Enzyme activity in the particulate fraction measured in the absence of Na^+ was $18 \pm 2 \mu\text{mol}$ of P/h per mg of protein in untreated animals and $20 \pm 2 \mu\text{mol}$ of P/h per mg of protein in animals killed during convulsions. This difference was not significant. In the presence of Na^+ the corresponding activities (corrected for activity with Mg^{2+} alone) were 21 ± 3 and $36 \pm 4 \mu\text{mol}$ of P/h per mg of protein, representing an increase as a result of electroschock of 68% ($P < 0.01$). An increase in Na^+ -stimulated activity of the same order was observed in the preparations made from cerebellar tissue.

However, no increase in $\text{Na}^+ + \text{K}^+$ -stimulated adenosine triphosphatase activity was observed in the unfractionated homogenates prepared from convulsing animals. In this case the Na^+ -stimulated activities were $11 \pm 1 \mu\text{mol}$ of P/h per mg of protein in control animals and $13 \pm 1 \mu\text{mol}$ of P/h per mg of protein during convulsions. Further, the increased activity in the particulate fraction was only obtained from tissues that had been frozen in liquid N_2 , either during the killing of the animals or subsequent to killing by decapitation. The increase was, however, observed to a smaller extent when tissues were homogenized without previous freezing in solution containing sodium deoxycholate. These are procedures that enhance the activity of the enzyme in normal tissue preparations (Samson & Quinn, 1967). Their requirement for the increase in activity associated with convulsions, and the failure to observe this increase in unfractionated homogenates, suggest that *in vivo* the enzyme activity may be controlled by membrane-bound factors that are partially released in a soluble form by freezing the tissue, and more completely by such treatment after exposure of the brain to the metabolic consequences of depolarizing electric currents.

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The Effects of Dietary Vitamin B_6 Deficiency on the Development of the γ -Aminobutyrate Shunt in Rat Brain

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Certain neurological signs of dietary vitamin B_6 deficiency (e.g. convulsions) are thought to be related to deranged γ -aminobutyrate metabolism (Roberts *et al.*, 1964). We have therefore undertaken a study of the development of the γ -aminobutyrate shunt in pyridoxine-deficient neonatal rats to investigate this relationship. Deficiency was induced by feeding mother rats on a pyridoxine-deficient diet during both gestation and lactation periods (Dakshinamurti & Stephens, 1969). The presence or absence of convulsions was difficult to ascertain. Brain development (represented by its weight) was retarded at birth and completely arrested in the second postnatal week.

The normal patterns of development of whole brain glutamate decarboxylase (EC 4.1.1.15) and γ -aminobutyrate transaminase (EC 2.6.1.19) in the first 3 weeks of life were similar to those obtained by Van den Berg *et al.* (1965), Sims *et al.* (1968) and Sims & Pitts (1970). Whole-brain γ -aminobutyrate concentration followed closely the increase in glutamate decarboxylase and γ -aminobutyrate transaminase activities. Pyridoxine deficiency markedly retarded development of the activities of glutamate decarboxylase and γ -aminobutyrate transaminase and lowered the concentration of γ -aminobutyrate. On the day 15 the γ -aminobutyrate concentration was lowered to 65% of the value of comparable controls. Glutamate decarboxylase and γ -aminobutyrate transaminase activities (assayed without the addition of pyridoxal phosphate to the incubation media) were decreased to 25 and 47% of control values respectively. This was due to a decrease in saturation of apoenzyme with coenzyme from 60 to 6% with glutamate decarboxylase and from 100 to 60% with γ -aminobutyrate transaminase, demonstrating the weak affinity of the decarboxylase for the coenzyme in contrast with the strong affinity of the transaminase. γ -Aminobutyrate transaminase maximal activity (assayed with pyridoxal phosphate added to the incubation medium) was decreased to 80%, whereas glutamate decarboxylase maximal activity rose to 230% of control values. The increase in glutamate decarboxylase maximal activity (which is probably indicative of enzyme protein concentration) could possibly be due to a feedback mechanism resulting in increased protein synthesis or in decreased degradation or in both.

