a ratio of 6900 moles per mole of opsin to a bleached frog retina at 27°C it caused full rhodopsin regeneration in 8 hours with a $t_{1/2}$ of 95 minutes. Rat rod outer segments have 3.5 times the surface to volume ratio of frog. Assuming a temperature coefficient (Q_{10}) of 2 for pigment regeneration [see Baumann (*14*)], one would expect rhodopsin regeneration to be seven times faster in rat than in frog. We found the rat retina at 37°C regenerated its rhodopsin completely in 1 hour (with a $t_{1/2}$ of 14 minutes) from equivalent amounts of reactants.

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Blood-Brain Neutral Amino Acid Transport Activity Is Increased After Portacaval Anastomosis

Abstract. *In rats after portacaval anastomosis (an animal model of chronic liver disease), transport of tryptophan and other members of the large neutral amino acid group from blood to brain was markedly enhanced. Increased transport activity was apparently restricted to the neutral amino acid transport system, since brain uptake of glucose, inulin, and tyramine was unaffected while blood-brain arginine transport was significantly reduced. These results strikingly confirm the hypothesis that carrier-mediated blood-brain transport is the limiting factor determining the availability of the neutral amino acids to the brain. The encephalopathy associated with cirrhosis may be the result of abnormal neurotransmitter metabolism and neurotransmission secondary to increased neutral amino acid transport activity and an increased brain content of members of the neutral amino acid group.*

Cirrhosis of the liver, usually related to alcohol abuse, is the sixth ranking cause of death in the United States. Chronic liver disease is frequently accompanied by symptoms of neurological impairment termed hepatic encephalopathy. The cause of the symptoms, which may progress to hepatic coma and death, is unknown. Encephalopathic episodes are associated with such diverse factors as protein ingestion, overdiuresis, or sepsis-induced catabolic states (*1*). In the cerebrospinal fluid of patients with cirrhosis, tryptophan (TRY) concentrations are two to three times normal and the serotonin metabolite 5-hydroxyindoleacetic acid is also elevated (*2*), suggesting that serotonin may play a role in hepatic encephalopathy. Fischer and Baldessarini (*1*) suggested that the other aromatic neutral amino acids, phenylalanine and tyrosine, may also be involved since their decarboxylated metabolites, phenethylamine and tyramine, can affect catecholamine metabolism. In a model of cirrhosis, the rat with a surgically constructed portacaval anastomosis (PCA), brain TRY and serotonin are markedly elevated, although total plasma TRY is unchanged (*3*). Since plasma leucine, isoleucine, and valine are frequently decreased in chronic liver disease, it has been suggested that blood-brain TRY transport is facilitated by decreased concentrations of the branched-chain neutral amino acid (NAA) competitors (*3, 4*). However, no evidence has yet been presented that brain TRY content may be influenced by modification of the blood-brain NAA transport system itself or that such alterations may play a role in any disease process.

It has been proposed that carrier-mediated transport of the NAA's across the blood-brain barrier may be the limiting factor in determining their availability for brain cell metabolism (*5*). The introduction of a single-injection, tissue sampling technique for studying blood-brain transport has permitted the tabulation of estimates of the maximum initial velocity, V_{max}, and the Michaelis constant, K_m, for transport of individual amino acids in rats (*6*). We have now used this technique in combination with amino acid analysis to investigate the blood-brain transport of various substances including TRY in rats after a PCA is formed. We find that the activity of blood-brain NAA transport is selectively increased after the PCA operation and that increased TRY transport activity is well correlated with increased brain TRY.

Portal blood flow was surgically diverted into the systemic circulation of female Sprague-Dawley rats (300 to 350 g) by creating a PCA, as previously described (*7*). Rats with a PCA and controls of the same age and weight were fed Purina rat chow and water ad libitum until they were killed by decapitation. The blood and brain concentrations of the large NAA's excluding TRY were determined by using a Beckman 121-MB amino acid analyzer in pentobarbital-anesthetized rats 14 days after PCA and in unoperated controls (Table 1). After PCA, plasma valine, isoleucine, and leucine decreased to about 80 percent of control values. Plasma methionine was not significantly elevated, while tyrosine, phenylalanine, and histidine all increased significantly. Plasma tyrosine, which showed the greatest increase after PCA, was about twice the control value. In contrast, brain valine, isoleucine, and

Table 1. Effect of portacaval anastomosis on plasma and brain neutral amino acids. Groups of 8 control rats and 11 rats with a PCA were anesthetized with pentobarbital and 10 minutes later were decapitated. Plasma and brain NAA's were determined by ion-exchange chromatography, using a Beckman 121-MB amino acid analyzer. Data are presented as mean ± standard error (S.E.) and were analyzed by Student's *t*-test. The last column shows the concentration of each amino acid in the PCA rats as a percentage of that in the control rats.

