Experimental protein–calorie deficiency

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Many investigations into the effects of malnutrition have as their background the severe calorie and protein deficiencies associated with underdeveloped countries (McCance & Widdowson, 1968). Mr Miller has already demonstrated that the protein value of a diet is best expressed as net dietary-protein calories % (NDpCal%), a value that can be obtained by biological assay (Platt & Miller, 1959). The value of a diet is reduced when it is diluted with fat or carbohydrate, or, as is less widely appreciated, from the consumer’s point of view when it is eaten in inadequate amounts (Platt, Miller & Payne, 1961). Both dietary conditions produce protein-calorie deficiency, as defined by the Joint FAO/WHO Expert Committee on Nutrition (WHO, 1962), but the severity of the various symptoms may differ widely. For example, a deficiency produced by feeding an animal on grossly inadequate amounts of a well-balanced diet may lead to a greater inhibition of weight gain than occurs in another animal given unlimited quantities of a diet of low protein value; but the second animal will have the greater amount of body water and fat and a lower concentration of albumin in its serum. The low serum albumin will reduce the transport of albumin-bound vitamin A and calcium (Friend, Heard, Platt, Stewart & Turner, 1961; Gutman, 1953).

From his experiences in China, Platt (1958) became convinced that there was an underlying ‘protein malnutrition’ in beriberi and probably in other vitamin deficiency states. Certainly, when animals are given diets containing insufficient amounts of vitamins or minerals, for instance thiamine (Cowgill, Deuell & Smith, 1925), retinol (Mellanby, 1950) and calcium (El-Maraghi, Platt & Stewart, 1965), there is often a severe loss of appetite, so that the original deficiency is complicated by some degree of protein-calorie deficiency. Platt believed also that there was an element of protein-calorie deficiency in most, if not all, diseased subjects. Overseas students working in his unit demonstrated that, in rats infected with Plasmodium berghei (Dema, 1959) and Nippostrongylus muris (Orraca-Tetteh, 1964) and in dogs carrying heavy loads of Toxocara canis (Al-Rabii, 1963), the value of the diet to the infected animal was about half that to the uninfected control. Part of this reduction could be related to a reduced intake of food, but the value to infected animals was also lower than to uninfected pair-fed controls. The precise nature of this phenomenon
is unknown; however, similar effects are produced by toxins such as β-amino-
propionitrile and aflatoxin (Lebshtein, 1965).

It is common in human patients to find one or more deficiencies—calories, protein,
vitamins or minerals—complicated by infections or infestations. The synergistic
effects of food deficiencies and infection have been fully reported by Scrimshaw
(1964). However, as protein-calorie deficiency may occur under so many widely
different conditions, it is essential that we should be able to recognize its effects and
differentiate them from the changes due to other, more specific, deficiencies or
infections.

I wish now to refer to the histological and chemical changes to which such protein-
calorie deficiency is related.

Animals which are maintained on diets that are deficient in protein or inadequate
in quantity are underweight and usually suffer from a normochromic, normocytic
or slightly microcytic anaemia (Platt, Heard, Stewart & Al-Rabii, 1962). They have
small bones which exhibit varying degrees of transverse trabeculation and rarefaction
(Platt & Stewart, 1962; Dickerson & McCance, 1961). The trabeculation and
rarefaction can be related to a calcification of the cartilage before the cells have
reached their full size and to a reduced deposition of bone matrix by the osteoblasts.
The cells of most of the body tissues are small, owing to a reduction in their cyto-
plasm, and this change can be seen in tissues as different as cartilage, hypophysis,
pancreas, muscle and liver, and although the cells of the latter often appear to be
enlarged, this is due to the inclusions of fat and glycogen (Platt, Heard & Stewart,
1964a). A reduced rate of cell proliferation in the alimentary tract (Munro &
Goldberg, 1964), combined with the small cell size, leads, in the small intestine, to
a shortening of the villi and a reduction in the volume of the mucous membrane
(Platt, Heard & Stewart, 1964b), to which the total amount of intestinal prolidase
(Heard, 1957), and probably other enzymes, is related. A reduced volume of mucous
membrane will therefore be paralleled by a reduction in the intestinal enzymes.
The deficient animals also have a reduced supply of digestive enzymes from the pan-
creas, the exocrine cells of which are small and contain few zymogen granules.
Although we have not found any pancreases devoid of zymogen granules, it is obvious
that the store of enzymes is very deficient. This reduced volume of mucous membrane
and poor production of enzymes appears to be a vicious cycle which can only be
broken by an adequate intake of a well-balanced diet.

