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THE EFFECTS OF SOME INHIBITORS AND ACCELERATORS OF SODIUM TRANSPORT ON THE TURNOVER OF ^{22}NA IN THE CEREBROSPINAL FLUID AND THE BRAIN

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SUMMARY

1. The purpose of the experiments was to discover whether the turnover of ^{22}Na in the c.s.f., which is largely determined by its rate of secretion, is affected in the same manner by inhibitors or accelerators of active transport as the turnover in the brain tissue since there is reason to believe that the composition of the extracellular fluid of brain is controlled by active processes.

2. Although acetazolamide (Diamox) inhibits rate of secretion of c.s.f. and the turnover of ^{22}Na in this fluid it does not appreciably affect the turnover of ^{22}Na in the brain tissue of either rat or rabbit, the small inhibition observed being probably secondary to the effects on the c.s.f.

3. Ouabain inhibits secretion of c.s.f. and turnover of ^{22}Na in this fluid, but it, also, has no effect on turnover of ^{22}Na in the brain tissue alone or in combination with Diamox.

4. Amphotericin B and amiloride, the anti-aldosterone spirolactone S.C. 114266, all inhibited secretion of c.s.f. without affecting turnover of ^{22}Na in the brain tissue; actinomycin D, puromycin and cycloheximide, however, had no effect on secretion of c.s.f.

5. Vasopressin inhibited secretion of c.s.f. and turnover of ^{22}Na in this fluid but *increased* the turnover in the brain by some 16 %.

6. In the ventriculo–cisternally perfused rabbit, replacement of 80 % of the NaCl in the perfusion fluid by choline chloride caused a slowing of the passage of ^{22}Na from blood into the perfusion fluid.

7. On the basis of these results it is concluded that the brain extracellular fluid is not renewed by appreciable bulk-flow, in contrast with the c.s.f.

INTRODUCTION

The cells of the central nervous system require for their adequate functioning a stable ionic environment, constituted by the extracellular fluid of this tissue; to some extent this is achieved by the blood–brain barrier, which tends to damp down the effects of variations in the composition of the blood plasma, but there is reason to believe that more active processes of control of the composition of the extracellular fluid are involved. Recent studies bearing on the relations between the cerebrospinal fluid (c.s.f.) and the extracellular fluid of the central nervous system have emphasized the similarity in their probable ionic make-up (see for example Wallace & Brodie, 1939; Davson, 1958, 1967; Pappenheimer, Fencl, Heisey & Held, 1965; Bito & Davson, 1966; Cohen, Gerschenfeld & Kuffler, 1968). Since this make-up is significantly different from that of a filtrate of blood plasma, this suggests that active transport mechanisms are required for elaborating both fluids. If this is, indeed, true, we may expect to find that the effects of inhibitors of ionic transport will be similar when these are measured on transport across the blood–cerebrospinal fluid barrier and across the blood–brain barrier.

In the present work we have examined the effects of a number of agents, known to affect the active transport of sodium, on the exchanges of isotopic sodium between the blood on the one hand, and c.s.f. and brain parenchyma on the other, in an attempt to determine whether the exchanges of the isotope between blood and the parenchyma are, indeed, controlled by similar mechanisms to those governing the exchanges between blood and the c.s.f. Earlier studies suggest that the penetration of sodium into the c.s.f. is primarily determined by a unidirectional flux from plasma across the choroidal epithelium, accompanied by water to give an isosmolar secretion. Thus the turnover of ^{22}Na in the normal c.s.f. in the rabbit has a rate constant of 0·0048 min^{-1}, i.e. some 0·48 % is renewed per minute (Davson, 1955); the rate of secretion of the fluid is 0·0088 ml. min^{-1} (Pollay & Davson, 1963) which, with a total volume of 1·8 ml., would give a renewal date of 0·48 %/min. Thus the net influx is almost completely accounted for by secretion of a fluid containing the same concentration as that in the existing fluid. A reduced rate of secretion of fluid should, on this basis, be measured by a reduced rate of passage of ^{22}Na into the c.s.f. from the blood. In general, however, there are two routes for exchange between plasma and c.s.f., namely this unidirectional flow from the choroid plexuses and, in addition, through diffusional exchanges between the c.s.f. and the adjacent parenchyma by way of the extracellular fluid (Fig. 1). According to the prevailing gradient of concentration, this latter process may lead to a net gain or loss of material by the c.s.f. The reason why the

net gains of ^{22}Na by the c.s.f. from the plasma are apparently completely accounted for by the unidirectional flow from the choroid plexuses is to be found in the absence of appreciable gradients of concentration between the extracellular fluid and c.s.f. during the approach to equilibrium; in other words, when a steady level of ^{22}Na was maintained in the plasma, the relative specific activities in c.s.f. and brain increased at approximately the same rate (Davson, 1955). With other substances, or under different experimental conditions, however, the net uptake by c.s.f. in a given time will depend on both influx through the choroid plexuses and exchanges with the adjacent brain tissue. This consideration applies to the studies

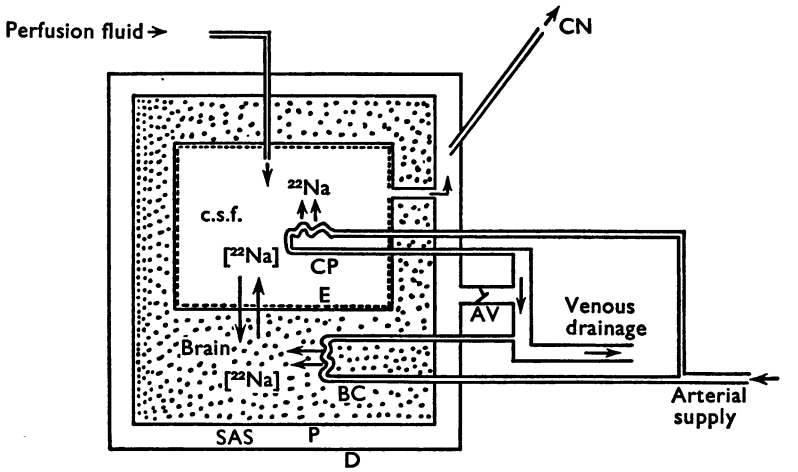

Fig. 1. A diagram of the routes of exchange of ^{22}Na between the blood, c.s.f. and extracellular fluid of brain. AV, arachnoid villi; BC, brain capillaries; CP, choroid plexuses; D, dura; CN, cisternal needle; E, ependyma; P, pia; SAS, subarachnoid space.

described in this paper; in most of these, the ventricular system was perfused at a rate high compared with the rate of secretion of c.s.f., and a steady level of ^{22}Na was maintained in the plasma; under these conditions the relative specific activity of the perfusion fluid was always less than that of the adjacent tissue, so that net influx was due to both the unidirectional flow from the choroid plexuses plus that due to exchanges with the tissue. In control animals it is possible to resolve the uptake by the perfusion fluid into two components; a relatively rapid initial uptake dominated by the unidirectional influx from the choroid plexuses, and a much slower one almost entirely determined by the influx from blood into the brain across the blood–brain barrier and thence across the ventricular walls into the perfusion fluid (Davson & Pollay, 1963). Under these condi-

tions, then, in which the ventricles are perfused with an artificial c.s.f. while a steady level of isotope is maintained in the plasma, the analysis of the effluent fluid and, at the end of the experiment, that of the brain, should enable us to measure the effects of inhibitors on the exchange processes between blood and c.s.f. on the one hand, and between blood and brain on the other. By including in the perfusion fluid a marker that is unable to diffuse out of the ventricular system across the ependymal walls, the rate of production of new fluid by the choroid plexuses may be assessed independently of measurements of turnover of ^{22}Na. Because it was desired to compare the effects of Diamox (acetazolamide) with those reported earlier (Davson & Luck, 1957; Koch & Woodbury, 1960) a number of experiments were carried out on the intact rat as well as the intact rabbit; under these conditions measurements of uptake by c.s.f. and brain in any animal were of necessity confined to single periods of time.

