glycoprotein bands were detected in fraction A, whereas in fraction D only four tritiated proteins were observed. It should be noted that there was no overlap in any of the membrane glycoproteins identified on sodium dodecyl sulphate/polyacrylamide gels.

To determine if glycoprotein synthesis is influenced by recognized inhibitors of eukaryotic and prokaryotic protein-synthesizing systems, synaptosomes were incubated with cycloheximide and chloramphenicol. In the presence of cycloheximide the uptake of fucose into synaptosomal protein was inhibited by 70%, whereas a 33% inhibition was observed for the chloramphenicol-incubated material. These results are very similar to those reported for the uptake of amino acids into synaptosomes (Wedege et al., 1977).

The question of whether isolated synaptosomes can carry out protein and glycoprotein synthesis is still controversial. The evidence reported in the present communication does suggest that there are two systems for glycoprotein synthesis/ modification in 'pinched-off' nerve endings: one mechanism linked to the intraterminal mitochondria and the other resembling the system normally associated with eukaryotic cells.

**Effect of undernutrition on brain-specific proteins during development**

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Undernutrition in early life seems to have a marked influence on the morphological, biochemical and functional development of the central nervous system (for review, see Balazs et al., 1979). The brain-specific proteins, D₁, D₂ and D₃ are enriched in synaptosomal-membrane fractions, being predominantly localized within, outside and inside the membrane respectively (Jørgensen, 1976). In the present communication the effect of undernutrition on the development of these proteins in the rat cerebellum is reported.

Rats of the Porton strain were undernourished from the sixth day of pregnancy throughout lactation and also after weaning, until they were killed as described previously (Patel et al., 1973; Lewis et al., 1977). At each age indicated in Fig. 1, three rats of each group were used. The cerebella were homogenized in 0.32 M-sucrose and the membrane proteins were solubilized by incubating with 3 vol. of 0.02 M-phosphate buffer, pH 8.5, containing 4% Triton X-100 and 1% aprotinin (a proteinase inhibitor) for 2h at 4°C. The samples were analysed directly for the antigenic proteins D₁, D₂ and D₃ by crossed immuno-

![Fig. 1. Developmental changes in the amount of D₁, D₂ and D₃ antigens in the cerebellum of normal and undernourished rats](image-url)

The concentration of these antigens was estimated by crossed immunoelectrophoresis using an anti-(synaptosomal plasma membrane) serum. The values were normalized by using adult forebrain homogenate as standard. The relative concentrations of the antigenic proteins were calculated per mg of cerebellar homogenate protein and the final results were expressed as a percentage of 35-day-old control values. The data were transformed logarithmically and analysed by analysis of variance with a two-way classification. The standard errors of the logarithmically transformed means were: D₁, 0.038; D₂, 0.042; and D₃, 0.049. The asterisk refers to the significant difference between the mean values of the normal and undernourished groups; P < 0.05. O, Mean control values; ●, mean values for undernourished animals.
were quantified with reference to known quantities of the phosphokinase; CK MM, the MM isoenzyme of creatine phosphokinase in synaptic membranes (generously given by Dr. O. S. Jørgensen, University of Copenhagen). For the detailed description of the method and apparatus, see Axelsen et al. (1973). The precipitin arcs of D1, D2, and D3 antigens were identified (Bock, 1972) and the amount of the antigen quantified. In 1- and 6-day-old cerebella, where the antigen precipitin arcs were very small, they were quantified with reference to known quantities of the antigens in an adult forebrain homogenate added as internal standard.

In normal rats, the results showed quantitative (Fig. 1) alterations in D1, D2, and D3 antigens and qualitative change in the D2 antigen during the early postnatal period. The amounts of D1 and D2 protein at birth (day 1) were about 30% of the 35-day value and increased progressively, reaching adult values by about 21 days of age. In contrast, the amount of D3 antigen was maximal at birth and declined progressively, to about 40% of the original value at day 35. The decrease in D3 antigen was relatively greater during the first 2 weeks than the latter 3 postnatal weeks. These quantitative changes in the developmental pattern of D1, D2, and D3 antigens in rat are similar to those previously observed in the mouse (Jacque et al., 1976). In addition, mainly one form of the D2 antigen ('anodic-immature') was detectable during the first postnatal week, but a slower moving form ('cathodic-mature') became apparent later and this progressively superseded the immature form, constituting nearly all the D2 antigen in 35-day-old rats.

