. The plexus was mounted, ventricular surface facing upwards, on a millipore

filter (0·22 μm pore diameter), which covered the window of the lower part of the chamber. Pins located at the points of the triangular window facilitated the positioning of the plexus over the window and the assembly of the two half chambers. Ringer solution (0·25–0·5 ml.) was placed in the upper compartment, and this was stirred and oxygenated by a fine stream of humidified gas injected through a port close to the surface of the tissue.

Following a pre-incubation period lasting about 30 min, the saline was replaced with a test solution containing [^{131}I] and [^{3}H]mannitol. At the end of the incubation period the tissue exposed to the fluid in the upper chamber was cut out using a punch. The punch, which was machined to fit the window between the chambers, was inserted from the lower part of the chamber to lift the tissue (supported by the filter paper) above the level of the incubation fluid. The tissue was then removed with fine forceps and rinsed three times with 'cold' Ringer solution in a millipore filter unit. About 1 ml. of rinse solution was used which did not contain I^- or mannitol. The plexus was weighed, dissolved in tissue solubilizer, and assayed for ^{131}I and ^{3}H. It required about 5 sec to terminate the experiment and rinse the plexus.

Mannitol was included in the test solutions as a marker for the film of solution that adheres to the ventricular surface of the plexus. Previous experiments (Wright, 1972*b*) have shown that there was no significant difference between the mannitol and inulin spaces in this tissue.

In most experiments choroid plexuses were pre-incubated in bicarbonate Ringer solution containing 100 μM NaI and, where appropriate, ouabain and perchlorate. Preliminary experiments showed that the uptake of I^- into the tissue was unaffected by the omission, or inclusion, of I^- in the pre-incubation solutions, i.e. a trans-concentration effect across the ventricular surface was not observed.

Solutions. Most experiments were carried out in a bicarbonate saline containing 85 mM-NaCl, 2 mM-KCl, 1 mM-$CaCl_2$, 1 mM-$MgSO_4$ and 25 mM-$NaHCO_3$ gassed with 95 % O_2/5 % CO_2. Some experiments (mentioned specifically in the text) were also carried out in a phosphate saline containing 104·5 mM-NaCl, 2 mM-KCl, 1 mM-$CaCl_2$, 1 mM-$MgSO_4$, 2·125 mM-Na_2HPO_4 and 0·375 mM-NaH_2PO_4 and gassed with 100 % O_2. In a few experiments the phosphate buffer was replaced with a 1·5 mM Tris buffer. The Na and/or Cl^- concentration in these solutions was varied by iso-osmotically replacing the NaCl with KCl, NaBr, LiCl, choline Cl, or mannitol, whereas the KCl concentration was varied by replacing the KCl with RbCl, LiCl or CsCl.

The anion composition of these solutions was varied by the addition of one or more of the following salts: NaI, $NaReO_4$, $NaBF_4$, NaSCN, $NaClO_4$, $NaNO_3$ and KSeCN to give final concentrations ranging between $2{\cdot}5 \times 10^{-5}$ and 5×10^{-3} M. Changes in ionic composition brought about by the addition of radioactive isotopes were taken into account during the preparation of the solutions and the calculation of the results.

Radioactive isotopes were obtained from the Cambridge Nuclear Radiopharmaceutical Corp., Cambridge, Mass. (Na^{131}I, carrier free; and Na^{82}Br, 1664 mCi/g), Amersham/Searle, Arlington Heights, Illinois (KS^{14}CN, 41 mCi/mmol) and New England Nuclear Corp., Boston, Mass. (^{3}[H]D-mannitol, [^{14}C]D-mannitol, 50 mCi/mmol). $^{m99}TcO_4^-$ was produced in the laboratory from a commercial ^{99}Mo source. Radioactive samples were assayed by conventional counting techniques using, where appropriate, a gas flow proportional counter, a liquid scintillation counter and a γ-scintillation counter. Unidirectional fluxes were expressed in mol/cm^2 h where the area refers to the area of the window between the Lucite flux chambers, and tissue accumulations were expressed as the Tissue/Medium (T/M) ratio, where

$$T/M = \frac{\text{concentration of solute per ml. of tissue water}}{\text{concentration of solute per ml. of incubation media}}.$$

As reported previously the serosal area of the plexus was 12–15 mm² (flux chamber window area 6·2 mm²), the wet weight of the tissue averaged 7 mg, the total tissue water accounted for 81 % of the total wet weight, and the inulin space (extracellular fluid) amounted to 48 % of the tissue water.

Experiments were carried out at room temperature (22–23° C). All calculations were carried out on a Hewlett Packard Calculator (Model 9100A). Experimental errors are quoted as the S.E. of the mean.

RESULTS

Transepithelial fluxes

As discussed previously (Wright, 1972*a*) the electrical potential difference (p.d.) across the frog choroid plexus was generally less than 1 mV, c.s.f. side positive, and the average conductance of the plexus was about 5 m-mhos/cm². In this series of experiments it was also observed that the p.d. across the plexus was unaffected by the presence or absence of anions, and that inhibitors of active transport changed the p.d. by less than 1 mV. Thus, in the absence of concentration gradients, it is not necessary to consider the transepithelial p.d. as a factor influencing the transport of anions across the plexus.

Iodide

Most of the experiments in this study were carried out using I^- owing to the fact that inexpensive, carrier-free, radioactive isotopes of this anion are available. A few experiments were also carried out with other commercially available radioactive anions, namely ^{82}Br, $S^{14}CN$, and $^{m99}TcO_4$. Pertechnetate was included owing to the growing clinical interest in the use of this anion. The specificity of the active anion transport process was also investigated by competition effects (p. 544).

The unidirectional fluxes of carrier free ^{131}I across the choroid plexus are shown in Fig. 2. In these experiments the rate of appearance of the ^{131}I in the serosal fluid was about fifty times greater than the appearance of the isotope in the ventricular solution, i.e. there was a net flux of I^- across the choroidal epithelium from the ventricular to the serosal solutions. Furthermore, these experiments show that the difference between the two unidirectional fluxes was eliminated by the addition of ouabain to the external solutions. There was a decrease in the ventricle to serosa flux whereas there was no significant change in the flux in the opposite direction.

As shown in Fig. 2 the time required for the unidirectional I^- fluxes to reach a steady state depends upon the direction of the flux. At an I^- concentration of 100 μequiv/l. the flux from the ventricle to serosa took about 65 min, but in the opposite direction only about 20 min were required. Two factors may contribute to the delay (see Hoshiko & Ussing, 1960; Diamond, 1962; Schultz & Zalusky, 1964): the first is the time necessary for the intracellular iodide to reach a steady-state

specific activity. Here the approach to the steady-state flux is an exponential function of time and is related to the intracellular I^- pool size; the second factor is the time necessary to reach a steady rate of isotope diffusion across the plexus. In this case the time intercept (t) of the line tangent to the steady-state slope is related to the diffusion coefficient (D) of I^- in the tissue by $t = \lambda^2/6D$ where λ is the thickness of the diffusion barrier. Four observations suggest that the reason for the delay is the time required for the tissue I^- pool to reach the steady-state specific activity: (1) the time delay depended on the direction of the flux, i.e. the active flux was

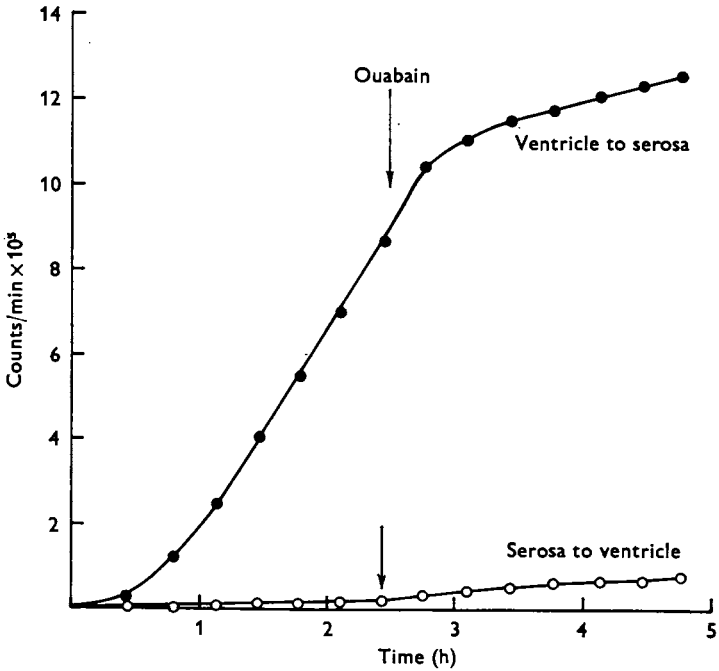

Fig. 2. Unidirectional fluxes of ^{131}I across the choroid plexus. In one experiment carrier-free ^{131}I was added to the ventricular solution and the appearance of the isotope in the serosal fluid was measured with time. In the other experiment the isotope was added to the serosal fluid and the flux was measured from the serosa to ventricle. At the time indicated ouabain ($6{\cdot}6 \times 10^{-5}$ M) was added to the external solutions. The counts on the initially non-radioactive side are plotted on the ordinate against time on the abscissa. The flux to the blood side of the preparation was much greater than the flux in the opposite direction and the difference was eliminated by ouabain.

about three times longer than the passive flux; (2) two inhibitors of active I^- transport, ouabain and ClO_4, reduce the time delay for the unidirectional flux from ventricle to serosa from 65 to 20 min; (3) the approach to the steady-state flux can be fitted by a single exponential curve; and (4) the thickness of the diffusion barrier in the choroid plexus is not sufficient to account for the delay in the flux (i.e. taking the sum of the effective unstirred layers on each side of the plexus as 800 μm (cal-

culated assuming free solution diffusion coefficients in the unstirred layers, Wright & Prather, 1970) and taking D as $1{\cdot}52 \times 10^{-5}$ cm²/sec, t is estimated to be 70 sec – about a factor of 20 less than that observed experimentally).

