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The Use of Zonal Centrifugation in the Preparation of Subcellular Fractions from Brain Tissue

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The first attempted separation of a brain homogenate into its subcellular components in a zonal centrifuge was by Barber, Rankin & Anderson (1966), who loaded whole brain on to a continuous density gradient of sucrose. Later, Cotman, Mahler & Anderson (1968) used a similar gradient to subfractionate material comparable with the crude mitochondrial fraction (P_2) of Gray & Whittaker (1962). A discontinuous gradient was used by Rodnight, Weller & Goldfarb (1969), who were able to prepare relatively large quantities of components of the crude mitochondrial fraction from ox brain. Suitably adapted discontinuous gradients in a zonal head offer a method of obtaining certain fractions more quickly and in larger amounts than is possible with the conventional bucket rotor and we have been investigating the possibilities.

The crude mitochondrial fraction obtained from whole rat brain or up to 15 g of cat cerebral hemispheres and prepared by the method described by Gray & Whittaker (1962) was loaded on to a discontinuous gradient of sucrose (0.4–1.7M) in a B-XIV aluminium rotor (capacity 650 ml) which was run in a modified MSE 50 ultracentrifuge. The separation was allowed to proceed for 2 h at 67 000g, after which the rotor was unloaded and fractions (20 ml) were collected. After the distribution of protein had been determined, from the extinction at 280 nm, the nature of the subcellular fractions in the different peaks was examined by a number of methods, particularly enzyme assay.

A fraction moving through a band of 1.4 M-

sucrose and clearly separated from mitochondria gave the orange fluorescence with Acridine Orange characteristic of lysosomes and, in several preparations examined, green fluorescence caused by mitochondria was not observed (Koenig, Gaines, McDonald, Gray & Scott, 1967). The succinate dehydrogenase concentration in this fraction was less than 0.025% of that of the mitochondria on a protein basis, a value that is comparable with that of Koenig *et al.* (1967). The method can be used to prepare a lysosomal fraction rapidly from 12–15 g of brain tissue. The synaptosomal fraction was also clearly defined and exhibited the high occluded free lactate dehydrogenase ratio characteristic of this material (Marchbanks, 1967). The distribution of 5'-nucleotidase and acetylcholinesterase in all fractions was also studied.

The methods employed and their probable value will be discussed.

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Biochemical Studies of Taurine in the Developing Rat Brain

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Taurine is found in high concentration in immature neural tissue although, unlike glutamate and γ -aminobutyrate, there is a striking decrease in its concentration during development of the brain (Agrawal, Davis & Himwich, 1968). Since taurine is probably formed through decarboxylation of cysteinesulphinic acid, we have examined the activity of cysteinesulphinic acid decarboxylase during development of the rat brain in relation to taurine concentration. Surprisingly, there is a steady increase of decarboxylase activity from after birth to 20 days *post partum*, closely resembling the increase in glutamate decarboxylase activity seen during the same period.

The distribution of both taurine and cysteine-sulphinatase decarboxylase has been studied in subcellular fractions of adult rat brain. Much of the taurine (70%) was found in the soluble fraction, as has been found for glutamate and γ -aminobutyrate (Ryall, 1964; Mangan & Whittaker, 1966). Some 20% of the total taurine was present in the crude mitochondrial fraction, of which two-thirds was localized in synaptic endings and the rest in myelin. After 45% of the cysteine-sulphinatase decarboxylase was localized in the crude mitochondrial fraction, where its distribution in synaptosomes and myelin is similar to that of taurine. Both taurine and cysteine-sulphinatase decarboxylase activity could be released from the crude mitochondrial fraction by osmotic shock. The distribution of taurine, cysteine-sulphinatase and glutamate decarboxylases was examined in different anatomical regions of the brain. No direct relationship was found in each area between the concentration of taurine and that of cysteine-sulphinatase decarboxylase, as has also been observed for the γ -aminobutyrate-glutamate decarboxylase system (Roberts, 1962).

There are some structural resemblances between taurine, γ -aminobutyrate and glycine, and in addition there is physiological evidence to suggest that taurine, like γ -aminobutyrate, may have depressant action on neurones (Curtis & Watkins, 1960). The presence of cysteine-sulphinatase decarboxylase and taurine in the nerve-ending particles and the increase in decarboxylase activity during development raises the question as to whether or not taurine is a neurotransmitter or a modulator.

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Undernutrition and the Development of Certain Enzymes in Rat Brain

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There is much current interest in the possible effects of experimental undernutrition on the chemistry and metabolism of the developing brain. Previous studies have mainly involved measurement of content of nucleic acids, lipids or total protein after growth retardation of suckling animals (Dobbing, 1964; Howard, 1965; Culley & Mertz, 1965; Winick & Noble, 1966; Dobbing, 1968). Apart from two reports (Sereni, Principi, Perletti & Sereni, 1966; Chase, Dorsey & McKhann, 1967), the first of which was based on small numbers of animals, there are no results on brain enzyme activities under such nutritional conditions.

The developmental profiles of four enzymes representative of different metabolic pathways or subcellular organelles have been determined in the homogenates of whole brains of control and undernourished rats aged 0-21 days. Undernutrition was achieved by underfeeding the dams during gestation and lactation such that the weight of the young at 21 days was only 34% of that of controls.

The enzyme activities (per g wet wt. of tissue) rose three- to five-fold between birth and 21 days in control animals. The development of succinate dehydrogenase (EC 1.3.99.1; acceptor ferricyanide), fructose diphosphate aldolase (EC 4.1.2.13) and acetylcholinesterase (EC 3.1.1.7) was retarded by undernutrition. The activity deficits at 21 days were respectively 21, 14 and 11%, compared with a deficit in brain weight of 27%. The developmental profile of β -N-acetylglucosaminidase (EC 3.2.1.30), which is probably a lysosomal enzyme (Sellinger, Rucker & Verster, 1964), was unaffected by undernutrition.

The question is being investigated whether these enzymic deficits persist into adult life after feeding *ad libitum* beyond weaning.

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