METHODS

General

Rabbits of mixed strains weighing 2·5–5 kg and rats of the hooded Lister strain weighing 250–350 g were used. Anaesthesia was induced by intravenous Nembutal, 30 mg/kg (intraperitoneal in rats), and supplemented by open-drop ether for surgery. Anaesthesia was maintained, so that the blink reflex was just abolished, by further injections of Nembutal. ^{22}Na was used for the majority of the experiments, but some of the later experiments were performed using ^{24}Na. Experiments in which both isotopes were injected into the same animal, and the ^{22}Na determined after decay of the ^{24}Na, gave the same results, thus confirming that the isotopes may be used indifferently. For simplicity in describing and discussing the results, the isotope will be described as ^{22}Na. A suitable activity was established in the blood by an initial injection and the level was kept constant, to within $\pm 3\%$, by infusing a solution at a gradually reducing rate. Blood samples were taken at 15 min intervals from the central ear artery of the rabbit and the femoral artery of the rat in order to monitor the level of the isotope. Drugs were administered, wherever feasible, on both sides of the blood–brain barrier, i.e. both ventricularly and intravenously. If a drug was rapidly metabolized, it was infused continuously into a carotid loop.

Drugs used

The route by which each drug was given is indicated by the following code: I/V, intravenous injection; V/C, ventriculo–cisternal perfusion, C/L, interarterial via carotid loop, I/V.P., intravenous continuous infusion.

Experiments with intravenous infusion of ^{22}Na

Drug	Route	Dose
Acetazolamide	I/V	50 mg/kg at 30 min before the start
(Diamox Lederle)		25 mg/kg at the start
Neptazane (Lederle)	I/V	100 mg/kg

Experiments with ventriculo–cisternal perfusion and intravenous infusion of ^{22}Na

Drug	Route	Dose
Ouabain (Diamox)	V/C	10^{-6} M
	I/V	100 mg/kg, V/C 20 mg/100 ml. mock c.s.f.
Ouabain + Diamox (Ouabain)	V/C	10^{-6} M
Spirolactone (SC 114266 Searle)	V/C	50 mg/100 ml. mock c.s.f.
	C/L	0·5 mg/min
Amphotericin B (Squibb)	V/C	1 mg/100 ml. mock c.s.f.
	I/V.P.	10^{-5} M solution ½ ml./min for 30 min then ¼ ml./min for rest of experiment
Amiloride	V/C	20 mg/100 ml. mock c.s.f.
	C/L	25 mg/kg. 15 ml. saline at 0·1 ml./min
Pitressin (Parke, Davis)	V/C	20 u./100 ml. mock c.s.f.
	C/L	0·3 u./min
Synthetic Vasopressin (Calbiochem)	V/C	20 u./100 ml. mock c.s.f.
	C/L	0·3 u./min
Actinomycin D (Calbiochem)	V/C	250 mg/100 ml. mock c.s.f.
	C/L	1 mg/min
Puromycin (Koch Light)	V/C	10 mg/100 ml.
	C/L	0·01 mg/min
Cycloheximide (Sigma)	V/C	100 mg/ml. mock c.s.f.

Intravenous infusion: intact animal

A steady level of ^{22}Na was maintained in the plasma and, after the appropriate interval, a sample of c.s.f. (0·8 ml. in the rabbit and 0·2 ml. in the rat) was withdrawn and the animal was decapitated; the brain was removed, lightly blotted and hand-homogenized; 1 g aliquots were taken for counting; it has been shown that, if the carotid arteries and the jugular veins are cut before decapitation, contamination of samples by blood is less than 1% (Davson & Spaziani, 1959; Cameron, Segal & Davson, 1969). The steady-state distribution of ^{22}Na between blood on the one hand and brain and c.s.f. on the other was determined by giving a single injection of isotope and removal of samples 36–48 hr later. No increase in the relative specific activity of brain was found between 36 and 48 hr, so that the former interval was used for most experiments.

Intravenous infusion + ventriculo–cisternal perfusion

This was performed as described by Davson & Pollay (1963), modified so that the perfusion was bilateral. The rate of perfusion was 60 µl./min, 30 µl. through each ventricle. The mock c.s.f. was composed of Na^+ 153, K^+ 2·81, Mg^{2+} 1·7, Ca^{2+} 2·18, Cl^- 131, SO_4^{2-} 1·7, PO_4^{2-} 1·48, HCO_3 27·4 m-equiv/l.; to 100 ml. of this was added 100 mg glucose and 100 mg Blue Dextran 2000 (Pharmacia). The animals were perfused through the ventricles 15 min before the cisternal cannula was inserted; during this time outflow was through the normal channels. After insertion of the cisternal cannula the system was allowed to drain for a further 15 min before a steady level of ^{22}Na was established in the blood. Drugs were always given from the beginning

of this 30 min period. Samples of the effluent perfusion fluid were collected during 10 min periods, except in the early stages of the experiment when the collection period was shorter.

Isotope counting

The ^{14}C was counted by liquid scintillation in an alcohol, toluene, PPO/POPOP mixture. A Beckman C.P.M. 100 counter was used. The sodium isotopes were counted by crystal scintillation in an Ekco N 683a well-type counter, to an accuracy wherever possible of 1 %.

Carbonic anhydrase activity

The carbonic anhydrase activity of blood and brain was estimated by the method of Philpot & Philpot (1936) modified by Kielin & Mann (1940) and by Ashby & Chan (1943).

Expression of results

The experimental parameters determined were the activities of ^{22}Na in c.p.m., in unit volume of plasma, c.s.f. or perfusion fluid and in unit weight of brain, together with the concentrations of Blue Dextran (or other marker) in the inflowing and outflowing perfusion fluid. Uptake of ^{22}Na into the brain was expressed as a percentage of the activity at infinite time:

$$\text{uptake } (\%) = R_t/R_\infty \times 100; \quad \text{here } R_t = \frac{\text{c.p.m./g brain}}{\text{c.p.m./g plasma dialysate}}.$$

R_∞ is the same ratio after 36–48 hr.