In undernourished cerebellum, the concentrations of D1 and D3 antigens were higher at 6 days; thereafter, the developmental increase for D1 was markedly delayed and consequently at 21 days the amount of D1 was significantly lower than in controls (Fig. 1). Undernutrition had no appreciable effect on the developmental increase of D1, the values remaining consistently higher than in controls at 12 and 21 days. Even though the undernutrition was continued after weaning, the amounts of both antigens approached normal values by 35 days. In contrast, the concentration of D2 was normal at 6 days, but due to marked retardation in the maturational pattern in 12-, 21- and 35-day-old undernourished rats the values were 143, 120 and 123% of the controls respectively. The developmental switch in terms of 'anodic-immature' type to 'cathodic-mature' type was also retarded in undernutrition. Furthermore, when whole developmental curves were considered, unlike D1 and D2, the D3 was not restored to normal concentrations at 35 days. To test the reversibility of the effect of undernutrition, a group of experimental rats were given food ad libitum after 21 days. At 35 days, the amount of D3 antigen in these rats rehabilitated for 2 weeks was not different from that of undernourished animals of similar age and was consistently higher than normal. Therefore, the effect of undernutrition on the concentration of D3 antigen, which is believed to play an important role in synaptogenesis (Jørgensen et al., 1980), seems to be more persistent than its effects on the concentrations of either D1 and D3 antigens or on other biochemical parameters of brain maturation examined in our previous studies ( Patel et al., 1975; 1978).


A two-site immunoradiometric assay for the BB isoenzyme of human creatine kinase

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The BB isoenzyme of creatine kinase, CK BB, appears from a recent immunological study (Thompson et al., 1980) to be confined largely to nervous tissue in humans. This localization could provide a useful marker for damage to nervous tissue: amounts of CK BB as measured by radioimmunooassay appear elevated in sera of patients with a variety of neurological disorders (Thompson et al., 1980) as well as in metastatic disease (Silvermann et al., 1979).

Although radioimmunooassay provides a higher sensitivity than other assay methods (e.g. immunoprecipitation, ion-exchange chromatography) and does not rely on the presence of catalytically active enzyme, it is not specific for the BB isoenzyme, since the B-subunit in CK MB can also cross-react to an extent dependent on the particular antisem used.

CK BB was purified from human brain and antisemur to the enzyme was raised in sheep, as described by Thompson et al. (1980). Antibodies to CK BB were then purified by elution from an immunosorbant containing 6 mg of covalently bound CK BB in 20 mg of cellulose (produced as described by Miles & Hales, 1968). After incubation for 48 h at 4°C, elution was achieved by sequential washes with 50 ml of HCl, pH 3.0, and 10 ml of HCl, pH 2.0. Polypropylene microcentrifuge tubes (Sarstedt U.K., type LW 2070) were then coated at 20°C for 15 min with 0.2 ml of a 1:6 dilution in 50 mM-bicarbonate buffer, pH 8.2, of the high-affinity antibody fraction eluted at pH 2.0, aspirated, and then stored dry at -70°C before use. Anti-(CK BB) antibodies were labelled, as described by Miles & Hales (1968), while bound to an immunosorbant and eluted with pH 3 and pH 2 washes as described above. The purified labelled antibody was stored bound to immunosorbant at -20°C.

The two-site immunoradiometric assay was carried out as follows. Antibody-coated tubes were washed three times in 60 mM-Veronal buffer, pH 8.1, containing 5 g of bovine serum albumin/mlitre (Buffer A). To duplicate tubes were added 0.1 ml portions containing increasing amounts of purified CK BB, then 0.1 ml portions containing 125I-labelled anti-(CK BB) sufficient to give 6000 c.p.m. After an 8 h incubation, the tubes were aspirated, washed three times with Buffer A and counted for radioactivity.

Polypropylene tubes coated with anti-(CK BB) antibodies.