Transport of 1-Aminocyclopentanecarboxylic Acid from Feline Cerebrospinal Fluid

Abstract. *1-Aminocyclopentanecarboxylic acid was cleared from cerebrospinal fluid of the cat by a saturable mechanism. Clearance was inhibited by naturally occurring neutral amino acids. Carrier transport may explain the low ratio of amino acid in spinal fluid to that in plasma.*

The concentration of many amino acids in mammalian cerebrospinal fluid (CSF) is only a fraction of that in plasma (*1*). The mechanisms regulating amino acid concentration in CSF are unknown, but may include carrier-mediated transport from fluid to plasma. Transport mechanisms serving to maintain low concentrations of many organic acids, ions, and glucose in CSF are well known (*2*). Studies of CSF transport in vivo correlate qualitatively with studies of accumulation in the choroid plexus of the same solutes in vitro (*3*). An analogy between CSF transport and renal tubular transport has been suggested (*2*). In both kidney slices (*4*) and choroid plexus (*5*) amino acids accumulate against a concentration gradient in vitro. These observations have prompted a study of the clearance from CSF of a nonmetabolized neutral amino acid, 1-aminocyclopentanecarboxylic acid (cycloleucine) (*6*).

Ventriculocisternal perfusions were performed in adult cats anesthetized with pentobarbital (45 mg/kg) intraperitoneally (*7*). Artificial CSF containing ^{125}I-albumin, ^{14}C-cycloleucine (specific activity 4 mc/mmole), and various concentrations of unlabeled cycloleucine was perfused at a rate of 90 μl/min into the left lateral ventricle and collected at the cisterna magna. A steady concentration of each isotope in the cisternal effluent was reached after 1 hour of perfusion. After this time, three 20-minute samples of effluent were collected, weighed, and assayed in duplicate for ^{125}I and ^{14}C concentrations. Values obtained during the 1-hour period were averaged for the calculation of rates of formation and reabsorption of CSF, and CSF clearance of cycloleucine (*8*). Continuous perfusion for 6 to 7 hours resulted in no greater than a 10-percent change in the CSF clearance of cycloleucine when calculated for successive hourly intervals. Therefore, it was possible to obtain steady-state clearance values for three concentrations of cycloleucine in an individual animal. Perfusions were always performed in the order of increasing concentration of cycloleucine, and 1 hour was allowed for a new equilibrium to be reached before the effluent was collected for assay. The velocity of transport of cycloleucine from CSF was calculated as the product of the clearance value and the perfusate concentration.

Increasing perfusate concentrations of cycloleucine from 0.01 to 10 mmole/liter resulted in a gradual decline of clearance (K_0) of cycloleucine from 0.034 to 0.006 ml/min (Fig. 1A). The relation between total transport velocity (V) and substrate concentration suggested that cycloleucine was removed from CSF by a saturable and a nonsaturable component (Fig. 1B). The value 0.0046 (K_{0_D}), representing clearance by the nonsaturable component, was given by the slope of the curve between 2 and 10 mmole of substrate per liter. When transport by this component was subtracted from the values for total transport (V), the interrupted line (Y) resulted; this line represents the saturable component of cycloleucine transport. These data were then plotted by the Lineweaver-Burk method (*9*) (Fig. 1C) from which a V_{max} of 0.023 μmole/min and a K_t (affinity constant) of 1.4 mmole were calculated. Only 19 $\pm$ 3 percent (mean $\pm$ 1 S.E., n = 10) lost from the CSF during 6-hour perfusions was recovered in the brain, the remainder presumably having been transported to blood.

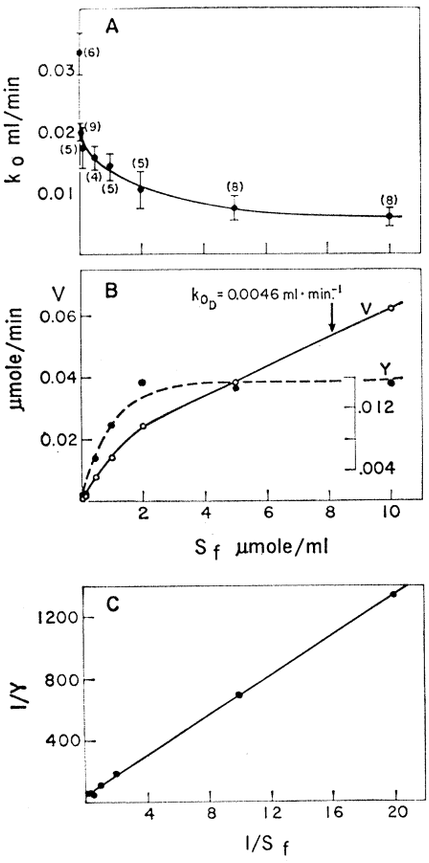

Fig. 1. Reduction of cycloleucine clearance from CSF with increasing perfusate concentration (S_f) is shown in (A). In (B), net transport of cycloleucine from CSF (V) is expressed as $K_0 \times S_f$. The nonsaturable clearance (K_{0_D}) was determined from the linear portion of V. The curve Y represents the saturable component of cycloleucine transport [$Y = (K_0 - K_{0_D}) \times S_f$]. (C) represents a reciprocal plot of Y and S_f values. Vertical bars represent standard error of the mean and numbers in parentheses refer to numbers of steady-state clearance values at each concentration.

