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Cerebrospinal Fluid Formation and Absorption and Transport of Iodide and Sulfate from the Spinal Subarachnoid Space

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INTRODUCTION

With introduction of the ventriculocisternal perfusion technique (ADAM *et al.* 1938) and its refinement and development (LEUSEN 1948; PAPPENHEIMER *et al.* 1962), numerous observations have been made concerning the rate of cerebrospinal fluid (CSF) formation and absorption (HEISEY *et al.* 1962; BERING AND SATO 1963; POLLAY AND DAVSON 1963; CALHOUN *et al.* 1967) and the transport of various organic (PAPPENHEIMER *et al.* 1961) and inorganic anions (DAVSON *et al.* 1962; DAVSON AND POLLAY 1963; CUTLER *et al.* 1968b) out of the CSF. To a large extent, most of these studies have been restricted to perfusions of ependymal-lined ventricles and choroid plexus resulting in limited knowledge concerning CSF dynamics in the non-ependymal-lined cavities such as the cranial and spinal subarachnoid spaces.

The study of BITO *et al.* (1966) represents one of the few studies in which an attempt was made to perfuse the cranial and spinal subarachnoid compartments. Perfusions were conducted either from the cisterna magna to the cranial subarachnoid space or from the lumbar subarachnoid compartment to the cisterna magna. However, the authors did not specify whether perfusions were limited to either of these compartments. Thus, from the results obtained during cranial subarachnoid perfusions it could not be determined whether the perfusion fluid came in contact with ependyma of the choroid plexus or ventricles. No results were given for the spinal subarachnoid

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perfusions. SATO AND BERING (1967) isolated the cranial subarachnoid compartment from the ventricular compartment by injecting Kaolin into the cisterna magna. Perfusion of the cranial subarachnoid compartment revealed that at least 40% of the CSF formed by dogs originated from this compartment. Finally, COBEN AND SMITH (1969) investigated iodide clearance, CSF formation and absorption during perfusion of the spinal subarachnoid compartment isolated by laminectomy or extradural ligation. While this approach assured isolation of the spinal subarachnoid space, it is possible that under these circumstances circulation to the spinal cord was compromised.

In this study we attempted to perfuse the spinal subarachnoid space under more physiological conditions in order to extend our knowledge of CSF dynamics within the subarachnoid space. Measurements included the rates of formation and absorption of cerebrospinal fluid and the clearance of iodide and sulfate from the spinal subarachnoid space. Values obtained were compared with similar data obtained during ventriculocisternal perfusion.

METHODS

General procedure

Three types of CSF compartment perfusions were performed on adult cats anesthetized with pentobarbital. The methods used in these perfusions have been

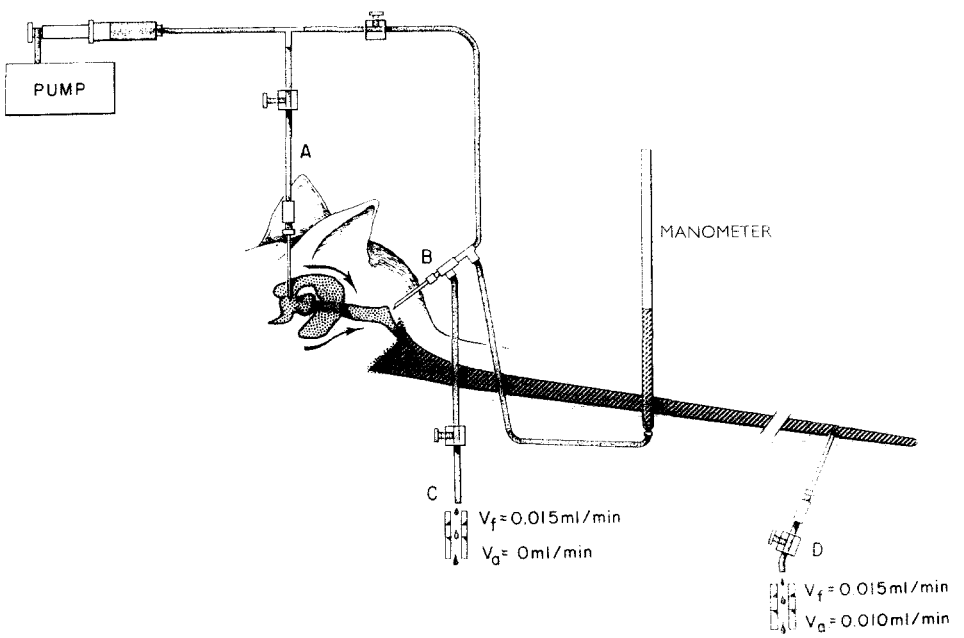

Fig. 1. Schematic version of the perfusion system used in this study. During ventriculocisternal (V-C) perfusions fluid delivered by the constant infusion pump entered the ventricular compartment at (*A*) and was collected at (*C*). The fluid entered the same site (*A*) during ventriculolumbar (V-L) perfusions but was collected at (*D*). Inflow of fluid during cisternal-lumbar perfusions (C-L) was at (*B*) and collection at (*D*). Manometric pressures were taken from the cistern or lumbar subarachnoid space with a water manometer connected in parallel to the efflux tubing. Mean rates of formation (V_f) and absorption (V_a) are shown for V-C perfusions at (*C*) and for V-L perfusions at (*D*).

