nism used, there is evidence that gyrase remains bound at the same site on the DNA, acting processively through cycles of sign inversion until binding is sufficiently weakened by the increased supertwist density that the enzyme is released (*25*).

In contrast to other models, supercoiling is necessarily quantized in the sign inversion scheme. The change in linking number per cycle is always two, even though the work required to change it increases with increasing supertwist density. Therefore, if the number of ATP molecules bound per cycle is constant, the thermodynamic efficiency of the reaction must progressively increase. A lower limit on the number of ATP molecules hydrolyzed per cycle can be estimated from the work (*19*) required to reduce by two the linking number of the most negatively supercoiled DNA molecule which can be further supercoiled by gyrase. Such an analysis suggests that at least two ATP's must be hydrolyzed for each round of sign inversion.

Most other models for supertwisting invoke an initial energy-requiring segregation of positive supercoils followed by their passive relaxation. Sign inversion is an active process in which breakage-and-rejoining is intrinsically coupled with the mechanical energy-requiring step of DNA traversal. The following is presented as a plausible sequence of events linking ATP binding and hydrolysis (*6*) to supertwisting. Gyrase binds to DNA forming a right-handed node. The subsequent binding of ATP by the *gyrB* protomers changes the conformation of gyrase (*21*) to one that stabilizes instead a left-handed node. To relieve the resultant strain, gyrase performs the remarkable feat of passing the front segment of DNA through a double-strand break in the back segment, and perhaps through the enzyme itself, while holding both ends of the break so that they cannot rotate. The energy of the broken phosphodiester bonds, conserved as a protein-DNA bond, is used to reseal the break after sign inversion. After resealing the break, gyrase catalyzes the hydrolysis of ATP. Release of adenosine diphosphate (ADP) and P_i allows the enzyme to return to its initial conformation so that a new cycle of sign inversion can begin.

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26. In Fig. 1, the axis is a line through the node perpendicular to the plane of the paper.
27. The size and structure of the loops of DNA determined by the node have no effect on the change in linking number by sign inversion. However, if the loops are intertwined so that they are topologically linked, a knot is produced in the DNA by the process of sign inversion.
28. Topoisomer standards were prepared by relaxing native p15A DNA in two separate reactions, each containing 0.5 μg of DNA and either 5 or 10 ng of ω protein. After incubation for 25 minutes at 37°C, the reactions were stopped and combined with 0.1 μg of native p15A DNA, to yield a mixture of relaxed and negatively supercoiled topoisomers covering the range resolvable by electrophoresis through a 1.2 percent agarose gel.
29. The sign inversion model was developed during conversations with our former colleague N. Patrick Higgins; we thank him for his help. Supported by NIH grant GM-21397 and (to P.O.B.) NIH fellowship GM-07281.

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Cerebral Vessels Have the Capacity to Transport Sodium and Potassium

Abstract. *The activity of Na^+,K^+-activated adenosinetriphosphatase and the uptake of a potassium analog, rubidium, were found to be similar in cerebral microvessels and choroid plexus when measured in vitro. This similarity suggests that sodium and potassium concentrations in the nascent brain extracellular fluid are determined by the same active process that regulates their concentration in nascent cerebrospinal fluid. The brain microvessels may thereby play an active role in brain potassium homeostasis and brain extracellular fluid formation.*

The endothelial cells lining the cerebral blood vessels are joined by tight junctions and contain few, if any, pinocytotic vesicles (*1*). This continuous endothelial membrane forms a limiting barrier, restricting the movement of many solutes between blood and brain. The study of movement of solutes through this barrier has been advanced by the recent development of techniques for isolation of metabolically active brain microvessels (*2, 3*). Experiments with preparations of these isolated vessels have shown that they contain specific transport systems for certain amino acids and sugars (*4, 5*). The mechanism by which the major cations Na^+ and K^+ cross this barrier has not been experimentally defined; however, the presence of a specific transport system may be inferred.