The addition of 5 mmole of L-alanine or L-valine per liter to the perfusate reduced the CSF clearance of 0.05 mmole of cycloleucine per liter by average values of 50 $\pm$ 6 and 93 $\pm$ 3 percent, respectively. L-Lysine reduced cycloleucine clearance by only 10 $\pm$ 1 percent. These values represent means ($\pm$ 1 S.E.) for three animals in each group. The apparent specificity of the cycloleucine carrier for neutral amino acids has been observed in other tissues (*10*). These results suggest that natural amino acids may be transported from CSF, which may account for the low ratio of amino acid in CSF to that in plasma.

Transport of amino acids from the CSF may influence the amino acid concentration in brain. The concentration in brain of a number of solutes has been shown to be regulated in part by the concentration in CSF (*11*). Davson (*12*) has proposed that the low CSF concentration of these solutes serves as a "sink" for the brain.

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The Moon: Time of Appearance and Nearest Approach to Earth

Cloud, in his recent article "Atmospheric and hydrospheric evolution on the primitive earth" (*1*), cites my report (*2*) as suggesting that sedimentary textures in younger rocks call for lunar origin in more recent times (times more recent than 2 aeons). This is a misinterpretation not only of my views but of Gerstenkorn's (*3*) capture mechanism on which they were based. This should be corrected, since there is no basic conflict between Cloud's conclusion that lunar tides appeared in the geologic record more than 2 aeons ago and my conclusion that the closest approach of the moon to the earth took place 0.7 aeon ago. According to Gerstenkorn's theory the moon in this interval of time was in retrograde orbit and was gradually approaching the earth. This is illustrated graphically in an article by MacDonald (*4*, Fig. 4) which is cited by Cloud.

The beginning of the Proterozoic about 2.5 aeons ago appears to be the most probable time for the capture of the moon. Pettijohn (*5*) notes the first appearance of the platform facies in the sedimentary record at this time, which involves the deposition of high-energy sediments. Gill (*6*) considers the change from graywacke and argillite to quartzose sedimentation at the Archean-Proterozoic boundary to be a real geologic event. This change indicates the appearance of a new source of energy in the oceans, with a worldwide increase in the amplitude of tides and currents. The amplitudes of stromatolites noted by Cloud give additional confirmation. The capture of the moon at this time affords a logical explanation for these observations.

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I did indeed misinterpret Olson's views in the sense that he (*1*) did not specify that the moon first appeared in orbit more recently than 2 aeons ago. He said only that it probably made its closest approach during the "Lipalian interval," seemingly interpreted by him as around 0.6 to 1 aeon ago. I took this to imply a belief that the moon appeared at about the same time (*2*), simply because it seems to follow from any capture mechanism that the difference in time between first appearance and nearest approach would be within limits too brief for geochronological resolution (*3*). If we assume, however, that a moon captured 2.5 aeons ago could delay its nearest approach to the earth until 0.7 aeon ago, as Olson now specifies, what would be the geological consequences?

If the moon approached the earth to within 2.89 earth radii, as Gerstenkorn suggests (*4*) and Olson seems to accept, very high temperatures would have been generated by tidal friction (*3*). Such temperatures, if they did not vaporize earth and moon, would probably have caused the extensive or complete loss of any then existing atmosphere and hydrosphere from the earth's gravity field. In any case, it is highly unlikely that life could have persisted. Such events would be visible in the geological record; but I, at least, am unable to see them in rocks younger than 3.5 aeons. If either the atmosphere or life (or even oxygen-releasing photosynthesis) started anew 0.6 to 1 aeon ago, we should see geochemical and paleontological evidence of a return to anoxygenous conditions, and then a new episode of evolution of oxygen in the atmosphere in more recent times. The geologic record shows nothing like this. It implies instead a continuous (though fluctuating) addition of oxygen to the atmosphere from about 2 aeons ago until now.

To me it is simply unbelievable that there were "tides with amplitudes of thousands of feet" (*1*, p. 461), called for by a very near approach of the moon, during any part of earth history for which we have a record in the form of either sedimentary rocks or erosion surfaces. Moreover, the presence of thick and extensive Molasse-type sandstones and conglomerates in the upper part of the Swaziland System (*4*), which is more than 3 aeons old, means that, by Olson's own criteria, the moon could not have been acquired as recently even as 2.5 aeons ago.

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Green Monkey Agent of Disease

With regard to our report on the agent of disease contracted from African green monkeys (*1*), we regret that prior to our publication we were not aware of a publication by Siegert *et al.* (*2*) in which these authors describe certain properties of an agent (which they call Marburg virus) isolated by them from patients suffering from the disease in question.

Their observations of the morphology and ether sensitivity of the agent are similar to ours. They also point out the possible relation of Marburg virus to the members of the stomatoviridae group. With the aid of immunofluorescence, they were able to demonstrate replication of the Marburg virus in Vero cells. It would appear that Siegert's group and ours at the National Communicable Disease Center are working with the same organism.

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