trypsin solution was added to the remaining tissue fragments, which were incubated for another 15 minutes and stirred. The supernatant was again transferred to test tubes containing an equal amount of culture medium with 20 percent calf serum. Both samples were centrifuged gently at 800 rev/min for 2 to 3 minutes. Pellets were resuspended in the culture medium and left standing to allow settling of undigested fragments; the supernatant was then decanted and isolated cells were counted in a hemocytometer. The suspension was adjusted to 10^5 cells per milliliter. Leighton tubes were filled with 1 ml of cell suspension each and incubated at 37°C in a gas phase of 95 percent air and 5 percent CO_2. The medium was replaced every 2 to 3 days.

After 1 week of incubation, complete monolayers were formed, and 1 week later, in all 20 cultures, multiple areas of lysis developed, lined by multinucleated syncytial giant cells. Their nuclei contained from one to five eosinophilic inclusion bodies (Fig. 2). Some primary cultures were subcultured on the 10th day and these secondary cultures showed the same cytopathic effect as did subsequent cultures, at present in the eighth passage, usually by the 4th day of incubation. With the use of electron microscopy, intranuclear and cytoplasmic aggregates of microtubules were demonstrated which had the same dimensions and a strikingly similar arrangement to those observed in the biopsy specimen (Fig. 3).

Fresh primary and secondary cultures of brain cells were fixed in absolute ethanol, incubated with the serum of the patient, monkey and goat antiserums to measles, and with the serum of another patient with SSPE having measles antibody titers of 1 : 128 (hemagglutination-inhibition) and of 1 : 1024 (complement-fixation). The slides were washed in phosphate buffer and incubated with fluorescein-labeled rabbit antiserum to human gamma globulin. The intranuclear and perinuclear inclusion bodies of the syncytial giant cells displayed selective fluorescence, except in goat serum preparations.

In fusion experiments, 10^5 isolated VERO and human embryonic kidney cells per milliliter were mixed with 10^3 infected brain cells per milliliter and cultured without addition of promoting agents. These experiments produced a cytopathic effect in the foreign cell lines after approximately 10 days (Fig. 4), but neither infectivity nor hemagglutination was shown in secondary cultures by transmission of the supernatant of brain cell and VERO or human kidney cell cultures. Hemagglutination of the red cells of green monkeys with a titer up to 1 : 32 occurred when the supernatant of infected cultures, repeatedly frozen and thawed, was used as the antigen, and the effect could be inhibited by dilute human, monkey, and goat antiserums to measles. Subcutaneous or intraperitoneal injection of disrupted cultured VERO cells produced hindleg paralysis and loss of hair in three of 18 gnotobiotic mice (*12*).

In the meantime, free infective virus, transmissible to HeLa cells, has been obtained from the brain biopsy of our second patient with SSPE by mixing brain cells, cultured in the same way as described, and fused with HeLa cells (*13*).

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8. Parke Davis, Pitman-Moore, and Chas. Pfizer vaccines, respectively.
9. Certified Blood Donors Service Inc., Woodbury, N.Y.
10. Grand Island Biological Corp., Grand Island, N.Y.
11. Diluted 1:250 in Puck's Saline A (Difco Laboratories).
12. We thank Dr. J. Lukemeyer, Indiana University Medical Center, for this information.
13. The procedure for isolation of infective measles virus will be published soon (L. Horta-Barbosa, D. A. Fucillo, J. L. Sever, W. Zeman, *Nature*, in press).
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Purines: Active Transport by Isolated Choroid Plexus

Abstract. *Purines are actively accumulated by the isolated rabbit choroid plexus by a specific saturable transport mechanism. In vivo this system probably serves to excrete the purine catabolities of brain from cerebrospinal fluid into blood.*

A specific deficiency of the hypoxanthine phosphoribosyltransferase (E.C. 2.4.2.8), which catalyzes the conversion of hypoxanthine to nucleotide, is associated with profound neurological and behavioral disturbances (Lesch-Nyhan syndrome) (*1*). It has been speculated that uric acid concentrations in blood (and brain) are positively correlated with intelligence (*2*). However, little is known of the mechanisms by which the amounts of purine in brain and cerebrospinal fluid are regulated.

The study of transport by isolated choroid plexus has provided evidence for the existence of specific transport processes which export drugs and certain anions from cerebrospinal fluid to blood (*3*, *4*). Our data obtained with the isolated choroid plexus of the rabbit indicate that this tissue possesses a specific, saturable transport system for purines of high capacity.

Choroid plexuses from the lateral ventricles of female rabbits (1 kg) were incubated at 37°C in Krebs-Henseleit bicarbonate buffer containing C^{14}-labeled purines and other additives. The incubation was performed at 37°C in small stoppered tubes. The tubes were rotated to promote mixing of the tissue

Table 1. Inhibition of xanthine accumulation by various chemical analogs.

Analog	Inhibition (%)
Hypoxanthine	89
Guanine	80
Adenine	76
6-Methyluracil	79
Uracil	66
Pentobarbital	0
Allantoin	0
Allopurinol*	35
Urate	20

* [4-Hydroxypyrazolo(3,4-d)pyrimidine].

with the medium and the atmosphere (95 percent O_2, 5 percent CO_2). At the end of the incubation period the tissue was removed, blotted, weighed, and hydrolyzed in potassium hydroxide; radioactivity was then counted by liquid scintillation. Tritiated inulin was included in the incubation medium, and its presence in the hydrolyzate was used to correct tissue content for adherent purine-containing medium. Metabolism of the radioactive purines was determined after analysis of the tissue and medium by paper chromatography (*5*). The data are expressed as micromoles of purine accumulated per gram wet weight of tissue in 30 minutes of incubation. The actual combined weight of the plexuses was 13.94 ± 1.38 mg (S.D.).