described in detail by CUTLER *et al.* (1968b) and HAMMERSTAD *et al.* (1969). Fig. 1, a schematic version of the perfusion system, illustrates the three different perfusion routes used in these studies. A 20 ml syringe driven by a constant infusion pump at a rate of 94 μl/min was connected with polyethylene tubing to the inflow needle and in parallel to a pressure transducer, or water manometer, used to monitor inflow pressure. Inflow during ventriculolumbar (V-L) and ventriculocisternal (V-C) perfusions was through a No. 20 caudal anesthesia needle placed stereotactically into the anterior horn of a lateral ventricle (*A*). That for cisternal-lumbar (C-L) perfusions was through an 18-gauge needle inserted percutaneously into the cisterna magna (*B*). An 18-gauge needle was also used during V-C perfusions to collect the outflow from the cisterna magna at (*C*). During V-L or C-L perfusions, outflow was collected from the lumbar subarachnoid space (*D*) through an 18-gauge spinal needle inserted percutaneously at the level of the superior iliac crest. The outflow was led by polyethylene tubing to a photo-electric drop counter and collected in fractions of 20 drops using a fraction collector. Normally, the opening of the outflow tubing was adjusted to a height providing a perfusion pressure of approx. "0" cm water with reference to the cisterna magna. Artificial CSF of the composition recommended by MERLIS (1940) was used as the perfusion medium.

Formation, absorption and volume of CSF

Dextran Blue (Pharmacia, Uppsala, Sweden), or ^{125}I-labelled human serum albumin ([^{125}I]HSA), was added to the CSF to act as an indicator of CSF formation and absorption, and to measure the CSF volume. During 4 C-L perfusions 2mg/ml of colloidal carbon (Pellikan Ink, C-11/1413A, Günther Wagner) was added to the perfusate to investigate the effect of particulate matter on absorption of CSF. The distribution of the colloidal carbon within the spinal compartment was determined by making serial histological sections, 4 μ thick, from paraffin-embedded blocks of cervical and lumbar cord which were stained lightly with hematoxylin and eosin. Histoautoradiograms were also made from these sections as described by CUTLER *et al.* (1964) to assess the distribution of [^{125}I]HSA.

CSF clearance of radioiodide and sulfate

In addition to Dextran Blue or [^{25}I]HSA, carrier-free sodium [^{125}I] iodide (0.3μC/ml) and/or sodium [^{35}S] sulfate (5μC/ml) was also added to the perfusate to study the clearance of these 2 anions from CSF. In order to evaluate the proportion of radioiodide and sulfate cleared from the ventricular and spinal subarachnoid compartments in the same animal, V-C perfusions were conducted in 4 animals following V-L perfusions. Experiments were also performed to determine the effect of adding high concentrations of the sodium salts of iodide, sulfate, thiocyanate, iodoacetate or dinitrophenol (DNP) on the clearance of radioiodide and sulfate from CSF.

Ureter-ligated animals which had received an intravenous injection of carrier-free sodium [^{131}I] iodide (0.75 mC/kg) or [^{35}S] sulfate (0.5 mC/kg) were used to investigate the effect of a plasma-to-CSF concentration gradient on CSF clearance of these two anions. In these experiments CSF perfusions were begun and collections made, during the 3rd and 5th hours after intravenous injection. Influx of radioiodide and sulfate

into CSF from blood was studied using the same procedure except that in these experiments the perfusate did not contain radioiodide or sulfate. Samples of blood (0.2 ml) were drawn during the CSF-collection periods for assay of blood ^{131}I and ^{35}S radioactivity.

To determine what proportion of the total radioiodide or sulfate cleared from CSF remained in brain, or spinal cord, at the end of a perfusion, the brain and/or spinal cord were removed from some animals, homogenized in distilled water. ^{125}I and ^{35}S radioactivity was determined in 1 ml aliquots.

Radioassay and calculation

Radioassay of ^{35}S was performed on aluminum planchettes using a Geiger Mueller Counter at 18% efficiency. ^{131}I and ^{125}I activity was assayed in a well-type scintillation counter at 10% and 40% efficiency respectively. Blue Dextran concentration was determined by measuring the optical density of 620 mμ.