The relationships between the unidirectional fluxes and I^- concentration are shown in Fig. 3. The serosa to ventricle flux was a linear function of the I^- concentration from carrier free ($I < 5 \times 10^{-10}$ M) up to 5×10^{-3} M. The slope of the line corresponds to an I^- permeability coefficient of $1{\cdot}0 \times 10^{-5}$ cm/sec. In contrast, the flux from ventricle to serosa was composed of both diffusional and saturable components. Above 250 μM-NaI the flux increased with concentration in a linear fashion with a slope parallel to the unidirectional flux from serosa to ventricle. The net flux of iodide across the plexus, the difference between the unidirectional fluxes, is plotted against the iodide concentration in Fig. 4. This shows that the maximum net flux amounted to 36×10^{-9} mol/cm² hr.

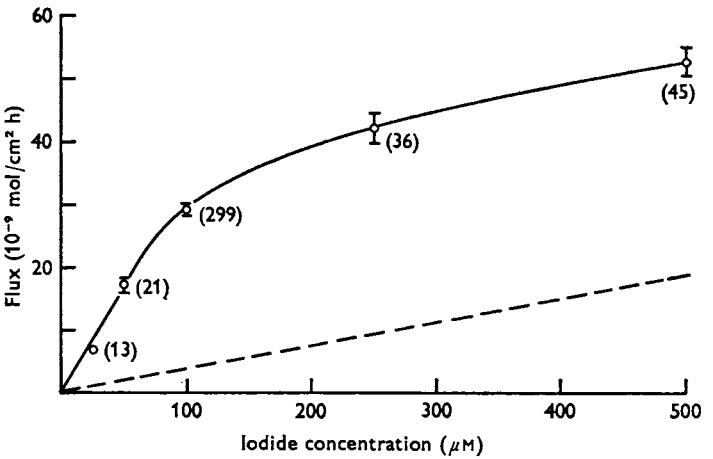

Fig. 3. Iodide unidirectional fluxes from ventricle to blood as a function of the external I^- concentration. At each concentration the mean flux, the S.E. and the number of estimates are indicated. Note that the flux was composed of non-linear and linear components. The slope of the linear component is identical with the slope of the unidirectional flux from serosa to ventricle (the interrupted line).

Some consideration must be given to unstirred layer effects since it is difficult to achieve perfect mixing of the solutions adjacent to biological membranes. The effective thickness of the unstirred layers at the ventricular and serosal surfaces of the choroid plexus, under the present experimental conditions, are about 300 and 500 μm respectively (from Wright & Prather, 1970). The true membrane permeability coefficients (P_m) are related to the observed permeability coefficients (P) and the thickness of the unstirred layers (δ_v and δ_s) by the relation

$$\frac{1}{P} = \frac{1}{P_m} + \frac{\delta_v}{D} + \frac{\delta_s}{D},$$

where D is the iodide-free solution diffusion coefficient. In the case of the I^- fluxes from serosa to ventricle, this equation shows that P_m is greater than P by 6%, i.e. the resistance to I^- diffusion across the unstirred layers is small relative to the resistance of the epithelium.

Owing to the concentration dependence of the unidirectional I^- flux from ventricle to serosa, the importance of the unstirred layer effect is also concentration dependent. At low concentrations, 25–50 μM, the fluxes correspond to an apparent permeability coefficient of 8×10^{-5} cm/sec and when corrected for the presence of the unstirred layers this increased to $1{\cdot}6 \times 10^{-4}$ cm/sec. In other words, the resistance to I^- diffusion across the unstirred layers is about equal to the resistance offered by the epithelium. However, the contribution of the unstirred layers to the resistance to the I^- flux across the plexus decreases with increasing I^- concentration, e.g. at an I^- concentration of 0·5 mM the I^- permeability coefficient only increased from $2{\cdot}9 \times 10^{-5}$ to $3{\cdot}5 \times 10^{-5}$ cm/sec when corrected for unstirred layers.

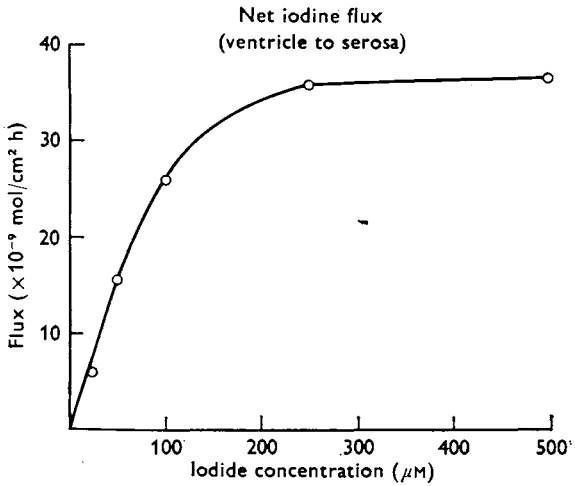

Fig. 4. The net flux of I^- across the choroid plexus as a function of the external I^- concentration. This curve was obtained from the unidirectional fluxes shown in Fig. 3. The net transport of I^- from the ventricular to the serosal solutions reached a maximum when the external I^- concentration was 250 μM. These fluxes were uncorrected for unstirred layer effects which effectively moves the curve to the left (see text).

Consequently, to obtain the true kinetics of active fluxes across biological membranes, it is necessary to take this unstirred layer effect into account. In general the presence of unstirred layers do not affect estimates of V_{max}, but they lead to underestimates of K_m. In the choroid plexus the corrected K_m for I^- transport is about 40 μm as opposed to an uncorrected value of 60 μM.

A few experiments were also conducted with arachnoid membranes isolated from the frog brain. These showed that there was no net transport of I^- across this membrane ($P_I \sim 5 \times 10^{-6}$ cm/sec) and that the unidirectional fluxes were unaffected by the addition of ouabain to the Ringer solutions.

Bromide, thiocyanate and pertechnetate

In a series of experiments the unidirectional fluxes of these anions were compared to the unidirectional fluxes of I^-. The results are summarized in Table 1, where it can be seen that there was a significant net transport of all four anions across the plexus from the ventricle to serosa. The passive permeability of these anions, as judged from the magnitude of the unidirectional fluxes from the serosal to the ventricular fluids were

$$P_I\ 9{\cdot}2 \pm 0{\cdot}6 \times 10^{-6}, \quad P_{Br}\ 8{\cdot}5 \pm 0{\cdot}5 \times 10^{-6}, \quad P_{SCN}\ 6{\cdot}9 \pm 1{\cdot}4 \times 10^{-6}$$
$$\text{and} \quad P_{TcO_4}\ 3{\cdot}5 \pm 0{\cdot}1 \times 10^{-6}\ \text{cm/sec}.$$

TABLE 1. Unidirectional fluxes of anions across the choroid plexus

Anion	Concentration	Flux (n-mol/cm² h) J_{sv}	J_{vs}
I	100 μm	3·3 ± 0·2 (56)	29·1 ± 0·7 (299)
Br	96 μm	2·95 ± 0·2 (10)	5·1 ± 0·3 (24)
SCN	81 μm	2·0 ± 0·4 (8)	13·7 ± 1·4 (34)
TcO_4	Carrier free	1·26 ± 0·2 (16)*	13·2 ± 1·0 (27)*
I	Carrier free	3·5 ± 0·7 (12)*	17·5 ± 2·4 (18)*

The fluxes quoted are steady-state values obtained 90–180 min after addition of the isotopes to the Ringer in the flux chamber. The Br and SCN concentrations were calculated from the specific activities and amounts of the radioactive isotopes added to the Ringer solutions in the chamber.

* For the presentation of these results it was assumed that the I^- and pertechnetate concentrations were 100 μM even though they were used at carrier-free concentrations.

In a previous study (Wright, 1972*a*) Cl^- unidirectional fluxes under these experimental conditions yielded a permeability coefficient of $6{\cdot}6 \pm 0{\cdot}3 \times 10^{-6}$ cm/sec. Thus the passive permeability sequence was

$$P_I > P_{Br} > P_{SCN} > P_{Cl} > P_{TcO_4}.$$

The unidirectional flux ratios (J_{vs}/J_{sv}) were I = 8·8, SCN = 6·8 and Br = 1·7 at a concentration of about 100 μM and I = 5·0 and TcO_4 = 10·4 at carrier-free concentrations.

It is expected that the affinity of the anion pump for Cl^- is substantially less than Br^- since, (i) previous experiments, under identical conditions, showed that the Cl^- flux ratio was not significantly different from I^- (Wright, 1972*a*), and (ii) I^- transport was not increased when the NaCl in the Ringer solution was replaced with Na isethionate.

These results suggest that the selectivity for active anion transport is $TcO_4 > I > SCN > Br > Cl$. It should be noted that this selectivity sequence does not bear any simple relation to the passive permeability sequence.

