that Po halos can be positively identified by ring structure studies alone. That x-ray fluorescence analyses also provide quite convincing evidence is seen in Fig. 3c, where I show for the first time the x-ray spectra of a Po halo radiocenter (specifically, a ^{218}Po halo). Comparison of Fig. 3, b and c, reveals that the Pb in the Po halo radiocenter in fluorite did not arise from in situ decay of U. [Longer runs have shown small amounts as Se as well as U in some Po halo radiocenters (*18*).] On the other hand, the presence of Pb is to be expected in a ^{218}Po halo radiocenter because the decay product is ^{206}Pb. That the parent nuclide was ^{218}Po and not a β-decaying isomer precursor (*13, 20*) follows from half-life considerations of the U halo U/Pb ratio (> 10); the proposed isomer, if formed at nucleosynthesis, should now be detectable in Po halo radiocenters. No trace of this isomer has yet been found, and I thus view the isomer hypothesis as untenable.

The x-ray data in Fig. 3c are unambiguous and should remove any doubt that previously reported ^{206}Pb/^{207}Pb mass ratios (*3, 13*) actually are Pb isotope ratios, and in fact represent a new type of Pb derived specifically from Po α-decay. In summary, the combined results of ring structure studies, mass spectrometric analyses, and electron induced x-ray fluorescence present a compelling case for the independent existence of Po halos. The question is, can they be explained by presently accepted cosmological and geological concepts relating to the origin and development of Earth?

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16. From α-decay theory, $d\lambda/\lambda \simeq (3/2)(ZR)^{1/2}(dR/R) + (2Z/E^{1/2})(dE/E)$, where Z is the atomic number, R is the nuclear radius in 10^{-15} m, and E ($= E_\alpha$) is the α-decay energy in million electron volts. A particle of mass m and charge z has a range r (halo radius), given by the expression $r = \text{constant} \times E^2/mz^2$. Then $d\lambda/\lambda \simeq 43(dR/R) + 46(dr/r)$. If the difference between the halo radius and the coloration band size at 4.2 Mev is real, then $\Delta r = -0.4$ μm and $d\lambda/\lambda \simeq 46(-0.4/13) = -1.4$. Since the minimum uncertainty in making comparative range measurements is $\Delta r = 0.1$ μm, it is actually impossible to establish the constancy of λ (for ^{238}U) from radiohalo data any better than $d\lambda/\lambda \simeq 46(0.1/13) = 0.35$. Also, if $dE/E = 0$ while $dR/R \neq 0$, then $d\lambda/\lambda \neq 0$. In such a case, halos furnish no proof that λ is constant.
17. Some inner ring coloration in Fig. 1f results from other α-emitters in the U decay chain. Fission track analysis shows that the dose of α-particles from ^{238}U is only about 10^{13} per square centimeter, about ten times less than the ^{4}He ion dose for medium coloration.
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γ-Glutamyl Transpeptidase in Brain Capillaries: Possible Site of a Blood-Brain Barrier for Amino Acids

Abstract. *A fraction containing capillaries and rich in γ-glutamyl transpeptidase was isolated from homogenates of bovine brain cortex by density gradient centrifugation. The enzyme was localized in the endothelial cells by a histochemical procedure. γ-Glutamyl transpeptidase may function in the transfer of some amino acids across the blood-brain barrier.*

γ-Glutamyl transpeptidase catalyzes transfer of the γ-glutamyl residue of glutathione to amino acids according to the following reaction (*1*)

Glutathione + amino acid ⇆
γ-glutamyl amino acid + cysteinylglycine

It was proposed that this reaction, which makes the degradation of glutathione dependent on amino acids, functions in amino acid transport (*2*).

The hypothesis is supported by the finding that γ-glutamyl transpeptidase is associated with cell membranes, especially in those cells where a high rate of amino acid transport is anticipated. Thus, histochemical studies have shown that the enzyme is heavily concentrated in the brush border of the proximal convoluted tubules of the kidney, the apical portion of the intestinal epithelium (*2, 3*), the choroid plexus, and brain capillaries (*4*). The reaction catalyzed by γ-glutamyl transpeptidase was later integrated into the γ-glutamyl cycle (*5*), proposed as a system for amino acid transport. After the amino acid is converted to its γ-glutamyl derivative by γ-glutamyl transpeptidase at or near the cell surface, a translocation step occurs bringing the γ-glutamyl amino acid into the cell (*5*). The amino acid is then released through the action of γ-glutamyl cyclotransferase (*6*) with the concomitant formation of L-pyrrolidonecarboxylic acid (*7*). Conversion of pyrrolidonecarboxylic acid to glutamate (*8*) and synthesis of glutathione complete the cycle. Evidence for the participation of the γ-glutamyl cycle in amino acid transport has been reviewed (*5, 9–11*).

γ-Glutamyl transpeptidase was de-

termined with L-γ-glutamyl-*p*-nitroanilide as the substrate (*12*). We also measured alkaline phosphatase (*13*) because it is localized in brain capillaries (*14*) and 5′-nucleotidase (*15*) because it is present in membrane fractions (*16*).