For computing the activity in the plasma dialysate, the experimentally determined Gibbs–Donnan ratio of 0·99 was used (Davson, 1955). When the uptake by c.s.f. was compared with that by brain, a comparable percentage was used. In perfusion experiments, rate of secretion of c.s.f. was computed from the steady-state dilution of Blue Dextran or other marker:

$$\text{flow} = \frac{C_{in} - C_{out}}{C_{out}} \times P,$$

where P is the rate of perfusion and C_{in} and C_{out} are the concentrations entering and leaving the ventricles. To describe uptake of ^{22}Na by the perfusion fluid, C_{out}/C_{pl} was plotted against time, where C_{pl} is the steady level in the plasma.

RESULTS

Measurement of the rate of secretion of the c.s.f.

If the ventriculo–cisternal system is perfused with a fluid containing a suitable marker, the rate of secretion of the natural fluid can be measured by the dilution of this marker in the effluent, when a steady state has been reached. The accuracy of the estimate will be reduced if the marker molecule can escape from the system by diffusion into the blood via the choroid plexuses and the brain parenchyma. Inulin has been used extensively in this work (Pappenheimer, Heisey, Jordon & Downer, 1962; Pollay & Davson, 1963), but Rall, Oppelt & Patlak (1962) have shown

that it can cross the ependyma, and Welch & Sadler (1966) have shown that the choroid plexus is also permeable to it; losses in this way would result in erroneously high values. A more suitable marker is the Blue Dextran 2000, which has a molecular weight of 2×10^6 and has a blue dye moiety firmly attached to the molecule. Ten experiments, in which both markers were present in the perfusion fluid, gave rates of secretion of $12{\cdot}0 \pm 1{\cdot}1$ (S.E.) and $8{\cdot}1 \pm 1{\cdot}0$ μl./min respectively according as the dilution of inulin or dextran was used as the experimental parameter. In all subsequent work dextran has been used as the indicator in assessing the rate of secretion.

TABLE 1. The steady-state distribution of ^{22}Na between plasma and c.s.f. ($R_{c.s.f.}$) and brain (R_{Br}), 36–48 hr after a single intravenous injection, together with water contents. $R = \frac{\text{c.p.m./g brain or ml. c.s.f.}}{\text{c.p.m./ml. plasma dialysate}} \times 100$. Limits are S.E. Numbers of experimental animals in parentheses

Species	$R_{c.s.f.}$	R_{Br}	H_2O content (g/100 g)
Rabbit	Control $107{\cdot}3 \pm 2{\cdot}2$ (7)	$36{\cdot}5 \pm 0{\cdot}3$ (7)	$78{\cdot}3 \pm 0{\cdot}7$ (5)
	Diamox $109{\cdot}4 \pm 1{\cdot}7$ (7)	$37{\cdot}7 \pm 1{\cdot}7$ (7)	$78{\cdot}5 \pm 0{\cdot}4$ (5)
Rat	Control $107{\cdot}6 \pm 2{\cdot}2$ (3)	$34{\cdot}0 \pm 0{\cdot}6$ (4)	—

Equilibrium distribution of ^{22}Na in plasma and brain

As indicated under *Expression of results*, the final steady-state distribution of ^{22}Na in normal and in treated animals is required. Results for the rabbit and the rat are shown in Table 1; it will be seen that the normal '^{22}Na-space' is some 36·5 % in rabbits and 34 % in rats; Diamox was without effect on this in rabbits, nor yet did it influence the total water-content of the brain.

Effect of Diamox on uptake of ^{22}Na in the intact animal

Earlier studies over periods of time from 15 min to 5 hr (Davson, 1955) had indicated that the penetration of ^{24}Na from the blood into the c.s.f. and brain followed a simple logarithmic course, so that the penetration during, say, 1 hr could be used as an index to the rate of equilibration. The results on the control and Diamox-treated animals are shown in Table 2. The reduction in rate of uptake by the c.s.f. of the rabbit is some 35–44 %, whilst the reduction in uptake by the brain, although considerably smaller, namely 15 %, is significant for the 30 min period, but not significant at the 60 min period ($P < 0{\cdot}01$). In the rat the decrease in uptake by the c.s.f. was 36 % and there was a small but statistically insignificant decrease in uptake by the brain. The present results thus confirm those of Davson & Luck (1957) in showing a considerable reduction in uptake by the c.s.f.; they differ

from this work in showing a small but statistically significant decrease in uptake by the brain in the rabbit (for the 30 min period). The absence of any significant decrease in uptake by the rat's brain is in conflict with the results of Koch & Woodbury (1960) on the nephrectomized animal, which showed both a decreased rate of uptake by brain and a decrease in the steady-state level reached after 24 hr.

TABLE 2. Effects of Diamox and Neptazane on the penetration of ^{22}Na from blood plasma into brain, R_{Brt} and cerebrospinal fluid $R_{c.s.f.t}$. Results are expressed as the percentage equilibration achieved in the stated time (*see Expression of results*). P is the probability that the observed difference would have occurred by chance

Species	Expt.	t	R_{Brt}	Change	P	$R_{c.s.f.t}$	Change	P
Rabbit	Control	30	$15{\cdot}4 \pm 0{\cdot}7$			$12{\cdot}3 \pm 0{\cdot}9$		
				-15%	0·01		-35%	$< 0{\cdot}001$
	Diamox	30	$13{\cdot}0 \pm 0{\cdot}2$			$7{\cdot}1 \pm 0{\cdot}6$		
Rabbit	Control	60	$25{\cdot}4 \pm 0{\cdot}9$			$24{\cdot}1 \pm 1{\cdot}0$		
				-15%	0·025		-44%	$< 0{\cdot}001$
	Diamox	60	$21{\cdot}7 \pm 1{\cdot}0$			$13{\cdot}5 \pm 1{\cdot}0$		
Rabbit	Control	60	$25{\cdot}4 \pm 0{\cdot}9$			$24{\cdot}1 \pm 1{\cdot}0$		
				-2%	0·7		-36%	$< 0{\cdot}001$
	Neptazane	60	$24{\cdot}9 \pm 1{\cdot}1$			$15{\cdot}1 \pm 1{\cdot}0$		
Rat	Control	60	$37{\cdot}7 \pm 0{\cdot}5$			$57{\cdot}9 \pm 1{\cdot}1$		
				-10%	0·05		-36%	
	Diamox	60	$34{\cdot}2 \pm 1{\cdot}5$			$37{\cdot}0 \pm 3{\cdot}0$		

The number of animals in each sample was six except for the estimates of $R_{c.s.f.t}$ in the rat, when the number was three owing to failure to withdraw suitable samples of c.s.f. before removing brain.

It may be argued that Diamox is ineffective in inhibiting transport across the blood–brain barrier because it penetrates only slowly into the brain (Maren, 1967), and therefore does not inhibit carbonic anhydrase activity of the brain fully. As Table 2 shows, however, Neptazane, a lipid-soluble carbonic anhydrase inhibitor, was no more effective in inhibiting uptake by the brain, in fact there was no significant reduction. Measurements of carbonic anhydrase activity by the isolated brain at the end of experiments in which Diamox and Neptazane had been given parenterally gave the following results: Diamox, 47% less than in the control brains; Neptazane, complete inhibition. When Diamox was given by combined ventriculo–cisternal and intravenous routes, as in the experiments to be described below, there was also complete inhibition.

