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14. We acknowledge with pleasure stimulating conversations with M. A. Sadovsky, I. L. Nersesov, and S. K. Nigmatullaev. C. H. Scholz suggested to us earlier the possibility (which we then discounted) that displaced sources in a spherical isotropic anomalous region could produce the observed effect. Y. P. Aggarwal provided a preprint of an important paper (*13*) and critically reviewed our manuscript. J. W. McCormick gave valuable help in computer problems. One of us (D.T.G.) wishes to acknowledge the Hewlett-Packard Co. for creating a handy desk calculator which can easily manage these complex calculations. Supported by NSF grant GA-36077x. Publication No. 1373 of the Institute of Geophysics and Planetary Physics, University of California at Los Angeles.

* We regret that David T. Griggs died 31 December 1974 before seeing proofs for this report.

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Folate Transport by the Choroid Plexus in vitro

Abstract. *Reduced folates are transported from blood into cerebrospinal fluid against a concentration gradient. In vitro, folates were transported into and released by isolated rabbit choroid plexuses. The choroid plexus uptake mechanism was specific for folates, energy dependent, and depressed by cold temperatures. In vivo, the choroid plexus may transport folates from blood to cerebrospinal fluid.*

Reduced folates, which are present at higher concentrations in brain and cerebrospinal fluid (CSF) than in plasma (*1*), play an essential role in brain metabolism (*2*). Yet, dihydrofolate reductase, the enzyme that catalyzes the reduction of folic acid (FA) to tetrahydrofolic acid (THF), is not present in mammalian brain (*3*). The high concentrations of reduced folates in CSF and brain are due to transport of reduced folates from blood—principally (+)L-5-methyltetrahydrofolate [(+)-MTHF] (*4*), which is the major folate in plasma (*5*). This transport system from blood is saturable; that is, increasing concentrations in plasma do not proportionately increase those in CSF (*6*). Levitt *et al.* suggested that a transport mechanism for reduced folates from blood into CSF through the blood-CSF barrier might reside in the choroid plexus (*4*). This study with the isolated choroid plexus shows that (i) there exists a specific, saturable, energy-dependent uptake system for folates in the choroid plexus; (ii) there also exists a mechanism for release of reduced folates from the choroid plexus; and (iii) the kinetic characteristics of this system of uptake and release are compatible with the choroid plexus being a locus of transport of reduced folates from blood to the CSF (*7*).

(±)[^{14}C]MTHF (60 mc/mmole) and [3′5′,9-^{3}H]FA (16 c/mmole) were obtained from Amersham/Searle. The naturally occurring stereoisomer [(+)L-[^{3}H]MTHF (0.17 c/mmole)] was biosynthesized by injecting a rat with 0.2 mc of [^{3}H]FA and isolating the (+)-[^{3}H]MTHF from the fresh liver the following day (*8*).

The choroid plexuses, obtained from brains of New Zealand white rabbits (1.5 to 2.0 kg) that were killed with intravenous pentobarbital (*9*), were individually placed in 3 ml of artificial CSF (*9*) containing [^{3}H]FA, or (±)-[^{14}C]- or (+)[^{3}H]MTHF. The artificial CSF also contained 1.0 m*M* thiourea and 2.0 m*M* sodium ascorbate to protect the reduced folates from oxidation (*4, 10*). The incubations were carried out in a metabolic shaker at 37°C under 95 percent O_2 and 5 percent CO_2 for 15 or 30 minutes. At the end of the incubation, each choroid plexus was wiped on a glass slide, weighed, and homogenized in 0.5 ml of H_2O. The radioactivity in tissue homogenates and media was determined, and the ratios of tissue to medium (T/M) were calculated (*9*). Substances added to the medium or conditions that depressed the ratios of [^{3}H]FA or (±)[^{14}C]MTHF are indicated in Table 1. Substances added to the medium or conditions that did not significantly affect the T/M ratio of (±)[^{14}C]MTHF included 0.17 μ*M* methotrexate, 1.0 m*M* probenecid, 2.0 m*M* glutamic acid, 2.0 m*M* choline chloride, omission of glucose from the medium, or omission of glucose and incubation under 95 percent N_2 and 5 percent CO_2.

In order to establish whether altera-

Table 1. In virto uptake (expressed as T/M ratios) of [^{14}C]MTHF (left) or [^{3}H]FA (right) by rabbit choroid plexus. Choroid plexuses were incubated for 15 minutes unless otherwise indicated. Values are means ± standard error. The number of experiments at each point is indicated in parentheses.