On the day 15 pyridoxine hydrochloride was administered intraperitoneally to some of the de-

ficient neonatal animals. At 24h later the saturation of glutamate decarboxylase and γ -aminobutyrate transaminase with coenzyme returned to normal, as did γ -aminobutyrate concentrations.

The dramatic effect of pyridoxine deficiency on the development of the γ -aminobutyrate shunt, reported here, is very likely to be associated with some of the characteristic neurological signs of the deficiency. However, a direct relationship cannot be claimed.

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Effects of Portocaval Anastomosis on the Metabolism of [$1\text{-}^{14}\text{C}$]Acetate and on Metabolic Compartmentation in Rat Brain

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The cerebral disturbances in chronic liver disease have been attributed to the high blood ammonia concentration (Bessman & Bessman, 1955). Portocaval anastomosis in man leads to similar cerebral disturbances (McDermott & Adams, 1954), and in the rat it reproduces the changes in astrocyte morphology found constantly in human chronic liver disease (Cavanagh & Kyu, 1971a,b). Further, a rise in glutamine concentration related to plasma ammonia concentration occurs regularly in the rat brain in these circumstances (Williams *et al.*, 1972). Most brain glutamine is normally formed from a small pool of glutamate that is preferentially labelled by certain precursors, such as [$1\text{-}^{14}\text{C}$]acetate (Berl & Clarke, 1969). To investigate this model system further the cerebral metabolism of [$1\text{-}^{14}\text{C}$]acetate was therefore studied in rats with portocaval anastomosis.

Six experimental animals 6 weeks after the operation (Kyu & Cavanagh, 1970) and six weight-matched controls were injected subcutaneously with [$1\text{-}^{14}\text{C}$]acetate (20 $\mu\text{Ci}/100\text{g}$ body wt.; specific radioactivity 59 mCi/mmol) and decapitated 10 min later. The basal grey areas of the brain (caudate nucleus, thalamus and hypothalamus) were studied because they show the most conspicuous astroglial changes.

Fractionation of tissue and separation of amino acids were done as described by Patel & Balázs (1971).

Portocaval anastomosis had no marked influence on the tissue weight, water content or concentrations of RNA and protein, but [$1\text{-}^{14}\text{C}$]acetate metabolism was significantly affected. Thus the tissue contained 50% less ^{14}C per g than did that of the controls, and the utilization of [$1\text{-}^{14}\text{C}$]acetate in biosynthetic reactions was severely inhibited. The proportion of total tissue ^{14}C in the lipid fraction was decreased from 20 ± 0.5 to $6.5 \pm 0.49\%$, and in the protein fraction from 2.8 ± 0.20 to $1.8 \pm 0.31\%$. Although the percentage conversion of [$1\text{-}^{14}\text{C}$]acetate into 'tricarboxylic acid-cycle' amino acids was only little affected, the distribution of ^{14}C among the various amino acids differed markedly from that in the controls.

The concentration of glutamine increased by 400% and that of glutamate by 20%, whereas that of aspartate did not change significantly from the control value. The specific radioactivities of glutamate and glutamine, adjusted to a total ^{14}C content of 1×10^5 d.p.m/g, were significantly diminished, and those of aspartate were increased. Hence the aspartate/glutamate specific-radioactivity ratio increased from 0.77 ± 0.03 to 1.42 ± 0.03 , indicating that a new type of metabolic compartmentation had developed. On the other hand, the glutamine/glutamate specific-radioactivity ratio decreased from 2.86 ± 0.20 to 1.17 ± 0.09 . Nevertheless the results showed that the overall rate of glutamine synthesis was increased; relative to the specific radioactivity of glutamate, 75% more ^{14}C was incorporated into glutamine per g wet wt. in the experimental than in the control animals.

The mechanism of these effects is not yet clear. The chronic influx of ammonia into the brain may lead to an accumulation of glutamine at sites that are relatively metabolically inactive, and this 'inert' glutamine would mask the accelerated synthesis of glutamine at the active sites. Alternatively, the rates of acetate oxidation and glutamine synthesis may be increased in metabolic compartments that are normally relatively inactive.

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