Amino acid	Concentration		Percentage of control
	Control rats	PCA rats	
	Plasma (nmole/ml)		
Valine	242 ± 21	187 ± 11	77.3*
Methionine	60 ± 4	70 ± 4	116.7
Isoleucine	99 ± 7	77 ± 6	77.8*
Leucine	180 ± 17	144 ± 11	80.0
Tyrosine	61 ± 8	120 ± 8	196.7†
Phenylalanine	69 ± 4	119 ± 8	172.5†
Histidine	67 ± 5	107 ± 5	159.7†
	Brain (nmole/g)		
Valine	95 ± 6	92 ± 7	96.8
Methionine	51 ± 5	84 ± 3	164.7†
Isoleucine	39 ± 1	37 ± 2	94.9
Leucine	79 ± 3	91 ± 5	115.2
Tyrosine	53 ± 5	185 ± 8	349.1†
Phenylalanine	47 ± 2	151 ± 8	321.3†
Histidine	58 ± 3	166 ± 6	286.2†

*$P < .05$ compared to control values. †$P < .001$ compared to control values.

Table 2. Effect of portacaval anastomosis on rat brain/plasma concentration ratios of the large neutral amino acids. Data are presented as the mean ± S.E. for the brain/plasma ratio (nanomoles per gram divided by nanomoles per milliliter) of each amino acid in the groups of 8 control rats and 11 rats with a PCA. Statistical significance was determined by Student's *t*-test.

Amino acid	Brain/plasma ratio	
	Control rats	PCA rats
Valine	0.42 ± 0.04	0.51 ± 0.04
Methionine	0.88 ± 0.12	1.28 ± 0.08*
Isoleucine	0.41 ± 0.03	0.49 ± 0.02*
Leucine	0.46 ± 0.03	0.65 ± 0.04†
Tyrosine	0.91 ± 0.06	1.55 ± 0.09†
Phenylalanine	0.70 ± 0.05	1.30 ± 0.08†
Histidine	0.89 ± 0.06	1.58 ± 0.09†

*$P < .05$ compared to control values. †$P < .01$ compared to control values.

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leucine were not significantly altered after PCA, while brain methionine, tyrosine, phenylalanine, and histidine all rose to a far greater extent than in the plasma. In addition, whereas the brain/plasma ratio of each NAA was less than 1 in the control group, it exceeded 1 for methionine, tyrosine, phenylalanine, and histidine in the rats with a PCA (Table 2). These results do not support the hypothesis that altered plasma NAA/competitor ratios are sufficient to explain altered brain NAA levels after PCA.

Blood-brain transport of various substances was investigated by determining the brain uptake index (BUI), as described by Oldendorf (*8*). The BUI of TRY, phenylalanine, tyrosine, and leucine was significantly increased after PCA (Table 3). The BUI of phenylalanine was significantly increased over the control value both 7 and 28 days after PCA; all other experiments were performed on rats 14 ± 2 days after PCA. The BUI of all NAA's tested at the latter time was increased by nearly the same percentage over control values. These results imply a sustained increase after PCA in the activity of the NAA transport system. It has been shown that all these amino acids compete for uptake into the brain mediated by this blood-brain transport system (*9*).

No increase was observed in the BUI of tyramine or of inulin (molecular weight, 5000 to 5500), which are normally excluded from the brain. The BUI of glucose, for which a hexose-specific transport system has been demonstrated (*10*), was also unchanged after PCA. In contrast, the BUI of arginine, which is transported by a separate system specific for basic amino acids (*9*), was significantly decreased after PCA. Therefore, it is unlikely that increased transport of the NAA's after PCA was due to altered cerebral blood flow or to nonspecific effects on blood-brain barrier permeability, such as widening of the tight junctions between capillary endothelial cell membranes.

The effect on the BUI of tryptophan of increasing the concentration of unlabeled TRY in the injection solution was determined in both control and PCA rats. The results (Table 3) show that TRY transport continued to show saturability after PCA, and that brain TRY uptake was higher in PCA rats at all TRY concentrations up to 4 m*M*. As previously demonstrated (*6*), it is possible to calculate the K_m and V_{max} of transport from these data. After PCA, both the K_m and the V_{max} of transport were increased (control K_m = 0.061 m*M* and V_{max} = 9.7 nmole/g-min; PCA K_m = 0.103 m*M* and V_{max} = 44.6 nmole/g-min). For these calculations, it was assumed that cerebral blood flow was unaltered after PCA, as reported by Eklof *et al.* (*11*). These results may imply that the affinity of TRY for the transport system, as reflected by K_m, decreased after PCA, while the number of transport sites, as reflected by the transport V_{max}, increased; however, further study of the significance of K_m and V_{max} in transport is required for the interpretation of these data.