Our main interests have been in the effects of protein-calorie deficiency on the
endocrine, reproductive and nervous systems (Platt et al. 1964a; Platt, Pampiglione

Mulinos & Pomerantz (1940) suggested that protein-deficient or starved animals
should be regarded as being pseudo-hypophysectomized and that the reduction
in growth was related to a poor supply of growth hormone. Certainly the hypop-
physeal stores of growth hormone are reduced in protein deficiency (Srebnik &
Nelson, 1962), but the reduction is not great and the stores of some other hormones
are hardly affected. In protein-calorie deficient pigs and dogs the size, and possibly
the number, of cells containing acidophil granules was reduced. There were,
however, no exhausted glands, the usual appearance being of cells with inactive nuclei and a scanty cytoplasm filled with secretory granules. This is in keeping with a 'feed-back' from the high circulating concentrations of growth hormone found in some malnourished animals and children (Heard, Henry, Hartog & Wright, 1968; Pimstone, Wittmann, Hansen & Murray, 1966). Chromophobes and basophils are similarly affected and the modifications have to be considered in conjunction with other endocrine glands. In malnourished pigs there were normal urinary levels of corticosteroids or their derivatives, although the individual cells of the adrenal cortices appeared to be small and less active than in the normal. The more marasmic animals showed an elevated corticosteroid output, relative to body-weight, and the level of excretion was not increased in response to adrenocorticotrophic hormone, suggesting a hypertrophy of the gland. Stress is known to lead to a similar response, the further stages being a sudden drop in the corticosteroids and death. Perhaps this was the cause of the sudden, unexpected deaths which occurred in some of the deficient pigs.

Unfortunately, we have no knowledge of the circulating concentrations of thyroxine and its derivatives in our animals. The thyroid glands in the severely deficient pigs appear to be relatively inactive. The follicular cells are compressed, so that the width:height ratio, which is about 1.0 in the control animal, may be as high as 3 or 4 in the deficient pig. The number of Aron vacuoles is reduced and the vesicles are smaller than normal, though they tend to be angular and have the appearance of being distended and forced tightly together. In contrast, the glands of the deficient dogs exhibit only marginal modifications of the follicular epithelium, the most obvious change being an increase in the proportion of inter- or para-follicular cells. The change may be very marked in dogs born of malnourished mothers (congenitally malnourished) and themselves continued on the poor diets. In such animals the vesicles are also small and, although the follicular cells are still active, the area of the total interface between colloid and cells is greatly reduced. When pigs are re-fed, the glands respond dramatically; their proteolytic activity increases, the cells become columnar with a width:height ratio of < 1.0, and droplets of thyroglobulin can be seen within the cells.

The endocrine cells of the pancreas are reduced in volume in the protein-calorie deficient animals (see Table 1) and in the pig there are fewer cells containing β-granules, so that the ratio of non-α:α-cells is disturbed, a condition which is not found in the malnourished, even congenitally-malnourished, dog. The changes in β-cell appearance were found to be related to alterations in the regulation of blood glucose. To test this, pigs were given intravenous injections of glucose (0.4 g/kg body-weight) and samples of blood were collected at frequent intervals during the next 30 min. The logarithm of the blood glucose concentration plotted against time gave a straight line, the slope of which indicated the rate of uptake of glucose by the tissues, and therefore the 'glucose tolerance'. This rate increased steadily in normal pigs up to the 90th day of life, whereas in the protein-calorie deficient animals there was an early steep rise to a peak at 55 d followed by a fall to a value below that of the age controls. There was a similar pattern of change in the glucose
Table 1. Effects of diets of different protein value on the histological appearance of the pancreas

<table>
<thead>
<tr>
<th>Protein value of diet (NDpCal%)</th>
<th>No. of animals used</th>
<th>Zymogen granules*</th>
<th>Acinar arrangement</th>
<th>Cell volume as % of normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig 12</td>
<td>24</td>
<td>6</td>
<td>Good</td>
<td>100</td>
</tr>
<tr>
<td>8·3</td>
<td>3</td>
<td>6</td>
<td>Good</td>
<td>105</td>
</tr>
<tr>
<td>6·6</td>
<td>5</td>
<td>4</td>
<td>Fair</td>
<td>83</td>
</tr>
<tr>
<td>3·5</td>
<td>13</td>
<td>1</td>
<td>Very poor</td>
<td>68</td>
</tr>
<tr>
<td>2·5</td>
<td>8</td>
<td>1</td>
<td>Lost</td>
<td>64</td>
</tr>
<tr>
<td>3·5-12†</td>
<td>2</td>
<td>6</td>
<td>Good</td>
<td>99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mother’s Pups’</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog 10-10</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>

*Mean values obtained from arbitrary ratings of 0-6.
†Given diet of NDpCal%=12 for 4 weeks after 14 weeks on diet of 3·5 NDpCal%.