Rates of CSF formation (V_f) and absorption (V_a) in ml/min and [^{125}I]HSA volume of distribution (VD) in ml were determined by the equations developed by HEISEY *et al.* (1962) and KATZMAN *et al.* (1965).

$$(1)\ \text{CSF formation},\ V_f = V_i\left(\frac{C_i - C_o}{C_o}\right)$$

$$(2)\ \text{CSF absorption},\ V_a = V_i + V_f - V_o$$

$$(3)\ \text{Volume of distribution, VD} = \sum_{0}^{n}\left[\frac{V_i C_i - V_o C_o}{C_{ss}}\right]\Delta t - \text{DS}$$

where: V_i and V_o refer to influx and efflux flow rates in ml/min, C_i and C_o refer to the concentration of [^{125}I]HSA or Dextran Blue in the influx and efflux respectively; n is the number of samples collected; Δt is the time in min required to collect each sample; C_{ss} is the steady-state concentration of [^{125}I]HSA in the efflux; DS is the dead space of the outflow tubing in ml.

Equations used to calculate the efflux coefficient (k_o), or CSF clearance in ml/min, and the influx coefficient (k_i), or plasma clearance by CSF in ml/min, are as follows:

$$(4)\ \text{Efflux coefficient},\ k_o = \frac{V_i c_i - V_o c_o}{\bar{c}_v}$$

$$(5)\ \text{Influx coefficient},\ k_i = \frac{V_o c_o - k_o \bar{c}_v}{Cpl}$$

where: c_i and c_o are the concentrations in counts/min/ml of ^{35}S, ^{131}I or ^{125}I in the influx and efflux respectively; c_v is the exponential mean CSF compartment concentration in counts/min/ml $= c_o + 0.37\,(c_i - c_o)$; Cpl is the concentration in counts/min/ml of ^{35}S or ^{131}I in plasma.

RESULTS

Formation, absorption and volume of CSF

The rate of CSF formation was the same (0.015 ml/min) during V-L, V-C, and C-L

perfusions (Table 1). However, although the 3 types of perfusions were conducted at "0" cm of water pressure, with respect to the cisterna magna, CSF absorption differed depending on the CSF compartment perfused. With the addition of colloidal carbon to the perfusate (4 animals) the rate of absorption fell drastically from 0.010 ± 0.002 ml/min to 0.002 ± 0.001 ml/min. Serial histological sections of the cervical and lumbar cord revealed accumulations of colloidal carbon in pouch-like projections of arachnoid, often with a villous appearance, near the sites of dural reflections over posterior nerve roots. No carbon particles were found within the central canal of the spinal cord, nor did histoautoradiograms of spinal cord sections disclose radioactivity within the central canal following perfusions with [^{125}I]HSA.

TABLE I

SUMMARY OF CSF RATES OF FORMATION, ABSORPTION AND VOLUME OF DISTRIBUTION FOR [^{125}I]HSA IN CATS[a]

	Pressure at cistern mmH_2O	*VL*	*VC*	*CL*
V_f (ml/min)	"0"	0.015 ± 0.002[b] (10)	0.015 ± 0.001 (23)	0.015 ± 0.001 (16)
V_a (ml/min)	"0"		0.005 ± 0.001 (64)	0.010 ± 0.002 (10)
VD_r (ml)	"0"	4.0 ± 0.7 (9)	0.8 ± 0.1 (5)	3.2[c]

[a] VL: ventriculolumbar, VC: ventriculocisternal, CL: cisternal-lumbar perfusion; V_f: CSF formation; V_a: CSF absorption; VD: volume distribution.

[b] Values are means ± S.E. Number of animals used is given in parentheses.

[c] Value is the difference between the volume of distribution obtained during VL and VC perfusions, *i.e.* $VD_{(VL)} - VD_{(VC)} = VD_{(CL)}$.

Since perfusions were all conducted at "0" pressure referable to the cisterna magna it would be expected that during V-L or C-L perfusions CSF from the ventricles, basal cisterns and cranial and spinal subarachnoid spaces would be drained from the lumbar outflow. Presumably, all the CSF would act to dilute the [^{125}I]HSA in the influx and thus provide a measure of the CSF compartment. Experimental values obtained for the V-L and V-C compartment and a derived value for the C-L compartment are given in Table 1.

CSF clearance of radioiodide and sulfate

The average radioiodide clearance obtained during V-L perfusions of 0.125 ml/min was almost equal to the sum of the clearances obtained during V-C (0.059 ml/min) and C-L perfusions (0.057 ml/min) of 0.116 ml/min (Table 2). In the 4 animals where a V-L perfusion was followed by a V-C perfusion, by changing the outflow needle from the lumbar subarachnoid space to the cisterna magna, radioiodide clearance decreased from 0.125 ml/min to 0.057 ml/min. The difference, resulting from exclusion of the spinal subarachnoid space, was 0.068 ml/min which was somewhat similar to the value obtained during perfusions of this space.