Experimental condition	Uptake 15-minute (T/M)	MTHF control* (%)	Experimental condition	Uptake 15-minute (T/M)	FA control* (%)
Control, 80 n*M* (15)	11.9 ± 1.1		Control, 32 n*M* (3)	28.6 ± 1.7	
1°C (6)	0.6 ± 0.1	5	1°C (3)	0.5 ± 0.2	2
MTHF, 50.2 μ*M* (3)	0.7 ± 0.1	6			
FA, 32.0 n*M* (3)	5.5 ± 0.4	46	FA, 0.91 μ*M* (3)	2.9 ± 0.1	10
FA, 0.75 μ*M* (3)	2.0 ± 0.3	17	FA, 61.0 μ*M* (3)	2.0 ± 0.1	7
FA, 0.75 m*M* (3)	0.6 ± 0.1	5	MTHF, 0.10 μ*M* (3)	5.5 ± 0.4	19
Dinitrophenol, 1 m*M*; iodoacetate, 2 m*M*; no glucose (12)	7.5 ± 0.7	63	Dinitrophenol, 1 m*M*; iodoacetate, 2 m*M*; no glucose (3)	9.2 ± 1.3	32
Methotrexate, 1.0 μ*M* (3)	5.2 ± 0.6	44			
30-minute incubation, 80 n*M* (5)	26.3 ± 2.4	221	30-minute incubation, 32 n*M* (2)	55.1	193

* All the 15-minute percentage values indicated in the MTHF and FA columns differed significantly from their respective controls ($P < .05$) by Scheffe's method for multiple comparisons in the Gaussian analysis of variance (*13*).

tion of the (±)[^{14}C]MTHF or [^{3}H]FA had occurred in the tissue or medium during the incubations, the choroid plexuses were incubated for 15 minutes in 0.8 μM (±)[^{14}C]MTHF or for 30 minutes in 0.03 μM [^{3}H]FA. After the incubation, the choroid plexuses from each rabbit (~25 mg) were homogenized in 0.2 ml of H_2O with 2 percent mercaptoethanol, covered, heated at 75°C for 30 minutes, and centrifuged at 1560g for 10 minutes. Of the total radioactivity, more than 90 percent was recovered in the supernatant. Portions of the supernatant and incubating media were chromatographed on a DEAE-Sephadex column (*8*), on thin-layer cellulose plates in two systems (*11*), and on Whatman No. 1 paper (*12*). For (±)[^{14}C]MTHF, in all the above systems, more than 90 percent of the total ^{14}C radioactivity recovered [92 ± 5 percent (S.E.; $n = 5$) for the choroid plexus supernatant, and 91 ± 1 percent (S.E.; $n = 15$) for the incubating media] was associated with the peak for (±)MTHF. For [^{3}H]FA, in the two thin-layer systems, more than 88 percent of the recovered radioactivity in all cases [90 percent ($n = 2$) for the choroid plexus supernatant, and 65 percent ($n = 2$) for incubating media] was associated with the peak for folic acid. Less than 5 percent of the ^{3}H in the medium was volatile.

The relative affinity of FA, (±)MTHF, and (+)MTHF for, and the stereospecificity of, the folate concentrating system were determined by incubating the choroid plexuses for 15 minutes in medium containing 8 nM (±)[^{14}C]MTHF and 25 nM [^{3}H]FA or 25 nM (+)[^{3}H]MTHF. The T/M ratio of [^{3}H]FA was 2.25 ± 0.09 (S.E.; $n = 3$) times that of (±)[^{14}C]MTHF in the choroid plexuses. The T/M ratio of (+)[^{3}H]MTHF was 0.95 ± 0.06 ($n = 9$) that of (±)[^{14}C]MTHF. Thus, FA appeared to have a greater affinity for the folate concentrating mechanism of the choroid plexus than MTHF, and the concentrating mechanism was not stereospecific.

The kinetics of [^{14}C]MTHF uptake are indicated in Fig. 1. Increasing the concentration of MTHF in the medium from 8.2 nM to 50.2 μM resulted in a decline in the T/M ratios from 42.7 to 0.7 (Fig. 1A). The relation between total transport velocity (V) and substrate concentration suggested that MTHF uptake depended on a saturable (Y_s) and nonsaturable (Y_{ns}) component. The nonsaturable T/M ratio was assumed to be the T/M ratio (0.7) with 50.2 μM MTHF in the medium (*9*). When transport by the nonsaturable component was subtracted from the values for total transport, the line Y_s resulted and represented the saturable component of MTHF transport. These data were plotted by the Hofstee method (*13*) (Fig. 1C) from which a $Y_{max} = 65.6 \pm 14.0$ pmole min^{-1} ml^{-1} and a K_t (transport constant) of 18.0 nM were calculated (*9*).

