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The influence of protein–calorie deficiency on the central nervous system

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As apathy, listlessness, irritability and general misery are such constant manifestations of protein-calorie deficiency disease in man (Clark, 1951; Platt, 1954–5; Trowell, Davies & Dean, 1954) it is surprising that until recently there had been few investigations into the effect of protein-calorie deficiency on the nervous system. The fact that the brain is large relative to the rest of the body (Brown, 1965) and that recovery from neurological disturbances appears to be complete has been accepted by many observers as evidence of a favoured status for the brain. Food restriction, however, has been shown to produce changes in the central nervous system of man (Jackson, 1925), mice (Andrew, 1941), cats (Ferraro & Roizon, 1942), rats (Dobbing, 1964) and pigs (Dickerson, Dobbing & McCance, 1967). Changes have also been described in pigs fed *ad lib.* on diets of low protein value (Platt & Stewart, 1960; Lowrey, Pond, Barnes, Krook & Loosli, 1962; Platt, Pampiglione & Stewart, 1965).

Most of the changes would appear to be reversible but the gliosis found in deficient pigs (Meyer, Stewart & Platt, 1961; Platt *et al.* 1965) and the impaired maze-solving performance of rehabilitated pigs (Barnes, 1967) raise doubts about a complete return to normality. It has become clear that the severity of the changes could be modified by (a) the protein value of the diet, (b) the amount of diet consumed and (c) the age at which the deficiency was established. For instance, pigs given diets of low protein value from the 19th day of age showed less severe changes than those given similar diets at 14 days but more severe changes than those given

the diets from the 26th day. Could more severe and permanent changes be produced by initiating a deficiency even earlier, i.e. during intra-uterine life?

Production of congenital protein-calorie deficiency

Beagle bitches of known ancestry were mated to pedigree males and, from the offspring, pairs of female litter-mates 6–7 weeks of age were selected. One member of each pair was given a diet of low (NDpCal%=7) and the other a diet of high (NDpCal%=10 or 12) protein value. NDpCal% indicates the ratio of the retained protein, expressed in kcal, to the total metabolizable energy in the food consumed (Platt, Heard & Stewart, 1964). Both diets are adequate for the maintenance of adult dogs, but that of lower protein value is deficient during periods of rapid growth, gestation and lactation.

Animals given the diets of low protein value showed changes similar to those previously described in pigs; they grew less quickly than their normal litter-mates, showed some stiffness of the hind legs and changes in the electrical activity of the central nervous system. At about 13–16 weeks of age the stiffness became less marked and the electrical activity of the central nervous system passed into a relatively normal adult pattern. The weight of these animals continued to increase after that of their litter-mates had reached a plateau, so that by 12 or more months of age they might be as heavy as the better-fed controls. The animals might, therefore, on a superficial examination appear to be little different from the controls; however, the long bones were short (Stewart, 1965), carbohydrate metabolism was abnormal (Heard & Turner, 1967) and their central nervous systems still showed some morphological changes.

At 18–24 months of age the animals in each pair were mated to the same pedigree male. The lengths of the gestation periods were similar, but there were considerable differences in the size of the offspring. Pups from the deficient mothers had an average weight of about 280 g whilst those from the well-fed mothers averaged 350 g (see Table 1). As gestation periods did not vary the small pups should be regarded as immature or 'small for dates' and not as 'premature'.

Table 1. *Effect of protein value of bitch's diet on weight and number of offspring*

Protein value of bitch's diet (NDpCal%)	Mean weight of litter (g)	Ratio, weight 'amniotic fluid plus placenta': litter weight	Mean number of pups per litter	Birth weight of pups (g)*	Neo-natal deaths (%)	Weight of 6-week-old pups* (g)
10	1930	1·064	5·86	352±4	23	2071±65
7	1375	0·766	4·90	280±8	47	1598±57

There were significant differences between the two groups ($P \leqslant 0.05$) in numbers of pups per litter, birth weights and weights at 6 weeks.

*Mean value with its standard error.