Clearance of [^{131}I] iodide from the spinal subarachnoid space (C-L perfusions)

TABLE 2

CLEARANCE OF RADIOIODIDE AND SULFATE FROM THE CSF[a]

	Pressure at cistern (mm H_2O)	*VL*	*VC*	*CL*
k_o I (ml/min)	"0"	0.125±0.017[b] (4)	0.059±0.006 (29)	0.057±0.005 (16)
k_o I (ml/min) (against plasma concentration)	"0"	—	—	0.068±0.015 (3)
k_i I (ml/min)	"0"	—	—	0.006±0.001 (5)
k_o SO_4 (ml/min)	"0"	—	0.023±0.002 (29)	0.015±0.002 (16)
k_o SO_4 (ml/min) (against plasma concentration)	"0"	—	0.021±0.003 (7)	—
k_i SO_4 (ml/min)	"0"	—	0.003±0.006 (6)	—

[a] VL: ventriculolumbar, VC: ventriculocisternal, and CL: cisternal-lumbar perfusion; k_o: efflux coefficient; k_i: influx coefficient.

[b] Values are means ±S.E. Numbers in parentheses indicate number of animals used.

remained unaltered (0.068 ml/min) when a 2.8-fold plasma to CSF radioiodide concentration gradient was established. In these experiments plasma [^{131}I] iodide remained fairly stable, falling only 5%/h, and freely diffusable, since only 5% of the total activity was bound to protein.

The mean value for radioiodide influx, from plasma to CSF, during C-L perfusions was only a fraction of the efflux coefficient (Table 2). This value was obtained from animals which had received [^{131}I] iodide intravenously and were then perfused with artificial CSF containing no isotope. Following a steady state collection period, [^{125}I] iodide was added to the perfusate and a value for [^{125}I] efflux was obtained. Using the efflux coefficient obtained for each individual animal resulted in a more accurate determination of the influx coefficient.

Radiosulfate clearance from the CSF was studied during V-C and C-L perfusions and was in both instances considerably lower than that of radioiodide. Its clearance during V-C perfusions remained unaltered in the presence of an established plasma-to-CSF [^{35}S] sulfate concentration gradient. Moreover, as with radioiodide, the influx coefficient (k_i) was smaller than the efflux coefficient (k_o).

Analysis of residual amounts of [^{131}I] iodide or [^{35}S] sulfate left in whole brain after V-C perfusions indicated that only 4.7–4.9% of the net iodide cleared from CSF and 14.7%–16.5% of the sulfate remained in brain. After C-L perfusions only 6.5% of the total iodide cleared from CSF could be detected in spinal cord.

The percent change in the clearance of tracer [^{125}I] iodide and [^{35}S] sulfate during V-C and C-L perfusions as a result of adding various anions to the perfusate is shown

TABLE 3

PERCENT CHANGE IN CLEARANCE OF TRACER [^{125}I] IODIDE AND [^{35}S] SULFATE

Anion added to perfusate	*Percent change*			
	[^{125}I] Iodide		*[^{35}S] sulfate*	
	VC[a]	*CL*	*VC*	*CL*
4m*M* iodide	−62[b]	−62	−12	—
4m*M* sulfate	−2	+1	−85	+11
4m*M* thiocyanate	−62	−59	−8	—
1m*M* iodoacetate	—	−62	—	−5
4m*M* dinitrophenol	0	—	+40	—

[a] VC: ventriculocisternal perfusion, and CL: cisternal-lumbar perfusion.

[b] Values are means of the clearances obtained during perfusion with added inhibitors expressed as a percent of the mean clearances obtained during perfusions without inhibitors.

TABLE 4

COMPARISON OF CRANIAL AND SPINAL CSF COMPARTMENTS

	Cranial	*Spinal*
Volume distribution (ml)	0.75	3.2
Bulk formation of CSF	yes	none
Choroid plexus	yes	no
Bulk reabsorption of CSF	yes	yes
Arachnoid granulations	yes	yes
Carrier transport of iodide	yes	yes
Carrier transport of sulfate	yes	no

in Table 3. Normally, 4–5 h were necessary to perform perfusions on the same animals in which steady state periods had to be established without (control period) and with (experimental period) added inhibitor in the perfusate. In earlier experiments we had shown that the clearance of radioiodide and sulfate remained relatively constant over a period of 4–5 h. Radioiodide clearance from CSF was markedly inhibited by 4 m*M* DNP. However, following the inhibitory period, of some 15 min duration observed with the addition of iodoacetate during C-L perfusions, removal of iodide from the spinal fluid increased beyond that observed during the initial control period. A similar and parallel increase in the removal of sulfate was also observed during C-L perfusions with the addition of iodoacetate, except that unlike the clearance of iodide no inhibition was observed to precede this final phase. Radiosulfate clearance was inhibited by 4m*M* sulfate during V-C perfusions but not during C-L perfusions. An appreciable increase in the sulfate clearance was also observed during V-C perfusions when 4m*M* DNP was added to the perfusate. The results of the experiments are summarized in Table 4.