The possibility that choroid plexus uptake of (±)MTHF was due to extensive intracellular binding was investigated by three techniques. First, the choroid plexuses were incubated for 15 minutes in 0.22 μM (±)[^{14}C]-MTHF. The tissue was homogenized in 1.5 ml of H_2O with 2 percent mercaptoethanol. After centrifugation at 1560g for 10 minutes, 65 percent ($n = 2$) of the ^{14}C in the homogenate was present in the supernatant. In a second series of experiments, identically prepared homogenates were ultrafiltered at 5 pounds pressure (nitrogen) through a Millipore (PSED 1310) ultrafilter (*14*) with a molecular weight cutoff of 25,000. Only 30 percent ($n = 2$) of the ^{14}C was ultrafilterable. However, after the homogenates were heated for 30 minutes at 75°C, more than 90 percent in all cases ($n = 4$) of the ^{14}C appeared in the supernatant, and the ^{14}C was all ultrafilterable. Third, after homogenizing the choroid plexuses (~25 mg) in 0.2 ml of H_2O with 1 percent mercaptoethanol, the choroid plexus homogenates and nonhomogenized controls were incubated for 30 minutes in 0.15 μM [^{14}C]MTHF. The T/M ratios for the homogenates were obtained by centrifuging at 50,000g for 10 minutes at 4°C, weighing the pellet, and analyzing the ^{14}C in the pellet and medium (*15*). The ratios for the unhomogenized controls incubated at 37°C, and for homogenized choroid plexuses incubated at 37° or 1°C, or at 37°C with 25 μM FA in the medium were 11.5 ± 1.4 ($n = 3$), 2.8 ($n = 2$), 0.9 ($n = 2$), and 1.4 ($n = 2$), respectively. These results showed that a significant amount of the intracellular (±)MTHF was bound. Moreover, these binding results and the weak, although definite, dependence of (±)MTHF uptake by choroid plexus on conditions that impair intracellular energy production (Table 1) raised the possibility that an irreversible, saturable, specific intracellular binding system might account for a major portion of the (±)MTHF uptake by the isolated choroid plexuses.

However, the possibility that the intracellular (±)[^{14}C]MTHF was irreversibly bound inside the choroid plexus became untenable, as shown in the following studies. After incubation for 30 minutes in 0.08 μM (±)[^{14}C]-MTHF, the choroid plexuses were rinsed in artificial CSF (2 seconds) and transferred for a second incubation into artificial CSF containing thiourea and ascorbate without ^{14}C for 20 or 40 minutes. After the second incubation, the percentage of ^{14}C in the medium divided by the total ^{14}C in the tissue and medium was determined. At 20

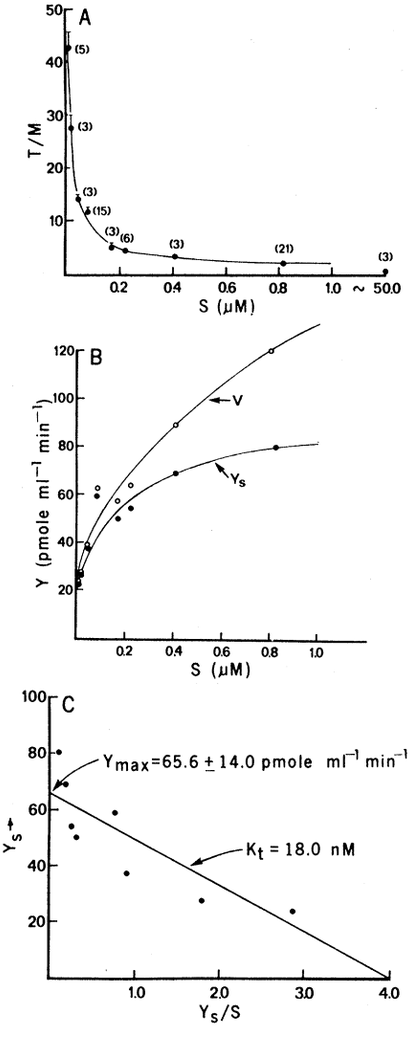

Fig. 1. (A) Reduction of [^{14}C]MTHF T/M ratios with increased medium concentration (S). (B) Total transport (V) and saturable transport (Y_s) as a function of concentration. (C) A Hofstee transformation of Y_s versus Y_s/S. Values represent means ± standard error and numbers in parentheses refer to numbers of choroid plexuses incubated at each concentration. Each choroid plexus in (A) was incubated for 15 minutes in artificial CSF containing various concentrations of (±)[^{14}C]MTHF at 37°C under 95 percent O_2 and 5 percent CO_2.

