labelled whole mouse brain, and the DNA was extracted and then chromatographed on hydroxy-apatite. In the preparations from scrapie-affected brain a small peak eluted at about 50°C was found. This may represent a small single-stranded specific scrapie-agent DNA.

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Deoxyglucose and Brain Glycolysis

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Injection or infusion of large doses of 2-deoxyglucose is known to cause elevated plasma concentrations of glucose and free fatty acids in man and laboratory animals, probably as a result of increased secretion of adrenaline. The deoxy sugar has also been suggested to act as an inhibitor of glycolysis in the brain. It is phosphorylated by brain hexokinase, so that both deoxyglucose and its phosphate might be expected to interact with glycolytic enzymes. Samples of guinea-pig cerebral cortex were homogenized in 0.32M-sucrose and centrifuged at 100000g for 1 h. The supernatant fraction was examined for activities of enzymes involved in the normal metabolism of glucose and glucose 6-phosphate. Enzymic specific activities ($\mu\text{mol}/\text{min per g}$) at 37°C and 2 mM substrate concentrations were: hexokinase (glucose: 5.9); glucose 6-phosphate dehydrogenase (glucose 6-phosphate: 4.1); 6-phosphogluconate dehydrogenase (6-phosphogluconate: 0.75); phosphoglucomutase (glucose 1-phosphate: 40.8); hexose phosphate isomerase (glucose 6-phosphate: 169; fructose 6-phosphate: 284). Inclusion in the reaction mixtures of 2-deoxyglucose or its phosphate at 2–4 mM had no effect on any activity except that of hexokinase. Deoxyglucose inhibits hexokinase by competition with glucose (K_i 0.25 mM; Bachelard *et al.*, 1971), but the phosphate was found to be inactive. Deoxyglucose thus has considerably less affinity for hexokinase than has glucose (K_m 0.04 mM) and also a lower affinity for the uptake process (Bachelard, 1971). Hexose phosphate isomerase is inhibited by deoxyglucose 6-phosphate in kidney and ascites-tumour cells (Wick *et al.*, 1957; Nirenberg & Hogg, 1958), but not in diaphragm (Kipnis & Cori, 1959). In the brain, only the transport and phosphorylation of

glucose seem to be affected. In order for accumulation of concentrations of deoxyglucose sufficient to cause significant inhibition of cerebral hexokinase, with resultant decreased glucose utilization and depletion of ATP, the circulating deoxyglucose concentration would have to be maintained at least one order higher than glucose. The possibility of action on enzymes acting at stages of metabolism remote from glucose 6-phosphate, although unlikely, has not been excluded. The recognition of deoxyglucose by the glucose-binding site on hexokinase, and the non-recognition of deoxyglucose 6-phosphate by the glucose 6-phosphate-binding site, provided further evidence for the allosteric properties of the enzyme.

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Increased Activity of the Sodium-plus-Potassium Ion-Stimulated Adenosine Triphosphatase in Rat Brain during Electrically Induced Convulsions

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Increased activity of the $\text{Na}^+ + \text{K}^+$ -stimulated adenosine triphosphatase of rat brain during convulsions induced by lethal doses of Metrazol (6,7,8,9-tetrahydro-5-azepotetrazole) has been reported by Bignami *et al.* (1966) and De Robertis *et al.* (1969). During an investigation of the effect of experimentally induced thyroid disorders on energy utilization in rat brain we have observed a similar increase in $\text{Na}^+ + \text{K}^+$ -stimulated adenosine triphosphatase activity in brain tissue from rats made to convulse by electric currents.

Male Wistar rats (100g) were stimulated through electrodes applied to the ears by monophasic square waves (85 V; 150/s; duration 1.5 ms) applied for 1 s. The animals were killed after 10 s during the tonic phase by total immersion in liquid N_2 . Homogenates (10%, w/v) of cerebral-cortex tissue and whole cerebellum were prepared in water (Samson & Quinn, 1967). $\text{Na}^+ + \text{K}^+$ -stimulated adenosine triphosphatase activity was determined in the unfractionated homogenates and in the total particulate fraction (obtained by centrifuging the homogenate at 10⁶g for 60 min) by the procedure of Rodnight & Lavin (1966).

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.

R. C. is in receipt of a Fellowship from Universidad Central de Venezuela, Caracas, Venezuela.

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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-