Lipids, Malnutrition & the Developing Brain

A Ciba Foundation Symposium
jointly with the Nestlé Foundation
in memory of Sir Norman Wright

1972

Elsevier · Excerpta Medica · North-Holland
Lipids, Malnutrition & the Developing Brain
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Editors: KATHERINE ELLIOTT and JULIE KNIGHT
In this volume the unsaturated fatty acids are designated by the carbon atom where the first double bond appears, counting from the methyl end of the molecule. For example:

\[
\begin{align*}
\text{n–9 series:} & \quad 18:1 \ (n-9) \ & \text{oleic acid} \\
& \quad 20:3 \ (n-9) \ & \text{eicosatrienoic acid} \\
& \quad 22:3 \ (n-9) \ & \text{docosatrienoic acid} \\
\text{n–6 series:} & \quad 18:2 \ (n-6) \ & \text{linoleic acid} \\
& \quad 20:4 \ (n-6) \ & \text{eicosatetraenoic acid} \\
& \quad 22:4 \ (n-6) \ & \text{docosatetraenoic acid} \\
& \quad 22:5 \ (n-6) \ & \text{docosapentaenoic acid} \\
\text{n–3 series:} & \quad 18:3 \ (n-3) \ & \text{linolenic acid} \\
& \quad 20:5 \ (n-3) \ & \text{eicosapentaenoic acid} \\
& \quad 22:5 \ (n-3) \ & \text{docosapentaenoic acid} \\
& \quad 22:6 \ (n-3) \ & \text{docosahexaenoic acid}
\end{align*}
\]
Plans for this symposium had from the beginning the encouragement and interest of Sir Norman Wright, Deputy Director-General of the Food & Agriculture Organization of the United Nations from 1959 to 1963. With his help the idea for a joint meeting with the Nestlé Foundation (of whose Council he was a member) became reality. At his death in 1970, both Foundations grieved to lose a wise and valued friend. With sincere affection and respect we offer this book in honour and remembrance of Norman Wright, whose life was given to the struggle against malnutrition.
The developing brain and the damage inflicted by malnutrition: an introduction

ALEXANDER VON MURALT

Nestlé Foundation, Lausanne

Wherever malnutrition prevails—and unfortunately this happens in vast areas of our world—the children are always most severely affected. Children are so dependent on the insight of their mothers, on the socio-economic background, and on the hygienic conditions of their small world, that they easily get caught by a vicious circle beginning with malnutrition and ignorance, leading up to a premature death. Malnutrition is the primary cause of the high rates of infant mortality in developing countries, even if the ultimate cause which the death certificate states may be something else, often an infectious disease like measles, which in malnourished children is apt to take a dangerous turn.

In recent years we have become more and more aware of the high vulnerability of infants, mainly for protein-calorie malnutrition. From animal experiments we have learned that growth is not only retarded by early malnutrition, but even stunted (see Widdowson and McCance 1960; Widdowson and Kennedy 1962; McCance and Widdowson 1962; Winick and Noble 1966, 1967; and also Miller 1969). What happens to the brain of a malnourished infant and what are the chances for the later intellectual development of a child who has been subjected to malnutrition at a critical period in his early life?

Our symposium deals with one aspect only, the lipids and the developing brain, but the problem has such an overwhelming importance for developing countries that it should be the Leitmotiv of the symposium!

Vulnerability

Prolonged starvation of human adults, leading finally to death, had the following effects on the weight of the various organs: brain and heart lost only 3% of their bulk; the muscles, liver and spleen lost 31%, 54% and 67%,
respectively. Wright (1945) concluded that 'the essential organs are thus spared as far as possible', or to put it in another way, that the tissues are sacrificed in inverse proportion to their importance. Is one entitled to conclude from these sad experiences of World War II that the brain of the adult is less vulnerable towards starvation than other organs? One can argue just as convincingly that special homeostatic mechanisms protect the brain during starvation, so-called 'brain sparing', just because it has a high vulnerability.

It appears that one must distinguish between intrinsic causes and extrinsic causes of vulnerability. Let me mention an example to explain this. Babies are vulnerable to water deprivation. Intrinsic cause: the immature kidney produces a hypotonic urine (in the adult it is hypertonic) and the baby loses comparatively more water. Extrinsic cause: the baby is unable to mobilize the fluid reserves in its tissue spaces in order to replace the loss of water because the homeostatic mechanisms of water balance have not yet been developed. This example may illustrate the two aspects of vulnerability.

