Enzyme changes in malnutrition

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Since this symposium is concerned with enzymes in medicine, I shall consider only the work that has been done in human malnutrition. It would be impossible to deal with the very large literature on enzyme changes in animals under different dietary conditions. I shall also confine myself to work which is related to protein and calorie deficiency because this is the field with which I am familiar, although since most of the B-complex vitamins act as co-factors for one or more enzymes of intermediary metabolism or of the respiratory chain, certain enzyme measurements may be of value in the diagnosis of vitamin deficiencies, eg, the measurement of red-cell transketolase (EC 2.2.1.1.) in thiamine deficiency and of glutathione reductase (EC 1.6.4.2.) in riboflavin deficiency.

Studies on enzyme changes in human subjects with protein-calorie malnutrition have, broadly speaking, followed three paths, with different aims and different underlying assumptions. These may be summarized as follows: (1) the study of enzymes as 'protein markers' to give information about the functional state of protein metabolism in the body as a whole, or in one tissue or organ; (2) the search for increases in enzyme concentration in blood or urine as evidence of cell damage; (3) the study of enzyme changes in tissues, in order to identify the underlying biochemical lesion in any given state of malnutrition.

Serum Enzymes as an Index of Protein Deficiency

Twenty years ago it was thought, perhaps naively, that, since enzymes are proteins, a fall in the amount of dietary protein might be reflected in a reduction in the amounts of enzymes in blood or tissues, and hence that measurements of enzyme activity might provide a useful tool for assessing the state of protein nutrition. It seemed also that, since many enzymes which appear in the serum are tissue-specific, their activity might reflect the functional state of the organ from which they are derived.

By 1950 it had been recognized that one of the cardinal biochemical changes in protein-calorie malnutrition is a reduction in the concentration of

plasma albumin which is synthesized in the liver. The first enzyme to be studied from a nutritional standpoint was also a protein derived from the liver, namely, cholinesterase (EC 3.1.1.8). At about the same time McCance and his colleagues working on adults in Germany (Hutchinson, McCance, and Widdowson, 1951) and I working on infants in West Africa (Waterlow, 1950) showed that in malnutrition plasma cholinesterase levels followed fairly closely those of albumin. In the malnourished infants the activity of this enzyme was reduced in the liver as well as in the plasma.

In the next few years, measurements of this kind were extended to a number of other enzymes which can be detected in the plasma, eg, alkaline phosphatase (EC 3.1.3.1), in children largely derived from bone, lipase (EC 3.1.1.3) and amylase (EC 3.2.1.1) from the pancreas, and carboxylase (EC 3.1.1.1) which is another liver enzyme. As Table I shows, in general the activities in serum or plasma are low in children with established protein malnutrition.

It is interesting that, when rickets is accompanied by protein deficiency, alkaline phosphatase levels are low instead of high; in fact, the florid signs of rickets do not appear until protein is given and the child begins to grow. The low levels of amylase and lipase in serum are accompanied by a reduced output of pancreatic enzymes into the gut (Thompson and Trowell, 1952). However, there appears to be an adequate functional reserve, since the digestion of carbohydrate and protein is not impaired, although there may be some reduction in the splitting of fat. Similarly the levels of the enzymes produced in the liver may be low without any other evidence of abnormal liver function. It seems, therefore, that these reductions in the activity of enzymes in the serum may be regarded as metabolic effects which precede functional impairment. Therefore they might be regarded as sensitive indicators of protein malnutrition, but we do not really know whether they are sensitive enough to indicate early malnutrition before other clinical or biochemical changes develop. The only studies on this point appear to be those of Arroyave, Feldman, and Scrimshaw (1958) and of

Enzyme	Activity in Serum or Plasma	Authors
Cholinesterase	Reduced	Waterlow (1950) Dean and Schwatz (1953) Baron (1960) Edozien (1961) Schendel <i>et al</i> (1962) Montgomery (1963)
Alkaline phosphatase	Reduced	Dean and Schwartz (1953) Edozien (1961) Sandstead <i>et al</i> (1965)
Carboxylase esterase (nonspecific)	Reduced in kwashiorkor Slightly reduced in marasmus	Dean and Schwartz (1953) Srikantia <i>et al</i> (1964) Srikantia <i>et al</i> (1964)
Amylase	Reduced in kwashiorkor Unchanged in marasmus	Dean and Schwartz (1953) Edozien (1961) Srikantia <i>et al</i> (1964) Srikantia <i>et al</i> (1964)
Lipase	Reduced in kwashiorkor; unchanged in marasmus	Srikantia <i>et al</i> (1964) Srikantia <i>et al</i> (1964)
Creatine kinase	Reduced in kwashiorkor; less reduced and sometimes elevated in marasmus	Balmer and Rutishauser (1967)

Table I *Some enzyme changes in protein calorie malnutrition which may indicate the nutritional state*

Behar, Arroyave, Flores, and Scrimshaw (1960) who found that plasma cholinesterase levels in children from poor families, who might be regarded as marginally malnourished, did not differ from those in better fed controls.