Of the amino acids in plasma, TRY is unique in that most of it is bound to the plasma albumin, which alters its availability for transport (*12*). Total plasma TRY, plasma "free" TRY (the fraction not bound to albumin), brain TRY, and the brain uptake index of TRY were all determined in the same group of rats. Tryptophan concentrations were determined in the animals used for the BUI determinations at 0.020 m*M* TRY. Plasma free TRY was determined in 50-μl portions of an ultrafiltrate of plasma prepared by centrifuging 1.5 ml of plasma in a Centriflow membrane cone (Amicon Corporation) at 1000*g* for 20 minutes. Brain TRY was determined in the supernatant of a homogenate in 5 percent trichloroacetic acid of the hemisphere contralateral to the injection. Tryptophan was determined by the method of Dencla and Dewey (*13*).

Brain TRY, plasma free TRY, and the brain uptake index of TRY, but not total plasma TRY, were significantly elevated in rats after PCA (Table 4). A significant positive correlation was found between plasma free TRY and brain TRY (r^2 = .662, N = 22, $P < .001$; control and PCA rats together). A significant positive correlation was also observed between brain TRY and the BUI of TRY (r^2 = .732, N = 23, $P < .001$; control and PCA rats together). This correlation strongly suggests that experimentally obtained BUI values are a valid index of the physiological activity of the blood-brain amino acid transport system. Since BUI data represent the transport of a trace amount of TRY in Ringer solution

Table 3. Effect of portacaval anastomosis on brain uptake index of amino acids, glucose, inulin, and tyramine. Groups of rats (numbers shown in parentheses) were anesthetized with pentobarbital and were given intracarotid injections of ^3H_2O plus ^{14}C-labeled substances at the concentrations shown. The rats were decapitated 15 seconds later and brain ^{14}C and ^3H were determined for the BUI calculation. Except as noted for phenylalanine, all experiments were conducted 14 ± 2 days after the PCA. The last column shows the BUI value for each test substance in PCA rats as a percentage of that in control rats. Data are presented as mean ± S.E. and were analyzed by Student's *t*-test.

^{14}C-Labeled test substance	Injected concentration (m*M*)	BUI (%)		Percentage of control
		Control rats	PCA rats	
Phenylalanine				
1 week after PCA	0.002	47.7 ± 2.0 (4)	69.6 ± 4.5 (4)	145.9*
4 weeks after PCA	0.002	49.4 ± 2.5 (3)	80.8 ± 3.7 (4)	163.6*
Tyrosine	0.002	29.7 ± 1.7 (7)	44.8 ± 0.9 (5)	150.8*
Leucine	0.003	37.9 ± 0.7 (4)	56.0 ± 1.3 (7)	147.8*
Tryptophan	0.020	33.2 ± 1.6 (11)	53.8 ± 1.6 (12)	162.1*
	0.100	15.2 ± 0.7 (6)	30.2 ± 1.2 (6)	198.7*
	0.500	8.9 ± 0.8 (4)	15.5 ± 1.3 (4)	174.2*
	1.000	5.5 ± 0.5 (4)	9.7 ± 1.3 (4)	176.4†
	4.000	3.7 ± 0.4 (4)	6.2 ± 0.4 (3)	167.6*
Arginine	0.003	12.8 ± 0.7 (8)	9.1 ± 0.8 (6)	71.1*
D-Glucose	0.005	30.9 ± 1.3 (5)	27.8 ± 1.8 (3)	90.0
Inulin		2.0 ± 0.1 (4)	2.2 ± 0.3 (3)	110.0
Tyramine	0.033	1.3 ± 0.1 (4)	1.4 ± 0.3 (4)	103.8

*$P < .01$ compared to control values. †$P < .05$ compared to control values.

Table 4. Effect of portacaval anastomosis on plasma total, plasma free, and brain tryptophan. Tryptophan levels were determined in the rats used for measurements of the brain uptake index for 0.02 m*M* TRY. Brain TRY was determined in the hemisphere contralateral to the injection. Data are presented as mean ± S.E. for 11 controls and 12 rats with a PCA and were analyzed by Student's *t*-test.

Rats	Tryptophan		
	Plasma total (nmole/ml)	Plasma free (nmole/ml)	Brain (nmole/g)
Control	107.8 ± 7.8	5.4 ± 0.5	17.6 ± 0.5
PCA	110.3 ± 9.3	11.3 ± 2.0	43.6 ± 3.9
Percentage of control	102.3	209.3*	247.7*

*$P < .01$ compared to control values.

without competing amino acids or albumin, the uptake index is presumably unaffected by plasma NAA competitors or by the TRY binding capacity of albumin. Recent studies of the physiological regulation of brain TRY have focused on determining the relative importance of the latter two factors. Our data suggest that not only brain TRY but also brain methionine, tyrosine, phenylalanine, and histidine may be influenced by the activity of the blood-brain transport system.