Effects of Diamox and ouabain on the penetration of ^{22}Na into the perfused ventricles

In these experiments a steady level of ^{22}Na was maintained in the plasma, and the ventricles were perfused with an artificial c.s.f. containing no isotope; in Fig. 2 the rise in concentration of ^{22}Na in the perfusion fluid, measured by $R_{c.s.f._t}$ = concn. in fluid/concn. in plasma, has been plotted against time. The control curve shows the initial rapid rise, followed by

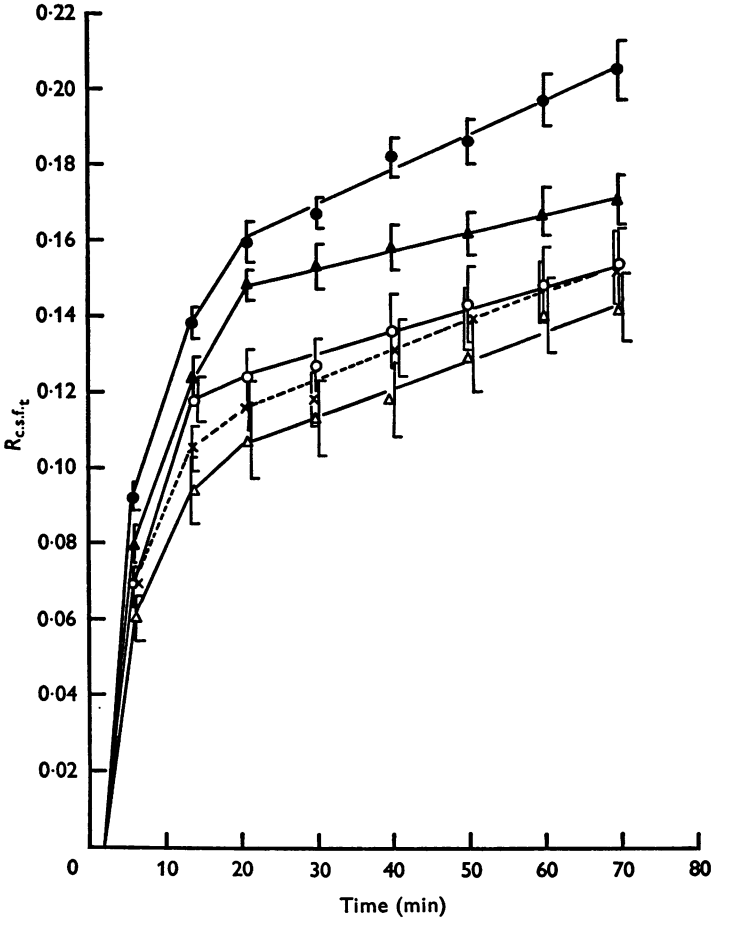

Fig. 2. The effect of Diamox, ouabain and inhalation of 18% CO_2 on the penetration of ^{22}Na from the blood into the ventriculo–cisternal perfusion fluid. Bars are twice S.E. of mean. Ordinates: concentration in emerging perfusion fluid/concentration in plasma. Abscissae: time (min) from beginning of intravenous infusion. ●, control; ▲, 18% CO_2; ○, ouabain; ×, Diamox + ouabain; △, Diamox.

the much slower rise which is determined by the passage of the isotope from blood into brain and secondarily into the ventricles. Both Diamox and ouabain reduce the initial rapid uptake, but have little apparent effect on the slope of the slower phase; a combination of the drugs seems no more effective than single application.

TABLE 3. Effects of various inhibitors on the rate of secretion of c.s.f. Limits are S.E. n equals number of animals. P is the probability that the observed difference would have occurred by chance.

Species	Inhibitor	n	Secretion rate (μl./min)	Inhibition (%)	P
Rabbit	Control	23	$12{\cdot}9 \pm 0{\cdot}7$		
	Diamox	10	$4{\cdot}6 \pm 1{\cdot}1$	64	$> 0{\cdot}001$
	Ouabain	6	$5{\cdot}8 \pm 0{\cdot}7$	55	$> 0{\cdot}001$
	Diamox + ouabain	7	$4{\cdot}0 \pm 0{\cdot}5$	69	$> 0{\cdot}001$
	Spirolactone	5	$3{\cdot}7 \pm 0{\cdot}8$	71	$> 0{\cdot}001$
	Amiloride	8	$6{\cdot}4 \pm 0{\cdot}9$	50	$> 0{\cdot}001$
	Amphotericin	10	$3{\cdot}8 \pm 0{\cdot}8$	70	$> 0{\cdot}001$
	Vasopressin	8	$6{\cdot}4 \pm 0{\cdot}7$	50	$> 0{\cdot}001$
	Choline chloride	6	$9{\cdot}6 \pm 1{\cdot}1$	26	0·05
	18% CO_2	5	$10{\cdot}0 \pm 1{\cdot}6$	22	0·1
	Puromycin	5	$11{\cdot}0 \pm 1{\cdot}0$	8	0·3
	Actinomycin D	5	$12{\cdot}7 \pm 1{\cdot}4$	1	0·9
	Cycloheximide	5	$10{\cdot}4 \pm 1{\cdot}4$	19	0·2
Rat	Control	6	$2{\cdot}1 \pm 0{\cdot}9$		
	Diamox	3	$0{\cdot}9 \pm 0{\cdot}1$	57	0·02

In these experiments the rates of secretion of c.s.f. were measured by dilution of Blue Dextran, whilst at the end of the perfusion period the brain was analysed for ^{22}Na, so that the effect of the drugs on net uptake by the brain during a period of 75 min was measured. The results on rates of secretion are shown in Table 3, and it will be seen that ouabain, as well as Diamox, reduces the rate of secretion of c.s.f.; and this accounts for the reduced rate of rise of activity in the early phases of the curves of Fig. 2. The failure to influence the slower phases of uptake by the perfusion fluid suggests a failure to influence the penetration into the brain, and the analyses of the brain, shown in Table 4, confirm this. A better comparison of the slow phases of penetration is given by a semi-logarithmic plot, in which the ordinates are the difference between the ratio C_{out}/C_{pl} at the steady state, R_∞, and the corresponding ratio at a given time, t, namely R_t. Because of the reduction in rate of secretion caused by the inhibitors, the value of R_∞ will be lower than that in controls. To determine both control and experimental values of R_∞ requires very lengthy perfusion, so that it is preferable to 'work backwards', the animal being injected with ^{22}Na some 36 hr before perfusion; after this period the brain

TABLE 4. Effects of various inhibitors on the penetration of ^{22}Na from the blood into the brain during 75 min of ventriculo–cisternal perfusion. Limits are S.E. *n* equals number of animals. *P* is the probability that the observed difference would have occurred by chance

Inhibitor	*n*	Equilibration achieved at 75 min (%)	Difference (%)	*P*
Control	23	24·10 ± 0·96		
Diamox	5	22·26 ± 1·37	−8·3	0·25
Ouabain	6	21·62 ± 1·85	−10·3	0·15
Diamox + ouabain	7	21·16 ± 0·94	−12·2	0·05
Spirolactone	5	21·24 ± 0·86	−11·9	0·10
Amiloride	7	24·19 ± 1·62	−0·4	0·75
Amphotericin B	9	23·78 ± 0·79	−1·3	0·5
Vasopressin	7	28·47 ± 1·57	+18·1	0·08
Choline	6	20·80 ± 1·41	−13·7	0·05
18% CO_2	5	24·2 ± 1·90	+0·4	
Puromycin	5	24·0 ± 1·1	−0·4	0·2
Actinomycin D	5	21·0 ± 0·9	−12·9	0·1
Cycloheximide	5	23·4 ± 1·8	−0·3	0·5