DISCUSSION

To date, most cerebrospinal fluid perfusion studies have dealt almost exclusively with the cranial CSF compartment, with the result that little is known concerning bulk

formation, absorption and solute transport from the spinal subarachnoid fluid. During C-L perfusions we attempted to limit our perfusion to the spinal subarachnoid space by maintaining the perfusion pressure, relative to the cisterna magna, from 0 to 1 cm of water. Under these conditions we expected that the perfusate would flow caudally from the cisterna magna, with little if any flowing in a cephalad direction along extraventricular spaces. The adequacy of this method in isolating physiologically the spinal subarachnoid space was evaluated from the radioiodide and sulfate clearance data presented in this study. Presumably, at this pressure the cranial compartments and choroid plexus bathed during V-L perfusions should be the same as those bathed during V-C perfusions. Thus any difference arising in the clearance of radioiodide between these two routes of perfusion could be attributed to extracranial clearance. The clearance of radioiodide during V-L perfusions was 0.125 ml/min, that obtained during V-C perfusions was 0.057 ml/min. The difference of 0.068 ml/min should represent the amount cleared in extracranial sites or in the spinal subarachnoid space. The fact that during C-L perfusion a clearance of 0.057 ± 0.005 ml/min was obtained argues that under these circumstances the spinal subarachnoid space was physiologically isolated from the cranial compartment.

It has also been demonstrated that the cat choroid plexus of the posterior fossa, incubated *in vitro*, concentrates radiosulfate by a mechanism which is self-saturable (ROBINSON *et al.* 1968b). Since the choroid plexus normally represents a major site for the transport of solutes out of CSF, we would expect that if the perfusion fluid, during a C-L perfusion, came into contact with the choroid plexus, the clearance of radiosulfate would have become saturated, or at least reduced, in the presence of high concentrations of sulfate. However, no reduction was observed indicating that little if any of the perfusate came in contact with the choroid plexus of the 4th ventricle.

The principal site of CSF formation is generally acknowledged to be the choroid plexus. However, evidence for extrachoroidal ependymal formation has been presented by JACOBI AND MAGNUS (1925) and more recently by POLLAY AND CURL (1967). In addition, BERING AND SATO (1963) have presented evidence for formation of CSF in the cranial subarachnoid space. The finding in our studies that CSF formation was identical during V-L, V-C and C-L perfusions, indicated that in the cat there was no formation of CSF in the spinal subarachnoid space. A similar observation has been reported for the dog (COBEN AND SMITH 1969). The conclusion is further supported by data published by BERING (1965) showing CSF formation for the cat to be 0.017 ml/min, of which 41% was estimated to be derived from extraventricular sources. Since this rate of formation is almost identical to the one we observed during V-C perfusions (0.015 ± 0.001 ml/min) it would appear that in the cat all of the CSF originates in the cranium.

The assumption that most of the CSF is absorbed through arachnoid villi located in the cranial subarachnoid space (WEED 1914; DAVSON 1963), has been questioned by DANDY (1929) and others (BEDFORD 1955; BERING AND SATO 1963) who suggested that other sites may be more important. Our results showed that a detectable amount of absorption occurred in the spinal subarachnoid compartment. Structures resembling arachnoid villi of cerebral meninges have been demonstrated to extend through the dura mater and project into the vascular lumen of veins associated with spinal nerve

roots (ELMAN 1923; WELCH AND POLLAY 1963). In our study the addition of colloidal carbon to the perfusate reduced spinal fluid absorption. Since carbon particles were found to accumulate in the arachnoid villi of the spinal roots it was likely that spinal fluid absorption occurred through these structures. Spinal fluid flow through the central canal, which had been demonstrated in the rabbit (BRADBURY AND LATHEM 1965) did not seem likely since in the cat neither carbon particles nor [^{125}I]HSA was found in the central canal of the spinal cord. Absorption of fluid in the spinal subarachnoid space could explain why a small amount of CSF absorption observed by us and HOCHWALD (1967) occurred during V-C perfusions conducted at a negative pressure. This loss could represent fluid which had flowed past the cisternal needle and was absorbed in the spinal subarachnoid space. However, we cannot exclude the possibility that in our study and HOCHWALD's some of the perfusate entered basal cisterns and was absorbed by arachnoid villi associated with cranial nerves (SHANTHAVEERAPPA AND BOURNE 1964). In this respect, it is of interest that an almost identical absorption rate (0.012 ml/min) was observed in dogs whose spinal subarachnoid space had been isolated by laminectomy (T-9-8) or by extradural ligation (COBEN AND SMITH 1969).

The volume of [^{125}I]HSA distribution obtained in these studies during V-L or C-L perfusions was assumed to represent a measure of the total CSF compartment for the adult cat. The value obtained of 4.0 ± 0.7 ml was almost identical to the 4.4 ml volume obtained by CSF drainage in this species (FLEXNER 1933). Since the volume of the cranial CSF compartment measured during V-C perfusions, was approximately 0.8 ml, it appears that in cat the largest proportion of CSF (3.2 ml) is contained within the spinal subarachnoid space.