The pups were suckled for 6–7 weeks and those from the well-nourished mothers grew well and developed normally. Those from the protein-calorie deficient mothers

grew slowly and the death rate during this period was about twice that of the normal group. The surviving animals developed abnormally; their tongues were often protruded and tremulous and at about 3–4 weeks of age fine head tremors could be felt. By 6 weeks these had usually coarsened to athetoid movements of the head and neck. The deficient pups were, at this age, often more active than the normal animals, but 'followed' less readily. The wide-based waddling gait, normal in the very young pup, persisted with age and, with the stiffness that usually developed in the hind legs, led to a 'kangaroo-like' gait.

At weaning some animals of each litter were given diets of high protein value (NDpCal% = 10) and the others diets of low protein value (NDpCal% = 5 or 7). Animals born of normal mothers and given the diets of high protein value continued to develop normally. Litter-mates given the low-protein diets were similar to those described on p.96 except that when given diets of NDpCal% = 5 they usually developed, in addition, tremors of the head and limbs.

When pups from the deficient mothers were weaned on to the better diets (NDpCal% = 10) the abnormalities which had been present during the suckling period regressed, but did not completely disappear; the animals were small for their breed (Fig. 1) and tended to be nervous and fearful, so that their behavioural reactions were uncertain.

The most dramatic changes occurred when animals from the protein-calorie deficient mothers were weaned on to diets of low protein value (NDpCal% = 5 or 7); they continued to grow slowly (Fig. 1), and at about 8 or 9 weeks of age there was often an exacerbation of the other signs of abnormality. They had a wide-based gait, the hind legs were very stiff, with an external rotation giving the appearance of subluxation of the head of the femur, although no such abnormality was found. The tremors became marked and the animal progressed with a slow, staggering gait. Those jerky, poorly co-ordinated movements were intensified by excitement, such as that caused by the presence of a strange dog. When lifted up and supported around the thorax or by the loose skin at the rear of the neck, the deficient animal extended its forepaws and thrust its head forward, whereas normal pups held in a similar fashion hold their heads up and allow their legs to hang freely (see Pl. 2, Platt & Stewart, 1968). Some animals stand with the back curved and the hind legs brought forward as if about to defaecate and this may be exaggerated until they assume a sitting posture (see Pl. 2 of Platt & Stewart, 1968). During the period of exacerbation quite gentle exercise causes further intensification of the signs of abnormality. The animals show a reluctance to walk for more than 5 or 6 min but, if they can be persuaded to continue, a proportion become agitated, micturate, defaecate, froth at the mouth, run in circles (stiffness is not so obvious at these times) and then fall on to their sides. The running movements may be continued for some time, but eventually the animals lie still and show no interest in their surroundings. After some minutes they stagger to their feet looking frightened and strained and from this point may return to the pre-exercise state or suffer further convulsions. Several animals have died in convulsions and others have had to be killed because of repeated seizures.