Fresh bovine brains were obtained from the local slaughterhouse and transported on ice to the laboratory. Unless otherwise stated, all operations were carried out at 0°C. The brain was freed from the meninges, and the cortex was homogenized in ten volumes of 0.32*M* sucrose with a Potter-Elvehjem glass homogenizer equipped with a motor-driven Teflon pestle. Three upward and downward strokes of the homogenizer were applied. The homogenate was centrifuged at 1000*g* for 10 minutes (RC 2-B Sorvall refrigerated centrifuge). The supernatant fraction (S_1) was discarded and the pellet (P_1) was resuspended in two volumes of 0.32*M* sucrose and placed on a sucrose density gradient prepared from layers of 5 ml each of solutions of 2.3*M*, 2.0*M*, 1.7*M*, 1.5*M*, 1.2*M*, and 0.8*M*. The tubes were centrifuged at 53,000*g* for 2 hours (SW 25.1 swinging bucket rotor of the model L Beckman ultracentrifuge).

More than 60 percent of γ-glutamyl transpeptidase was sedimented by low-speed centrifugation (1000*g*) (Table 1). By comparison less than half of the alkaline phosphatase activity sedimented under the same conditions; the bulk of 5′-nucleotidase remained in the supernatant fraction. Density gradient centrifugation yielded five bands. In one of these, fraction 5, which sedimented between 1.7 and 1.5*M* sucrose, the specific activity of γ-glutamyl transpeptidase was more than eightfold higher than that in the starting homogenate and contained more than 20 percent of the total transpeptidase activity. The same fraction showed about a fourfold enrichment of alkaline phosphatase while the specific activity of 5′-nucleotidase was less than that in the crude homogenate.

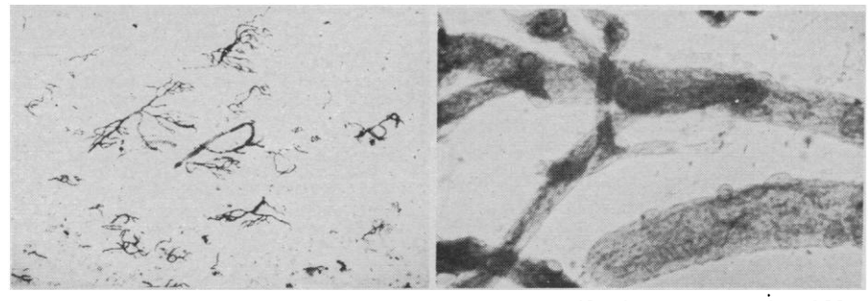

Fig. 1. Isolated capillaries from bovine brain at two magnifications (× 11 and × 355) stained for γ-glutamyl transpeptidase activity.

The particulate material in fraction 5 was further examined under the light microscope after preparation of smears on microscope slides. The slides were dried and fixed in ice-cold acetone for 12 hours. The localization of γ-glutamyl transpeptidase was determined by a modification of a procedure described earlier (*3*). The slides were incubated for 60 minutes at 25°C in a mixture (40 ml) consisting of phosphate buffer (0.1*M*, *p*H 7.2), glycylglycine (0.01*M*), fast garnet GBC (15 mg), and L-γ-glutamyl-2-naphthylamide (*17*) (0.62 m*M*); 0.5 ml of a solution containing 100 μmole of L-γ-glutamyl-2-naphthylamide and 200 μmole of HCl in 2.5 ml of acetone were added to the incubation mixture. After incubation the slides were rinsed with distilled water and dried in air.

The fraction consisted mainly of fragments of capillaries or larger vessels branching out into capillaries (Fig. 1). The endothelium of the capillaries showed dark areas (actually brown-red) indicating the localization of γ-glutamyl transpeptidase activity (*3*). The intensity of the reaction is far greater in the capillaries than in the larger vessels. This difference is especially visible in places where a larger vessel gives off capillary branches. The glial cells and single neurons that contaminated the fraction did not show any transpeptidase activity. These findings, while showing that brain capillaries can be isolated, confirm histological studies that localized the γ-glutamyl transpeptidase in the cytoplasm of endothelial cells of brain capillaries (*4*).

The existence of a blood-brain barrier for amino acids has been suggested by various kinds of studies. Many amino acids do not readily enter the brain even when their concentrations in the bloodstream are substantially increased, and their rates of entry vary widely (*18*). Studies with labeled amino acids have shown that there is a rapid exchange between brain and blood amino acids even in he absence of their net movement into the brain (*19*). That the movement of amino acids between blood and brain occurs through mediated transport processes is indicated by stereospecificity of uptake (*20*) and competition among amino acids for entry into the brain (*20, 21*). This competition among groups of amino acids led to the postulate that several different systems exist, each specific for certain groups of amino acids (*21*).

One or more of these systems may be associated with γ-glutamyl trans-

Table 1. Summary of fractionation of γ-glutamyl transpeptidase and two phosphatases in bovine brain cortex. Analyses are based on 1 g of bovine cortex.

