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Organic Acid Movement Out of the Central Canal.* (31995)

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The function of ependymal cells has long been under consideration and diverse views have been advanced in ascribing a biological role to the ependyma. Because ependymal cells are ciliated, it has been suggested that these cells can circulate cerebrospinal fluid and indeed such has been reported for *Xenopus* larvae(1). Evidence exists that the choroid plexus is involved in the production of cerebrospinal fluid and, by implication, its ependymal cells(5,13,14,15,21). More recently, a specific function of the ependymal cells of the choroid plexus has received support, *i.e.*, that these cells are involved in the absorption of organic acids and other anions(4,7,14,20).

Although it is technically convenient to study transfer from brain ventricle to blood, interpretation of results is complicated by the fact that the ventricular lining is a structurally mixed cellular layer. Since the central canal of the spinal cord is lined almost exclusively by ependymal cells, investigation of translocation of solute and solvent into and out of the central canal should more clearly define the functional role of ependymal cells. To this end, experiments involving perfusion of the central canal have been carried out.

Methods. In the first developmental experiments, rabbits were chosen as the experimental animals, while later studies were performed exclusively on cats. Both species were anaesthetized with nembutal. Laminectomies were performed at a low thoracic and a lumbar region of the cord. V cuts

were made at both levels. A fine polyethylene catheter connected to a Sage infusion pump was inserted into the thoracic canal for delivery of perfusate. In all experiments, delivery was at a rate of 3 λ /min. A second catheter was inserted at the lumbar region for collection. Sampling of perfusate was done by inserting a glass capillary tube into the collection catheter and allowing it to fill for fixed times. Periods varied from 15 minutes to one hour depending on the rate at which fluid was collected. The perfusate contained radio inulin (C^{14}) and had the following composition in mM/l: NaCl 150; $NaHCO_3$ 2.4; KCl 5.6; $CaCl_2$ 2.2; glucose 2.5. Appropriate test substances were then added to the perfusates.

It was not possible to collect fluid at every insertion of the collection catheter. It is likely that at least two factors were responsible. 1) It was found in some experiments that the catheter had passed out of the canal into the cord and had become plugged with bits of neural tissue. 2) The canal itself was blocked by cellular debris. Under such conditions, the lumbar laminectomy was extended caudally, the cord recut and collection tried again. Such procedure often led to a successful experiment.

The following analytical procedures were used: 1) Volume recovery was estimated by weight difference of the collection capillary tube. 2) Chloride was determined using an electro-titrator (Aminco). 3) Radio inulin was determined with an automated thin-window gas-flow counter (Nuclear, Chicago). 4) Chlorophenol red [CPR] was measured spectrophotometrically in micro cuvettes at

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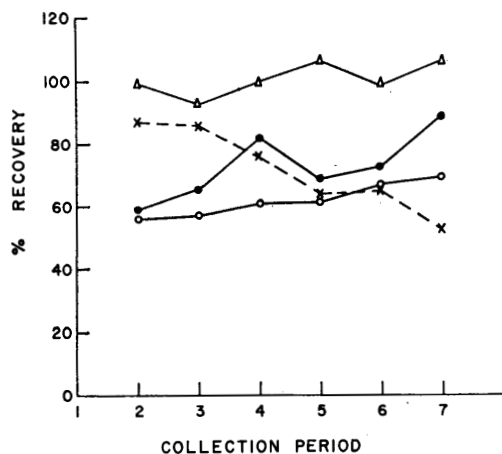


FIG. 1. Characteristics of fluid collected from a perfused cat central canal. Closed circles show CPR data. Open circles show radio inulin. Volume recovery is shown by crosses; triangles indicate relative chloride concentrations. Collection periods were 20 minutes.

520 $m\mu$ after alkalization and appropriate dilution. 5) The micro-method of Deetjen and Sonnenberg(8) was used to estimate para-aminohippuric acid [PAH].

Results. Results of a typical experiment designed to study CPR movement are shown in Fig. 1. In these studies, CPR concentration was 0.1 mM/l. Several points are to be noted: 1) Inulin and CPR concentrations are reduced. 2) Although the average concentration of the collected fluid for CPR is a little higher than that of inulin, for the most part the reductions in inulin and CPR concentrations are about the same. 3) Chloride concentration is maintained. 4) Volume recovery is incomplete. Data from two other experiments are shown in Table I. In a second series of experiments, the perfusate contained PAH (0.5 mM/l) as a test substance. Summary data comparing the final concentrations of PAH to that of inulin

TABLE I. Comparison of Inulin and PAH or CPR Final Concentrations of Perfusates.

Inulin	PAH	Inulin	CPR
78*	105	62	57
93	97	94	95
86	59		
92	90		
90	82		

* All values given as percent perfusate concentration.

TABLE II. Effect of Probenecid on Chlorphenol Red Movement.