Effects of competitive anions

From the earlier studies of accumulation in the thyroid gland (see Wolff, 1964) and choroid plexuses (Welch, 1962*a*; Becker, 1961) it is well known that certain anions competitively inhibit active I^- accumulation. Consequently the effect of these anions on active I^- transport across the frog choroid plexus has been investigated. Fig. 5 shows the effect of per-

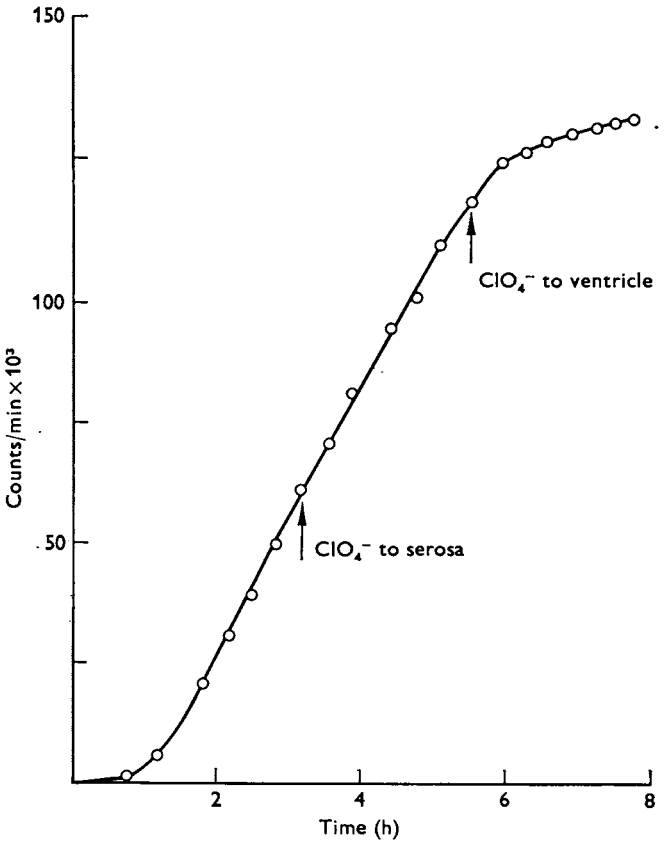

Fig. 5. The effect of ClO_4 on the unidirectional flux of I^- from ventricle to serosa. ^{131}I was added to the ventricular solution and the rate of appearance of the isotope in the serosal solution was measured before and after the addition of ClO_4 (20 μM) to the serosal and ventricular solutions. The iodide concentration in this experiment was 100 μM. Note that ClO_4 was only effective in the ventricular solution.

chlorate (ClO_4) on the I^- flux. Addition of ClO_4 (20 μM) to the serosal solution had no effect on the rate of appearance of ^{131}I in the serosal fluid, but when ClO_4 was added to the ventricular solution the I^- flux was reduced to the level of the passive component within 1 hr. In five experiments the addition of 20 μM-ClO_4 to the ventricular solution reduced the flux of 100 μM-I^- from the ventricular solution to the serosal solution by 25×10^{-9} mol/cm^2 h, whereas in two experiments the unidirectional flux in the opposite direction was unaffected. Further experiments showed that (i) the effect of ClO_4 was reversible (two experiments), (ii) higher concentrations of ClO_4 (100 μM) were required to abolish the net I^- flux when the I^- concentration was 500 μM (seven experiments), and (iii) ClO_4 (20 μM) blocks the net transport of SCN^- (two experiments) and TcO_4^- (one experiment) across the tissue.

TABLE 2. Effect of anions on I^- and SCN^- fluxes across the choroid plexus

A. I^- unidirectional flux J_{vs}

Anion	Concentration	% Inhibition
ClO_4	20 μM	61 ± 4 (5)
ReO_4	50 μM	84 ± 3 (3)
BF_4	50 μM	64 ± 4 (3)
SCN	50 μM	40 ± 6 (2)
SeCN	50 μM	39 ± 6 (2)
NO_3	100 μM	33 ± 23 (2)
Br	104·5 mM	27 ± 10 (2)

B. SCN unidirectional flux J_{vs}

ClO_4	50 μM	72 ± 8 (2)
I	50 μM	5 ± 5 (2)

The I^- and SCN^- fluxes were carried out at concentrations of 100 μM and 81 μM respectively. Unidirectional fluxes from ventricle to serosa were monitored for 3 h, before the addition of the competing anion to the ventricular fluid.

Table 2 summarizes the effects of other anions on the fluxes of I^- and SCN^-. The concentrations of anions required to produce detectable effects on the I^- and SCN^- fluxes ranged from 2×10^{-5} to 1×10^{-1} M, and at least for ClO_4^- (two experiments) and SCN^- (two experiments) these inhibitory effects were reversible. From the degree of inhibition of the fluxes one can obtain an index of the affinity of the transport mechanism for the various anions, namely,

$$ClO_4 > ReO_4 \sim BF_4 > SCN \sim SeCN > I > NO_3 > Br.$$

The position of I^- was assigned on the basis of the effect of SCN^- on I^- transport on one hand and the effect of I^- on the transport of SCN^- on

the other. In addition, it may be concluded that the affinity of the transport system for ClO_4^- is at least a factor of 250 greater than the affinity for Br^-.

Effect of inhibitors

(i) *Ouabain.* As shown in Fig. 2 and Table 3 ouabain blocks the net transport of I^- across the choroid plexus. At an I^- concentration of 100 μequiv./l., ouabain concentrations as low as 1×10^{-7} M produced significant inhibitions of transport; whereas $6{\cdot}6 \times 10^{-6}$ M ouabain in either the ventricular solution (four experiments) or in both the ventricular and serosal solutions (three experiments) was sufficient to abolish the

TABLE 3. Effect of ouabain on anion fluxes across the choroid plexus

Anion		(J_{vs} n-mol/cm² h) Control	Ouabain
I (100 μM)	*a*	27·2 ± 3·0 (3)	5·4 ± 1·7 (4)
	b	29·2 ± 1·6 (3)	6·3 ± 1·7 (4)
SCN (81 μM)	*a*	8·4 ± 0·8 (3)	2·6 ± 0·3 (4)
	b	10·4 ± 0·8 (3)	2·9 ± 1·1 (4)
Br (98 μM)	*a*	7·1 ± 0·3 (4)	5·9 ± 1·7 (5)
	b	5·5 ± 0·6 (4)	2·6 ± 0·2 (4)
	c	5·7 ± 0·6 (5)	2·8 ± 0·5 (4)
TcO_4 (carrier free)	*a*	10·1 ± 0·7 (3)	6·3 ± 1·3 (3)

This Table shows individual experiments where ouabain ($1{\cdot}6 \times 10^{-5}$ M) was added to both Ringer solutions after monitoring J_{vs} about 2·5 h. The fluxes in the presence of ouabain, which were obtained 1 h after addition of the inhibitor, should be compared with the unidirectional fluxes from the serosal to the ventricular solutions shown in Table 1.

net transport of I^- across the tissue. The time course of the inhibition followed roughly the same time course as ouabain binding to the c.s.f. side of the preparation (Quinton *et al.* 1973), i.e. the half-time for both inhibition of transport and binding was about 25 min. Ouabain in the serosal solution alone (three experiments) failed to affect active I^- transport, and ouabain present in either the serosal or ventricular solutions did not affect the backflux from the serosal to the ventricular solutions. This inhibitor was also shown to abolish (*a*) the net I^- flux at all concentrations tested (carrier-free, 100, 500, 2,500 and 5000 μequiv/l.), and (*b*) the active fluxes of SCN^-, Br^- and TcO_4^- (Table 3). The effect of ouabain on transport was judged to be irreversible since there was no significant recovery of transport upon washing the chambers repeatedly with ouabain-free solutions.

(ii) *Oligomycin.* This inhibitor of Na/K ATPase, also inhibited I^- transport when added to the external solutions to give a final concentration of 10 μg/ml. (two experiments).

(iii) *Phloretin and phlorrhizin.* The active transport of I^- across the choroid plexus was abolished upon addition of 0·5 mM phloretin to the ventricular solution, but there was no effect when the inhibitor was added to the serosal solution only (two experiments each). Phlorrhizin (0·5 mM) on the other hand did not inhibit I^- transport (four experiments).

(iv) *Reducing agents.* Catechol (2 mM), pyruvate (3 mM), tyrosine (1 mM, pH 7·8) and the antithyroid agents, methylthiourical (1 mM) and 2-mercapto-1 methyl-imidazole (1 mM) all failed to block the transport of I^- across the plexus (two experiments each). These results indicate that trace amounts of I_2 and I_3^- in the solutions do not contribute significantly to the fluxes of the ^{131}I across the tissue.

(v) *Anoxia.* In three experiments changing the gas mixture from 95 % O_2/5 % CO_2 to 95 % N_2/5 % CO_2 failed to produce any significant change in the I^- unidirectional fluxes. In one experiment changing the CO_2 content of the gas mixture from 5 to 12 %, i.e. changing the pH by 0·4 u., was also without effect.

(vi) *2,4-dinitrophenol.* Addition of 5×10^{-4} M-2,4-DNP to the incubation media reduced the active unidirectional flux by 70 % whereas the backflux was unaffected (two experiments each).

(vii) *Other compounds.* Diamox ($1{\cdot}5 \times 10^{-3}$ M), glucose (10 mM), furosemide (1×10^{-4} M), 2,4,6-trinitro-*m*-cresolate (1 mM) and amiloride (6×10^{-3} M) all failed to produce any significant effect on the unidirectional I^- fluxes.