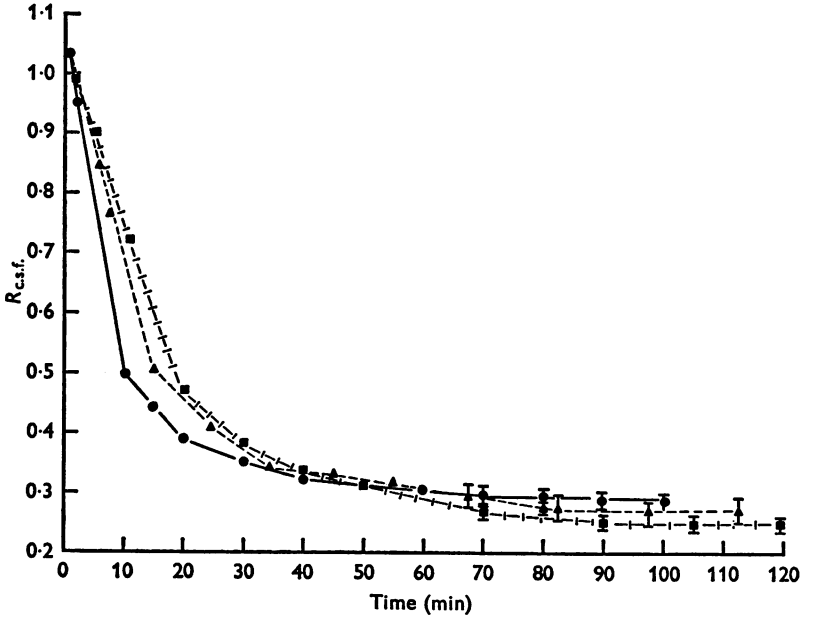

Fig. 3. The efflux of ^{22}Na from the brain and c.s.f. into the ventriculo–cisternal perfusion fluid. Each curve is the mean from four animals, which were loaded with ^{22}Na for 36 hr before the perfusion. Ordinates and abscissae same as for Fig. 2. ●, control; ■, ouabain; ▲, Diamox.

is in equilibrium with plasma and, on perfusing with a 'cold' perfusion fluid, the concentration in the effluent falls from a high initial value (because the ^{22}Na in the first drops of fluid has the same activity as that in the plasma) to a final steady-state value (Davson & Pollay, 1963). The curves for control, Diamox- and ouabain-treated animals are shown in Fig. 3, the points being the means of four experiments; the approximate steady-state values were 0·29 for control, 0·275 for Diamox-treated and 0·25 for ouabain-treated animals. The smaller values for the treated

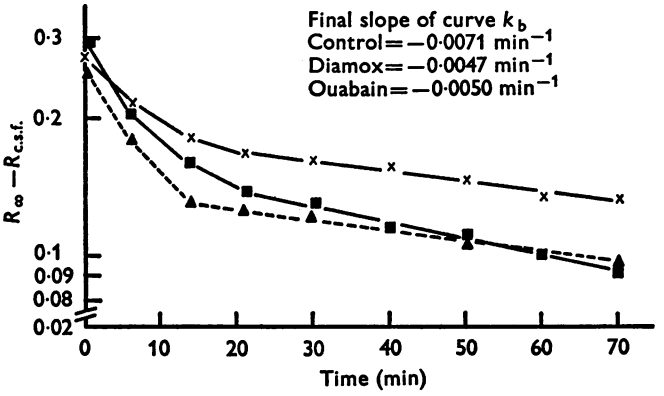

Fig. 4. Effect of Diamox and ouabain on the rate of penetration of ^{22}Na from the blood into the ventriculo–cisternal perfusion fluid. This is a semi-logarithmic plot of the results shown in Fig. 2 to determine the slope of the final part of the penetration curve, which represents the slower phase of penetration from the brain extracellular space. Ordinates and abscissae as in Fig. 2. ■, control; ×, Diamox; ▲, ouabain.

animals are to be expected, in view of the reduced rates of secretion, but the differences are probably not statistically significant. The computed ratios, based on inserting the measured parameters into eqn. (3) of Davson & Pollay (1963), are 0·31, 0·245 and 0·255 for control, Diamox-, and ouabain-treated animals respectively.

In plotting the results semi-logarithmically, as in Fig. 4, the experimentally determined values were used. The slopes for the late phases of penetration in controls and treated animals are not significantly different, since a value of $P > 0·3$ was obtained; this value was derived from a t test on the slopes of all individual experiments, and not from the mean curves plotted. Thus the results of these perfusion experiments are consistent with the studies on the intact animal in showing a reduced rate of secretion of c.s.f. caused by Diamox.

As we shall see in the Discussion, they are consistent with an absence of significant effect on the blood–brain barrier to ^{22}Na, the small reduction in

uptake by the brain in both the intact and perfused animal (Tables 2, 4) being due to loss from brain to the c.s.f. or perfusion fluid, resulting from the reduced influx in the secretion.

Unilateral treatment with ouabain. To improve the precision of the comparison between control and ouabain-treated animals, one lateral ventricle was perfused with artificial c.s.f. and the other with the same fluid containing 10^{-6} M ouabain. The penetration of ^{22}Na from blood into both cerebral hemispheres was compared, but there was no significant difference between the two sides (Table 5).

TABLE 5. Effects of unilateral treatment of brain with 10^{-6} M ouabain. Artificial c.s.f. was perfused into one lateral ventricle and the same fluid containing 10^{-6} M ouabain into the other. A steady level of ^{22}Na was maintained in the plasma and, after 120 min, the brain was removed and divided sagitally into halves. The activity of ^{22}Na in each (R_{Brt}) is expressed as the percentage of the steady-state value achieved within this period. Each value is the mean of five animals. Limits are the S.E. of the mean difference.

	Control half	Ouabain half
R_{Brt}	27·7 %	30·4 %
Mean difference	$2·6 \pm 1·5$	

The probability that this value differs significantly from zero is 0·2.

Effects of hypercapnia. Since Diamox causes the animal to become acidotic (Maren, 1967; Brzezinski, Kjällquist & Siesjö, 1967) by the inhibition of carbonic anhydrase, the effects of a hypercapnia-induced acidosis were studied. Eighteen per cent CO_2 was given across a T-shaped tracheal cannula at 5 l./min; this volume of gas would give a blood pH of 7·1–7·2, just slightly more acidotic than with Diamox (Maren, 1967; Cameron *et al.* 1969). Table 3 shows that the hypercapnia reduced the rate of secretion by a statistically insignificant amount, and Table 4 shows that it had no effect on the uptake by the brain. The penetration into the perfusion fluid has been included in Fig. 2; the flatter slope of the late phase suggests a diminished rate of penetration into brain but this is not borne out by the analyses of Table 4. It is unlikely, then, that the effects of Diamox on rate of secretion of c.s.f. are due to the acidosis *per se*, whilst the absence of a significant increase in uptake by brain suggests that sodium does not participate in the increased permeability of the blood–brain barrier induced by hypercapnia, as measured with slowly penetrating substances such as iodinated serum albumin (Cutler & Barlow, 1966), and sucrose (Cameron *et al.* 1969).