Serum creatine kinase (EC 2.7.3.2) is the latest addition to the list of tissue specific enzymes found to be reduced in malnutrition. The low levels found in children with kwashiorkor were thought by Balmer and Rutishauser (1967) to reflect the reduction in muscle mass. This was confirmed experimentally by Reindorp (1970) who showed that in rats deprived of protein there was a close relationship between serum creatine kinase activity, creatinine output, and muscle weight. In marasmus Balmer and Rutishauser (1967) found that creatine kinase levels tended to be higher than in kwashiorkor, and some very high values were found just before death in fatal cases. They concluded that when there is active breakdown of muscle tissue, and perhaps when muscle protein is being catabolized to provide energy, there may be an increased release of creatine kinase into the blood stream.

In fact most of the enzymes listed in Table I are reduced less in marasmus than in kwashiorkor, and serum albumin behaves in the same way. The hypothesis usually advanced to explain this is that when protein catabolism, particularly in muscle (Millward, 1970), is increased to make good a calorie deficiency, more amino acids are available for the synthesis of essential proteins such as enzymes. Thus the activity of the enzymes in serum is the resultant of two opposing factors, protein deficiency

tending to lower the levels and calorie deficiency tending to increase them. This makes interpretation of serum levels difficult.

A further difficulty about the use of creatine kinase as an index of muscle protein depletion is that serial measurements showed very large fluctuations, probably due to infection or to physical activity (Reindorp, 1970). It seems likely that the levels of other enzymes in the serum will also be affected by non-nutritional factors, but this point has not, to my knowledge, been adequately explored.

A general criticism of this whole approach is that it is largely empirical. There does not seem to be any particular reason why some enzymes but not others appear in serum in detectable amounts, and one cannot draw any general conclusions from the behaviour of these particular enzyme proteins. We now know that the same dietary stimulus may alter the activity of different enzymes in different, even in opposite, ways. In the liver, protein deficiency causes a reduction in the rate of synthesis of albumin, and presumably also of cholinesterase, but this does not apply to liver proteins as a whole (Waterlow and Stephen, 1968). There are enzymes in the liver whose activity is extremely sensitive to the level of protein intake, eg, enzymes of amino acid catabolism, such as serine dehydratase, (EC 4.2.1.13) the activity of which may change 100-fold with an appropriate stimulus (Fallon, Hackney, and Byrne, 1966). Unfortunately, serine dehydratase has not been detected in the blood, so that advantage cannot be taken of the sensitivity with which it responds to the level of protein intake.

For these reasons, the measurement of enzymes in serum has not in practice proved particularly useful, either for assessing the severity of protein malnutrition or for detecting an impending deficiency state. However, further work may well reverse this judgment, as the field has not been fully explored.

Leakage of Enzymes from Tissues

We tend to think of protein-calorie malnutrition as causing a loss of cell substance which is reversed when food is provided. However, a starved cell must eventually die. When this occurs it might be expected that enzymes will leak into the serum just as in other diseases where there is cell destruction. In kwashiorkor fatty infiltration of the liver is a common feature and may be so severe that the child develops jaundice and dies, probably of hepatic failure. The microscopical appearance suggests that there may be very little circulation of blood through these livers, and it is not surprising if there is extensive hepatic cell damage.

Table II summarizes the results of a number of studies which have been made of this point. In

Enzyme	Activity in Serum or Plasma	Authors
Aminotransferases	Increased	Baron (1960) Edozien (1961) McLean (1962)
	Unchanged	Smith (1962)
Isocitrate dehydrogenase (EC 1.1.1.42)	Increased	McLean (1962)
Lactate dehydrogenase (EC 1.1.1.27)	Increased	Sandstead <i>et al</i> (1965)

Table II Some enzyme changes in protein-calorie malnutrition which may indicate cell damage

general, raised enzyme levels have been found in the serum of patients with protein malnutrition. The findings of McLean (1966) are of particular interest. He showed that the activity of alanine aminotransferase (EC 2.6.1.1) in serum was increased, and that the greater the increase the greater was the mortality (Table III). In the liver, however, the opposite was found, namely, a decrease in the aminotransferase, which was more marked in the more severe cases (Table IV). Leakage no doubt plays a part in this, but McLean showed that there is also an adaptive fall of enzyme synthesis in response to protein deficiency, exactly like that produced experimentally by low protein diets in rats (Rosen, Roberts, and Nichol, 1959; Muramatsu and Ashida, 1962). Presumably this adaptive change must tend to minimize the increase in serum aminotransferase produced by cell damage.

Serum ALAT	No. of Patients	No. of Deaths	Mortality (%)
0-100	63	0	0
101-200	44	3	7
201-250	8	2	25
251-500	18	5	28
501 ×	4	4	100

Table III Serum alanine aminotransferase (ALAT) on admission and mortality rate in malnourished children¹

¹In normal Jamaican infants; GPT = 45 ± 24 (McLean, 1966).