Apart from the lack of effect of Diamox and anoxia, these results are essentially the same as those reported for the accumulation of anions in the *in vitro* preparations of the rabbit choroid plexus (Becker, 1961; Welch, 1962*a*). Negative effects with anoxia and carbonic anhydrase inhibitions have also been obtained previously in investigations of amino acid and sodium transport by the frog choroid plexus (Wright, 1972*a*, *b*). The explanation for the lack of effect of carbonic anhydrase inhibitors in the frog may be that the uncatalysed rate of CO_2 hydration is rapid enough to meet the demand for H^+ and/or HCO_3^- by the tissue.

Ionic requirement for active anion transport

The sensitivity of the active transport process to ouabain and oligomycin suggests that a Na/K ATPase is directly, or indirectly, involved. This was investigated further by studying the effect of cations on I^- fluxes.

Na^+ replacement

Some of these experiments are illustrated in Fig. 6 where it can be seen that the active unidirectional flux from ventricle to serosa was abolished upon omitting sodium from the Ringer solutions. Replacing the NaCl with KCl (Fig. 6*a*), LiCl (Fig. 6*b*), choline Cl (not shown) and mannitol

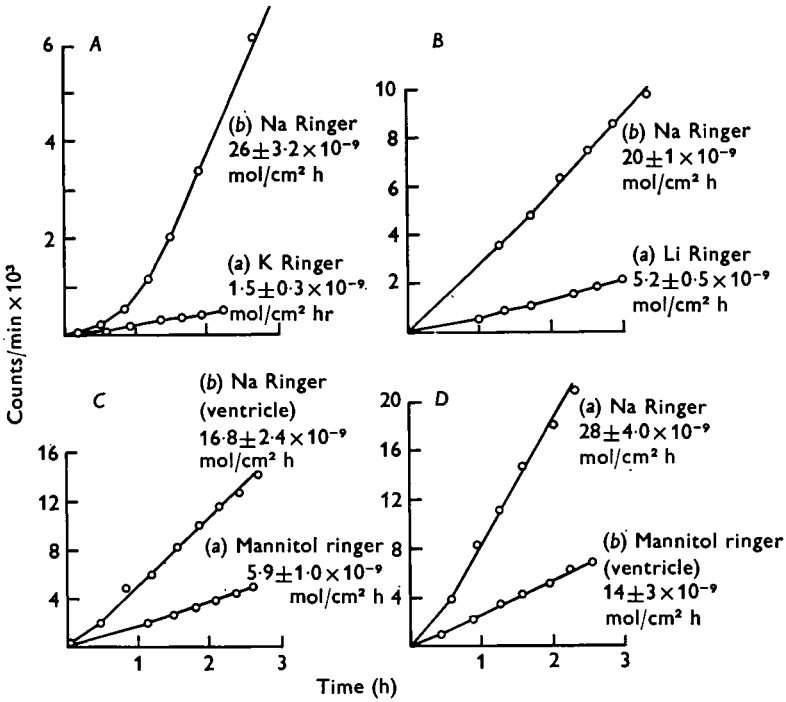

Fig. 6. The Na dependence of iodide transport across the choroid plexus. The unidirectional fluxes of I^- from ventricle to blood were determined when the NaCl of the external saline was replaced with KCl (*A*), LiCl (*B*) and mannitol (*C* and *D*). In experiments *A*, *B* and *C* the choroid plexuses were incubated overnight at 0° C in the solution to be used in each experiment. The following day they were mounted in the chamber and were allowed to equilibrate at room temperature for 1 h before measurement of the unidirectional fluxes. After a further 2–3 h NaCl Ringer solution was restored to either both sides of the tissue (*A* and *B*) or to the ventricular solution only (*C*). It should be noted that when NaCl was replaced with KCl, LiCl or mannitol, the ventricle to blood flux was reduced to the level of the passive component, and upon return to the NaCl solutions the active flux was restored. In experiment *D* the flux was measured across a fresh tissue in normal Ringer solution before replacement of the NaCl in the ventricular compartment with mannitol. Note that the inhibition of the I^- flux was incomplete. In all experiments the I^- concentration was 100 μM and the Ringer solutions were buffered with phosphate.

(6*c* and 6*d*) reduced the unidirectional flux to the level of the diffusional component. It is also shown that the active flux was restored upon addition of Na to the Ringer solution. (When the NaCl in HCO_3 Ringer was replaced with mannitol, final Na^+ concentration 25 m-equiv/l., only a 40% inhibition of I^- transport was observed (three experiments).) The final point to be drawn from Fig. 6 is that the I^- flux was only reduced about 50% when the NaCl in either the ventricular or the serosal compartment was replaced iso-osmotically with mannitol. Similar results were also obtained when the NaCl in the ventricular or serosal solutions was replaced with LiCl. That is NaCl in the solution bathing either face of epithelium was sufficient to maintain active I^- transport at about half the normal rate.

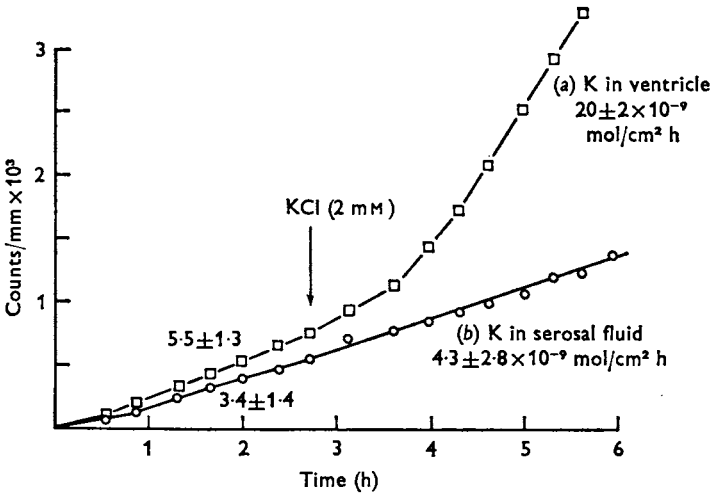

Fig. 7. Effect of K on I^- fluxes across the choroid plexus. In these two experiments the tissues were incubated overnight in K-free saline at 0° C before mounting in the flux chambers. The fluxes of I^- (J_{vs}), were then measured in the absence of K, and after K (2 mM) was added to either the ventricular or serosal fluids. Note that in the absence of K the unidirectional fluxes are at the level of the passive component, and that the active flux was restored upon addition of K (2 mM) to the ventricular, but not the serosal fluids.

These experiments suggest that (i) Na^+ is required for active I^- transport, (ii) the requirement for Na^+ is specific, i.e. K^+, Li^+, choline$^+$ or mannitol, cannot substitute for Na^+, and (iii) Na^+ on either the ventricular or the serosal faces of the epithelium can stimulate I^- transport.

K^+ replacement

Preliminary experiments demonstrated that simply omitting K^+ from the Ringer solutions inhibited the active flux of I^- across the plexus by

about 40% (4), and that the subsequent addition of KCl (2 mM) to the ventricular solution alone was sufficient to overcome this partial inhibition.

Complete inhibition could be obtained by pre-incubation of the plexus overnight in a K^+-free solution at 0° C. This is shown in Fig. 7 where it is also shown that the subsequent addition of 2 mM-KCl to the ventricular solution, but not the serosal solution, restored the active I^- flux to the normal control value within 1 hr.

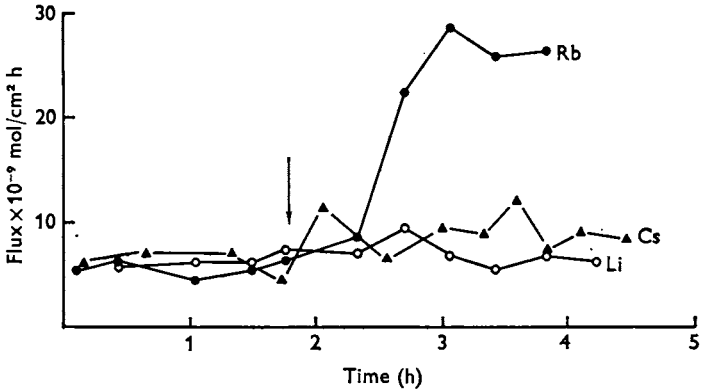

Fig. 8. The specificity of the K requirement for iodide transport across the choroid plexus. In these three experiments the plexuses were incubated overnight in K-free saline at 0° C before mounting in the flux chambers as in Fig. 7. The flux of I^-, from ventricle to blood, was measured in the absence of K, and after addition of 2 mM-Rb (●), Cs (▲) or Li (○) to the ventricular fluid. It can be seen that Rb, and to a much lesser extent Cs, can mimic the effect of K on I^- fluxes. Li on the other hand was unable to stimulate the I^- flux. The I^- concentration in all experiments was 100 μM.

The ability of other cations to substitute for K^+ was also investigated. Fig. 8 shows that Rb^+, was able to mimic K^+, whereas Cs^+ and Li^+ had little or no effect. In a total of three experiments each, the I^- flux increased by 2×10^{-9} mol/cm² hr on addition of 2 mM-CsCl, whereas there was no significant increase in flux with 2 mM-LiCl. It was also observed that K was unable to stimulate the active transport of I^- when the NaCl in a PO_4 Ringer was replaced with either mannitol (three experiments) or LiCl (three experiments).

