phenol was added to the perfusate or Diamox (100 mg/kg) given intravenously. These results support our interpretation that dilution of perfusate is brought about by ependymal activity. Such dilution could occur secondarily to a transfer of salt into the perfusate, with water following because of osmotic forces as in the kidney(18) or by co-diffusion(10). The maintenance of a high chloride concentration in the perfusate when the concentrations of the added test substances were decreasing is consistent with such a proposal. In other experiments, we have obtained evidence that salt does in fact move into the central canal perfusates (19).

Summary. A method for perfusing the central canal of cats and rabbits is described. The reduction of concentration of PAH or CPR added to the perfusate is about the same as the reduction in concentration of added radio inulin. Reduction of CPR concentration is not influenced by probenecid or by the presence of another organic acid (PAH). The data are interpreted to indicate that the ependymal cells lining the central canal do not transport organic acids. It is suggested that the parallel reduction in concentration of inulin and added organic acid results from dilution of the perfusate.

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Sodium and Chloride Movement into the Central Canal of Cat Spinal Cord.* (31996)

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In the previous report, a perfusion method was used to study egress of organic acids from the central canal(4). Results of the studies indicated that the perfusate was diluted as it passed down the central canal. It was suggested that the dilution could be secondary to salt transfer into the central canal with water following the movement of salt. The experiments to be reported were designed to explore more fully this possibility.

Methods. Cats, anesthetized with sodium pentobarbital I.P. (35 mg/kg) were used exclusively in this study. The method of

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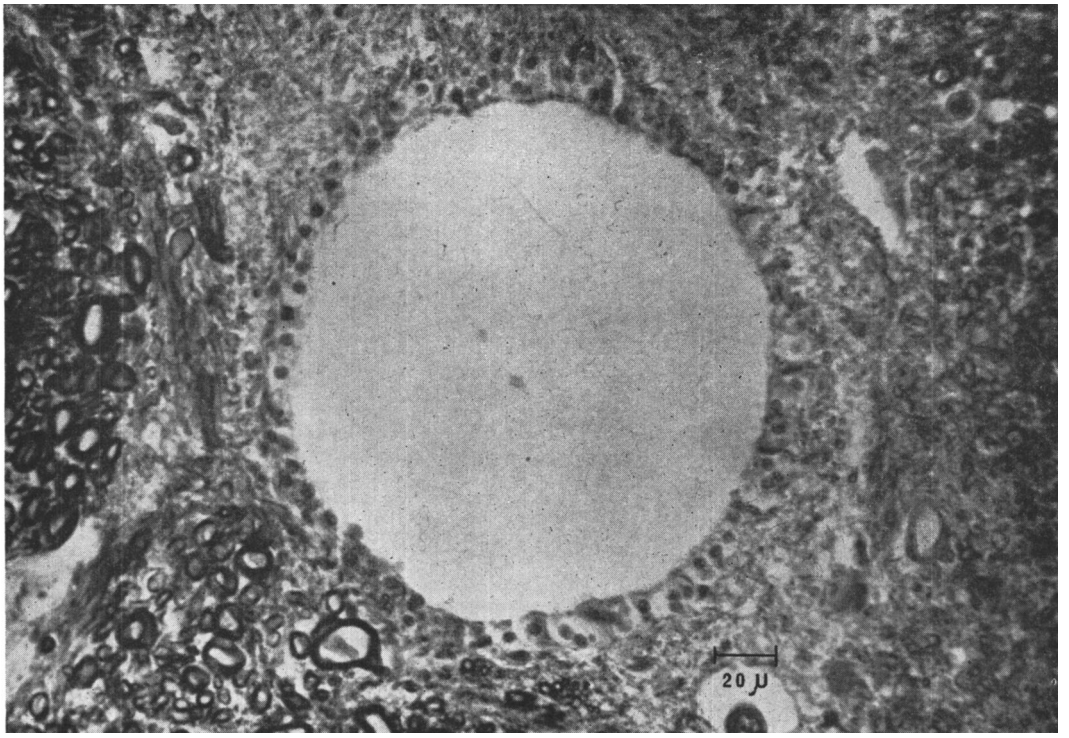

FIG. 1. A light micrograph showing area of central canal of cat spinal cord. Canal was perfused *in vivo* with osmium tetroxide.

perfusion of the central canal was essentially the same as that used previously(4). The only modification was that the cannulae were held in place by 2% agar in Ringer's which was first heated and then applied at just above setting temperature. Two different kinds of perfusion solutions were used: In one, chloride concentration was reduced relative to CSF values(1) by partial replacement with sulfate. In the other, sodium was partially replaced by choline. Concentrations of Na^+ and Cl^- are indicated in the text and tables. Ca^{++} (1.1 mM/l) K^+ (5.6 mM/l) and HCO_3^- (2.4 mM/l) approximated CSF values in both solutions (1). Buffered osmium tetroxide was added to the perfusate in two experiments. After fixation, the area of the central canal was excised, imbedded in Epon and sectioned. Fig. 1 represents a light microscope projection of a one micron section.

