Transport of organic acid dyes by the isolated choroid plexus of the spiny dogfish *S. Acanthias*

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RECENT studies have indicated that organic acids are actively transported from the cerebrospinal fluid (CSF) to the blood.[1] This process appeared to occur within the area of the fourth ventrical and cisterna magna. It was suggested that the choroid plexus of the fourth ventricle is the site of this transport process,[1] since embryonal choroid plexus grown in tissue culture transported organic acid dyes.[2, 3] It seemed of interest to determine if active transport of organic acid dyes could be demonstrated to occur *in vitro* in isolated choroid plexus tissue of a mature animal, as well as in cultured material from embryos. This is a preliminary report presenting evidence that the choroid plexus of the spiny dogfish can transport chlorphenol red across the choroidal epithelium. In this process the dye is concentrated in the lumen of the capillaries of the choroid plexus.

The brains of dogfish caught in Frenchman's Bay were rapidly dissected and placed in cold balanced salt solution. Fragments of choroid plexus about 1 mm square were then obtained from all the ventricles and were placed in a solution of the following composition, kindly suggested by Forster: in m moles/l., NaCl, 280; KCl, 12; $CaCl_2$, 10; $NaHCO_3$, 4·5; NaH_2PO_4, 0·5; $MgCl_2$, 5; urea, 360.

The drugs studied were incorporated into this basic solution. At least four tissue fragments from each of three dogfish were studied in each experiment. The usual concentration of chlorphenol red was 3×10^{-5} molar. Each tissue fragment was incubated at 18 °C in the well of a glass slide in about 0·2 ml of medium, and was observed periodically through a microscope at a magnification of 50–100 ×. When the tissue was added to the basic medium containing chlorphenol red, the lumen of the capillaries became bluish-red within 10–15 min. The color inside the capillaries unquestionably was more intense than the color of the surrounding solution, thus indicating concentration of the compound within the capillaries.* The cuboidal cells of the choroid plexus remained uncolored. The bluish-red color of the chlorphenol red was easily distinguishable from the occasional orange-brown clump of hemoglobin which remained in the capillaries.

The following experimental results define the active nature of this process. Incubation of the tissue at 2 °C prevented the uptake of the dye and caused a run-out of that previously accumulated at 18 °C. 2,4-Dinitrophenol, 2×10^{-4} molar, inhibited the uptake of the dye. Competition with another organic acid, *p*-aminohippurate (PAH), was demonstrated. Further, addition of 5×10^{-3} molar PAH to a tissue which already had concentrated chlorphenol red resulted in run-out of the lumenal dye. Inhibition of dye uptake was complete when the medium was K-free, and was partial when Mg^{++} or Ca^{++} were omitted.

Phenol red was concentrated in the same manner as chlorphenol red. Bromphenol blue, on the other hand, was taken up by both the cells and, to a lesser extent, the lumen; this seemed independent of the metabolic activity of the tissue, since chilling to 2°C did not alter the uptake of the dye. It is interesting that the isolated choroid plexus of the dogfish behaved similarly to the isolated proximal tubule of the flounder kidney and, therefore, only the tubule and the choroid plexus have been shown to possess this activity.[4, 5]

The important point is that the isolated dogfish choroid plexus moved chlorphenol red from the external medium, representing the CSF, across the choroidal epithelial cells into the capillary lumen in a manner consistent with an active transport process. These observations demonstrate that the choroid plexus is a site of active removal of organic acids from the CSF. There exist, therefore, at least three different routes by which compounds leave CSF,[6–8] i.e. passive diffusion of lipoid-soluble substances; bulk flow of CSF, including all its solutes; and, finally, at the choroid plexus, active transport.

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* Preliminary micropuncture studies have shown that the lumenal concentration of bromphenol blue was at least 2·5 times that of the medium.

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The enzymic oxidation of certain folic acid antagonists

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WE HAVE previously demonstrated[1] that dichloromethotrexate* (DCM), a folic acid antagonist, undergoes oxidative deamination in animals and humans. Based on chemical, physicochemical and synthetic[2] evidence, the structure of 4,7-dihydroxy-DCM* is proposed for this metabolite. We wish to report that the oxidative deamination is effected *in vitro* by an enzyme system which occurs in the soluble fraction of the liver of several animal species.

Apart from simple pteridines,[3, 4] the enzymic oxidation of folic acid derivatives has not hitherto been described. Attempted oxidation of DCM by the ascorbate-ferrous system[5] and by milk† or calf liver‡ xanthine oxidase was unsuccessful in our hands. This failure, together with the differences in distribution between xanthine oxidase and our enzyme system in various species and organs, suggests they are dissimilar. When 2 μmoles of DCM were aerobically incubated for 3–4 hr with rat liver homogenate equivalent to 1 g of tissue at 37°, with shaking, oxidative deamination was virtually complete. Homogenates were prepared in 3 parts of isotonic KCl, using a Potter homogenizer. For the incubation a simple technique was employed: a measured amount of various liver (or other organ) fractions was placed inside a cellulose (Visking Corp.) sac in a flask containing substrate dissolved in 0·1 M Tris buffer [tris-(hydroxymethyl)-aminomethane] of pH 8·2. A blank containing no substrate was likewise prepared. At the end of incubation, the outside solutions were examined by differential spectrophotometry. Oxidation of DCM was indicated by disappearance of the substrate, as well as by appearance of the metabolite. If oxidation was complete, then the spectrum observed was that of 4,7-dihydroxy-DCM alone. The product of the enzymic oxidation was identical with a specimen isolated from the bile of a DCM-treated rabbit,[6] not only spectrophotometrically and chromatographically, but also with respect to mobility in high-voltage electrophoresis.[7] Also, monochloromethotrexate, monobromomethotrexate, chlorobromomethotrexate, dichloroaminopterin, difluoromethotrexate, monochloroaminopterin, and monofluoromethotrexate all appeared to be similarly oxidized. On the other hand, although gradual disappearance was noticed, folic acid, aminopterin, and methotrexate (amethopterin) afforded no well-defined product. In contrast with both DCM and dichloroaminopterin, neither N^{10}-methyl-dichlorofolic acid nor dichlorofolic acid appeared to be affected by the enzyme system, indicating, perhaps, that the first step in the oxidation is hydroxylation of the 7-position, followed by oxidative deamination of the 4-amino group; however, conclusive evidence must await further investigation.

No oxidation occurred if incubation was carried out with liver homogenate previously heated to about 80° for 3 min, thus implicating an enzymic reaction. Additional supporting evidence was supplied by studies on intracellular localization and the optimal pH-value for the reaction; although

* Generic name for N-{3,5-dichloro-4-[(2,4-diamino-6-pteridinylmethyl)-methylamino]benzoyl} glutamic acid, the corresponding chemical name for 4,7-dihydroxy-DCM is therefore N-{3,5-dichloro-4[2-amino-4,7-dihydroxy-6-pteridinylmethyl)-methylamino] benzoyl}-glutamic acid.

† Worthington Biochemical Corporation, Freehold, N.J.

‡ Courtesy of Dr. Ruth K. Kielley of the National Cancer Institute.