Animal	Before probenecid	After probenecid
Rabbit	93*	95
Rabbit	96	98
Cat	69	65
Cat	79	84

* All values expressed as percent of control concentrations.

are also shown in Table I. Again, the reduction in concentration was about the same as that for inulin, although there was a greater scatter in these data when compared to that obtained in the CPR experiments.

The fact that volume recovery is less than 100% does not necessarily reflect fluid reabsorption. Pools of fluid could sometimes be seen to form at the site of the collection pipette. When the perfusate contained CPR, the pools had the magenta color characteristic of the dye. When samples of these pools were aspirated and plated, inulin C^{14} was detectable. It therefore seems that the reduced volume recovery is a result of leaks.

The reduction of CPR concentration could result from a carrier mediated movement of the dye out of canal by a system analogous to the organic acid transporting systems of kidney(11) or brain ventricle(14,15). Two tests of this possibility were made. In one series of experiments, the effect of probenecid on CPR movement was studied since this substance has been found to suppress translocation of organic acids(2). Results of 4 experiments using both rabbits and cats are shown in Table II. After control collections wherein the perfusate contained the dye alone, the central canal was then perfused with a solution of the same composition, but containing probenecid at a concentration of 0.4 mM/l. As can be seen, no meaningful change in the reduction of CPR concentration was observed.

The second test of carrier mediated organic acid movement was based on competitive effects between PAH and CPR transport. Movement of an organic acid which is transported by kidney(11,12) and by the ventricle is suppressed by the presence of another transported species(14), and in the kidney, kinetic studies have indicated that

TABLE III. Effect of Addition of PAH on CPR Concentrations of Perfusates of Cat Central Canals.

CPR	PAH	Inulin
79*	62	72
68	53	66
89	84	91
71	†	75
67	66	82

* All values expressed as percent of control concentrations.

† No steady concentrations attained in this experiment. PAH concentrations fell gradually from 80% to 35% of control levels over a period of four hours.

the inhibition occurs by organic acids competing for transport(12). If a similar system was operative in spinal cord ependymal cells, one would predict that the addition of another transported acid would result in suppression of the reduction of CPR concentration. Central canals were perfused with test solution to which PAH had been added at a concentration of 5 mM/l. Results of these experiments are shown in Table III. Comparison of the first and third columns shows that CPR and inulin concentrations fall to approximately the same level. In one experiment, constant PAH concentrations of the perfusate were not found, but rather there was a gradual decline in PAH concentration with time. We have no explanation for this one experiment. In the others, PAH concentration falls to about the level of CPR and inulin.

Discussion. The question arises whether the cord is functional under the conditions of these experiments. In control tests, it was possible to record electrical activity at one end of the perfused segment when the other end was stimulated. It appears then that neuronal function at least is maintained in these preparations despite the surgical and perfusion procedures. Evidence has been presented that the incomplete volume recovery can be attributed to gross leaks at the site of the collection catheter. Additional leaks through connections of the central canal with the subarachnoid space do not seem likely. Although Bradbury and Lathem did describe such connection in the rabbit(3), they were not found in the cat. Moreover, in the rabbit, the connections were confined to the

region of the filum terminale, but were not seen in the thoraco-lumbar region, which was the level perfused in these experiments.

Chlorphenol red was chosen as the test organic acid in these studies because 1) its chemical structure is closely related to phenol red, a dye whose movement from cerebrospinal fluid to blood has been studied in the cat(14). 2) In a different species, the dogfish, chlorphenol red has been found to be transported across the isolated choroid plexus(16). 3) In other organic acid transport systems it behaves the same as does phenol red(9). Since the reduction in CPR concentration of the perfusate is not affected by probenecid or the presence of an organic acid transported by the same path as is CPR in other systems, it does not seem likely that this substance is actively moved out of the central canal or translocated by a carrier mediated process. Since chlorphenol red is transported across the choroid plexus, it may be that the ventricular ependyma of the choroid plexus is qualitatively different from the ependyma lining the central canal.

The observation that CPR, PAH and inulin concentrations are reduced to about the same extent suggests that dilution of the perfusate could account for the reduction in concentration of test substances. It has been shown by Rall *et al*(17) that inulin can diffuse from ventricular fluid into brain tissue. Although this leak is considered to be small(22), one might argue that in our experiments the reduction in inulin concentration as well as CPR and PAH represents loss of these substances rather than dilution. However, since the molecular sizes of the 3 test substances are so much different, they should diffuse out of the central canal at vastly differing rates. The finding that the reduction in concentration is similar for all 3 substances implies a process other than simple diffusion. Utilizing an aqueductal-anterior IV ventricular perfusion system which is devoid of choroid plexus, Curl *et al* (6) were able to show a net volume flow across the living ventricular ependyma. The accretion of fluid was in the order of 0.33 μ l/min/cm² of ependymal surface. This flow was markedly attenuated when 2,4 dinitro-

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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