Trans-cord electrical potential difference (P.D.) between the central canal and the exterior of the cord was measured in some experiments. Fine polyethylene leads filled with 2.7 M KCl were used as electrical conduits. One lead was threaded into the collection catheter, the other inserted into the agar block around the cord. Insertion into either the caudal or rostral agar had no effect on the magnitude of the P.D. The polyethylene leads fed to calomel half cells which in turn were connected to the input of a high impedance voltmeter (Keithley 610A). Electrical asymmetry of the system was determined by inserting both polyethylene leads into the agar block. The measured values were corrected for any asymmetry (always equal to or less than 1 mv).

Analytical methods for inulin and chloride have been described(4). Sodium was analyzed by direct flame photometry using the flame attachment to a Beckman Spectrophotometer.

Results. It can be seen from Fig. 1 that a patent central canal exists and that the layer of ependymal cells lining the canal maintains its integrity during perfusion. Table I summarizes data obtained from experiments with the different perfusion solu-

TABLE I. Ionic Concentrations of Plasma, CSF, Perfusate and Collected Samples.

Chloride*				Sodium*					
P	CSF	C	E	P	CSF	C	E	% Control inulin (E/C × 100)	N
—	125	100	112	—	164	160	175	83	13
115	132	100	114	150	156	160	181	95	8
126	142	100	119	150	158	160	172	72	10
120	139	100	117	153	163	160	174	99	10
113	134	100	165	145	152	160	191	—	5
—	140	150	151	138	150	135	149	99	7
112	142	150	143	144	160	135	165	92	3
115	143	155	153	128	151	135	138	94	4
117	139	155	156	146	164	135	145	98	5
113	140	150	151	138	152	128	136	87	4

* All concentrations are given as mEq/l.

P column = plasma concentrations; CSF = concentrations of cerebrospinal fluid; C = initial concentration of perfusate; E = concentration of collected samples, and N gives number of samples.

tions. The data show the following: 1) Inulin concentrations are reduced. 2) Sodium concentrations are increased despite the fact that the original perfusate may have had concentrations of this ion above that of plasma. 3) Chloride concentrations increase only when low Cl^- solutions are perfused. 4) The final concentrations of sodium can be greater than found in cisternal or subdural CSF collected before the start of the experiment as well as being higher than plasma concentrations. These changes indicate a movement of salt and water into the perfusates. The accretion of fluid per unit area of ependymal surface was calculated using the following equation:

$$\frac{V = R - \frac{R}{I} \times 100}{\pi \; d \; l}$$

where V is the accretion rate (μl/min/cm^2), R the initial perfusion rate (μl/min), I the percent control inulin and d and l the diameter and perfused length of the central canal (cm). The diameter of the central canal was determined from micrographs of osmium perfused cords (see Figure 1). The calculated volumes ranged from 0.12 μl/min/cm^2 to 3.67 μl/min/cm^2 with a mean of 1.45 μl/min/cm^2.

If salt movement into the perfusate involves an active process for translocation of sodium, one can predict that sodium movement can be reduced by interfering with the metabolism of the system or by treatment with classical transport inhibitors. Because of the geometry of this experimental system, the use of added inhibitors presents difficulties (see *Discussion*). In order to estimate the contribution of metabolism, sodium movement was compared in animals before and after administering lethal doses of nembutal. Measurements were made from 15 minutes up to 3 hours after death. Data summarizing the results of these experiments are shown in Table II. It can be seen that in a dead animal, the capacity to move sodium into the perfusate is lost.

Although the experiments considered above are consistent with an active transport of sodium, they could also be explained on the basis of a passive movement of sodium down a metabolically dependent electrical gradient. Since the limiting membrane of the central canal consists of ependymal cells, it would be desirable to measure the trans-ependymal potential. However, it does not seem likely that an ependymal sheet can be isolated. It was decided, therefore, to measure the trans-cord potential and to see how it was altered when sodium movement was inhibited. Such measurements do not indicate absolute values, since the values obtained are a function of the sum of all potentials generated across structures lying between the electrodes. Data from experiments of this design are given in Table III. It can be seen that when the movement of sodium into the perfusate is inhibited, the inside of

TABLE II. Changes in Sodium and Inulin Concentrations Before and After Death.

Control [Na]* (mEq/l)	Living†		Dead†	
	[Na] (mEq/l)	Inulin (% control)	[Na] (mEq/l)	Inulin (% control)
160	174	99	159	109
150	163	93	155	99
135	149	99	134	110
135	145	98	135	116

* Initial concentration in perfusate. † Average concentration in collected samples.

the canal becomes less positive to the grounded outside of the cord. These results support the concept of active transport rather than passive diffusion of sodium.