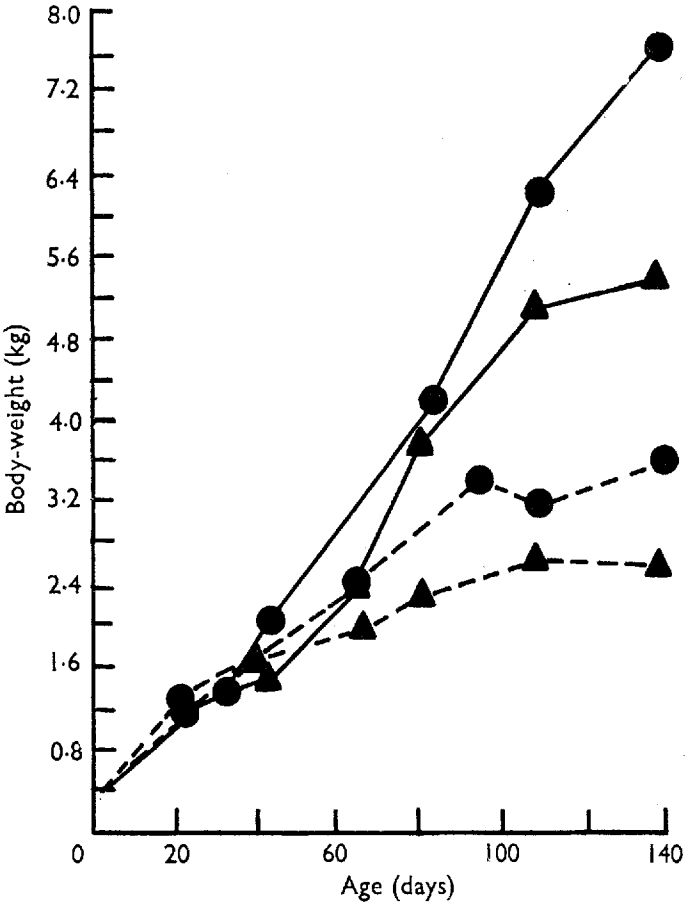

Fig. 1. Weight curves of pups born of normal (●) or malnourished (▲) bitches and maintained from weaning on diets having a protein value of NDpCal% 10 (———) or NDpCal% 5 (— — —).

The intensification of the abnormal appearances during weeks 8–13 is followed by a partial recovery, but the congenitally malnourished animals continue to exhibit more marked abnormalities than those subjected to protein-calorie deficiency only after weaning (p. 96).

The development and phases of the electrical activity of the dog's brain have been described by Pampiglione (1963). The malnourished dogs exhibit changes which, whilst constant in type, vary greatly in degree. Deviations in the development of the electroencephalogram can be discerned from an early age but the most marked changes occur during the period 8–13 weeks—a time corresponding to the exacerbation of clinical signs. In a normal dog (3 months old) the rhythmic activity is regular, is more marked in the posterior than the anterior half of the head, is of about 5–6 cycles/sec and has an amplitude of 20–40 μv with some further components of lower amplitude. In the malnourished animal the rhythmic activity is slower, of large amplitude (up to 300 μv), there are sharp waves and multifocal spikes, whilst

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the faster low-amplitude waves are reduced (Meyer, Pampiglione, Platt & Stewart, 1961; Stewart, 1967; Stewart & Platt, 1967).

The brains of the malnourished animals are large in relation to body-weight in the young, have approximately the normal relation to body-weight in the adult, but at all ages weigh less than those from age controls (Stewart, 1965; Platt & Stewart, 1968). Even when adult, the brains of congenitally malnourished animals fall below or are in the lower portion of the normal range of weight for age.

Histological changes are seen in the cells of the spinal cord. The cells have a reduced chromatin content, there is often a peripheral chromatolysis with an increase in perineuronal oligodendrocytes. Astrocytes are reactive and there is an increase in both the number and calibre of their fibres. The changes are not restricted to any one group of cells but are less obvious as one passes from the lower to the higher levels of the central nervous system. Satellitosis becomes rare, the common changes being loss of chromatin, a reduced number of cells and an increase in the astrocytic fibres. A few fibres degenerate, but there is no widespread degeneration of myelin. The ventral and lateral funiculi, especially at the periphery, are somewhat pale when stained by the Weigert Pal (Kultchitsky) technique. It seems probable that this pallor is due to inadequate myelinization rather than to degeneration of formed myelin, for in the deficient animals there are many small and few large myelin sheaths and the myelin walls are thin.

The cortex shows few positive changes; the gyri seem less plump than in the normal, owing in part to a reduced amount of 'white matter', the number of cells is reduced, but those present show little abnormality beyond a reduced quantity of chromatin.

The most consistent changes in the congenitally malnourished pups are tremors, athetoid movements and disturbances of gait and posture. Such manifestations are, in man, often associated with disturbances in the basal ganglia, and in experimental protein-calorie deficiency changes have been found in the thalamic, caudate and lenticular nuclei; they comprise loss of Nissl granules, an increased satellitosis, ghost cells and probably some loss of cells.

