Observations on the mechanisms of adaptation to the low protein intakes

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SUMMARY

Experiments are described which attempt to throw light on the mechanism by which animals and man adapt to low protein intakes.

In the rat, studies by constant infusion of a labelled amino acid have shown that in the protein depleted animal there is a small reduction in total protein turnover: this, however, is not enough to account for the great reduction in urinary nitrogen output.

Constant infusion and single injection experiments agree in showing that in rats on a low protein diet there is a change in the pattern of protein turnover: synthesis of carcass protein (muscle and skin) is reduced, while that of liver protein is well maintained.

The preservation of synthesis in liver seems to depend partly on increased re-utilization of amino acids liberated by the catabolism of tissue protein. This economy may be brought about by adaptive enzyme changes —decreased activity of the urea cycle enzymes and increased activity of amino acid activating enzymes in the liver. These changes, previously described by others in the rat, have been shown to occur in the human liver also.

Studies in human infants with ^{75}selenium-labelled methionine provide some support for the concept that when the protein intake is limited, turnover is preferentially maintained in the liver. However, not all liver-produced proteins behave in the same way; studies of albumin kinetics in infants show that when the protein intake is altered, there is a rapid change in the rate of albumine synthesis, together with a redistribution of albumin between intra and extravascular spaces. Later and more slowly occurs a change, presumably compensatory, in the rate of albumin catabolism.

Hormonal changes may play a part in these adjustments. Increased

Received for publication October 14, 1966.

cortisone and decreased insulin activity would have the effect of promoting amino acid uptake by liver at the expense of muscle.

It is concluded that the net nitrogen loss which occurs when the protein intake is reduced results simply from the time-lag before the adaptive mechanisms come into play, and therefore cannot logically be regarded as the loss of reserve protein. The practical implications of this concept are discussed.

A basic characteristic of protein metabolism in mammalian organisms is that nitrogen balance can be maintained over a wide range of nitrogen intake. The situation is quite different for energy metabolism: as far as we know, energy expenditure is not automatically adjusted to equal the level of intake. Nitrogen equilibrium is such a familiar property that we have come to take it for granted, yet virtually nothing is known about how it is maintained — that is, the nature of the homoeostatic mechanisms. This is far from being an academic question; in fact, it has an important bearing on practical problems of protein nutrition, as we shall try to show.

Fig. 1 illustrates diagrammatically the urinary N excretion day by day when a subject changes from a high to a low protein intake. In the human adult this adaptation takes 6-8 days (1); we have found that in the infant it is much quicker, and is complete in 2-3 days (2). During the period of adaptation there is a net loss of N from the body, which in the adult amounts to between 3 and 5% of total body N (3). Again, in the infant this loss is much smaller — only about 0.7% of total body N (2). Therefore it seems that the child is much more economical of protein when supplies are restricted. The protein lost during adaptation to a low intake, and regained when the diet is altered in the opposite direction, has been described as 'labile protein' (3). There is much controversy about the significance of labile protein. Should it be regarded as a protein store, which for normal health must be kept filled to the highest possible level? Alternatively, are the losses and gains of labile protein merely by-products of a change in the level of metabolic activity, and of no significance in themselves? The answer to this question is of importance, because if it is considered necessary for health that "protein stores" should be well filled, then protein requirements must be set at much higher levels than those previously recommended (4). We believe that this question cannot be answered until

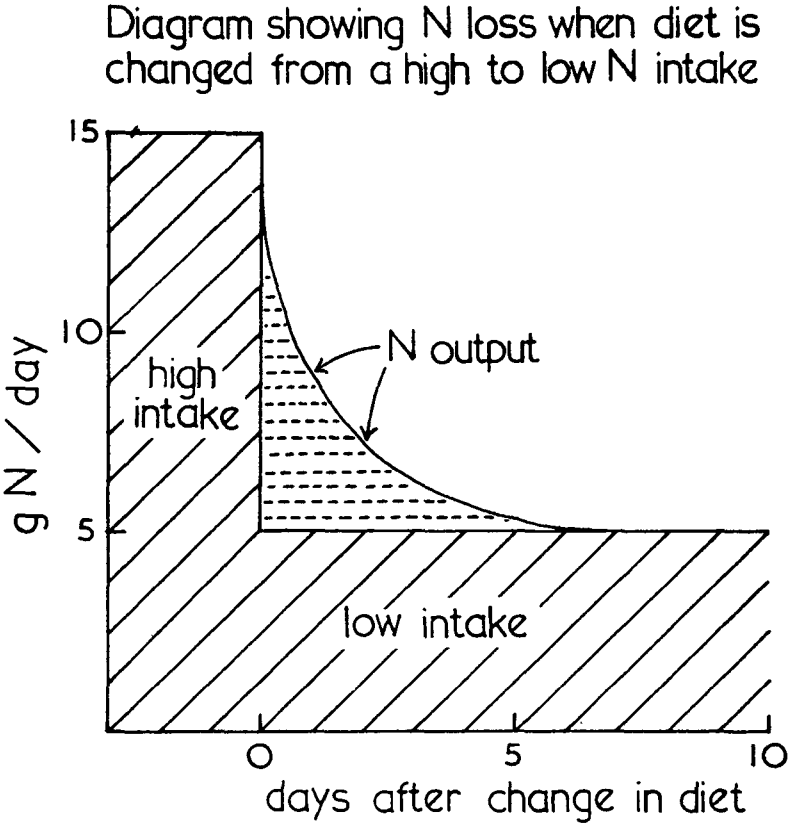

Fig. 1.—Diagrammatic representation of change in urinary nitrogen output when the protein intake is altered from normal to maintenance level.

we understand more about the mechanism by which the urinary N output adapts to match the intake.