Increases in the K^+ concentration above 2 m-equiv/l. in the ventricular fluid, at a constant Na^+ concentration, did not further increase active I^- transport. In fact, increases in the K^+ concentration to 10 m-equiv/l. produced a 25% inhibition of the I^- flux, and the degree of inhibition increased to about 50% when the K^+ concentration was raised to 20 m-equiv/l.

The passive unidirectional flux of I^- across the choroid plexus from serosa to ventricle was unaffected by changes in the cation composition of the Ringer solutions.

The following conclusions can be drawn from these experiments, (i) K^+ is required for active anion transport across the choroid plexus, (ii) Rb^+, and to a lesser extent Cs^+, can substitute for K^+, but Li^+ cannot, (iii) K^+ does not stimulate I^- transport in the absence of Na^+, (iv) K^+ is only effective when present in the ventricular solution, (v) high concentrations of K^+ inhibit active iodide transport. This latter conclusion is consistent with the observations that high K^+ concentrations inhibit Na/K ATPase activity and the active Na^+ transport across epithelial membranes (see Bonting, 1970).

TABLE 4. Steady state Tissue/Medium (T/M) ratios

Anion	Concentration	Saline	T/M
I	100 μM	HCO_3	$7{\cdot}7 \pm 0{\cdot}7$ (9)
SCN	82 μM	HCO_3	$3{\cdot}0 \pm 0{\cdot}4$ (3)
I	100 μM	PO_4	$4{\cdot}4 \pm 0{\cdot}7$ (8)
Br	96 μM	PO_4	$0{\cdot}88 \pm 0{\cdot}08$ (3)
Mannitol	5 mM	PO_4	$0{\cdot}4 \pm 0{\cdot}1$ (4)*
I	Carrier free	HCO_3	$9{\cdot}9 \pm 0{\cdot}7$ (3)
TcO_4	Carrier free	HCO_3	193 ± 36 (3)

Steady-state ratios were measured 2·5 h after addition of the isotopes to the incubation media, which was either phosphate or bicarbonate Ringer solution.

* Taken from Wright (1972*b*).

Accumulation of anions within the choroid plexus

The uptake of anions into the choroid plexus was measured by incubation of the plexus in 3–5 ml. physiological saline for periods ranging from 30 sec to 3 hr. At the end of the incubation period the amount of anions in the tissue was estimated by the same procedure used previously for amino acids and LSD (Wright, 1972*b*, *c*). The results were expressed as the T/M ratio. There was a rapid accumulation of I^- in the tissue over the first 30 min of the incubation and then a steady state was reached over the following hour. The steady state T/M ratios obtained are included in Table 4. In HCO_3 saline the I^- T/M ratio was about twice that obtained in PO_4 saline, which in turn was about an order of magnitude higher than the mannitol ratio. Using mannitol uptake as an index of the extracellular space of the tissue, it is calculated that the intracellular I^- concentration is 2 mM when the tissue was incubated in saline containing 100 μM-NaI. Ouabain (6×10^{-6} M) reduced the uptake of I^- into the plexus by 50% (two experiments). I^- was not accumulated within the arachnoid membrane.

Two observations strongly suggest that I^- is accumulated within the epithelial cells of the plexus; (i) Coben (1969) has reported that in preliminary experiments isolated epithelial cells from the choroid plexus accumulate I^-, and (ii) preliminary experiments in this laboratory indicate that bullfrog red blood cells, which represent a significant proportion of the total number of cells in the plexus, do not accumulate iodide.

Included in Table 4 are the T/M ratios obtained with Br^-, SCN^- and TcO_4^-. It is doubtful from these experiments whether or not Br^- is actively accumulated within the epithelium as the T/M ratio did not exceed 1. The deduced selectivity sequence for anion accumulation within the plexus is TcO_4 (20) > I (1) > SCN (0·4) > Br (0·2). It should be noted (i) that this selectivity sequence is essentially the same as that obtained for the active transport of these anions across the plexus (see p. 544), and (ii) these T/M ratios are comparable to those obtained for I^-, SCN^- and TcO_4^- with *in vitro* preparations of the rabbit choroid plexus (Becker, 1961; Welch, 1962*a*, *b*; and Oldendorf, Sisson & Iisaka, 1970).

The uptake of I^- into the plexus was also determined at the termination of a few unidirectional flux experiments. In control experiments where the unidirectional flux from the ventricular to the serosal fluid was monitored, the amount of I^- within the tissue corresponded to $1{\cdot}1 \times 10^{-7}$ mol/cm^2. This is about 100 times greater than the amount of mannitol in the tissue under these conditions (see Wright, 1972*b*). Ouabain (7×10^{-5} M) and ClO_4 (5×10^{-5} M) reduced the I^- uptake from the ventricular solution by 75%. On the other hand I^- uptake from the serosal solution was only about $4 \pm (5)$% of the uptake from the ventricular solution, and this was unaffected by ouabain and ClO_4.

It can be concluded that anions are accumulated within the choroidal epithelium during the active transport of these anions from the c.s.f. to blood, and that anions in the serosal compartment are unable to equilibrate with the transport pool within the epithelium. This, together with the observations that the passive unidirectional fluxes reach a steady state much faster than active unidirectional fluxes (Fig. 2 and p. 539), strongly suggests that passive anion fluxes bypass the epithelial cells and cross the tissue largely through a shunt. As discussed previously (Wright, 1972*a*) there is evidence to suggest that tight junctions represent the high conductance pathway across this epithelium.

In two experiments, which were carried out in flux chambers, the efflux of I^- into the ventricular and serosal solutions was measured after the tissue had reached a steady state with ^{131}I in the external solutions. More than 90% of the isotope that had accumulated within the plexus washed out within 3 hr, and like the amino acids (Wright, 1972*b*), but unlike LSD (Wright, 1972*c*), more than 70% of the isotope appeared in the ventricular

compartment. These two experiments suggest that I^- is not irreversibly bound within the plexus, and that the apical surface is more permeable to I^- than the serosal surface.

Fluxes across the brush border

Unidirectional influxes across the brush border of the choroidal epithelium were measured with the chamber illustrated in Fig. 1. Fluxes of mannitol and iodide as a function of time are shown in Fig. 9. Mannitol,

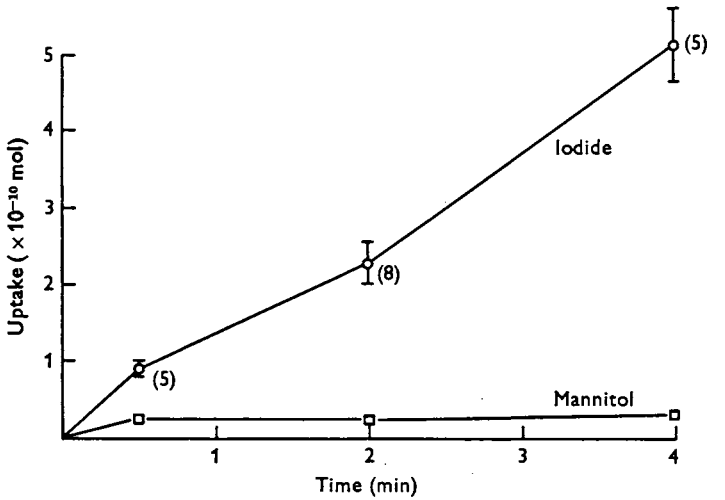

Fig. 9. The unidirectional influx of I^- (J_{vc}) across the brush border membrane of the choroidal epithelium. The ventricular surface of the choroid plexus was exposed to saline containing ^{131}I and [^{3}H]mannitol for periods ranging from 30 sec to 4 min, in the chamber illustrated in Fig. 1. The graph shows the amounts of I^- and mannitol in the plexus after the incubations. For each incubation period the mean uptake, the S.E. and number of estimates are shown. (In the case of mannitol the S.E. are smaller than the size of the squares used to indicate the mean.) Note that the extracellular compartment as judged by the size of the mannitol space achieved a steady state within 30 sec, and that the I^- uptake was linear fr 30 sec to 4 min. The slope of the I^- uptake gives a unidirectional flux of $1{\cdot}2 \times 10^{-10}$ mol/6·2 mm^2 min. The I^- concentration in all cases was 100 μM, and the mannitol uptake was normalized to this concentration.

which was used as an extracellular marker, equilibrated with the film of fluid adhering to the tissue within the first 30 sec; there was no significant increase in the mannitol 'space' for incubations lasting up to 4 min. Thus the $t_{\frac{1}{2}}$ for mannitol entry into the ventricular unstirred layer must be about 15 sec. (This half-time is substantially shorter than that obtained previously from streaming potential measurements, 52 sec (Wright &

Prather, 1970), and the explanation probably lies in the more efficient stirring achieved in the present chamber.) The half time is equivalent to an unstirred layer thickness of 120 μm; i.e. $t_{\frac{1}{2}} = 0{\cdot}38\ \delta^2/D$, where δ is the unstirred layer thickness and D the mannitol diffusion coefficient (Diamond, 1966). Since the diffusion coefficient for I^- is twice as high as that for mannitol, ^{131}I should also have reached a steady state with the ventricular unstirred layer within 30 sec.