Effects of other pharmacological agents

Amphotericin B. As Table 4 shows, this antibiotic caused no significant increase in uptake by the brain, whilst the rate of secretion of c.s.f. was markedly reduced (Table 3). The curve of penetration into the ventricular perfusion fluid (Fig. 5) is consistent with these findings.

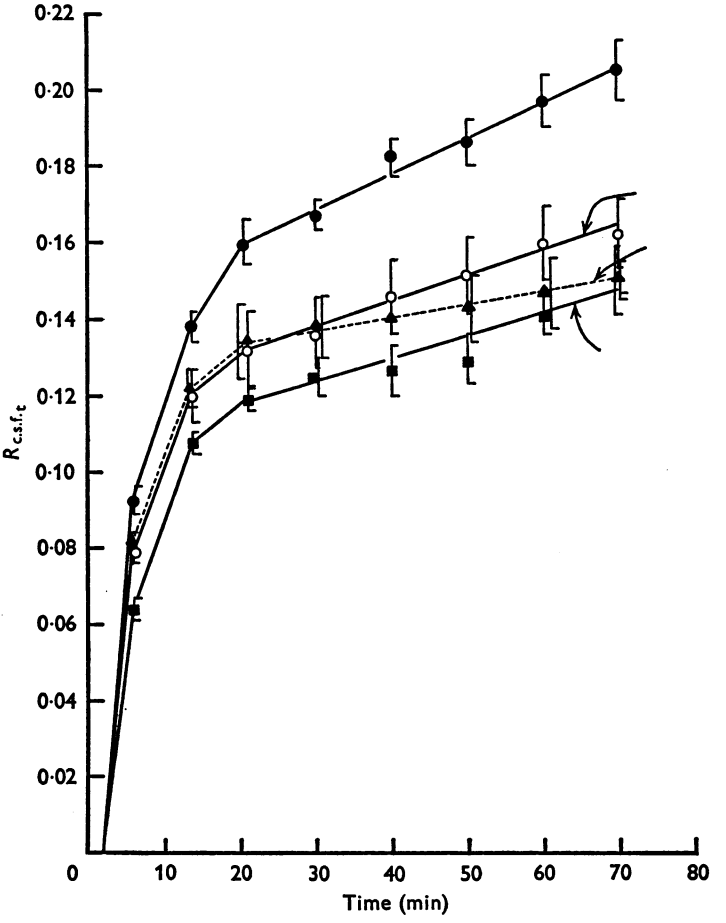

Fig. 5. Effects of amiloride, spirolactone, and amphotericin B on the penetration of ^{22}Na from the blood into the ventriculo–cisternal fluid. Ordinates and abscissae as in Fig. 2. ●, control; ○, spirolactone; ▲, amiloride; ■, amphotericin B.

Amiloride. This compound is a potent diuretic and inhibits sodium transport across the toad bladder (Bentley, 1968). When this drug was given intravenously, and into the fluid perfusing the ventricles, it had

little effect on sodium turnover; however, when given directly into a carotid loop, rate of secretion of c.s.f. was inhibited by 50 % (Table 3) but, as with other inhibitors of secretion, there was no significant change in uptake by the brain. The effects on penetration of ^{22}Na into the perfused

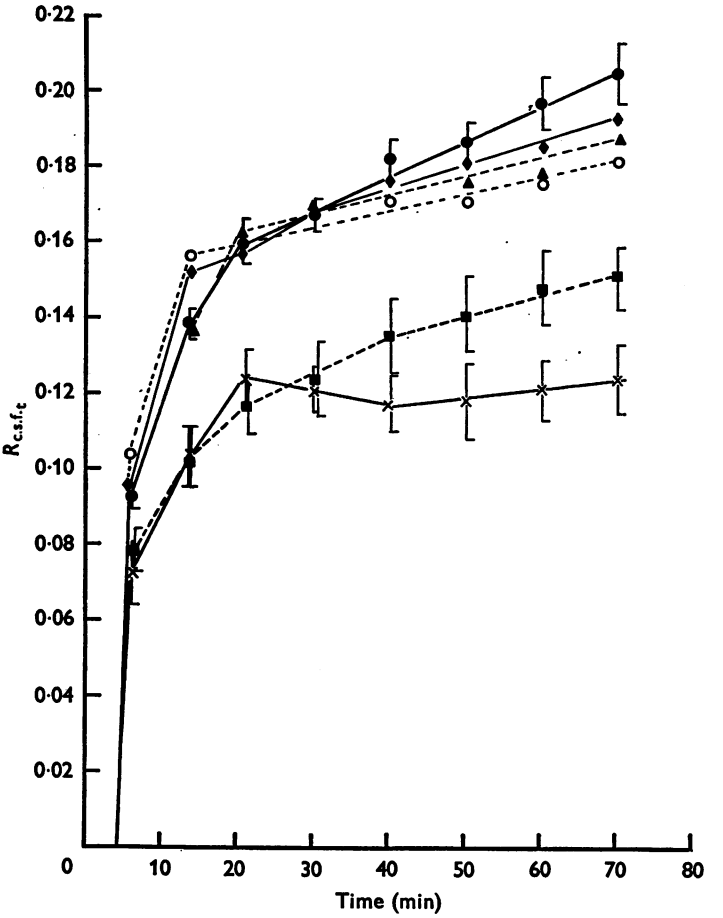

Fig. 6. The effect of vasopressin and of the replacement of 80 % of the sodium chloride by choline chloride on the rate of penetration of ^{22}Na from the blood into the ventriculo–cisternal perfusion fluid. Included are the results of experiments using inhibitors of protein synthesis. Ordinates and abscissae as for Fig. 2. ●, control; ◆, puromycin; ▲, actinomycin D; ○, cycloheximide; ■, vasopressin; ×, choline chloride.

ventricles are included in Fig. 5; the slowing of the early phase of uptake is consistent with the diminished rate of secretion, whilst the flatter slope of the later phase would suggest a diminished penetration through the

blood–brain barrier, which, however, is not confirmed by the analyses of the brain at the end of the experiments (Table 4).

Aldosterone inhibitors. The fluorinated spirolactone, S.C. 114266, was administered via a carotid loop to enable a sufficient concentration of drug to reach the choroid plexuses. It caused a 68% reduction in the rate of secretion of c.s.f. (Table 3), whilst there was only an insignificant decrease in uptake by the brain (Table 4). The curve of penetration into the perfused ventricles is included in Fig. 5, and both phases of penetration are consistent with these findings.