The results of the perfusion studies demonstrated that during V-C and V-L perfusions radioiodide and sulfate were cleared from the CSF by mechanisms other than just bulk flow. It is likely that a large proportion of the clearance was to blood, since only a small proportion of the total radioiodide and sulfate cleared from CSF could be recovered from brain. Since the clearance of both these anions, during V-C perfusions, was self-saturable, occurred against a concentration gradient, and was reduced by known competitive antagonists or metabolic inhibitors, it was concluded that an appreciable portion of the clearance involved an active transport mechanism. Possibly the failure of DNP to inhibit iodide and sulfate clearance could be attributed to the rather high concentration used. DAVSON AND POLLAY (1963), using a much lower concentration (0.05mM) in their ventricular perfusate, observed a 27% reduction in iodide transport in the rabbit. The possibility that use of high concentrations led to general interference of membrane function resulting in increased diffusion from CSF to blood, was suggested by the results obtained during C-L perfusions with 1 mM iodoacetate.

The study also demonstrated that during C-L perfusions radioiodide and sulfate were cleared from the spinal subarachnoid space. The fact that iodide clearance was self-saturable, occurred against a concentration gradient and was inhibited by competitive and metabolic inhibitors, suggested that a significant portion of the total iodide cleared involved a carrier mechanism. The same conclusion was drawn for iodide clearance from the isolated spinal subarachnoid space of the dog (COBEN AND SMITH 1969). By contrast, that portion of radiosulfate clearance that exceeded albumin

clearance appeared to be associated with diffusion since high concentrations of sulfate did not affect its overall clearance.

The fact that during V-C perfusions the clearance of radioiodide and radiosulfate could be inhibited independently of each other indicated that these anions were removed from CSF by independent carrier mechanisms (CUTLER *et al.* 1968b; LORENZO *et al.* 1968). Accumulation of these anions by the cat choroid plexus incubated *in vitro* also occurred through mutually exclusive mechanisms (ROBINSON *et al.* 1968b), which in addition had different patterns of ontogenetic development (ROBINSON *et al.* 1968a). The autonomy of these transport mechanisms was further substantiated by the results obtained during C-L perfusions showing the presence of an iodide but not a sulfate carrier mechanism in the spinal subarachnoid space.

The probable site for CSF transport carriers have generally been attributed to the choroid plexus or ependyma (PAPPENHEIMER *et al.* 1961; DAVSON *et al.* 1962; POLLAY AND DAVSON 1963). The ability of the choroid plexus to accumulate tracer iodide and sulfate against a concentration gradient *in vitro* (BECKER 1961; WELCH 1962; ROBINSON *et al.* 1968b) has been acknowledged to indicate that *in vivo* the choroid plexus constitutes an important site for the transport of these anions from CSF to blood. The fact that neither ependyma (except in the central canal) nor choroid plexus is found within the spinal subarachnoid compartment suggests that other tissues or cell types must be involved in the transport of iodide from spinal fluid to blood. Cells or tissues which are suspected to be capable of transport activity are arachnoid cells, which contain various oxidative phosphorylating enzymes (SHANTHAVEERAPPA AND BOURNE 1965) and glial cells, which project through the pia and constitute part of the limiting membrane of the subarachnoid space (RAMSEY 1965; WAGGENER AND BEGGS 1967). However, pia-arachnoid is known not to transport iodide *in vitro* (WELCH 1962) and to date no direct evidence has been presented demonstrating glial transport. The possibility that transport could take place at the blood-cord interface has been investigated by isolating the spinal cord (BITO *et al.* 1966; COBEN AND SMITH 1969). Although neither group excluded this possibility entirely, BITO *et al.* (1966) suggested that it was not a likely site. The evidence presented by us tends to support this conclusion. If the carrier mechanism was associated with the endothelial cell of the capillary within neural tissue, inhibition of this mechanism should lead to increased levels of radioiodide within the spinal cord. This was not observed. Lastly, subarachnoid vessels could be involved; however, no evidence is available regarding the role of these vessels in transport function.

It has been suggested that the CSF may act as a "sink" to solutes in the extracellular space of the central nervous system (DAVSON 1963). Evidence supporting this hypothesis has been presented for inulin (WOODWARD 1967), sucrose (OLDENDORFF AND DAVSON 1967), mannitol and sulfanilic acid (CSERR *et al.* 1968) and iodide and sulfate (CUTLER *et al.* 1968a). The "sink" action of CSF requires the existence of a concentration gradient from brain to CSF. Presumably, active transport of solute out of CSF aids in maintaining this concentration gradient. In the ventricular "compartment" it is likely that the choroid plexus acts to transport solutes from CSF to blood and thereby to maintain such a brain-to-CSF gradient. However, since no choroid plexus is present in the spinal subarachnoid compartment the actual site of the transport

mechanism becomes quite significant with respect to the "sink" hypothesis. If the site is within the subarachnoid compartment then the concentration of solute within the spinal cord could be influenced by the surrounding spinal fluid. However, if the mechanism is within neural tissue then the "sink" hypothesis would have to be re evaluated with respect to the spinal cord.

