

STUDIES RELATING TO THE FUNCTION OF CEREBROSPINAL FLUID IN Squalus acanthias

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Many polar substances are found in much lower concentration in cerebrospinal fluid (CSF) than in plasma, an observation which has led to the suggestion that CSF may serve as a "sink" for polar substances which have accumulated in the brain. According to this hypothesis, materials are removed from brain by net diffusion from brain extracellular fluid (ECF) to CSF. Despite net uptake from brain, CSF concentration is maintained low relative to that in brain through continual renewal by bulk formation and reabsorption of fluid and, for some compounds, by specific transport systems.

In order to test the hypothesis that CSF may function as a "sink," we have looked for a diffusion gradient within the brain of the dogfish, Squalus acanthias, comparing the concentration of substances in brain tissue adjacent to CSF with that in tissue located further from the fluid. Five substances were examined: three (mannitol, inulin and sulfanilic acid) might be expected to demonstrate the "sink" action of CSF, as they are polar substances found in low concentrations in CSF; the fourth (antipyrine) served as a control, its concentrations in brain, plasma and CSF water being equal in the steady state; and the fifth was endogenous chloride.

Test substances were given by intravenous or intramuscular injection and plasma concentration maintained constant by repeated i.m. injections. At the conclusion of the experiment, samples of plasma, CSF, brain and extradural fluid (EDF) were obtained and analyzed. Distribution ratios between brain and plasma (R_{Br}), brain ECF and plasma (R_{ECF}), CSF and plasma (R_{CSF}), and EDF and plasma (R_{EDF}) are shown in Table 1. Comparison of values for R_{ECF} and

Table 1

UPTAKE OF SUBSTANCES BY BRAIN, CSF, AND EDF FOLLOWING
INTRAVENOUS ADMINISTRATION

Mean values of distribution ratios (# of observations) between brain and plasma (R_{Br}), brain ECF and plasma (R_{ECF}), CSF and plasma (R_{CSF}), and EDF and plasma (R_{EDF})

Substance	Δt , hr	R_{Br}^*	R_{ECF}^\dagger	R_{CSF}	R_{EDF}
Mannitol- C^{14}	4	.08 (3)	.40	.11 (5)	.08 (5)
	21	.15 (3)	.75	.35 (3)	.52 (3)
Sulfanilic acid	4.5	.14 (3)	.70	.17 (5)	.16 (5)
	20	.27 (5)	1.35	.28 (14)	.63 (15)
Inulin- C^{14}	24	.08 (4)	.40	.09 (4)	.28 (4)
Antipyrine- C^{14}	18	.83 (2)	-	.98 (2)	.96 (2)

* Values are for cervical spinal cord only. Values for medulla, optic lobe, and cerebellum were not significantly different.

† R_{ECF} was calculated assuming that test substances remain extracellular and that ECF constitutes 20% brain volume. R_{ECF} for sulfanilic acid of 1.35 probably indicates cellular uptake of drug.

R_{CSF} indicates that concentration in brain extracellular fluid is greater than that in CSF for mannitol, sulfanilic acid and inulin. This suggests that there is a gradient favoring net diffusion from brain to CSF for each of these three substances. In contrast, there are no concentration gradients between CSF, plasma and brain for antipyrine. (The value of .83 for R_{Br} agrees well with values for total brain water determined by comparison of wet and dry weights.)

R_{ECF} is also greater than R_{EDF} ; however, unlike CSF, EDF cannot serve as a sink for the brain. This is shown by the observation that as long as 24 hours after injecting either inulin- C^{14} or dye into EDF, there is no detectable uptake by brain.