It has been proposed that the NAA transport system of the blood-brain barrier transports the NAA's by facilitated diffusion into an extracellular fluid compartment (ECF), from which they are actively transported into brain cells (*5*). After PCA, increased capillary NAA transport would permit a greater influx of NAA's into the ECF, thus making the NAA's more available to the concentrative, active transport systems of the brain cells. The increased brain/plasma ratios of the NAA's after PCA thus probably do not reflect concentrative transport across the blood-brain barrier itself, but rather a decrease in the normal restrictions on blood-ECF transport. The relatively low brain/plasma ratios of leucine, isoleucine, and valine in both PCA and control animals may be explained if these amino acids are readily catabolized by brain. These results thus strikingly confirm the hypothesis that the rate-limiting factor in the availability of NAA's to the brain is the activity of carrier-mediated blood-brain (blood-ECF) transport.

These results also strongly support the postulated role of the NAA's in the pathogenesis of hepatic encephalopathy and demonstrate a previously unsuspected involvement of the liver in the regulation of blood-brain transport activity. Fischer *et al.* (*14*) described the use of a specially formulated intravenous feeding solution in the treatment of hepatic encephalopathy. This solution, which is low in aromatic amino acids and high in branched-chain amino acids compared to conventional parenteral nutrition formulas, was designed to permit increased protein tolerance in a group of patients traditionally incapable of ingesting adequate amounts of dietary protein without encephalopathy. If patients with cirrhosis and portal-systemic shunting of the circulation also have increased blood-brain NAA transport, then increasing their plasma levels of leucine, isoleucine, and valine should result in decreased brain uptake of the aromatic amino acids, which may interfere with normal neurotransmitter metabolism. Further study is required to determine whether increased NAA transport is a unique pathological response to impaired liver function or whether the normal physiological regulation of brain NAA content is achieved in some part by this mechanism.

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$$\frac{(\text{dpm } ^{14}\text{C}/\text{dpm } ^{3}\text{H})_{\text{brain}}}{(\text{dpm } ^{14}\text{C}/\text{dpm } ^{3}\text{H})_{\text{injectate}}} \times 100$$
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Chloroquine Resistance Produced in vitro in an African Strain of Human Malaria

Abstract. *After continuous cultivation in the presence of chloroquine, an African strain of the malaria parasite,* Plasmodium falciparum, *acquired resistance to the drug. The resistance was stable and comparable in vitro to that occurring naturally in a strain from Southeast Asia. This suggests that chloroquine resistance, absent until now in Africa, might arise in the future.*

Chloroquine-resistant strains of *Plasmodium falciparum* have occurred thus far only in Asia and Latin America (*1*). Since chloroquine is the antimalarial drug of choice, and since *P. falciparum* remains a major cause of morbidity and mortality in Africa (*2*), the emergence of such strains in this continent could have very serious consequences. We have now demonstrated in vitro the potential for chloroquine resistance in an African strain of *P. falciparum*.

Strain FCR-3 (FMG) was isolated in September 1976 from a patient in the Gambia, West Africa, and since then has been kept in continuous culture (*3*) in our laboratory. Using a petri dish test described by Trager *et al.* (*4*), together with a multiwells test, we determined its response in vitro to chloroquine (Fig. 1, A and B). Repeated experiments over an 8-month period showed a constant pattern of sensitivity to the drug. The parasite growth was completely inhibited by a 48-hour exposure to a concentration of 0.1 μg of chloroquine base per milliliter of medium. This was consistent with results reported from the Gambia by Smalley (*5*), who used a different in vitro method described by Rieckmann *et al.* (*6*).

To produce resistance, we maintained a line of FCR-3 in continuous culture by the petri dish–candle jar method of Jensen and Trager (*7*), in a medium containing progressively higher concentrations of chloroquine. We used 35-mm petri dishes with 1.5 ml of 8 percent red cell suspension, changing the medium daily and subculturing to fresh blood cells every 4 days. Beginning with a barely inhibitory concentration of 0.01 μg/ml, we increased the chloroquine level stepwise as the growth of the parasites permitted. After 1 month, or 15 cycles of asexual multiplication, the experimental line had been adapted to a medium containing 0.1 μg of chloroquine base per milliliter of medium, three times the effective mean plasma concentration for a drug-sensitive strain (*8*).

The experiment illustrated in Fig. 2 confirmed at that time the emergence of a new strain, unaffected by the same concentration of chloroquine (0.1 μg/ml) that proves lethal to the original African strain. Continuing the stepwise increase, we are now growing the new strain, R-FCR-3, in the presence of 0.13 μg/ml, and have maintained some lines derived from it for up to 8 weeks at 0.16 μg/ml. Multiplication of the parasite

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