Plasma concentrations of *N*-acetylneuraminic acid in severe malnutrition

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1. In rat studies, circulating concentrations of *N*-acetylneuraminic acid (NANA) have been shown to be an indicator of NANA concentrations in the brain and functional brain activity, in relation to nutritional state and stimulation. Abnormal behaviour can be improved with exogenous NANA. In the present study, the plasma NANA concentration has been measured in children with severe malnutrition and compared with that in controls.

2. NANA was measured colorimetrically in the plasma of twenty-three severely malnourished children (mean age 11·43 (SD 6·05) months) before and after recovery, and in thirty-four controls (mean age 14·28 (SD 7·32) months). In thirteen of the malnourished children, NANA was measured after infections had been treated with a course of antibiotics.

3. Mean plasma NANA concentration was significantly higher in protein–energy malnutrition (PEM) (2·89 (SD 0·58) μmol/ml; n 23) compared with controls (2·13 (SD 0·37) μmol/ml; n 34, $P < 0.001$). The levels remained high in PEM after infections had been treated (2·87 (SD 0·43) μmol/ml, n 13) but returned to control levels at recovery from PEM (2·14 (SD 0·24) μmol/ml).

4. In contrast to the findings in rats, in malnourished children plasma NANA concentrations were not reduced and did not relate directly to nutritional state or, by inference, brain function. These findings do not provide any support for the use of exogenous NANA supplements to improve brain function in humans.

Most studies have shown that severely malnourished children have a deficit in mental development (Lloyd-Still, 1976; Grantham-McGregor *et al.* 1982). Intervention which includes increased stimulation can offset this deficit to some extent (Grantham-McGregor *et al.* 1980). There has been considerable interest in understanding the biochemical basis of these changes and in identifying biochemical markers which would reflect the functional changes in the behaviour of severely malnourished children. The suggestion by Morgan (1980) that circulating levels of *N*-acetylneuraminic acid (NANA) might represent such a marker excited our interest. NANA is a component of the gangliosides and glycoproteins which play important roles in neurotransmission, being integral components of the synaptic vesicles (Rahmann *et al*, 1976). Hence, these compounds are found in particularly high concentrations in nervous tissue generally and nerve endings in particular (Ledeen, 1978); lower concentrations are found in plasma (Morgan, 1981).

Rats that have been given a low-protein diet and are therefore nutritionally deprived, demonstrate an abnormal pattern of behaviour and a reduction in the NANA content of the brain compared with controls (Morgan & Winick, 1981). There is a concomitant fall in the circulating concentrations of NANA, the magnitude of the fall correlating with the wet weight of the brain, ganglioside NANA and glycoprotein NANA concentrations in the brain (Morgan, 1980). The administration of exogenous NANA to malnourished rats not only increases the concentration of ganglioside and glycoprotein NANA in the brain, but also causes a reduction in the associated behavioural abnormalities (Morgan & Winick, 1980*a*). If malnourished animals are exposed to a regimen of early stimulation, there is a reduction in behavioural abnormalities which is associated with an increase in both ganglioside and glycoprotein NANA concentrations in the brain (Morgan & Winick, 1980*b*).

Table 1. *The anthropometric measurements of the children studied, weight expressed as a percentage of that appropriate for the child's age (wt/age), or a percentage of that appropriate for the child's height (wt/ht)*

(Mean values and standard deviations)

| Hospital... | | Admission | | | | Discharge | | | |
| | | wt/age | | wt/ht | | wt/age | | wt/ht | |
Type of PEM	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Marasmic	4	48·5	10·0	64·1	4·7	62·1	17·5	83·0	6·1
Marasmic–kwashiorkor	13	52·7	4·4	71·8	7·4	66·8	6·3	91·1	4·6
Kwashiorkor	6	68·5	12·4	81·8	6·9	71·4	9·0	88·7	4·7
Controls	34	99·6	13·5	—	—	—	—	—	—

PEM, protein–energy malnutrition.

Thus, in rats, the metabolism of gangliosides, as represented by circulating levels of NANA, can be associated with malnutrition, behaviour and the level of stimulation. The suggestion has been made that this association is causal (Morgan & Winick, 1980*a*). If the findings in rats were obtained in humans, this would provide a rational basis for giving supplements of NANA to children whose development is delayed as a result of malnutrition or limited environmental stimulation.

The glycolipid NANA concentrations in plasma of normal children aged 6 months to 12 years have been reported by Dacremont (1972). Patwardhan *et al.* (1971) found that serum glycoproteins were increased in malnourished children compared with healthy controls. We know of no study that has looked for associations between total plasma NANA concentration and malnutrition or behaviour in humans. If plasma NANA concentration is causally related to the functional activity of the brain one may expect to be able to demonstrate a change during rehabilitation from protein–energy malnutrition (PEM), as the behaviour improves, and a difference between recovered children and normal children (Grantham-McGregor *et al.* 1978). In the present study the total plasma NANA has been measured in severely malnourished children on admission to hospital and during and after recovery, and compared with the values obtained from normal healthy children.