Critical periods

As long as organogenesis goes on, the developing organism is much more defenceless against harmful external factors, such as malnutrition, than when it has reached maturity. During the period where the brain grows faster than any other organ of the body—the period Dobbing (1968a, b) called 'the growth spurt'—brain vulnerability is critical. But the timing of critical periods varies from animal to animal, and since we have to rely on animal experimentation the extrapolation of such results to the human brain is loaded with uncertainty. As far as we can guess now, the critical period for human babies begins with the second half of foetal life and ends about 18 months after birth.

Research has to go a long way before we can connect biochemical or structural abnormalities, due to malnutrition, with any specific dysfunction of the brain. Apathy of the children is one of the most constant signs of protein-calorie malnutrition; the loss of curiosity and the lack of desire for exploration, a progressive withdrawal from the environment, are other symptoms of acute protein deficiency. How can they be connected with deficiencies in the assembly of molecules into relevant brain structures or with a failure of forces that stabilize molecular conformations in the brain? There is a very deep gap between our knowledge of biochemical and structural brain maturity and what we call the process of achieving mental maturity—in a way the same gap that exists between the perfection of a computer at delivery from the factory and its performance after having been programmed.
Considering this example it becomes clear that what we want to know is this: does damage, inflicted on the brain of an infant at a critical period, heal with time and nutritional rehabilitation, or does it leave persistent effects which produce below-age-norm scores in all fields of behaviour 6-10 years later? Cravioto (1970) summarizes the situation as follows: 'From all quoted studies we can conclude that the existence of an association between protein-calorie malnutrition in infancy and retardation in mental development has been established beyond reasonable doubt'. A similar statement was made by Monckeberg (1971): 'Various studies made on both man and animals confirm that there really exists a critical period during the first months of life, when the damage produced by malnutrition has more serious and permanent consequences, which persist even when environmental conditions improve later on'.

Socio-economic influence

In animal experiments one can confirm the persistence of damage caused by early malnutrition after rehabilitation by chemical, structural and electrophysiological analyses over a lapse of time which is relatively short. Not so in human studies! There, the infants are exposed to poor housing, low levels of educational achievement, high infection rates and all sorts of taboos—nutritional and educational—during many years. The establishment of a truly causal relationship between malnutrition and deficient mental function is a very difficult task. Poor socio-economic conditions are almost always coupled with malnutrition, and both can have a significant deleterious effect on the growing child. This conjunction of factors makes it so difficult to determine the part which malnutrition may have played at the critical period—and yet, we should be able to overcome this ambiguity.

Two approaches seem to me of special interest: the study of siblings and the study of intersensory development.

Siblings. Cravioto and co-workers (1969) have carried out a study in a group of school-children who had suffered from severe protein-calorie malnutrition before their 30th month of life. The influence of the socio-economic factor was reduced by examining a group of siblings of similar age and identical sex. The children who had suffered from protein-calorie malnutrition in their early life scored consistently lower in all tests than their siblings who had no nutritional damage.

A similar study on siblings, but on a much larger scale, is now underway in Bogotá (Harvard—I.C.B.F.—Cornell Research Project). Dr Cobos is going to tell us about it in this symposium.
*Intersensory development.* Cravioto, together with Birch and collaborators (Cravioto, De Licardie and Birch 1966; Cravioto, Espinosa-Gaona and Birch 1967), studied the effect of early malnutrition on auditory-visual integration in school-age children of shorter stature, age by age. They showed poorer intersensory development than their taller equals in age. Such studies should be extended, especially with children who have been followed up from their critical period of early malnutrition until reaching school-age, in a vertical study. Intersensory disturbances are certainly more closely connected to internal factors due to malnutrition than to environmental influences.

*Separation of the influence of early malnutrition from environmental factors*

A clear experimental separation of the consequences of early nutritional damage of the brain from the disturbing socio-economic factors is of primary importance, because in the course of advising agencies or governments about the appropriate measures against mental retardation we must stand on solid ground with regard to its causes. Nutrient deficiencies during the critical period, together with low socio-economic factors, produce retardation of growth and of development of the intellect, and difficulties in learning and behaviour. This is not only true for infants who have passed through an attack of kwashiorkor or marasmus, but also for those who have suffered from moderate malnutrition, which is so abundant in developing countries. What we must know is whether malnutrition *per se* is sufficient to induce persistent effects on the functioning of the brain.