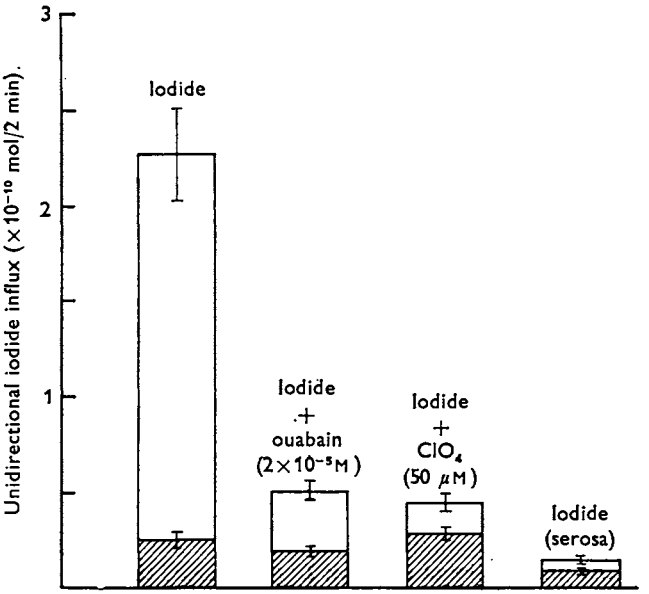

Fig. 10. The effect of ouabain and perchlorate on I^- unidirectional influxes across the brush border membrane. I^- influxes across the brush border were determined with the chamber shown in Fig. 1 as described for Fig. 9. The plexuses were preincubated for 1 h in (i) saline, (ii) saline + ouabain (2×10^{-5} M) or (iii) saline containing ClO_4 (50 μM). The fluxes were determined in an incubation period lasting 2 min. Shown in the Figure are the controls, the effect of ouabain, and the effect of ClO_4. Six experiments were carried out for each condition and shown in the Figure are the mean I^- and mannitol (hatched bars) uptakes with the S.E. Also shown in the Figure are the I^- and mannitol uptakes from the serosal surface of the tissue. The I^- concentration in all cases was 100 μM and the experiments were carried out in a random fashion on the same batch of frogs.

Following the first 30 sec of the incubation period the I^- flux increased linearly with time for at least 3·5 min. This flux in the steady state corresponds to the unidirectional influx of $1{\cdot}2 \times 10^{-7}$ mol/cm^2 hr of I^- across the apical surface of the tissue. Under these conditions the resistance to I^- diffusion across the ventricular unstirred layer is about 30% of that

offered by the apical membrane ($P = 3{\cdot}1 \times 10^{-4}$ cm/sec and $P_m = 4{\cdot}0 \times 10^{-4}$ cm/sec. See also p. 541). As shown in Fig. 10 this I^- flux is reduced by ouabain (2×10^{-5} M) and perchlorate (5×10^{-5} M) 85% and 90% respectively. The mannitol space was unaffected by these agents. Similar results were obtained when the NaCl in PO_4 saline was replaced with either mannitol or choline chloride. Finally, the I^- flux across the brush border was not linearly related to the I^- concentraion in the c.s.f., e.g. when the I^- concentration was increased from 25 to 200×10^{-6} M the influx only increased fourfold.

An estimate of the unidirectional influx across the apical surface of the epithelium can also be obtained from the measurement of tracer fluxes across the tissue, by the method described for Na fluxes across the frog skin by Curran, Herrera & Flanigan (1963) (see also Clarkson & Lindeman, 1969). This method is based on the kinetic analysis of unidirectional ion fluxes across a three compartment system (ventricular fluid-plexus-serosal fluid), and depends on the rate at which the ion flux across the tissue reaches a steady state (see Fig. 2 and p. 539), and the amount of isotope in the tissue at the steady state.

First consider the unidirectional flux of I^- from the ventricular fluid to the serosal fluid as it passes in turn across the apical surface of the epithelium, the intracellular compartment, and the serosal face of the tissue. The ^{131}I leaving the tissue to enter into the serosal fluid has the same specific activity as that in the intracellular pool, and so the flux across the tissue reaches a steady state when the specific activity of the pool reaches a steady state. The activity of the I^- in the intracellular pool, in turn, depends on the rate of efflux out of the pool across both the ventricular and serosal surfaces of the tissue.

Semilogarithmic plots of the approach of the unidirectional fluxes to the steady state could be fitted by straight lines, which in the case of the flux to the serosal side of the preparation (NaI 100 μM) had an average slope of 0·03 min^{-1}. Using this rate constant, the ratio of the ^{131}I in the plexus in the steady state to the ^{131}I in the ventricular fluid (0·05), and the total amount of I^- in the ventricular surface of the fluid ($1{\cdot}5 \times 10^{-7}$ mol), the unidirectional flux across the ventricular plexus was estimated to be about 2×10^{-7} mol/cm^2 h. This value compared well with that measured directly ($1{\cdot}2 \times 10^{-7}$ mol/cm^2 h).

For comparison with the unidirectional influxes across the ventricular surface of the choroid plexus influxes of I^- and mannitol across the serosal face of the tissue are included in Fig. 10. Although there is little difference in the serosal I^- and mannitol uptakes it can be seen that the I^- flux is less than 3% of the flux across the ventricular surface. As expected, neither the serosal mannitol space nor the serosal I^- influx was affected by the presence of ClO_4^-.

Finally, as judged by the magnitude of the unidirectional influx of anions across the brush border of the epithelium, the selectivity of the anion pump for anions was TcO_4 (2) > I (1) ~ SCN (1) > Br (0·2).

DISCUSSION

By all the commonly accepted criteria anions are actively transported across the frog choroid plexus, namely, (1) there is a net transport of I^-, SCN^-, TcO_4^- and Br^- across the epithelium, from the ventricular to the serosal surface, in the absence of external electrochemical potential gradients (Table 1), (2) the net flux of I^- across the tissue exhibits saturation kinetics with a V_{max} and apparent K_m of 35×10^{-9} mol/cm^2 h and 40×10^{-6} M respectively (p. 541), (3) net transport is blocked by metabolic inhibitors, for example, 2,4-DNP, an inhibitor of oxidative phosphorylation, and (4) other anions block the net transport of I^-, SCN^- and TcO_4^-, e.g. the addition of ClO_4^- to the ventricular solution reduces the unidirectional flux of I^- from the ventricle to the serosa to the level of the passive component (Fig. 5 and Table 2). However, as discussed below, there are a number of observations that raise the possibility that active anion transport may be secondary to the active transport of sodium and/or potassium across the plexus, e.g. ouabain, a specific inhibitor of Na/K ATPases and sodium transport, abolishes the net transport of anions (Fig. 2, Table 2 and p. 546).

Many of these characteristics of anion transport across the frog choroid plexus are shared by active anion transport processes in the thyroid gland, salivary gland, stomach, mammary gland, rabbit choroid plexus, ciliary body, small intestine, placenta, ova, salt glands of marine birds, and seaweed (see Brown-Grant, 1961; Wolff, 1964). Furthermore in the frog choroid plexus, as in most of these cells and tissues, the oxidation of I^- is not involved in the transport phenomena. Reducing agents and antithyroid agents (p. 547) are without effect, and fully oxidized anions such as ClO_4^- and ReO_4^- share the same common transport process. Thus, an understanding of anion transport across the choroid plexus is not only important with regard to the specific problem of anion distribution between c.s.f. and blood, but it bears on the more general problem of anion transport mechanisms.

This study, together with that reported by Pollay & Kaplan (1972), shows that the choroidal epithelium is, at least in part, responsible for active anion transport and the selective restriction of anion diffusion between blood and c.s.f.

Unidirectional fluxes across the cell membrane

Net transport of solutes across an epithelium such as the choroid plexus may occur either through the cellular route, where solutes in turn must pass across the brush border membrane, the cytoplasm and the basolateral membranes, or through the tight junctions, where solutes bypass the cells

and pass directly between the c.s.f. and the lateral intercellular spaces (see also Cereijido & Rotunno, 1968). The fact that net anion transport across the plexus is accompanied by anion accumulation within the tissue strongly indicates that the transcellular route is of prime importance, and that anions are actively transported across the brush border membrane. Quantitative analysis of the unidirectional fluxes has been undertaken to clarify the mechanisms involved.

Iodide

In the steady state the unidirectional fluxes across each cell membrane of the choroidal epithelium are related to the unidirectional fluxes across the epithelium by the following expressions:

$$J_{net} = J_{vs} - J_{sv} = J_{vc} - J_{cv} = J_{cs} - J_{sc}, \quad (1)$$

$$J_{vs} = \frac{J_{vc} \times J_{cs}}{J_{cv} + J_{cs}}, \quad (2)$$

$$J_{sv} = \frac{J_{sc} \times J_{cv}}{J_{cv} + J_{sc}}, \quad (3)$$

where J_{net} is the net I^- flux across the epithelium and J_{vs}, J_{sv}, J_{vc}, J_{cv}, J_{cs} and J_{sc} are the unidirectional fluxes across the epithelium and across

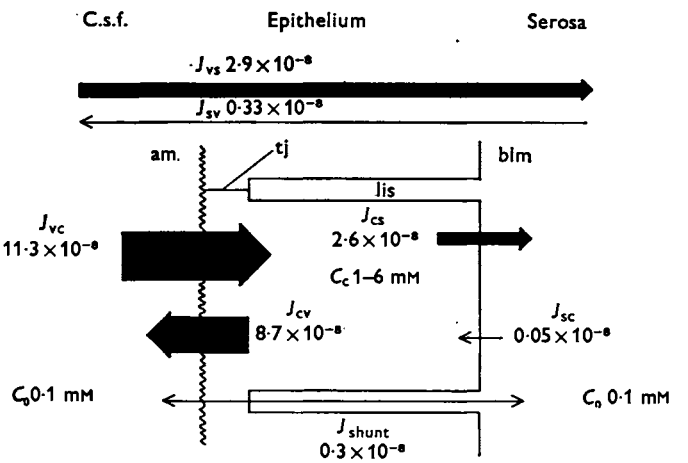

Fig. 11. Unidirectional I^- fluxes across the brush border and baso/lateral membranes of the choroid plexus epithelium. C_o = extracellular I^- concentration = 0·1 mM; C_i = intracellular I^- concentration: tj = tight junctions; lis = lateral intercellular spaces, am = apical, or brush border, membrane; and blm = baso-lateral membrane. Fluxes were not corrected for instirred layer effects; thus conclusions drawn from these fluxes refer to the properties of cell membranes and their attached unstirred layers (see text).

the apical and baso-lateral cell membranes. The subscripts v, s, and c, which refer to the ventricular, serosal and cellular compartments, indicate the individual unidirectional fluxes, e.g. J_{vc} = the unidirectional flux from the ventricular to the cellular compartments (see Wright, 1972*b*).