Cerebrospinal fluid (CSF) and brain extracellular fluid (ECF) are identical or virtually identical (*6*). The concentration of Na^+ and K^+ in the nascent CSF secreted by the choroid plexus has been shown to be dependent on a ouabain-sensitive process, indicating that Na^+, K^+-adenosinetriphosphatase is involved in the transport (*7, 8*) and that transport of Na^+ is a major energy-dependent step in the formation of the fluid. Although the CSF and brain ECF are contiguous at the ventricular wall, where movement of even large solutes is not impeded (*9*), it is unlikely that the secretory mechanisms

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of the choroid plexus have a controlling influence on the concentration of cations in the brain ECF. The implication then is that similar enzymatic machinery for the secretion and regulation of these cations in the nascent brain ECF exists at other sites. These sites are presumably at the barrier or adjacent to it. We found that the activity of Na^+,K^+-adenosinetriphosphatase and the uptake of rubidium, a potassium analog, were similar in brain microvessels and choroid plexus. This implies that these microvessels have cation transport systems like those in choroid plexus.

Tissues were taken from adult Sprague-Dawley rats of unselected sex. Cerebral microvessels were isolated (*10*) by a modification of the methods of Goldstein *et al.* (*3*) and of Mrsulja *et al.* (*5*). The isolated vessels contained many capillary segments and fewer segments of small arterioles and venules. No other cellular or subcellular elements were seen by light microscopy, and transmission electron microscopy showed that vessels were circumferentially complete and surrounded by an intact basement membrane (Fig. 1B).

The Na^+,K^+-adenosinetriphosphatase activity of the isolated cerebral microvessels, choroid plexus, and red blood cells (RBC's) was determined by measuring the inorganic phosphate released from adenosine triphosphate (ATP) in the absence and presence of ouabain (1 m*M*). It was the portion of the total adenosinetriphosphatase activity that was inhibited by ouabain. Na^+,K^+-Adenosinetriphosphatase activity was also measured in peripheral endothelial cells obtained from human umbilical veins by limited collagenase digestion (*11*).

The amount of inorganic phosphate released by the isolated microvessels increased linearly with time and was inhibited by cold (0°C). The specific Na^+,K^+-adenosinetriphosphatase activity of the isolated cerebral microvessels was similar to that measured in the choroid plexus (Table 1). This activity was much greater than that of RBC's. The activity derived from RBC's added little to the total Na^+,K^+-adenosinetriphosphatase activity determined in choroid plexus and isolated microvessels, only slightly lowering their specific activity. The activity in the microvessel isolate was more than 500 times greater than that measured in the human peripheral endothelial cells. The substrate (ATP) dependence of the microvessel Na^+,K^+-adenosinetriphosphatase and the kinetic parameters obtained from a computer fit of the data by linear regression analysis are shown in Fig. 1A. The Michaelis constant, K_m, calculated is similar to one that we had determined for C_6 rat astrocytoma cells; the maximum velocity, V_{max}, is approximately ten times higher (*12*).

Table 1. Activity of Na^+,K^+-activated adenosinetriphosphatase in cerebral microvessels, choroid plexus, umbilical vein endothelial cells, and red blood cells. Each value is the mean ± standard error; *N* is the number of experiments. The value for each experiment was the average of four determinations. Protein was determined by the method of Lowry *et al.* (*17*).

Tissue	Specific activity*	*N*
Cerebral microvessels	13.237 ± 3.175	7
Choroid plexus	8.260 ± 1.630	5
Umbilical vein endothelial cells	0.024 ± 0.003	4
Red blood cells	0.005 ± 0.002	4

*Expressed as micromoles of inorganic phosphate per milligram of protein per hour.