The original observations were made on the offspring of mongrel dogs purchased when adult. When it became clear that the clinical and histological changes brought about by protein-calorie deficiency might vary considerably under conditions which appeared identical, efforts were made to eliminate factors known to affect the central nervous system. Cammermeyer (1955) had reported on the abnormalities which might be found in the central nervous system of dogs purchased as normal adults; changes in neurological behaviour have been reported in deficiencies of, for instance, vitamin A, vitamin E and magnesium. In the more recent experiments, therefore, the animals are from stock maintained in the Mill Hill laboratories for several generations. The offspring of animals on high-protein diets are compared with the progeny of litter-mates on the low-protein diets and all are sired by the same male. The diets are compounded from dripping, starch, oats and casein with added vitamin and salt mixtures. The different protein values are obtained by varying the

proportions of casein and starch on a weight-for-weight basis, all other factors remaining constant.

The abnormalities found still vary from litter to litter and even within litters, but the offspring of the protein-calorie deficient bitches always exhibit some abnormalities whilst those from the well-nourished dogs appear to be normal. The changes described are probably a combination of deficiencies occurring *in utero* and during suckling. In rats, the suckling period has been shown to be important (McCance & Widdowson, 1962). Preliminary work (Payne & Stewart, unpublished) indicates that, in rats born of malnourished mothers and fostered from the day of birth by well-fed mothers, some damage has taken place before birth. Once the fact of intra-uterine deficiency is accepted then the variations found in the dogs are less puzzling; they may be related to factors such as position in the uterus (Wigglesworth, 1966) or the relative adequacy of the placenta. Table 1 shows a deficiency of the 'placenta plus amniotic fluid' relative to body-weight of the offspring, and in a few animals killed before term the ratio of placental weight to foetal weight was lower in malnourished than in normal dogs at similar stages of gestation.

Protein-caloric deficiency can be induced by the consumption of diets of low protein value or inadequate amounts of a good diet. There is also evidence that the pathology of some infections may in part be that of protein-calorie deficiency disease. However produced, such a deficiency during intra-uterine or immediate postnatal life leads, in animals, to physical stunting and behavioural changes which can be recognized in various tissues and systems of the body (Platt *et al.* 1964). Payne & Wheeler (1967) have presented evidence that, in primates, the metabolic stress of pregnancy is less in relation to maternal size than it is in non-primate mammals. Postnatal rates of growth are also low in the primates and, in keeping with this, their milk has a protein value of about NDpCal% = 8, whilst that of non-primates is nearer to NDpCal% = 12.

Diets having protein values similar to those given to the dogs could not, therefore, be expected to lead to gross neurological changes in man. Whether less severe changes would occur or whether the severe type of changes would follow the ingestion of diets of lower NDpCal% has not been established. There is, however, a great deal of evidence that malnourished mothers produce 'small for dates' infants (Burke & Stuart, 1952), that these have a high neonatal mortality and that the survivors have poor health and somewhat low intelligence quotients (Drillien, 1964). Cravioto (1962) and Cravioto, DeLicardie & Birch (1966) have tested many undernourished children and conclude that the intelligence scores are in inverse ratio to the weight deficit. Changes have also been described in the electrical activity (Nelson, 1959) and morphological appearances (Udani, 1960) of the central nervous system in protein-calorie deficient children. These, together with other changes in the blood and tissues, are similar to those found in the experimental deficiency (Platt *et al.* 1964). It seems likely, therefore, that man reacts as other animals but that the dietary deficiency must be relatively more intense.

Great efforts are at present being made to protect the preschool child from malnutrition, but it is essental that in our enthusiasm we should not ignore the

mother and thereby produce a somewhat different but possibly more permanent problem.

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Abnormalities of vitamin B_{12} and folic acid metabolism—their influence on the nervous system

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Recently Dr W. B. Castle of Harvard, one of the great figures of Medicine in this century, said, in the course of the first Sir Stanley Davidson Lecture of the University of Edinburgh, that little progress has been made in our knowledge of the causation of subacute combined degeneration of the cord, the neurological disorder that used to be such a striking complication of pernicious anaemia. Because of advances in therapy the condition is seldom seen now, but, despite all the progress that has been made in various fields of study of the megaloblastic anaemias, there