Most of the kinetic measurements were made with xanthine as substrate, since more than 95 percent of the radioactivity accumulated by the tissue was recoverable as chemically unaltered xanthine. In media containing 6.3×10^{-6} mole/liter xanthine, at 30 minutes the ratio of tissue to medium xanthine concentration was 2.81 ± 0.31 (S.E., 39 observations). Gradients were not established in the presence of cold or the metabolic inhibitor dinitrophenol, indicating the requirement of energy for the observed accumulation. Uptake of this purine by the tissue was linear for 1 hour, and the total amount accumulated at 30 minutes was taken as the initial rate of transport. A plot of the initial rate of uptake as a function of the medium concentration shows distinct saturation (Fig. 1). A reciprocal plot of the data gives a concentration for half-saturation (K_m) for xanthine transport of 0.17 mmole/liter and a maximum velocity (V_{max}) of 0.42 μmole per gram of tissue (wet weight) in 30 minutes. The observed K_m is in the vicinity of the known concentrations of hypoxanthine and xanthine in the cerebrospinal fluid: in man the total concentration of xanthine and hypoxanthine is 0.01 mmole/liter in normals, and 0.280 mmole/liter in patients with the Lesch-Nyhan syndrome (*6*).

The inhibition of xanthine accumulation by various purines and analogs was used to determine their affinity for the carrier mechanism. Of the paired plexuses obtainable from a single animal, one was incubated in medium which contained radioactive xanthine (6.3×10^{-6} mole/liter) and served as a control for the second, the medium for which also contained the test compound (1 mmole/liter). The average percentages of inhibition are indicated in Table 1. The purines in general show greater inhibition than the pyrimidines (pentobarbital is not inhibitory). Allopurinol and urate, which are only weakly inhibitory, show alterations in the imidazole portion of the purine molecule (*7*). It is suggested that the principal structural requirement for transport lies in the complete purine ring and particularly in the combination of an unsubstituted imidazole with the pyrimidine moiety.

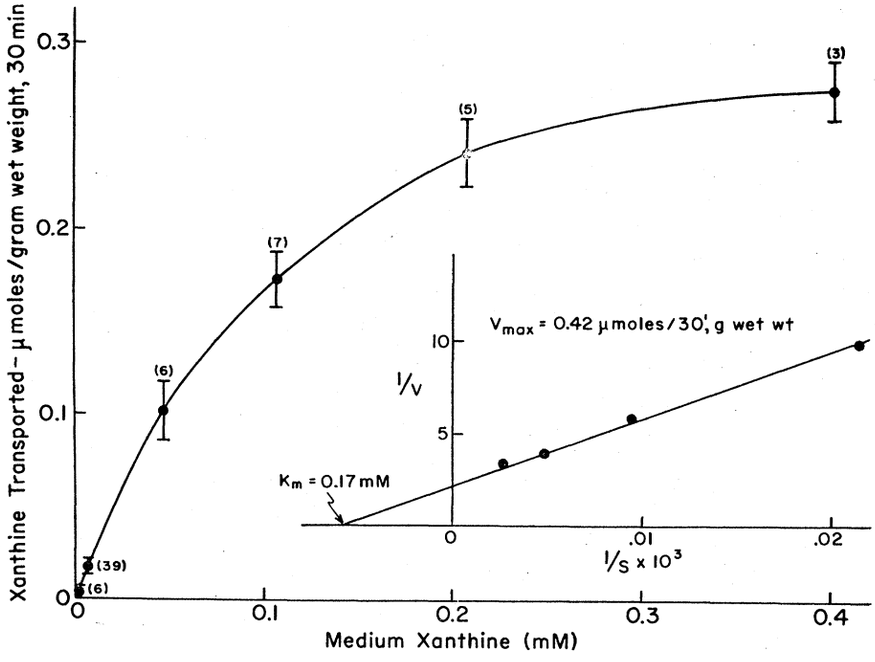

Fig. 1. Xanthine accumulated in tissue as a function of its concentration in the medium. The bars correspond to standard errors of the mean; the numbers of observations are given in parentheses. The inner figure is a reciprocal plot of the data.

Since urate is of particular physiologic interest, its relatively poor affinity for the xanthine carrier is especially noteworthy. Even at substrate concentrations as low as 10^{-6} mole/liter, no accumulation of urate to concentrations greater than the medium was observed (*8*). It may be that, as for erythrocytes, the carrier has affinity only for the undissociated uric acid (*9*). At the *p*H of the medium this constitutes but a small fraction of the total urate present. However, brain does not appear to contain the enzyme xanthine oxidase which catalyzes the production of urate from xanthine and hypoxanthine (*10*). It is the latter two compounds which are therefore the proper end products of purine metabolism by brain, and it is these which are most avidly accumulated by the choroid plexus. While it cannot be determined from studies of the isolated tissue whether the net transport of purines is from cerebrospinal fluid to blood, this is most probable since the same surface of the plexus which faces outward into the medium under experimental conditions is exposed to the cerebrospinal fluid in vivo. The choroid plexus would serve thus as an excretory mechanism for endogenous purine catabolites of brain, thereby regulating their levels in cerebrospinal fluid.

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7. *p*-Aminohippurate (10 mmole/liter), a substrate for the anion-organic acid transport system (*4*), and tetramethylammonium bromide (10 mmole/liter), presumed substrate of the quartenary amine system (*3*), were not inhibitory.
8. Over 95 percent of the radioactive urate added was recoverable as such at the end of the incubation. Hypoxanthine, which was extensively accumulated by the tissue, was also extensively converted to nucleotide.
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