*Lipids and their functional role in the brain*

One of the events in the maturation of the brain that has evoked a considerable amount of attention is myelination. Our knowledge of the structure of the myelin sheath was gained by studies on peripheral nerve, based on the pioneering work of Schmitt, Bear and Clark (1935), Fernández-Morán (1952), Geren (1954) and Geren and Schmitt (1955). These and other studies provide the following picture. The myelin sheath is a highly ordered, concentric and spiral layered structure, surrounding the axon in the internode. The lipids form a bilayer with their axes in radial orientation, between two protein sheaths, which originate from an invagination of Schwann cell membranes, wrapped around the axon in many layers (up to 100 and more). The axon at the centre is surrounded by its own axonal membrane, which at the node, where the myelin
The developing brain and the damage inflicted by malnutrition

Sheath is interrupted, is excitable. In the brain, myelin deposition is more complicated and the glial cells (oligodendroglia) produce the myelin 'coating'.

The electrophysiologist considers myelin as an insulating material of high importance, ensuring the correct functioning of the complicated electrical network of nerve cells, axons and dendrites, and he is rather surprised to hear that a deficiency in essential fatty acids has consequences not only for the myelin composition, but also for the functioning of the brain! Between nerve cells and glial cells there is a 'symbiotic contact', much more intimate and functionally important than the contact of our insulator and a conducting element.

The excitable membrane

This membrane creates the nervous impulse and is thus the basic element of the activity of the peripheral and central nervous system. The excitable membrane is the carrier of information.

Our conceptions of the structure of membranes have recently changed quite a lot and the Danielli-Davson (1935) and Robertson (1962) model is in conflict with recent new findings. No evidence could be found for the presence of proteins in β-conformation, as postulated by the model. On the contrary, in erythrocyte membranes half of the proteins are in α-helical conformation, the rest are coiled (see Zahler 1969). In most membranes studied, hydrophobic bonds could be demonstrated between lipids and proteins (see Chapman 1968; Chapman and Kamat 1968; Wallach and Gordon 1968; Zahler 1969; Zahler and Weibel 1970). It seems evident that the loosely bound lipids (cholesterol and portions of the phosphatides) are held within the membrane by hydrophobic bonds with the helical proteins. Lecithin and sphingomyelin are probably more tightly fixed by polar interaction with protein. How is this high degree of organization of the lipids achieved? There is a strong mutual interaction between the lipids and the protein backbone of the membrane. The assumption that the great variety of lipids of a given membrane are selected and incorporated by the membrane proteins seems plausible.

Changes of the membrane potential in squid and Maia axons produce rapid, reversible changes of the birefringence of the radially oriented lipoproteins of the membrane (see Cohen, Keynes and Hille 1968, 1971). In the olfactory nerve of the pike this optical change is very marked, due to the large proportion of radially oriented lipoproteins in this nerve (see von Muralt 1972).
Conclusions

Recalling the Leitmotiv of our symposium, the problem of intellectual development of a child who has been subjected to malnutrition at a critical period in early life, it is obvious that protein deficiency and deficiency of essential fatty acids can have persistent effects on the biochemical maturity of the brain. Since proteins act as the backbone of lipid incorporation in the brain and are probably responsible for the highly complicated lipid substructure, a protein deficit should have a longer-lasting deleterious effect on the brain than a transient deficit in essential fatty acids. It is hoped that the contributions to this symposium will shed new light on this fascinating subject.

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The developing brain and the damage inflicted by malnutrition

Vulnerable periods of brain development

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The manner in which the brain develops during the earlier stages of organogenesis and subsequently grows towards the mature state has always attracted much interest from neurobiologists. In recent years there has been increasing concern to investigate the possibility that there may be certain periods of brain development during which comparatively mild interference with growth may produce irreversible alterations in its final form, and whether these may be of consequence to higher mental function. In spite of the increasing attention which these ideas are attracting, the field remains extremely nebulous, a situation partly due to the technical difficulties of its proper investigation, but more particularly to our negligible knowledge of the physical basis of such important and less tangible brain functions. The existence and significance of 'critical' or 'vulnerable' periods is central to the discussion and forms the subject of the present paper.

CRITICAL OR VULNERABLE PERIODS

The idea of critical periods of brain development probably took root from two hitherto separated disciplines. The developmental behaviourists have described 'imprinting' phenomena in newly hatched birds and possibly analogous periods of 'socialization' in the postnatal development of mammals (Scott 1967; Fox and Stelzner 1966), while neurobiologists, more interested in the less behavioural aspects of brain development, have described a 'critical' period (Flexner 1955), when a large number of physical events are alleged to happen suddenly and in a meaningfully related way.

The critical period concerned with imprinting is more helpfully and more usually described as a 'sensitive' period, implying that developing behaviour is
more susceptible at this particular stage to certain lasting modifications of future performance, and the behavioural literature is full of similar, if less dramatic, examples of such periods of susceptibility in all species including man.