Sensitivity to insulin was tested by giving the animals a standard dose of insulin (0·1 i.u./kg body-weight), which was sufficient to swamp the amounts normally circulating. In normal animals there was a highly significant, positive correlation between insulin sensitivity and glucose tolerance; both were low in the very young and increased with age until adult values were reached (Heard & Henry, 1969). On the other hand, the protein-calorie deficient pigs showed a marked sensitivity to insulin even when the glucose tolerance was low, so that a deficiency of circulating insulin could be postulated. This was in keeping with the histological appearances of the pancreas at death; unfortunately we have no specimens of pigs killed during the early periods of high glucose tolerance, for at that time we might possibly have found a hyperactivity of the β-cells. In the deficient dogs, however, insulin-sensitivity correlated well with glucose tolerance, the early rise in the latter coinciding with a period of increased sensitivity to insulin and the later fall to a reduced sensitivity. An insulin antagonist was suspected and it was thought that growth hormone might be the factor. The answer is not quite so simple, for although high levels of circulating growth hormone might be the antagonist in dogs fed on diets of NDpCal%=5, glucose tolerance and insulin sensitivity are similarly disturbed in dogs fed on diets of NDpCal%=7, in which the circulating growth hormone is within the normal limits (Heard et al. 1968).

The malnourished pigs show changes in the chemical composition (Dickerson, Dobbing & McCance, 1967) and in the size and morphology (Platt et al. 1965) of the brain and some of the changes appear to be reversed with difficulty, if at all. For instance, Dobbing (1968) has shown that when malnourished pigs have been re-fed for 1 year there are still subnormal amounts of cholesterol and DNA in the brain. Our experience with dogs malnourished only after weaning indicates that
most of the changes produced after the brain has attained its full complement of cells are probably reversible; the importance of those that persist is disputed.

We have been interested for some years in the effect of poor diets on reproductive performance and have shown that animals maintained from weaning on suboptimal diets produce in later life fewer and smaller offspring than do well-fed animals of the same stock (see Table 2). The small congenitally-malnourished pups are produced

Table 2. Effect of restricted intake or poor quality of food on weight of offspring

(a) Food of low protein value during growth and gestation

<table>
<thead>
<tr>
<th>Mother's diet (NDpCal%)</th>
<th>Average no. in litter</th>
<th>Average weight of individual pups (g)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5.9</td>
<td>352</td>
<td>Stewart &amp; Platt (1968)</td>
</tr>
<tr>
<td>7</td>
<td>4.9</td>
<td>280</td>
<td></td>
</tr>
<tr>
<td>Rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>7.7</td>
<td>5.5</td>
<td>Stewart &amp; Sheppard (1971)</td>
</tr>
<tr>
<td>5-6</td>
<td>6.0</td>
<td>4.9</td>
<td></td>
</tr>
</tbody>
</table>

(b) Rats with restricted intake of food during gestation only

<table>
<thead>
<tr>
<th>Restriction (%)</th>
<th>Reduction in birth weight (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>16.0</td>
<td>Chow &amp; Lee (1964)</td>
</tr>
<tr>
<td>50</td>
<td>11.5</td>
<td>Berg (1965)</td>
</tr>
<tr>
<td>75</td>
<td>51.0</td>
<td></td>
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</tbody>
</table>

(c) Women of different socio-economic groups

<table>
<thead>
<tr>
<th>Socio-economic group</th>
<th>Average weight of offspring (g)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>3247</td>
<td>Udani (1963)</td>
</tr>
<tr>
<td>Medium</td>
<td>2975</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>2578</td>
<td></td>
</tr>
</tbody>
</table>

at full term, so must be regarded as ‘small for dates’ and not as prematures. Other workers (Naismith, 1969) claim that the mother’s tissues are sacrificed to provide for the young, in other words, that the embryo should be regarded as a parasite. This certainly occurs when the mother enters pregnancy in a well-nourished state and the deficiency is relatively mild, but when the deficiency is of long standing, so that the mother is malnourished when she becomes pregnant, further deficiencies are shared between mother and offspring (Platt & Stewart, 1967b).

In the congenitally-malnourished dogs, carbohydrate metabolism is disturbed and the transient, abnormally high, peak values for glucose tolerance appear even earlier than in dogs malnourished only after weaning. During the first few days of life, their blood glucose levels are low and, like their human ‘small-for-dates’, hypoglycaemic counterparts, the animals exhibit alterations in the central nervous system.