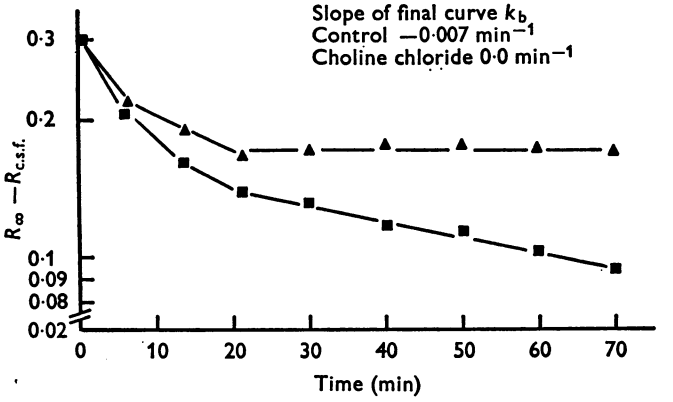

Fig. 7. A semi-logarithmic plot of the effect of replacement of 80% of the sodium chloride by choline chloride on penetration of ^{22}Na from blood into the perfusion fluid, demonstrating the marked reduction in the slow phase of penetration. Ordinates and abscissae same as for Fig. 2. ■, control; ▲, choline chloride.

Inhibitors of protein synthesis. If aldosterone acted by the same mechanism postulated for amphibian tissues, it would be reasonable to assume that inhibitors of protein synthesis (Clark & Yen Chang, 1965) might also block sodium transport into the rabbit central nervous system. Actinomycin D, puromycin and cycloheximide were administered by intravenous, carotid loop, and intraventricular routes for various periods of time, but no inhibition of secretion rate or of penetration of ^{22}Na into the c.s.f. and brain was detected (see Tables 3, 4).

Vasopressin. Pitressin was infused into a carotid loop. The initial phase of penetration of ^{22}Na into the ventricular perfusion fluid was slowed (Fig. 6), as was the rate of secretion (Table 3); but, as Table 4 shows, the uptake into brain from blood was *increased* by 16% ($P = 0{\cdot}08$). The use of Pitressin is complicated by the fact that 0·5% chlorbutol is included as a bacteriostat, so the experiments were performed using a synthetic vasopressin without any added bacteriostat; however, the same results

were obtained. Vasopressin does cause a raised blood pressure during the infusion, but this is not excessive, i.e. normal mean blood pressure in an anaesthetized rabbit was between 80 and 120 mm Hg, while that in animals receiving vasopressin was between 100 and 140 mm Hg, so that, in view of the general independence of central nervous blood-flow from arterial blood pressure, it is unlikely without further evidence that the raised blood pressure was a factor.

Partial replacement of NaCl by choline chloride. Fig. 6 shows the effect of replacing 80% of the sodium in the fluid perfusing the ventricles by choline chloride; there is a slowing of the penetration of ^{22}Na into the ventricles accompanied by a small inhibition of secretion, as measured by the dextran dilution (Table 3); semi-logarithmic plotting of the course of penetration into the perfusion fluid, as in Fig. 7, shows that the slope of the later phase is approximately zero, but the reduction in brain uptake is barely significant statistically (Table 4).

DISCUSSION

The purpose of this work has been to examine the turnover of sodium in the c.s.f. and the brain parenchyma in order to determine whether the two processes are governed by the same secretory mechanisms; to this end, the effects of inhibitors and accelerators of sodium transport in better-defined experimental systems, such as the toad bladder, have been measured. As indicated earlier, the turnover of any compound in either compartment cannot be viewed in isolation, since exchanges between the two must take place normally, and these will be accentuated if turnover in one or the other is inhibited independently. So far as sodium is concerned, the earlier study of Davson (1955) indicated that passage of the isotope from blood to the two compartments was well matched, so that during the approach to equilibrium it seems unlikely that appreciable gradients of activity between c.s.f. and brain are present.

With inhibition of the blood–c.s.f. barrier, however, gradients between the compartments will appear during the approach to equilibrium, and an appreciable amount of the influx into the brain may now pass into the c.s.f. This will give the specious appearance of an inhibition of the blood–brain transport.

With this point in mind we may briefly review the essential results of this work. So far as Diamox is concerned they confirm the earlier work of Davson & Luck (1957) and Fishman (1959) in demonstrating a considerable reduction in the rate of penetration of ^{22}Na into the c.s.f., and this reduction is associated with a larger reduction in rate of secretion of the fluid, as measured by the dilution of dextran in the perfused ventricles.

If, as suggested earlier, the influx of ^{22}Na is determined by a unidirectional flow in the newly secreted fluid, the change in the rate constant for penetration of ^{22}Na into the c.s.f. might be expected to be equal to the change in its turnover constant for the fluid as a whole. However, the rate constant for ^{22}Na is reduced by only about 35% compared with a 65% inhibition of secretion rate, but the discrepancy may well be due to the establishment of concentration gradients of ^{22}Na between brain and c.s.f. resulting from the failure of Diamox to inhibit appreciably the penetration into the brain across the blood–brain barrier. Thus, as we have seen, experiments on the intact animal suggest that penetration into the brain is either unaffected by Diamox (in the rat) or is reduced by a relatively small amount, as found in the rabbit. This small reduction in uptake of ^{22}Na by the brain may well have been secondary to the reduced penetration into the c.s.f., and the consequent gradients favouring passage from brain into c.s.f. Hence, so far as the intact rabbit is concerned, the results are consistent with a strong inhibition of secretion of c.s.f. with no effect on passage of ^{22}Na into the brain. The experiments with ventriculo–cisternal perfusion agree well with this conclusion, the slow phase of penetration from blood into the perfusion fluid, whose rate is determined by the passage from blood into brain and thence into the ventricles, being unaffected by Diamox.

In the rat, the finding that Diamox causes a small decrease in the rate of uptake by the brain agrees with the findings, but not the interpretation, of Koch & Woodbury (1960) on the nephrectomized animal. The rate of turnover of c.s.f. in the rat is considerably higher than in the rabbit, if this is expressed in terms of volume flow per unit weight of brain, i.e. 2·5 μl./min. g compared with 1 μl./min.g, so that we should expect an inhibition of secretion of the c.s.f. to be reflected in a decrease in net uptake by the brain, due to the losses from brain to c.s.f. that will presumably occur because of the gradients of ^{22}Na activity that follow from the inhibition of secretion. The observed reduction in net uptake by the brain can probably be accounted for on this basis.

The results on the rate of secretion of c.s.f. obtained with the other pharmacological agents are largely what might be expected of a process governed by an active transport of Na^+; ouabain, for example, reduced the rate of secretion of c.s.f. by some 55% and, when combined with Diamox, caused no greater inhibition than that caused by Diamox alone. The failure to achieve more than 70% inhibition by any of the agents employed might suggest that the residual 30% of fluid production occurred by a process independent of active transport of Na^+, possibly by passive filtration through the choroid plexuses. The striking effect of the anti-aldosterone drug (spirolactone) likewise brings the process of secretion of

c.s.f. into line with other systems involving active transport of Na^+, although the failure of inhibitors of protein synthesis to affect either the rate of secretion of c.s.f., or transport of ^{22}Na in this fluid, is in contrast to the studies of Crabbé & de Weer (1964) on the toad bladder. This could, however, be more a problem of lack of penetration of these substances and failure to reach a sufficient concentration at the site of action (i.e. the cell nucleus or the ribosome) to cause a detectable inhibition. In contrast to the above findings, a similar anti-aldosterone drug, spironolactone, has been reported by Domer (1969) to increase the rate of secretion of the c.s.f. in the cat.