By contrast the 'critical' period of physical brain development (Flexner 1955) is much more diffusely described and may be thought to stand up less well to detailed scrutiny. Furthermore it did not (at least originally) imply sensitivity, but merely a collection of sudden, dramatic and simultaneous events. In retrospect it may now only appear to have been the first awareness of the phenomenon of the brain 'growth spurt' to be described later, and much of the suddenness may be an artefact due to the use of animals having short lifespans with a very compressed growth programme.

Certainly at this distance of time the original accounts now read rather incoherently as a catalogue of processes examined in only one small area of cerebral cortex in the period from 41 to 45 days of gestation in the guinea-pig, corresponding to the 10th day or so of postnatal life in the rat. There seems to be little similarity in the direction being taken by these processes. Some of them are coming to an end at this time (e.g. the maturation of neuronal nuclear volume); some are in full spate (the growth of dendritic processes and the arrival of Nissl substance); some are beginning their upward journey towards adult levels (the activity of certain enzymes); some are beginning to fall from their elevated immature levels (the chloride space); and some are only detectable for the first time (cortical electrical activity). The latter presumably depends on the sensitivity of the electronic apparatus of detection.

In fact, as we know from much less sophisticated investigations, this 'critical' period from 41 to 45 gestational days is merely the earlier part of the brain growth spurt which is at its height in this species from 45 to 50 days. If earlier workers had looked at other parts of the brain for other processes at other times, they would have detected equally dramatic phases of glial cell division occurring at about 55 days and of myelination just before term (66th day of gestational period) (Dobbing and Sands 1970a).

From the point of view of neurobiology having an application to human populations, the older concept of critical periods has little relevance. It is more profitable to investigate the sensitivity or vulnerability of developmental phases to interference, and especially to look for long-term evidence of such susceptibility in the shape of detectable distortions of structure and function in the adult brain. The best-known examples of such vulnerable periods are in the field of teratology, where there are a number of examples of very precise moments of susceptibility during that period of organogenesis when the organ is acquiring its shape. Non-specific insults at this time may result in very gross disturbances of shape and form, which by their nature are irreversible. In
Vulnerable periods of brain development

humans such disastrous events are probably confined to the first trimester of gestation and it must be conceded that their detailed aetiology is often at best only suspected. In experimental animals viral disease, drugs, nutritional (vitamin) imbalance, X-irradiation and even mild pyrexia of short duration will produce deformities in the central nervous system, dependent on the precise timing of the insult in relation to embryological events.

Very little is known of any possibility of vulnerability during the second human trimester, although this is the period during which there seems to be a distinct phase of neuroblast proliferation, and adult numbers of neurons are virtually achieved by the time it is over (Dobbing and Sands 1970b). Perhaps within limits the number of neurons is functionally less important than their later dendritic development and the establishment of synaptic interconnections which are probably laid down during the third trimester and later. Perhaps the physiological circumstances of the second trimester render it less subject to foetal deprivation. Alternatively, it may even be that our academic ignorance of second trimester neuropathology is associated causally with our total ignorance of the nature and pathogenesis of vast numbers of cases of so-called unclassified mental retardation, whose brains the traditional neuropathologist finds such unrewarding objects of study.

The neuropathology of the third trimester of human gestation has until recently been mainly a matter of focal lesions, or patterns of focal lesions, inflicted by the circumstances of abnormal birth such as hypoxia, hypoglycaemia, or kernicterus. The consequences of these common lesions are just as lasting as the deformities of teratology, but their primary aetiology lies outside the nervous system and we need not postulate any particularly vulnerable period of brain development to account for them. Indeed, the brain is a great deal more susceptible to many of these insults in adult life than in the perinatal period.

THE BRAIN GROWTH SPURT

More recently the brain growth spurt has been identified as a period of vulnerability. This is the transient period during which increase in brain weight and a number of other important developmental processes proceeds at very high velocity. The nature of the vulnerability is different from that already described at other periods. There are no true deformities, nor is there any focal tissue destruction. However it seems that it is only necessary to retard growth during the period of the brain growth spurt for there to be irrecoverable distortions of pattern as well as quantitative deficits which are detectable in the
adult brain. In the human this growth spurt period appears to correspond to the last trimester of gestation and the first 18 months or so of postnatal life.