Data shown in Tables 1 and 2 demonstrate concentration gradients within two regions of brain—medulla and optic lobe. The medulla and optic lobe were separated from the rest of the brain, frozen on dry ice and cut, parallel to the ventricular surface of the brain, into two slices. The inner slice (tissue adjacent to CSF) constituted between 1/3 and 1/2 the total sample weight. Distribution ratios between outer and inner medulla, $[M_o]/[M_i]$, and between outer and inner optic lobe, $[OL_o]/[OL_i]$, are shown in Tables 1 and 2 respectively. For mannitol, sulfanilic acid and inulin, distribution ratios are greater than 1 following intravenous administration and less

Table 2

DIFFUSION OF TEST MOLECULES FROM BRAIN TO CSF (following i.v. administration)
AND FROM CSF TO BRAIN (following injection into CSF)

Distribution ratios (mean \pm SEM) between outer and inner medulla slices, $[M_o]/[M_i]$, and between outer and inner optic lobe slices, $[OL_o]/[OL_i]$

Substance	Δt , hr	(M_o/M_i)		(OL_o/OL_i)	
		Route of administration		Route of administration	
		Blood	CSF	Blood	CSF
Mannitol- C^{14}	4	1.43 \pm .07 (N=5)	.70 (.69, .72) (N=2)	1.23 (N=1)	-
Mannitol- C^{14}	21			1.26 (1.00-1.58) (N=3)	.76 (.71, .81) (N=2)
Sulfanilic acid	5	1.23 \pm .06 (N=4)			
Sulfanilic acid	20	1.25 \pm .07 (N=5)			
Inulin- C^{14}	4	-	.59 (.60, .58) (N=2)		
Inulin- C^{14}	24	1.48 \pm .22 (N=4)	.62 \pm .03 (N=6)		
Antipyrine- C^{14}	18	1.00 (1.01, 1.00) (N=2)			
Chloride	-	1.01 \pm .03 (N=5)			

than 1 following injection into the cerebellar cavity of brain. In other words, when these test substances enter brain via blood their concentrations (quantity/gm brain) are higher in the outer slice than in tissue located nearer CSF; whereas, following injection into CSF, concentrations are higher in tissue near the ventricular surface. Unlike mannitol, sulfanilic acid, and inulin, distribution ratios for antipyrine and chloride are 1. Since antipyrine distributes in total brain water and chloride is probably largely extracellular, the data suggest that inner and outer slices of medulla are similar with respect to distribution of fluid between the extracellular and intracellular compartments. Thus, deviation of the observed distribution ratios for mannitol, sulfanilic acid and inulin from 1 can be ascribed to regional differences in the concentrations of these substances within brain fluids, rather than to regional differences in the volumes of fluid compartments of the brain. Concentration gradients have also been observed within dog brain, as test molecules diffused from CSF to brain (Rall *et al.*, Life Sci. 1:43-48, 1962).

These results clearly demonstrate that CSF functions as a "sink" for mannitol, sulfanilic acid and inulin. When given systemically the concentration of each of these substances in brain ECF becomes greater than that in CSF (Table 1) and there is net diffusion from brain into CSF, as evidenced by the concentration gradient within the brain (Tables 1 and 2). In returning substances such as inulin to blood which have leaked across the blood-brain barrier from cerebral capillary blood to brain, CSF is functioning in a manner somewhat analogous to lymph in the peripheral tissue. It seems likely that CSF may also provide a means for transporting polar end products of neuronal and glial metabolism from brain to blood.

Our data also support the conclusion that the blood-brain barrier is not as well developed in lower vertebrates as in mature mammals (Cserr, Fed. Proc. 26:1024-25, 1967). Distribution ratios between brain and blood for sulfanilic acid, inulin and mannitol are considerably smaller in mature mammals than those reported here for the dogfish.

1967 #11

FINE STRUCTURE OF THE RECTAL SALT GLAND IN Squalus acanthias

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Slices of the rectal gland of dogfish were treated for one hour in appropriate incubation media at 5°-10° C and subsequently processed for electron microscopic examination. Previous work (Bulletin MDIBL 6:16, 1966) demonstrated that the procedures provide material favorable for correlation of fine structure with secretory activity and that structural integrity is maintained under a broad range of experimental conditions.

Osmotic and specific ionic conditions were varied during incubation and the slices were processed by histochemical methods for electron microscopy. Localization of the sodium precipitable by potassium antimonate, the localization and changes in ATP-ase activity and the morphology of the cytomembranes are of primary concern. The nature of the epicytoplasmic space (middle compartment of Curran and Kaye) will be explored on the basis of experiments with lanthanum and ruthenium red as particulate tracers applied following incubation. It is hoped that the results of these experiments will help resolve some of the controversy concerning the significance of the morphology of the lateral and basal cytomembranes in this and related organs involved in salt and water transport.