The behaviour of vasopressin in the cerebral system is in marked contrast to that in amphibian skin or gall bladder, since it causes a 50% decrease in rate of secretion of c.s.f. and, corresponding with this, a marked slowing of the rate of penetration of ^{22}Na into the perfused ventricles. This difference may be related to the structure of the choroid plexus since the flow of fluid occurs from base to apex of the epithelial cells, by contrast with the other tissues. In these other tissues, vasopressin is supposed to cause an increased permeability of the apical membrane to Na^+, and thus to favour transport into the cell and thence across the basal membrane by active processes. If vasopressin increased the permeability of the apical membrane of the choroid plexus epithelial cell, this would not necessarily favour transport of Na^+ out of the cell; in fact it might well do the reverse, the increased passive permeability militating against the efficiency of the active transport processes, presumably, in this case, operating across the apical membrane. The penetration of ^{22}Na into the brain was increased a little by vasopressin.

Amphotericin B has been shown by Lichtenstein & Leaf (1965) to increase sodium transport across the amphibian skin and bladder by virtue of an increase in the passive permeability of the epithelial cells to sodium at their outward facing sides; in the present work it *inhibited* the secretion of c.s.f., and the turnover of sodium in this fluid.

Amiloride, a diuretic and a potent inhibitor of sodium transport in the toad öocyte (Bentley, 1968), was only effective if given by arterial infusion into a carotid loop. Replacement of 80% of the Na^+ in the ventricular perfusion fluid by choline reduced the rate of secretion of c.s.f. by some 26%; this may well be related to the so-called substrate-facilitated transport in which the rate of transport of a labelled amino acid, for example, is accelerated by the presence of unlabelled molecules on the *trans*-side of the membrane, or by other molecules that share the same carrier mechanism (Levine, Oxender & Stein, 1965). In addition to slowing rate of secretion, and therefore the initial phase of penetration, there was a virtually complete blockade of the late slow phase, suggesting, if the analysis of

Davson & Pollay (1963) is correct, that passage of ^{22}Na from blood to brain is inhibited. However, measurements on the brain showed no reduction in uptake of the isotope at the end of the perfusion period. At the moment we have no explanation for this contradiction; the nature of the experiment restricted the analysis of the uptake by brain to one point in time, namely the end of the 75 min perfusion period; it may be that a knowledge of the uptakes at earlier periods would throw more light; thus there might have been an accelerated penetration of ^{22}Na into the brain in the early phase followed by more or less complete cessation in the later phase.

In general, the results may be summarized by stating that many of the various pharmacological agents examined have decreased the rate of secretion of c.s.f.; and this has been reflected in a reduced rate of turnover of ^{22}Na in the fluid. By contrast, the same agents have left the turnover of ^{22}Na in the brain parenchyma virtually unaffected; and this finding has an important bearing on the problem of the formation and possible circulation of the extracellular fluid of the central nervous tissue. As indicated earlier, there can be little doubt that the composition of the extracellular fluid is very similar to that of c.s.f. and, unless we assume that the c.s.f. imposes its own concentration on a passively formed extracellular fluid, this means that the extracellular fluid is a secretion with its composition determined by various pumps, Na^+, K^+, Cl^-, etc., acting across the blood–brain barrier, which is presumably the capillary endothelium (Reese & Karnovsky, 1967). If we accept that these pumps do, indeed, control its composition, we might go further and suggest that they, like the choroidal epithelial cell pumps, also cause a bulk-flow of fluid, secondary to the active transport of such solutes as Na^+; and some apparent support for this is provided by experiments by Bering & Sato (1963) on the dog's perfused ventricles, and those of Pollay & Curl (1967) on the rabbit's perfused aqueduct of Sylvius. To confine attention to the rabbit, Pollay & Curl found that fluid perfused through the aqueduct, without access to any choroid plexus, was diluted by fluid from the adjacent brain tissue; and, on the basis of an estimate of the total ependymal surface, they concluded that one third of the total measured production of c.s.f., approximately 4 μl./min, was derived from this non-choroid plexus source. They found, furthermore, that Diamox reduced the apparent flow from this source by some 60 %, suggesting a similar mode of formation. Our present results, showing no inhibition of turnover of ^{22}Na in the brain that could not be attributed to the reduction in choroid plexus secretion, are hard to reconcile with this claim of Pollay & Curl's. Thus the ^{22}Na-space of a rabbit's brain is approximately 33 %, so that with a 6 g brain there would be a pool of isotonic Na^+ of some 2000 μl., and if 4 μl./min of this fluid were

added to the c.s.f. and replaced from plasma, this would give a turnover, or renewal, rate of 0·2 % per min, i.e. a rate constant of 0·002 min^{-1}. The total turnover rate for ^{22}Na in the brain is approximately 0·004 min^{-1} (Davson, 1955) so that a half this turnover would be caused by the flow of extracellular fluid described by Pollay & Curl, whilst the remainder might be represented by a diffusional exchange not involving bulk-flow of fluid. If Diamox reduced the flow of 4 μl./min by 60 %, as found by Pollay & Curl, it would fall to rather less than 2 μl./min, and the turnover of ^{22}Na would fall by approximately 25 %; such a decrease should have been detected by the methods employed in this study.

It could be argued that the total Na^+-space of brain is made up of an intracellular and extracellular compartment, so that the renewal rate of the extracellular fluid would be considerably faster than given by the turnover rate in the whole pool of Na^+; this is probably true, since recent estimates for the rabbit suggest an extracellular space of 10–12 % (Oldendorf & Davson, 1967), but the kinetics of turnover of ^{24}Na in the brain, so far as they have been studied, do not reveal any obvious separation into two components, so that the pool may be considered kinetically as a single entity. This means, essentially, that if there is a bulk turnover corresponding to 4 μl./min, the ^{22}Na entering the extracellular space in this turnover exchanges relatively rapidly with the intracellular Na^+; thus the turnover of ^{22}Na in the brain has a rate constant of about 0·005 min^{-1} and this will be true of the rate of turnover of ^{22}Na in the extracellular fluid, but the bulk turnover of the extracellular fluid, expressed as a percentage value, will be some three times this because of its smaller volume.

It would seem, on the basis of the present results, that exchanges of ^{22}Na across the blood–brain barrier are not the result of unidirectional flow of Na^+, as seems to be true of the choroid plexuses, but are diffusional, in the same way, for example, that ^{22}Na in the plasma exchanges with Na^+ in the red cells under steady-state conditions. Such a system does not preclude the presence of a Na^+ pump across the blood–brain barrier, a pump that would probably have to be postulated by virtue of the rather higher concentration of Na^+ in the extracellular fluid than can be accounted for by a passive distribution (on the assumption that the extracellular fluid is similar in composition to the c.s.f.). But inhibitors of the Na^+ pump in this event need produce little measurable effect on the exchanges of ^{22}Na between blood and brain. Thus the extracellular fluid of the brain would be stagnant, relying on diffusion for the net flow of metabolites, etc., from cells to blood plasma, and vice versa.

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