The relationship of enzyme activity to cation uptake was studied in the two tissues, cerebral microvessels and choroid plexus, by measuring uptake of ^{86}Rb (*13*). The ^{86}Rb uptake by the isolated microvessels increased with time, was sensitive to ouabain, and was temperature-dependent (Fig. 1C). During a 10-minute incubation period ^{86}Rb uptake decreased 46 percent in the presence of 1 m*M* ouabain and 62 percent at 0°C. The uptake of ^{86}Rb per unit weight of tissue protein by microvessels was 2.8 times greater than that by choroid plexus (determined in two experiments, using mean values of quadruplicate samples).

Morphological localization of the Na^+,K^+-adenosinetriphosphatase in the microvessel isolate was determined by autoradiography, using [^{3}H]ouabain as a radioligand. The autoradiographs showed dense deposits of silver grains outlining the capillaries as well as the larger vessels. At the light microscope level these grains were uniformly present along the vessels (Fig. 1D).

The data demonstrate that isolated brain microvessels contain Na^+,K^+-adenosinetriphosphatase activity similar to that in choroid plexus; the uptake of a potassium analog by the isolated microvessels is by a ouabain-sensitive process and is quantitatively similar to the uptake by choroid plexus; and the enzyme Na^+,K^+-adenosinetriphosphatase is anatomically associated with microvessels including capillaries. These features may be unique to the blood vessels of the central nervous system, but a direct comparison with other blood vessels has not been possible. The specific activity of

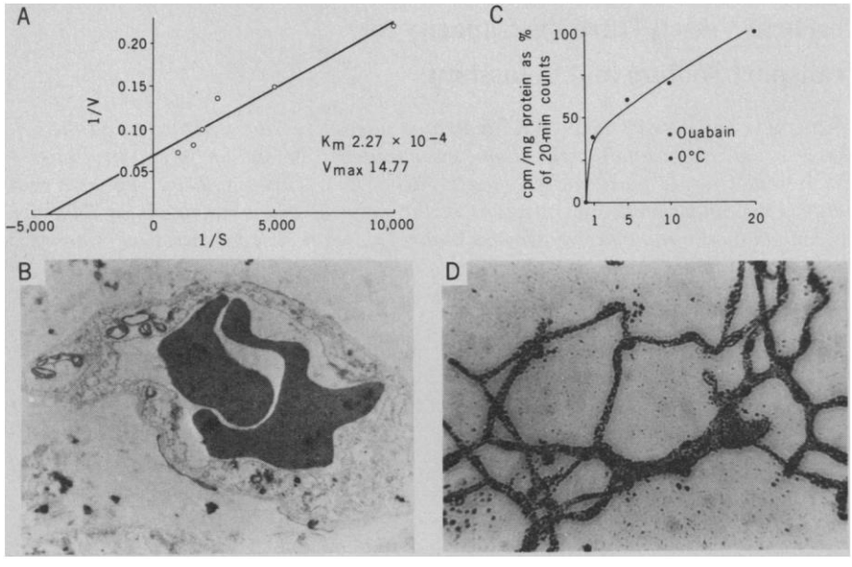

Fig. 1 (A). Relationship between Na^+,K^+-adenosinetriphosphatase activity (*V*; micromoles of inorganic phosphate per milligram of protein per hour) in cerebral microvessels and ATP concentration (*S*; moles per liter). Each point represents the mean of four determinations. The kinetic parameters shown were determined by linear regression analysis. (B) Electron micrograph showing an isolated cerebral capillary segment in cross section with a red blood cell in its lumen. The capillary basement membrane is intact (×5275). (C) Rubidium uptake by isolated microvessels expressed as a percentage of the uptake at 20 minutes, plotted against time (minutes). Each point represents the mean of three experiments, each experiment done in quadruplicate. The range of the 20-minute tissue counts per minute per milligram of protein was 1374 to 5831. The effects of ouabain (1 m*M*) and cold (0°C) are shown. (D) Autoradiograph of the microvessel isolate after incubation with [^{3}H]ouabain, showing dense deposits of silver granules outlining the capillaries as well as a larger vessel near the center (×200).

the enzyme in cerebral microvessels was much greater than that measured in vitro in peripheral endothelial cells, but the implication that a difference of such large magnitude would be present in whole vessel from another source is not yet warranted.