A high proportion (47%) of the congenitally-malnourished pups die before weaning and among the survivors some show incoordination of gait, peculiar postures and athetoid movements of the head and neck, and, when exercised, some pass into convulsive states.
The brains of the congenitally-malnourished pups are large relative to body-weight, but smaller than those of age controls, and in adult life are small relative to both age and weight (Stewart, 1965). Electroencephalic changes are present and these persist in animals which have been re-fed and, from a casual examination, would appear to be greatly improved (Stewart, 1968a). There are morphological modifications, the neurons have less chromatin, there is in the spinal cord a great increase in the neuroglial, especially the oligodendroglial, cells, and astroglial processes are increased in number and calibre. Within the cerebral cortex and cerebellum the neuroglial changes are less marked, but there appears to be a reduction in the total number of cells.

Congenitally-malnourished rats also exhibit changes in the central nervous system. The modifications are not as great as in the pigs and dogs, but there seems to be a reduced number of cells in the cerebral and cerebellar cortices and there is less myelin around individual fibres. This is in keeping with the low amounts of DNA, cholesterol and gangliosides found in rats malnourished from an early stage of development (Guthrie & Brown, 1968; Dobbing, 1968; Dickerson & Jarvis, 1970). Such changes are clearly more serious than many of those described in animals malnourished only after weaning, especially for DNA, which does not appear to reach normal levels even after prolonged re-feeding (Guthrie & Brown, 1968).

It seems likely that if the proliferation of cells is inhibited at the correct chronological time the deficit cannot be made good later.

Many of the changes found in protein-calorie deficient animals also occur in malnourished children. Thus, there are modifications in blood (Edozien & Rahim-Khan, 1968), bones (Platt, Stewart & Platt, 1963), alimentary canal (Stanfield, Hutt & Tunnicliffe, 1965), brain size (Brown, 1965), brain composition (Winick, Rosso & Waterlow, 1970), endocrine glands (Milner, 1970), EEG's (Nelson, 1959), limb control (Wayburne, 1968) and carbohydrate metabolism (Hadden, 1967). When such changes occur as a result of postnatal malnutrition, they can be seen to develop and can be measured, and are therefore accepted. Changes occurring before birth are more obscure and there is a great reluctance to accept them.

There is no doubt that mothers of low socio-economic groups produce babies of lower average weight than mothers of higher social status (Udani, 1963; Drillien, 1964). This has at various times been related to the mother's stature, her educational standard and other factors. Thomson & Billewicz (1963) pointed out that if small babies are produced by mothers who are small for their ethnic background, one then has to explain the poor growth of the mother. One possibility is that women in low socio-economic groups are the product of generations of mild malnutrition. If this is so, then any shortage of food during gestation might, on the basis of animal experiments, lead to the production of children incapable of reaching their physical and intellectual potential (Stewart, 1968b).

Platt (1954), said: 'Care beginning in prenatal life is likely to be a more successful means of achieving this objective [a healthy citizen] than the current attempts to repair the bodies of individuals already suffering from the effects of malnutrition, which are, indeed, often irreversible'. During the last two decades the work of his
unit has been built around this hypothesis, and I would like today to pay tribute to him and to all his former colleagues who have contributed to this work and have made possible the presentation of this paper.

REFERENCES


**Nutritional anaemias**

**BY A. W. WOODRUFF, N. R. H. EL-MARAGHI, L. E. PETTITT and R. J. C. STEWART, London School of Hygiene and Tropical Medicine and Hospital for Tropical Diseases, London**

It was to highlight the work of Ben Platt and his influence that this Meeting was conceived and the Council of the Royal Society of Tropical Medicine and Hygiene were very pleased when the Nutrition Society expressed willingness to join in this event. His influence on thought concerning nutrition throughout the world was very great and is exemplified by the many international committees on which he sat, particularly those of the WHO, UNICEF and FAO. If it were possible to assess quantitatively influence which nutritionists have had on a world-wide basis, I am sure that Ben Platt's name would be among the select group at the top. Particularly would this be so if influence on students and young research workers could be included. I have a personal reminiscence here; almost exactly 20 years ago I discussed with him my interest in anaemia associated with kwashiorkor; his reaction was warm and immediate, he invited me to his laboratories, then at Hampstead, and I will never forget the long afternoon and evening which he gave up to discussing and demonstrating to me work on anaemia. Much which we have been able to do stems from the kindly help received at that time. Throughout these 20 years Ben Platt's interest in it has been unfailing and culminated 3 years ago in collaboration between our two Departments in a series of experiments using dogs reared in and maintained on special diets within his Department. The results of some of these experiments I propose to put before you this afternoon.

The great prevalence of anaemia in the tropics needs no emphasis; many studies...