and 40 minutes, 20.4 ± 1.4 (S.E.; $n = 5$) and 38.6 ± 2.8 (S.E.; $n = 5$) percent of the ^{14}C had been released from the choroid plexuses. Moreover, when 1.0 μM FA was placed in the second medium, ^{14}C release at 20 and 40 minutes increased to 28.0 ± 3.4 (S.E.; $n = 4$) and 45.5 ± 1.0 (S.E.; $n = 4$) ($P < .05$). Moreover, when the second medium was kept at 1°C (without FA), only 15.7 ± 3.9 (S.E.; $n = 3$) was released after 40 minutes ($P < .05$). Thus, a significant portion of the ^{14}C was not irreversibly bound inside the choroid plexuses.

In conclusion, we have demonstrated in the choroid plexus a specific, saturable uptake system for folates that is distinct from the weak carboxylic acid, weak basic, and ascorbate transport systems of the choroid plexus (*9, 14*). That is, substances that completely saturated these systems had no effect on folate uptake. The affinity for the folate transport system is: FA > (±)-MTHF = (+)MTHF > methotrexate. Evidence in favor of the choroid plexus being a locus of folate transport from blood to CSF would include: (i) The observation that the K_t (18.0nM) for MTHF in the isolated choroid plexus (that is, the concentration of MTHF at which the uptake system is half-saturated) was approximately that of the blood (*1*). Moreover, this value was not significantly different from the K_t (12 nM) observed for folate transport from blood into human CSF (*6*). Thus, a slight increase in the concentration in the plasma would readily saturate folate entry into CSF as observed in vivo (*6*). The ascorbate transport system from blood to CSF is similar (*10*). (ii) The finding that, although there was significant intracellular binding of MTHF inside the choroid plexus, there was ready release of intracellular MTHF, a release that was decreased by incubation in the cold. However, questions as to whether reduced folates enter brain primarily from CSF via the choroid plexus rather than directly from blood need to be postponed until this folate transport system in the choroid plexus can be conclusively shown to transport reduced folates in significant amounts from blood to CSF in vivo.

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Toxicology and Pharmacological Action of Anabaena flos-aquae Toxin

Abstract. *Calves, rats, ducks, and goldfish given lethal oral doses of bacteria-free lyophilized cell suspensions of toxic* Anabaena flos-aquae *died as a result of respiratory arrest. Experiments with selected animals and pharmacological preparations showed that the main effect of the toxin was production of a sustained postsynaptic depolarizing neuromuscular blockade.*

Algal poisonings, caused by toxic blooms of the blue-green alga *Anabaena flos-aquae* (Lyngb.) de Bréb., have been an infrequent but repeated occurrence in several countries of the world (*1*). Most economic loss has been confined to livestock and waterfowl that have drunk wind-concentrated surface blooms, dominated by toxic strains of this alga, which develop in eutrophic freshwater lakes and sloughs during the summer months.

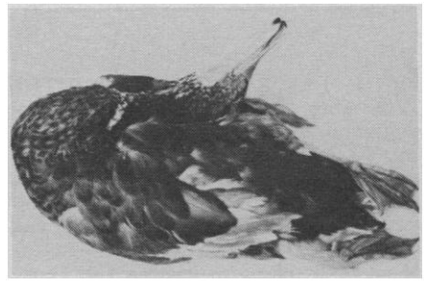

Fig. 1. Male mallard duck given an oral MLD (milligrams per kilogram) of *A. flos-aquae* lyophilized cells, showing opisthotonus. Death occurred in 15 minutes.

We have studied the toxic effects of lyophilized cells in calves, rats, mice, ducks, and goldfish and have also investigated the mechanism of action of toxin extracts of the algae. Bacteria-free cultures of *A. flos-aquae* NRC-44-1 were used (*2*). Mass cultures were concentrated by flash evaporation and lyophilized. Aqueous suspensions of lyophilized cells from different batches of the alga had a consistent minimum lethal dose (MLD) of 60 mg kg^{-1} administered intraperitoneally when bioassayed on male mice (15 to 23 g) or male rats (300 to 400 g).

A toxin extract was prepared from the lyophilized cells by a modification of the procedure of Stavric and Gorham (*3*). The algal powder was ex-