SUMMARY

Numerous studies have demonstrated transport mechanisms within the cerebral ventricles which regulate the concentration of solutes within the cerebrospinal fluid (CSF) *in vivo*. Furthermore, studies of choroid plexus transport *in vitro* indicate that ependymal cells play a major role in these active regulatory mechanisms. Unfortunately, less attention has been given to the possibility that such mechanisms may exist in extraventricular compartments such as the spinal subarachnoid space (SSS).

Ventriculo-lumbar, ventriculo-cisternal and cisternal-lumbar perfusions were performed in anesthetized cats to study bulk formation and absorption of spinal subarachnoid fluid (SSF), and the clearance of radioiodide and sulfate from the SSS. The results obtained are summarised in Table 4.

Finally, the results indicate that non-ependymal tissues or cells are capable of actively transporting iodide from the SSF to blood. The probable locus of this transport mechanism and its relation to the "sink" hypothesis are discussed.

REFERENCES

ADAM, H. M., R. A. MCKAIL, S. OBRADOR AND W. C. WILSON (1938) Acetylcholine in cerebrospinal fluid, *J. Physiol. (Lond.)*, 93: 45–46P.

BECKER, B. (1961) Cerebrospinal fluid iodide, *Amer. J. Physiol.*, 201: 1149–1151.

BEDFORD, T. H. B. (1955) The movement of cerebrospinal fluid over cerebral hemispheres of the dog, *J. Physiol. (Lond.)*, 128: 51–52P.

BERING, E. (1965) The cerebrospinal fluid circulation, In: C. MCC. BROOKS, F. F. KAO AND B. B. LLOYD (Eds.), *Cerebrospinal Fluid and the Regulation of Ventilation*, Blackwell, Oxford, pp. 395–412.

BERING, E. AND O. SATO (1963) Hydrocephalus: Changes in formation and absorption of cerebrospinal fluid within the cerebral ventricles, *J. Neurosurg.*, 20: 1050–1063.

BITO, L. Z., M. W. B. BRADBURY AND H. DAVSON (1966) Factors affecting the distribution of iodide and bromide in the central nervous system, *J. Physiol. (Lond.)*, 185: 323–354.

BRADBURY, M. W. B. AND W. LATHEM (1965) A flow of cerebrospinal fluid along the central canal of the spinal cord of the rabbit and communications between this canal and the sacral subarachnoid space, *J. Physiol. (Lond.)*, 181: 785–800.

CALHOUN, M. C., H. D. HURT, H. D. EATON, J. E. ROUSSEAU Jr., AND R. C. HALL, Jr. (1967) Rates of formation and absorption of cerebrospinal fluid in Holstein male calves, *Univ. Conn. Coll. Agr. Exp. Sta., Bull.* 401.

COBEN, L. A. AND K. R. SMITH (1969) Iodide transfer at 4 cerebrospinal fluid sites in the dog: evidence for spinal iodide carrier transport, *Exp. Neurol.*, 23: 76–90.

CSERR, H., J. D. FENSTERMACHER AND D. P. RALL (1968) Studies relating to the function of cerebrospinal fluid, *Fed. Proc.*, 27 (2): 516.

CUTLER, R. W. P., A. V. LORENZO AND C. F. BARLOW (1968a) Sulfate and iodide concentration in brain. The influence of cerebrospinal fluid, *Arch. Neurol. (Chic.)*, 18: 316–323.

CUTLER, R. W. P., R. J. ROBINSON AND A. V. LORENZO (1968b) Cerebrospinal fluid transport of sulfate in the cat, *Amer. J. Physiol.*, 214: 448–454.

CUTLER, R. W. P., G. V. WATTERS AND C. F. BARLOW (1964) I^{125} labelled protein in experimental brain edema, *Arch. Neurol. (Chic.)*, 11: 225–238.