Secretion of CSF by the choroid plexus has been shown to be directly related to the activity of Na^+,K^+-adenosinetriphosphatase in that structure (*14*), and ouabain binding sites have been shown to be abundant on the choroid plexus epithelial surface (*15*). In addition, the concentration of K^+ measured in the nascent fluid secreted by the choroid plexus is dependent on the activity of the enzyme (*7*). It was found in other tissues that the capacity to actively transport Na^+ or K^+ is directly proportional to the activity of Na^+,K^+-adenosinetriphosphatase in that tissue, and the ratio of cation transported to enzyme activity measured was nearly equal when six different tissues were studied (*16*). We have shown that Na^+,K^+-adenosinetriphosphatase activity and cation uptake in isolated microvessels are very similar to those in choroid plexus and we infer that the brain microvessels have the capacity to transport Na^+ and K^+ by a similar mechanism. We suggest that the regulation of K^+ in the nascent brain extracellular fluid and the movement of Na^+ into that fluid from microvessels is related to the activity of Na^+,K^+-adenosinetriphosphatase present in those vessels.

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Differential Competition with Cytotoxic Agents: An Approach to Selectivity in Cancer Chemotherapy

Abstract. *An approach to increasing the selectivity of cancer chemotherapeutic agents is presented in which noncytotoxic competitive substrates are used to discern the differences in structural requirements for transport of cytotoxic agents between tumor cells and a sensitive host tissue, the hematopoietic precursor cells of the bone marrow. Examples are given for two such systems, one responsible for the transport of nucleosides and another for the transport of amino acids. Cytidine is twice as effective in reducing the toxicity of showdomycin for murine bone marrow cells in culture as it is for murine L1210 leukemia cells. Conversely, homoleucine is twice as effective in reducing the toxicity of melphalan for L1210 cells as it is for bone marrow cells. These observations can serve as a basis for the development of bone marrow protective agents and for the design of cytotoxic agents that may be preferentially transported into tumor cells.*

A principal concern in cancer chemotherapy is the protection of sensitive host tissue, such as the hematopoietic progenitor cells of the bone marrow, from the action of cytotoxic agents. It may be possible to do this by taking advantage of the many differences in the architecture of the plasma membrane between normal cells and tumor cells (*1*), which could alter the specificity of transport sites. Such transport systems are present in mammalian cells for the uptake of nucleosides and amino acids and are mediated by a carrier, a component of the plasma membrane that often exhibits high affinity and limited capacity for the nutrient. The structural requirement for substrates to participate in carrier uptake depends on the individual transport system, and certain cytotoxic agents of value in cancer chemotherapy have been shown to enter cells by such transport systems (*2-5*). The limited capacity of transport carriers, termed saturability, makes them susceptible to competitive interaction, and thus the activity of cytotoxic agents that gain entrance to the cell through their agency may be con-

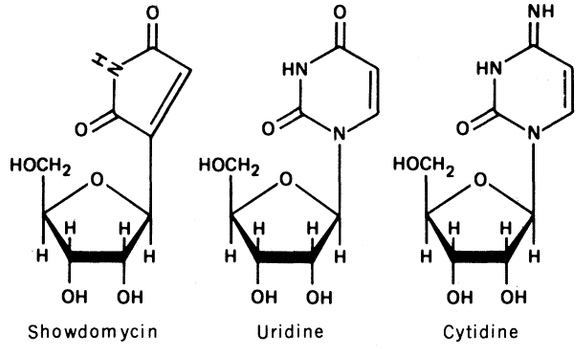

Fig. 1. Structures of showdomycin, uridine, and cytidine.

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