Fraction	Total protein (*28*) (mg)	γ-Glutamyl transpeptidase		Alkaline phosphatase		5′-Nucleotidase	
		Specific activity*	Enrichment†	Specific activity*	Enrichment†	Specific activity*	Enrichment†
Crude homogenate	98.0	0.1	1.0	0.58	1.0	2.2	1.0
Centrifugation (1000*g*)							
Pellet (P_1)	24.2	0.22	2.2	0.73	1.3	1.41	0.6
Supernatant (S_1)	75.2	0.04	0.4	0.29	0.5	1.69	0.8
Fraction 5 (from sucrose density gradient)	2.7	0.82	8.2	2.2	3.8	1.5	0.7

* Activity is measured in units, one unit being the amount of enzyme that catalyzes the formation of 1 μmole of the product per 30 minutes. Specific activity is measured in units per milligram of protein (*28*). † Enrichment represents change of specific activity relative to the specific activity of the homogenate arbitrarily set as 1.0.

peptidase and the γ-glutamyl cycle. Supporting this idea are the observations that methionine, a good substrate for the transpeptidase (*12*), is rapidly taken up by brain (*18*) and that glycine and aspartate, which are poor substrates (*5*), are hardly taken up at all (*18*). Both the transpeptidase (*22*) and amino acid uptake (*23*) are sensitive to monovalent cations. Also consistent with this idea is the localization of the enzyme in the endothelium of brain capillaries, which is the anatomical site of the blood-brain barrier (*24*). Finally, brain contains significant amounts of the three intermediates of the γ-glutamyl cycle—glutathione (*25*), pyrrolidonecarboxylic acid (*26*), and γ-glutamyl amino acids (*27*). It may be relevant that signs of brain damage were manifest in a patient excreting large amounts of pyrrolidonecarboxylic acid (*10*) and by two others with another block in the γ-glutamyl cycle, namely, a deficiency in the γ-glutamylcysteine synthetase (*11*).

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Regulation of Amino Acid Transport in Kidney Cortex of Newborn Rats

Abstract. *After incubation at 37°C the subsequent uptake of α-aminoisobutyric acid, cycloleucine, glycine, and* L-*proline by newborn (as compared to adult) rat kidney cortex slices is enhanced. The effect is abolished by the presence of cycloheximide, actinomycin D, and high concentrations of the above-mentioned amino acids in the medium during the 37°C incubation prior to measurement of uptake. The data suggest that there is an adaptive control mechanism which is expressed on incubation at 37°C and which can regulate amino acid transport in newborn rat kidney cortex.*

The examination of membrane transport systems in developing rat kidney cortex has aided in distinguishing the separate nature of the process for sugars and amino acids (*1*) and has served to delineate differences in the transport mechanisms of several amino acids (*2*). During further experiments with the newborn rat kidney cortex as a model system to explore the responsiveness in vitro of the tissue to hormonal stimulation, we have observed that merely incubating the cortical slices in buffer at 37°C enhances their ability to accumulate some neutral amino acids. We now report observations that indicate the presence of a time-dependent regulatory process for amino acid transport in renal cortical cells of newborn but not in those of adult rats, and that this process is associated with concomitant protein synthesis.

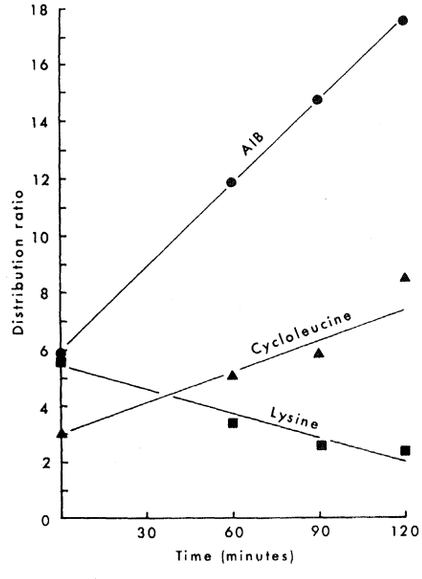

Fig. 1. The effect of the length of preliminary incubation on the subsequent uptake of amino acids by newborn rat kidney cortex slices. The abscissa is the duration of the incubation in buffer alone prior to addition of substrates for the 1-hour incubation to measure the uptake designated by the distribution ratio, that is, the number of counts per minute per milliliter of intracellular fluid to that per milliliter of medium.

The technique for determining the in vitro uptake and intracellular concentration of ^{14}C-labeled amino acids and sugars in kidney cortex slices from newborn and adult Sprague-Dawley rats has been described (*1–3*). The uptake is expressed as the distribution ratio—the ratio of the number of counts per minute per milliliter of intracellular fluid to the number per milliliter of medium. For all the substrates tested except proline, which is rapidly metabolized, the ratio is indicative of a concentration gradient. The total tissue water was 76 percent of the wet tissue weight, and the extracellular space of newborn cortical slices, as determined by inulin penetration, was 20 percent of the wet tissue weight. The experiment consisted of incubating the slices