DANDY, W. E. (1929) Where is cerebrospinal fluid absorbed? *J. Amer. med. Ass.*, 92: 2012–2014.
DAVSON, H. (1963) The cerebrospinal fluid, *Ergebn. Physiol.*, 52: 20–73.
DAVSON, H. AND M. POLLAY (1963) Influence of various drugs on the transport of I^{131} and PAH across the CSF-blood barrier, *J. Physiol. (Lond.)*, 167: 239–246.
DAVSON, H., C. R. KLEEMAN AND E. LEVIN (1962) Quantitative studies of the passage of different substances out of the cerebrospinal fluid, *J. Physiol. (Lond.)*, 161: 126–142.
ELMAN, R. (1923) Spinal arachnoid granulations with special reference to the cerebrospinal fluid. *Bull. Johns Hopk. Hosp.*, 34: 99–104.
FLEXNER, L. B. (1933) A note on the rate of circulation of cerebrospinal fluid, *Amer. J. Physiol.*, 106: 201–203.
HAMMERSTAD, J. P., A. V. LORENZO AND R. W. P. CUTLER (1969) Iodide transport from the spinal subarachnoid fluid in the cat, *Amer. J. Physiol.*, 216: 253–258.
HEISEY, S. R., P. HELD AND J. R. PAPPENHEIMER (1962) Bulk flow and diffusion in the cerebrospinal fluid system of the goat, *Amer. J. Physiol.*, 203: 775–781.
HOCHWALD, G. M. AND M. WALLENSTEIN (1967) Exchange of albumin between blood, cerebrospinal fluid, and brain in the cat, *Amer. J. Physiol.*, 212: 1199–1204.
JACOBI, W. AND G. MAGNUS (1925) Gefäss und Liquorstudien am Hirn des lebenden Hundes, *Arch. Psychiat. Nervenkr.*, 73: 126–138.
KATZMAN, R., L. GRAZIANI, R. KAPLAN AND A. ESCRIVA (1965) Exchange of cerebrospinal fluid potassium with blood and brain, *Arch. Neurol. (Chic.)*, 13: 513–524.
LEUSEN, I. (1948) Influence de la teneur en calcium et en potassium du liquide céphalo-rachidien sur le système vasomoteur, *Arch. int. Pharmacodyn.*, 75: 422–424.
LORENZO, A. V., J. P. HAMMERSTAD AND R. W. P. CUTLER (1968) The effect of anesthetic agents on the cerebrospinal fluid clearance of S^{35} sulphate and I^{125} iodide, *Biochem. Pharmacol.*, 17: 1279–1283.
MERLIS, J. K. (1940) The effect of changes in the calcium content of the cerebrospinal fluid on spinal reflex activity in the dog, *Amer. J. Physiol.*, 131: 67–72.
OLDENDORF, W. H. AND H. DAVSON (1967) Brain extracellular space and the sink action of cerebrospinal fluid, *Arch. Neurol. (Chic.)*, 17: 196–205.
PAPPENHEIMER, J. R., S. R. HEISEY AND E. F. JORDAN (1961) Active transport of Diodrast and phenolsulfonphthalein from cerebrospinal fluid to blood, *Amer. J. Physiol.*, 200: 1–10.
PAPPENHEIMER, J. R., S. R. HEISEY, E. F. JORDAN AND J. DEC. DOWNER (1962) Perfusion of the cerebral ventricular system in unanesthetized goats, *Amer. J. Physiol.*, 203: 763–774.
POLLAY, M. AND F. CURL (1967) Secretion of cerebrospinal fluid by the ventricular ependyma of the rabbit, *Amer. J. Physiol.*, 213: 1031–1038.
POLLAY, M. AND H. DAVSON (1963) The passage of certain substances out of the cerebrospinal fluid, *Brain*, 86: 137–150.
RAMSEY, H. J. (1965) Fine structure of the surface of the cerebral cortex of human brain, *J. Cell Biol.*, 26: 323–331.
ROBINSON, R. J., R. W. P. CUTLER, A. V. LORENZO AND C. F. BARLOW (1968a) Development of transport mechanisms for sulfate and iodide in immature choroid plexus, *J. Neurochem.*, 15: 455–458.
ROBINSON, R. J., R. W. P. CUTLER, A. V. LORENZO AND C. F. BARLOW (1968b) Transport of sulfate, thiosulfate, and iodide by choroid plexus *in vitro*, *J. Neurochem.*, 15: 1169–1179.
SATO, O. AND E. A. BERING Jr. (1967) Extraventricular formation of cerebrospinal fluid. *Brain and Nerve*, 19: 31–33.
SHANTHAVEERAPPA, T. R. AND G. H. BOURNE (1964) Arachnoid villi in the optic nerve of man and monkey, *Anat. Rec.*, 148: 335.
SHANTHAVEERAPPA, T. R. AND G. H. BOURNE (1965) Histological and histochemical studies of the choroid of the eye and its relations to the pia-arachnoid mater of the central nervous system and perineural epithelium of the peripheral nervous system, *Acta Anat. (Basel)*, 61: 379.
WAGGENER, J. D. AND J. BEGGS (1967) The membranous coverings of neural tissues: an electron microscopic study, *J. Neuropath. exp. Neurol.*, 26: 412–426.
WEED, L. H. (1914) The pathways of escape from the subarachnoid spaces with particular reference to the arachnoid villi, *J. med. Res.*, 31: 51–91.
WELCH, K. (1962) Active transport of iodide by choroid plexus of the rabbit *in vitro*, *Amer. J. Physiol.*, 205: 617–624.
WELCH, K. AND M. POLLAY (1963) The spinal arachnoid villi of the monkeys *Cercopithecus aethiops sabaeus* and *Macaca irus*, *Anat. Rec.*, 145: 43–48.
WOODWARD, D. L., D. J. REED AND D. M. WOODBURY (1967) Extracellular space of rat cerebral cortex, *Amer. J. Physiol.*, 212: 367–370.