Since J_{vs}, J_{sv} and J_{vc} were estimated experimentally, it is possible to compute J_{net}, J_{cv}, J_{cs} and J_{sc}. The results obtained for the fluxes at an external I^- concentration of 100 μM are shown in Fig. 11. In view of the mounting circumstantial evidence (see p. 552 and Wright, 1972*a*) indicating that a major route of passive ion permeation across this tissue is via the so called tight junctions, the results in Fig. 1 were calculated assuming that 90% of the passive I^- flux (J_{sv}) occurs through the tight junctions, J_{cv}, J_{cs} and J_{vs} were calculated from eqns (1) and (2) after correcting J_{vc} and J_{vs} for the amount passing through the shunt pathway. As seen from Fig. 11, the unidirectional fluxes across the apical surface of the cell are almost an order of magnitude greater than the fluxes across either the epithelium or across the baso-lateral membrane.

Apart from J_{sc}, the calculated unidirectional fluxes across the cell membranes were relatively insensitive to the precise magnitude of the shunt path, e.g. assuming that only 30% of the passive flux across the epithelium occurs by the shunt, J_{vc}, J_{cv} and J_{cs} increase by less than 10%, but J_{sc} increases about sevenfold. Thus estimates of the permeability of the serosal or baso-lateral membrane, and P_v/P_s, the ratio of the two cell membrane permeabilities, are very sensitive to the magnitude of the shunt.

An internal check of this analysis can be made with reference to the intracellular I^- concentration. Under these conditions the intracellular concentration (C_i) is about 2 mM as deduced from the T/M ratio and the mannitol extracellular space (p. 551). The intracellular concentration may also be estimated independently using the expression

$$C_i/C_o = 1 + J_{vc}/C_o(P_v + P_s),$$

where C_o is the I^- concentration in the ventricular and serosal solutions (see Wright, 1972*b*). The permeability of the serosal face of the epithelium (P_s) is given by

$$P_s = \frac{J_{sc}}{C_o}$$

and the permeability of the ventricular surface of the epithelium (P_v) by

$$P_v = P_s \times \frac{P_v}{P_s} \quad \text{assuming} \quad \frac{P_v}{P_s} = \frac{J_{cv}}{J_{cs}}.$$

Using these relationships an intracellular concentration of 6 mM is obtained. On the other hand if only 30% of the passive flux permeates across the epithelium via the shunt, the estimated intracellular concentration is only 1 mM.

This line of reasoning cannot be used to obtain a precise estimate of the magnitude of the shunt pathway, because the values of P_v and P_s, used to estimate the intracellular concentration, were calculated ignoring the possible contributions of mem-

brane potentials to the anion fluxes. Although there is no substantial p.d. across the epithelium (Wright, 1972*a*), the cell interior, at least in the rabbit choroid plexus, is negative by about 65 mV compared with the external bathing solutions (Welch, 1967). Assuming that the constant field equation applies under these circumstances and that permeabilities are voltage independent, then the apparent permeability coefficients must be multiplied by a factor to account for the influence of the p.d. on the iodide fluxes across the cell membranes, i.e.

$$P = P \text{ (apparent)} \times \frac{EF/RT}{1-e^{EF/RT}}.$$

The factors for the iodide influx and outflux are 4·8 and 0·4 respectively. In the situation where 90 % of the passive permeation occurs via the shunt, the estimated intracellular I^- concentration drops to 1 mM, and with a 30 % shunt drops to 0·3 mM upon taking into account the presence of membrane potentials. Uncertainty remains as to the actual effect of membrane potentials on the I^- fluxes as the constant field assumption may, or may not, be applicable. Nevertheless, these theoretical results are consistent with the experimental results, despite these uncertainties about the magnitude of the shunt and the effect of the p.d.'s.

SCN^-, TcO_4^- and Br^-

The relationship between the accumulation of anions within the choroid plexus and transport across the epithelium can be further clarified by comparing the behaviour of I^-, SCN^-, TcO_4^- and Br^-. In the case of SCN^- both the T/M and the net transport across the tissue are lower than that for I^-, despite the fact that the unidirectional influx of the two anions into the tissue across the apical membrane (J_{vc}) are similar (see Tables 1 and 5, and p. 555). Computation of the unidirectional fluxes across each cell membrane, cf. Fig. 14, reveals that the major reason for the discrepancy is that the permeability of the apical membrane to SCN^- is about three times greater than the permeability to I^-. On the other hand, the relatively low net TcO_4^- flux across the plexus (Table 1), (despite the very high T/M (Table 4) and high value of J_{vc} (p. 555)) is accounted for by the low permeability of the baso/lateral cell membrane. Finally the low Br^- T/M and net flux across the tissue is due to the low active Br^- flux (J_{vc}) across the apical membrane. These conclusions confirm the view expressed earlier that it is invalid to assume that accumulation of solutes within the choroid plexus parallels either the rate of solute transport across the epithelium, or the rate of active transport into the cell.

Effects of ouabain and ClO_4^-

The addition of ouabain or ClO_4^- to the ventricular fluid blocks the net transport of anions across the choroid plexus (Figs. 2 and 5 and Table 2), the influx of I^- across the apical cell membrane (Fig. 10) and the accumulation of anions within the tissue. Analysis of the data shows that all these effects are due to the inhibition of the anion influx into the cell

across the apical cell membrane. J_{vc}, J_{cv} and J_{cs} were all reduced to the same degree while there was little or no effect on J_{sc}.

These results suggest the following mechanism for active anion transport across the frog choroid plexus: anions are actively transported into the choroidal epithelium by a ouabain sensitive pump located on the brush border membrane. The rate of the pumping depends on the concentration of anions within the ventricular fluid (c.s.f.), the K_m and the V_{max}. The anion concentration within the epithelium depends upon (i) the rate of anion pumping, (ii) the permeability of the apical cell membrane, (iii) the permeability of the baso-lateral cell membrane and (iv) the cell volume, the final step in the transport process being the passage of anions down their electrochemical potential gradient from the cell interior to the serosal compartment.

Relations between anion pumping and the Na/K pump

There is substantial, albeit circumstantial, evidence that I^- pumping across the apical cell membrane is directly, or indirectly, related to the activity of a Na/K exchange pump in the apical membrane of the epithelium. It will be recalled that Na^+ is actively transported across the choroid plexus from the serosal to the ventricular surface (Wright, 1972*a*). Mainly on the basis of thermodynamic consideration, it was postulated that Na^+ enters the choroidal epithelium from the serosal solution down the electrochemical potential gradient, and is subsequently pumped into the ventricular solution, by a ouabain sensitive Na/K exchange pump in the brush border membrane. Recently this model was given more weight by a biochemical and autoradiographic study that demonstrated ouabain was preferentially bound to the apical surface of the epithelium (Quinton *et al.* 1973).

There are at least five lines of reasoning to link anion pumping to this Na/K exchange pump: (1) the addition of ouabain to the ventricular solution inhibits *both* Na^+ secretion and anion absorption. Ouabain in the serosal solution is without effect in both cases; (2) ouabain binding to the apical cell membrane and the inhibition of both Na^+ and I^- transport follow the same time course; (3) both ouabain binding to the epithelium and the inhibition of I^- transport are irreversible; (4) I^- transport requires the presence of Na. Mannitol, Li^+, K^+ and choline$^+$ are unable to substitute for Na^+ in the external solutions. The cation selectivity, i.e. $Na^+ > Li^+$ or K^+, resembles that for Na^+ transport; (5) the presence of K^+ is required for I^- transport. Rb^+, and to some extent Cs^+, can mimic the action of K^+ but Li^+ cannot. This ionic specificity, i.e.

$$K^+ \sim Rb^+ > Cs^+ > Li^+,$$

is also very similar to that for the Na/K exchange pump in cell membranes. Furthermore, as expected, the effect of K^+ on I^- transport is only seen in the presence of Na^+, and high K^+ concentrations cause inhibition of I^- transport.