Biopsy Taken	No. of Biopsies	ALAT (units/mg protein; mean ± SD)
From Dead Patients		
Within 30 minutes after death	11	1.8 ± 0.5
From Living Patients		
2-3 days after admission	8	3.4 ± 2.1
6-14 days after admission	8	4.3 ± 2.1
15-20 days after admission	4	10.1 ± 2.2
>40 days after admission	15	11.8 ± 2.2
After low protein diet given two weeks after recovery	7	6.5 ± 1.2
From Adults, Well Nourished and without Liver Disease	6	12.5 ± 2.5

Table IV Alanine aminotransferase (ALAT) in liver biopsies from malnourished children and adult controls (McLean, 1966)

Ittyerah, Dumm, and Bachhawat (1967) and Begum and Ittyerah (1970) found that in India children with kwashiorkor had raised serum levels and increased urinary excretion of the lysosomal enzyme arylsulphatase (EC 3.1.6.1), and suggested that there is a specific lesion of the lysosomes. However, in the malnourished patients there was no change in other lysosomal enzymes, β-glucuronidase (EC 3.2.1.31) and acid phosphatase (EC 3.1.3.2). Moreover, the excretion of arylsulphatase was greatly increased by infection, so that the effect was not specific for malnutrition.

It seems probable that these increases in serum and urinary enzymes are relatively late events in the progression of malnutrition; they may be of value for prognosis, but not for assessment or diagnosis in the early stages.

Cellular Enzymes: the Search for Biochemical Lesions

Measurements of enzymes after death are not likely to be of much value, and therefore for work on tissue enzymes we are confined to biopsies or white blood cells, and this in turn means that micro-methods have to be used. These difficulties have limited both the scale and the scope of what has been achieved.

In the decade from 1950 to 1960, several groups studied a range of liver enzymes in malnourished

infants. The results have been reviewed before (Waterlow, 1959; Waterlow, Cravioto, and Stephen, 1960) and need not be repeated here. It is enough to recall that the enzymes were selected largely because it was possible to measure them by micromethods and that, although some were reduced, the majority were well preserved even in severe malnutrition.

This work was entirely empirical; later work was at least guided by some more definite objective or hypothesis. For example, our studies of oxidative phosphorylation in the liver (Waterlow, 1961) were based on the idea that protein deficiency might interfere with an overall reaction which depended upon the structural integrity of subcellular elements, even though all the individual enzymes might be well preserved. In fact, in malnourished infants oxidative phosphorylation was reduced in liver homogenates and it was also less stable than in controls. Clearly a defect in oxidative phosphorylation would be a very serious matter; however in interpreting these results it was difficult to separate artefacts caused by excess fat in some of the malnourished livers.

Metcoff, Frenk, Yoshida, Torres Pinedo, Kaiser, and Hansen (1966) have made extensive studies in severely malnourished infants of enzymes and intermediates of carbohydrate metabolism in muscle, with the aim of explaining the mechanism of the disturbances in electrolyte balance which often prove fatal. Their salient finding in the present context was a decrease in pyruvate kinase (EC 2.7.1.40) activity. The results are consistent with the idea that there is a failure of the energy supply for the 'sodium pump', so that sodium accumulates in the cell.

Children with protein-calorie malnutrition often present the paradoxical condition of hypoglycaemia with reduced glucose tolerance. Alleyne and Scullard (1970) investigated the possibility that the low blood sugar might be caused by a reduction in hepatic glucose 6-phosphatase (EC 3.1.3.9). In fact, however, the activity of this enzyme was found to be increased when the children were malnourished, and fell on recovery. In a later study, Alleyne, Flores, Trust, and Waterlow (1971) obtained muscle samples by punch biopsy and tested the peripheral utilization of glucose by measurements of glycolysis in homogenates. With several substrates glycolysis was reduced in both malnourished and recovered children when compared with children who had never been malnourished. In another series insulin secretion also remained low long after clinical recovery had occurred (James and Coore, 1970). These findings suggest that malnutrition may cause long-lasting, possibly even permanent, metabolic changes.

A final example of the application of enzyme measurements to problems of human nutrition is our study of adaptive changes in enzymes of amino acid

metabolism in the liver (Stephen and Waterlow, 1968). As the children recovered, the activity of the amino acid synthetic system fell, and that of argininosuccinate lyase (EC 4.3.2.1), an enzyme of the urea cycle, rose. Thus it appeared that in the malnourished state adaptations had occurred which would reduce the catabolism of amino-acids to urea and promote their uptake into protein.

Conclusion

The ultimate practical objective of the enzyme studies which have been described is to help in the prevention of protein-calorie malnutrition. It is often supposed that this is a practical problem for which new scientific knowledge is not needed. That is a superficial view. We are faced with the possibility that 'marginal malnutrition' in childhood may cause a permanent handicap throughout life, and yet we cannot recognize or define this 'marginal malnutrition' with any precision. This is not the place to discuss the semantics of what is meant by the phrase. The point on which I wish to end is that progress depends on a better understanding of the processes of nutritional adaptation, and of when and why they break down. Enzyme changes must play a fundamental part in these processes, and this is the justification for the work which has been summarized here.

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