METHODS

The study comprised an index group (*n* 23) and a control group (*n* 34), both with children aged 6–36 months. Children in the index group suffered severe PEM and were consecutive admissions to the Tropical Metabolism Research Unit, University Hospital of the West Indies. Blood samples (1 ml) were collected from all index children within 48 h of admission and before discharge at clinical recovery. In a sub-sample (*n* 13), blood was also taken following treatment of intercurrent infections 6–20 d after admission. The children in the control group were attending the Well Baby Clinic of the same hospital. Blood was drawn from these on a single occasion. Their mothers answered a questionnaire on their past and current health status, to ensure that they had no history of serious illness, and were free from infection at the time of the study. Those children of low birth-weight were excluded.

Age, sex and weight were recorded for all the subjects. The malnourished children also had their heights measured, and the presence or absence of oedema was noted.

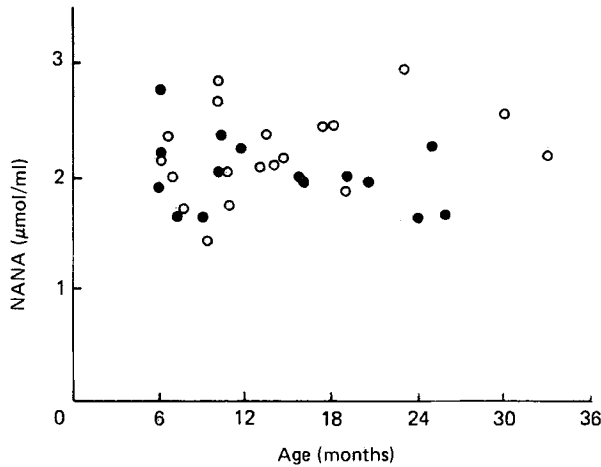Informed consent was obtained from the parents or guardians of all participants, and the

Fig. 1. Plasma *N*-acetylneuraminic acid (NANA) concentrations (μmol/ml) in plasma from normal males (○) and females (●) between 6 and 36 months of age. Over 15 months of age the males had a significantly higher NANA concentration than the females ($P = 0.01$).

study had the approval of the Ethical Committee of the University Hospital of the West Indies.

Blood was collected by venepuncture into sodium EDTA, centrifuged and the plasma separated. Plasma was stored between -10 and $-20°$ until assayed. Plasma NANA was determined in triplicate by the thiobarbituric acid assay, against appropriate standards, by the method of Warren (1959) as modified by O'Kennedy (1979).

Statistical analysis was carried out using the Student's '*t*' test.

RESULTS

The mean age of the malnourished children was 11·43 (SD 6·05) months and that of the healthy children 14·28 (SD 7·32) months. Four of the malnourished children were diagnosed as suffering from marasmus, thirteen marasmic–kwashiorkor, and six kwashiorkor (Anon., 1970). Table 1 shows that the mean weight-for-age (% of standard) of the healthy children was 100 (SD 13), 48 (SD 10) for those with marasmus, 53 (SD 4) for marasmic–kwashiorkor and 69 (SD 12) for kwashiorkor. At discharge from hospital the values were 62 (SD 18) for marasmus, 67 (SD 6) for marasmic–kwashiorkor and 71 (SD 9) for kwashiorkor.

The mean plasma NANA concentration in the normal children was 2·13 (SD 0·37) μmol/ml (Fig. 1). There was no difference in plasma NANA concentration by age or sex for the control group as a whole (Fig. 1). However, over 15 months of age males had a significantly higher concentration than females (2·4 *v.* 1·9 μmol/ml, $P = 0.01$), which was probably a reflection of the relatively small numbers in this age group. There were one male and five females aged more than 15 months in the malnourished group. Consideration of the results in these children as a separate group did not modify the conclusions. On admission to hospital (Fig. 2) the mean NANA concentration in malnourished children was 2·89 (SD 0·58) μmol/ml, significantly higher than that in the normal children ($P < 0.001$). There was no difference between the malnourished children with marasmus, marasmic–kwashiorkor or kwashiorkor (Table 2). However, the numbers in each group were small. In those malnourished children who had a specimen taken during recovery, following the treatment of infections, the NANA level was not significantly different from values on
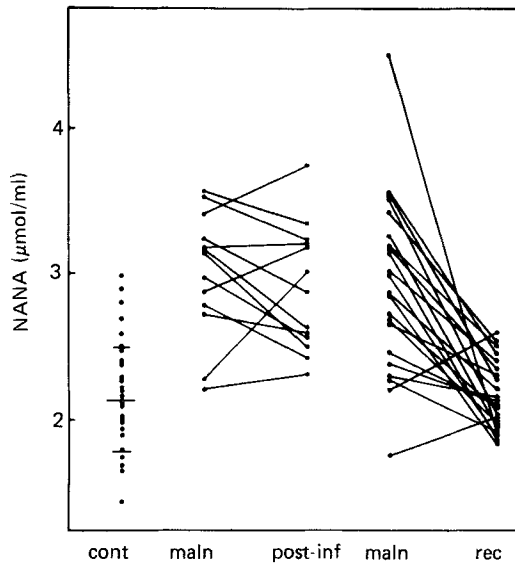
Fig. 2. *N*-acetylneuraminic acid (NANA) concentrations in plasma of control children (cont) and malnourished children at admission to hospital (maln), following the treatment of infection (post-inf) and at recovery (rec).