It is generally accepted that Na^+ transport across cell membranes, including the choroid plexus, is related to the activity of membrane bound Na/K ATPases (for reviews see Glynn, 1968; Bonting, 1970 and Skou, 1971). The hydrolysis of ATP by the membrane bound ATPase can be summarized by the reaction sequence:

$$\begin{array}{ccc} ATP + E_1 & \overset{Na,\,Mg}{\rightleftharpoons} & E_1 \sim P + ADP \\ \Big\downarrow\Big\uparrow & & \Big\downarrow\Big\uparrow{\scriptstyle Mg} \\ P_i + E_2 & \underset{K}{\rightleftharpoons} & E_2 \sim P + H_2O \end{array}$$

where ATP, Mg^{2+} and Na^+ are required on the internal surface of the cell membrane, and K^+ is required on the outer surface. ADP and P_i are liberated within the cell, and ouabain, which also acts from the external surface, can react with either E_2 or $E_2 \sim P$ to form ouabain-enzyme complexes. There is a remarkable similarity between the Na/K pump and the membrane Na/K ATPases, for example, the half-maximal activation concentrations of the cations are the same for both transport and the hydrolysis of ATP. Thus the correlation between active anion and cation transport in the choroid plexus may have two interpretations, namely (1) active anion transport is directly or indirectly coupled to active Na/K pumping, and (2) active anion transport is energized by the membrane bound Na/K ATPase system.

There are four classes of interaction that could account for the apparent relationship between anion and cation pumping.

(1) *The co-transport hypothesis.* There is a marked similarity between the active transport of anions across the apical cell membrane of the choroid plexus, and the active transport of amino acids and sugars across the brush border membrane of the intestinal epithelium (see Schultz & Curran, 1970), e.g. non-electrolyte accumulation within the intestinal epithelium depends upon the presence of Na^+ and is inhibited by cardiac glycosides. This coupling between Na^+ and non-electrolyte transport is frequently explained in terms of the co-transport theory. This proposes that Na^+ and the non-electrolytes enter the cell via a common carrier, and that the energy for the accumulation of the non-electrolytes within the cell is derived from the Na^+ electrochemical potential gradient across the brush border membrane. Thus, inhibitors such as ouabain are thought to act indirectly on non-electrolyte accumulation by dissipating the ionic gradients across the apical membrane. Such a theory might also explain

anion transport in the choroid plexus, but this seems improbable. Unlike sugar transport across the brush border of the intestinal epithelium, ouabain blocks the influx of anions across the apical surface of the choroid plexus. In the intestine it is envisaged that ouabain blocks the net transport of sugars and amino acids into the epithelium by increasing the backflux of the non-electrolytes out of the cell across the brush border membrane.

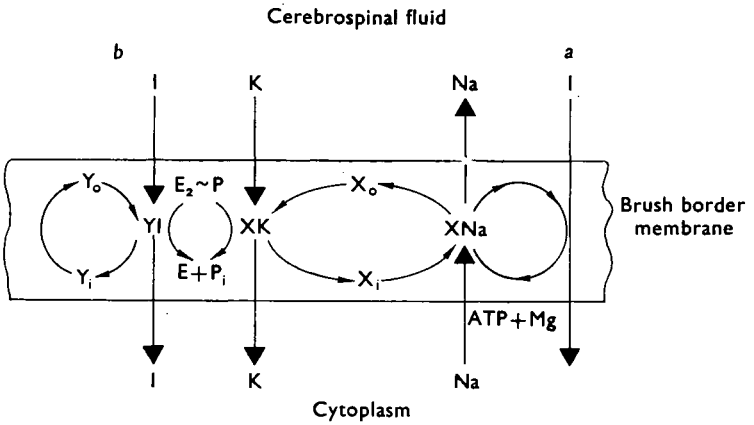

Fig. 12. Two models for active I^- transport across the apical membrane of the choroidal epithelium. I^- transport is linked either to the Na/K exchange pump (*a*), or to membrane Na/K ATPases (*b*). For further details see the text.

(2) *Electrical coupling.* At this juncture it is also appropriate to eliminate one other possible mechanism of I^- coupling to Na/K transport, namely, electrical coupling. The electrical potential difference across the apical membrane of the choroid plexus has the opposite polarity (-65 mV; Welch, 1967) to that required to draw anions into the epithelium against their electrochemical gradients. Furthermore, the influx would be expected to increase, not decrease, in the presence of inhibitors such as ouabain.

(3) *Linked sodium/iodide pumping.* There are two different kinds of interactions which are likely to explain the relationship between I^- pumping and the Na/K pump and/or Na/K ATPases. These are schematically shown in Fig. 12. The first (12*a*) is where the active I^- transport is coupled to the Na/K pump by some, yet unknown, mechanism. Such a direct coupling implies a stoichiometric relationship between I^- and Na^+ (K^+) pumping. The rates of Na/K pumping across the apical surface are not yet known, but these are at least equal to the rate of Na^+ pumping

across the plexus, namely, 1·5 μmol/cm^2 hr. The I^- flux is less than 10% of this (120 n mol/cm^2 hr), and so, if there is a linked Na/I pump the coupling ratio must be very small.

(4) *Na/K ATPase driven I^- pumping.* The second scheme shown in Fig. 12*b* is where the Na/K ATPase is directly coupled to an anion carrier system, i.e. the energy inherent in the $E_2 \sim P$ complex, formed during the hydrolysis of ATP by membrane bound Na/K ATPases, can be used to energize carrier mediated anion transport. The energy liberated could be used to bring about translocation of this anion carrier and reduce its affinity for anions at the interior of the cell membrane. This scheme is not unlike that proposed by Kimmich (1970) and Kimmich & Randles (1973) to account for sugar (and amino acid) transport across the intestinal brush border membrane (amino acids are also accumulated across the brush border membrane of the choroid plexus by a sodium dependent pump (Wright, 1972*b*)). Furthermore, in bacterial systems it is recognized that more than one transport process may be driven by a single reaction. For example, in *E. coli*, it has been shown that a number of amino acid and sugar carrier systems are coupled to a membrane bound D-lactate dehydrogenase (see Kaback, 1972). In this case carriers are thought to be electron transfer intermediates with specific binding sites which undergo reversible oxidation-reduction. In the oxidized state these have a high affinity for the substrate and upon reduction undergo possible conformational changes to cross the membrane and lower the affinity for the substrate. In animal cells the hydrolysis of $E_2 \sim P$ could provide the energy for carrier mediated transport across cell membranes.

In the case of anion transport across the apical membrane of the choroidal epithelium, such a mechanism could account for the relationship to cation transport and the Na/K ATPases, namely, with a common energy supply, maintained by the membrane bound ATPases, both cation and anion transport should be affected by those factors influencing the activity of the ATPases. Experiments are in progress to attempt to distinguish between these two models.

Anion selectivity

The results presented here demonstrate that both passive permeation and active transport of anions across the frog choroid plexus are selective processes. The passive permeability sequence, as judged from unidirectional fluxes from serosa to ventricle, was

$$I\ (1) > Br\ (0{\cdot}9) > SCN\ (0{\cdot}75) > ClO\ (0{\cdot}7) > TcO_4\ (0{\cdot}4).$$

This sequence is similar, but not identical, to the sequences observed in the thorium treated gall bladder (Machen & Diamond, 1972) and in

barnacle muscle fibres (Hagiwara, Toyama & Hayashi, 1971). The affinity of the choroid plexus pump for anions, as judged from fluxes and the competition effect, was

$$ClO_4 > ReO_4 > BF_4 > SCN \sim SeCN \sim I > NO_3 > Br > Cl.$$

This is similar to that reported by Becker (1961) and Welch (1962*a*) for the accumulation of anions within the rabbit choroid plexus.

The affinity of the pump for anions is, to a first approximation, correlated with the inverse of the anions free energy of hydration. Detailed analysis of the anion selectivity exhibited by biological systems provides strong evidence that Eisenman's theory of ion selectivity (see Diamond & Wright, 1969) can be extended to include the polyatomic anions (E. M. Wright, in preparation). (However, under some circumstances, steric factors may play an important role in determining polyatomic selectivity.) In Eisenman's terminology the active transport of anions across the choroid plexus is controlled by weak field strength sites, but the field strength is not so weak that the free energy of anion interaction with the membrane sites may be ignored.

Conclusion

In this study it has been demonstrated that anions are actively transported across the frog choroid plexus, and that this is linked to either a Na/K exchange pump, or a Na/K ATPase in the apical cell membrane. The anion pump has a wide specificity in that a large variety of anions are transported across the epithelium. Many of the characteristics of active anion transport across the plexus are shared by active transport processes in a variety of other cells and tissues. However, the active transport of anions by tissues such as the choroid plexus and thyroid gland appear to be quite distinct from passive anion transport in the red cell. For instance, (i) anions are not actively transported across red cell membranes (Tosteson, 1959); (ii) the selectivity of red cell anion transport, Cl > SCN > I (Wieth, 1972) is quite different from active anion transport; (iii) the 'K_m' of Cl^- transport across the red cell membrane is about three orders of magnitude greater than the 'K_m' for active iodide transport (see Gunn, Dalmark, Tosteson & Wieth, 1973); and (iv) 2,4,6-trinitro-*m*-cresolate does not inhibit active iodide transport although it is a potent inhibitor of anion transport in the red cell (Gunn & Tosteson, 1971).

However, there is a similarity between the two transport processes, in that phloretin inhibits anion transport in both the choroid plexus and red cell (see Gunn *et al.* 1973).

It is my pleasure to acknowledge Patricia Ilg for her meticulous technical assistance, and Drs Julio H. Moreno and Jared M. Diamond for valuable discussions

and their critical review of the manuscript. This investigation was supported by grants from the Life Insurance Medical Research Fund and the National Institutes of Health (USPHS NS 09666).

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