Discussion. Evidence has been presented indicating that CSF is produced by the ependymal lining of the choroid plexus and that the formation of CSF involves an active transfer of Na^+ up an electrochemical gradient (2,6,7,11,12,14). The experiments reported here indicate that salt transfer and fluid production are not unique to the ependymal lining of choroid plexus, but also occur across the ependyma of the central canal. Welch has found choroid plexus of rabbit to produce an average of 0.37 μl/min/mg (11), and the rates in dog and in goat have been reported to be 0.5 μl/min/mg(7) and 0.4 μl/min/mg(8), respectively. For comparison with our findings, these values were recalculated utilizing the relationship between weight and area derived by Welch and Sadler(13). The resulting values for choroid plexus of 1.03 μl/min/cm^2 in rabbit, 1.39 μl/min/cm^2 in dog and 1.11 μl/min/cm^2 in goat agree well with the average value of 1.45 μl/min/cm^2 reported in this study for the central canal. Curl *et al*(3), using an aqueductal-anterior IV ventricular perfusion system devoid of choroid plexus, have shown a net volume flow across the ventricular ependyma of 0.33 μl/min/cm^2. Although this value is somewhat lower than that for the ependymal lining of the central canal, it is of the same order of magnitude. It does seem likely, therefore, that the entire ependymal lining of the central nervous system contributes to the production and modification of CSF.

TABLE III

P.D. live	P.D. dead	Difference
+0.5*	−5.8	−6.3
0	−7.0	−7.0
0	−6.0	−6.0
+4.8	−0.8	−5.6

* Sign is that of the potential in mV inside of the canal with respect to the grounded outside of the spinal cord.

The interpretation of our findings is based on the assumption that changes in inulin concentration can be used as an index of dilution. This assumption may be challenged, since inulin has been shown to move from ventricular fluid to brain tissue(9,10). According to Rall *et al*(9), this loss of marker is reduced after death. Although a leak of inulin and its reduction after death is consistent with the directional changes in inulin concentration found in our experiments, this in itself is not sufficient to explain all of the data: 1) In 3 of 4 experiments (Table II), inulin concentration after death rose considerably above 100%, a finding which implies a mechanism other than reduction of an inulin leak. 2) The increase in sodium concentration above values in either plasma or CSF is not predictable on the basis of an inulin leak. A simultaneous water loss could be postulated; however, it is difficult to conceive of a mechanism by which inulin and water are lost while sodium is retained. Granted that a small leak of inulin could occur, this does not appear to be the major factor in the changes of inulin concentration in these experiments. An explanation which is consistent with all of the data is an active transport of sodium into the lumen of the canal, followed by inflow of fluid; the transport capacity is lost after death, and some sodium and water diffuse out of the canal, thus raising inulin concentration.

The electrical measurements show that the inside of the canal becomes less positive to

the outside of the cord when salt movement is abolished by death of the animal. Although absolute potential difference between the site from which sodium is moved and the internal fluid is not known, measurements of the directional changes are valid and are consistent with the existence of an "electrogenic pump" for sodium. The existence of such a transport system would correlate well with the observation of a high concentration of a Mg^{++} activated ATPase localized in the ependymal lining of the cord(5).

Further evidence for sodium transport by the ependyma could be obtained if inhibition by ouabain or digitoxin were demonstrable. Experiments of this type are complicated in this system since one predicts that the concentration of inhibitor decreases along the length of the central canal. If one used short lengths of cord to counteract this problem, then the amount of sodium transported may not be enough to be measured significantly. Nevertheless, experiments attempting to demonstrate inhibition were tried using 10^{-4} M concentrations of the inhibitors. No inhibition by ouabain was found in agreement with the observation that ouabain does not inhibit cerebrospinal fluid formation by the choroid plexus(7). With digitoxin as an inhibitor, clear cut inhibition of salt and water movement was detected in 2 out of 4 experiments (unpublished observations). Under conditions of inhibition, the canal again showed a reduced electrical positivity. These preliminary observations also support the idea that a luminally directed sodium pump exists in the ependymal lining of the central canal.

Summary. Net fluxes of sodium, chloride and water across the ependymal lining of tral canal and that a sodium transport system is involved in this process. the central canal of cats have been inferred from changes in concentration of the 2 ions and radioinulin in perfusates. Sodium, chloride and water move into the central canal. Translocation is abolished in dead animals. Changes in trans-cord electrical potentials show that the lumen of the central canal becomes less positive to the outside when solute movement is inhibited by death. The data are interpreted as indicating that the ependymal lining moves fluid into the cen-

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