Table 2. *Plasma* N-*acetylneuraminic acid* (*NANA*) *concentrations in children with protein–energy malnutrition* (*PEM*)

(Mean values and standard deviations at admission to hospital)

Type of PEM	*n*	Plasma NANA (μmol/ml)	
		Mean	SD
Marasmic	4	3·09	1·09
Marasmic–kwashiorkor	13	2·86	0·47
Kwashiorkor	6	2·83	0·45

admission (2·87 (SD 0·43) μmol/ml) (Fig. 2). During rehabilitation there was a progressive fall in the NANA concentration, so that by the time children were discharged the levels were not different from those of the healthy children (2·14 (SD 0·24) μmol/ml).

DISCUSSION

Based on the results of the studies carried out in rats (Morgan *et al.* 1980*a*,*b*, 1981) we had expected to find a reduction in NANA concentration in malnutrition, rising to near normal levels during recovery. Not only were we unable to demonstrate the expected relationship between plasma NANA concentration and malnutrition, but actually found the reverse. We considered possible confounding variables which could account for this relationship

where NANA levels in the malnourished children admitted to hospital were higher than normal values.

Circulating levels of NANA, in particular the glycoprotein components, have been shown to be elevated in a number of disease states (Sharma & Sur, 1967). Infection is commonplace in malnutrition, and it has been suggested that an elevation in NANA may be due to associated infection (Maghrabi & Waslien, 1976). The majority of the malnourished children in the present study had a clinically obvious infection on admission, and all received antibiotic therapy. Therefore, after it was clear that NANA levels were high on admission, a series of samples were collected from children after their infections had been treated. The finding that there was no change in the NANA level would suggest strongly that infection by itself could not account for the high NANA levels. In a smaller group of children it was possible to follow the NANA levels throughout recovery, and invariably there was a progressive fall into the normal range. Hence there was an inverse relationship between the weight-for-height and the plasma NANA concentration. High levels of circulating NANA have also been found in childhood rickets. (Sharma & Sur, 1969). Although there are marked changes in bone development in malnutrition, rachitic changes are not a constant feature, although they may develop during the recovery phase (Alleyne *et al.* 1977). Most of the children in the present study had radiological boney changes of a non-specific nature. The most marked skeletal change was varying degrees of stunting (height-for-age: malnourished 87·2 (SD 4·9), recovered 86·8 (SD 4·9)). We were unable to demonstrate any correlation between NANA levels and height-for-age on admission or following recovery.

Knowledge of the normal metabolism of NANA and its control is incomplete. So far as is known NANA is released from glycoconjugates in the lysosome and then diffuses to the cytoplasm where glycoconjugates are again formed (Renlund *et al.* 1983). This is thought to occur mainly at nerve endings and to a lesser extent in other tissues such as liver, kidney and spleen. The measurement of plasma concentrations yields no information on the relative rates of production, utilization or catabolism. It may well be that the level of NANA in the brain does relate to its function, but if so this is not reflected in the plasma concentration of NANA in our children, and would lead us to one of two conclusions. The model used for producing malnutrition in experimental rats (Morgan & Winick, 1981) is inappropriate for human malnutrition. In the clinical setting the complex interplay of multiple nutrient deficiencies presents a picture which has a different pathophysiology to that of simple protein deprivation (Landman & Jackson, 1980). Alternatively, there are important differences in the metabolism of NANA between humans and rats.

Originally we had expected to find low circulating levels of NANA in PEM as predicted by the rat model. Had this been the case then it would have been appropriate to consider the effect of supplemental dietary NANA on mental development. Our findings would suggest that this type of intervention is not indicated at the present time. However, the NANA levels in malnourished children were abnormal, but in the opposite direction to that predicted by the rat model. This is worthy of investigation and further work needs to be done on the relationship between NANA and development in humans.

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