These ideas arose from testing the hypothesis that the vulnerability of the developing brain (in the present sense) may be related to its rate of growth. Thus the period of slow growth preceding the growth spurt should be as relatively invulnerable as is the mature brain when the growth period is over. In very general terms this proposition, even though it takes far too simple a view of the growth of such a complex organ, can indeed be shown to be true. When one considers the heterogeneity of brain structure compared with most other parenchymatous tissues, as well as the complicated temporal sequence and spatial distribution of most of its developmental processes, it is surprising how well such a hypothesis can be tested using such crude indices as estimates of cell number, degree of myelination and fresh weight of large complex regions.

Fortunately the general pattern of events in brain development seems to be similar from one mammalian species to another, so that the hypothesis can be tested experimentally in animals, provided the species differences in the timing of birth are taken into account (Fig. 1). Unlike the respiratory and cardiovascular systems, normal birth is of no significance to brain development, and it matters little that the growth spurt is foetal in guinea-pigs, perinatal in pigs,
Vulnerable periods of brain development

predominantly postnatal in humans and entirely so in rats (Davison and Dobbing 1968). It is only necessary to abandon such expressions as 'foetal brain' or 'neonatal brain', unless the species be specified and the brain growth characteristics of that species known.

The major brain growth spurt may be said to begin in all species at about the time the adult number of neurons is virtually achieved: by the end of the second human trimester or at birth in the rat. There is some neurogenesis later than this time, especially amongst microneurons in the cerebellum.

Among the major identifiable processes during the brain growth spurt period are the following:

1. Very considerable glial cell multiplication. This in terms of numbers is much greater than the pre-growth spurt multiplication of neurons (Dobbing and Sands 1970b, 1972a) and it is therefore quite wrong to regard the end of cell multiplication in, for example, the rat as occurring at birth, as is often stated (Davison and Dobbing 1968).


3. The development of several enzyme systems (Adlard and Dobbing 1971a, b, 1972).


5. Development of certain reflex patterns (Smart and Dobbing 1971a, b).

6. Changes in water and cation content (de Souza and Dobbing 1971).

EVIDENCE FOR SELECTIVE VULNERABILITY OF THE BRAIN GROWTH SPURT PERIOD

This has accumulated from a number of different experimental designs, mostly exploiting the postnatal timing of the growth spurt in rats during their suckling period.

In the first and most widely used design, growth is retarded by increasing the size of the suckling litter, so that rats are weaned at about half the normal weight. They are then given food ad libitum until maturity and the mature brains of permanently stunted animals are examined (Fig. 2). These are compared with brains of animals well fed until weaning and undernourished later, or with those of severely undernourished adult rats. Deficits and distortions are confined to those animals underfed in the suckling period. It is noteworthy that growth retardation confined to the bodily growth spurt (which occurs later than that of the brain) does not induce permanent stunting of either whole body or brain (Dobbing 1968; Dobbing and Sands 1972a).

A second experimental design which may be more satisfactory, especially from the behaviourist's point of view, involves underfeeding the mother during
the pregnancy. Stunted offspring as well as normal control newborns are then cross-fostered at birth to well-fed and undernourished lactating females, the litter size being standardized. Thus there are four groups according to whether the gestational period or the suckling period or both or neither was nutritionally restricted. Any of these groups can be weaned to a restricted or an adequate diet, and in this way all combinations and permutations of growth restriction can be imposed in the pre-growth spurt, growth spurt or post-growth spurt periods. Figs. 3a and b show the bodily growth curves when this is done and emphasize the dominating influence of growth during the brain growth spurt on ultimate attainment.

In experiments at present in progress an attempt is being made to imitate the case of the human baby of low birth weight for its gestational age, known to clinicians as 'small-for-dates'. On the assumption that a 5-day-old rat is at a comparable stage of brain development to the human full-term newborn, animals from underfed mothers are left with their mothers until 5 days after birth and then cross-fostered in the same manner as in the previous design.
Vulnerable periods of brain development

Fig. 3 (a) and (b). Body weights of rats (a) before and (b) after 21 days of age reared in standardized litter sizes from mothers underfed during gestation (G-) and/or lactation (L-) and cross-fostered at birth. Diet was ad libitum from 21 days onwards. The birth weight was significantly less in progeny from underfed mothers (Smart and Dobbing 1971b).
Preliminary results again show (Figs. 4a and b) the dominance of the brain growth spurt period in deciding the final outcome.

The results of all these experimental manipulations have led to the general conclusion that permanent, irrecoverable distortions and deficits can be prod-

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**Fig. 4** (a) and (b). Body weights of rats (a) before and (b) after 25 days of age reared as in Fig. 3 except that cross-fostering and standardization of litter size occurred at 5 days after birth, to provide a model for human 'small-for-dates' babies (B. P. F. Adlard and J. L. Smart, in preparation)