The pioneer work of Addis and others (5, 6) showed that in the rat acutely deprived of protein a large proportion of the protein lost is derived from liver and intestinal mucosa. The liver may lose up to 40% of its cytoplasmic protein. The fact that two such vital organs lose so much protein naturally strengthens the case of those who believe that the loss of labile protein is of physiological significance. However, one must be cautious about applying to man these results obtain-

ed on the rat. As far as we know, there is no evidence that in man a short period of protein deprivation causes such a huge loss of protein from the liver.

TABLE 1

NITROGEN CONTENT OF LIVER AND BODY IN UNDERNOURISHED AND STARVED RATS

	PERCENTAGE OF CONTROL		
	Body weight	N in liver	N in rest of body
Rats acutely starved	73	61	88
Rats chronically undernourished	70	68	72

Data from Widdowson and McCance, Brit. J. Nutr. (1956), 10, 363.

Moreover, the pattern of protein loss is not static, and is not the same when low protein diets are continued for longer periods. This is well shown by the results of Widdowson and McCance (7) in table 1. After a short period of starvation the main loss of protein was from the liver, but in animals kept for a longer period on a reduced intake there was a parallel reduction in the protein content of the carcass. Similar results obtained by us (8) are shown in table 2. Young growing rats were acutely depleted after 3 days on a protein-free diet. The absolute deficits of nitrogen in various tissues were calculated by comparison with the amounts found in controls of the same age on a normal diet. The skin lost relatively more protein than any other organ. This again is a result which may not be applicable to man; in the rat, with its large surface to volume ratio, the skin contains a larger proportion of total body protein and is probably metabolically more important than in human beings.

TABLE 2

NITROGEN CONTENT OF WHOLE BODY AND OF TISSUES IN PROTEIN-DEPLETED RATS, AS A PERCENTAGE OF THE AMOUNTS IN CONTROLS

	NATURE OF DEPLETION		
	Acute	Chronic	Acute on chronic
Whole body	81	51	35
Liver	68	57	50
Other viscera	71	53	32
Brain	96	96	97
Muscle	105	62	42
Skin + hair	77	44	30

Acute = 3 days protein-free diet.
Chronic = 6 weeks 6% casein diet.
Acute on chronic = 6 weeks 6% casein followed by 3 days protein-free.

Data from Waterlow and Stephen, Brit. J. Nutr. (1966), 120, 461.

We also divided the skin, by chemical extraction, into three fractions: hair, cellular protein and residual protein, mainly collagen. The cellular fraction was rapidly reduced in amount by acute depletion, the 'collagen' fraction more slowly. This illustrates the point that the pattern of protein loss varies with the duration and severity of depletion. At the level of the cell also it is probable that different proteins are affected differently, judging by the way in which some enzymes are selectively preserved, others rapidly lost (9).

These, then, are the facts to be explained: that adaptation to a low protein intake involves first, an adjustment of output to match the intake; secondly, an alteration in the protein make-up of the body at all levels of organisation. We have tried to gain some insight into how these changes are brought about by various types of expermental approach, which of

necessity have had to be employed mainly on animals. Preliminary results obtained on human subjects are also presented.

The effect of protein depletion on protein turnover

An adult man, on a nitrogen intake of 15 g per day, will have urinary N output of perhaps 12 g per day. If he changes to an intake of 5 g N per day, the urinary output will fall to about 3 g N per day. The differences between intake and output are, of course, due to faecal and dermal losses. On the basis of Folin's distinction between exogenous and endogenous urinary N, one might suppose that on the high protein intake most of the extra 10 g N was simply diverted to the urine to form the exogenous fraction. Schoenheimer's discovery that food N mixs immediately with body N makes this view untenable. The available evidence indicates that in the normal human adult the total rate of protein turnover is of the order of 3-5 g protein per kg per day, equivalent to about 45 g N per day for an average man (10-16). By total turnover we mean the over-all rate at which N is incorporated into body protein, or liberated from protein by catabolism. These two rates must, of course, be equal in the steady state. The turnover rate of N seems therefore to be about three times as great as the rate of dietary N intake. It is reasonable to suppose, as a first approximation, that amino acids derived from the food and amino acids liberated by catabolism of body protein mix in a homogeneous pool. If that is so, we have the situation summarized in table 3: on a normal diet, N excretion = 20% of the total amount of N entering the pool. When the N intake is reduced, there are two possibilities, if we consider extreme cases. One alternative (table 3, colum C) is that N turnover is reduced, so that of the N entering the pool, the same proportion is excreted. The other possibility is that the total turnover remains the same, and the proportion excreted falls (column B). This would imply a very sensitive regulatory mechanism: when the N intake is reduced, the total amount of N entering the pool would fall from 60 g to 50 g per day — a reduction of 16 per cent, but the urinary N falls from 12 g to 3 g — a reduction of 75 per cent. It seemed to us important to distinguish between these alternatives: is the response to a low protein intake a reduction in total turn-

TABLE 3

HYPOTHETICAL RELATIONSHIP BETWEEN NITROGEN INTAKE, TURNOVER AND EXCRETION

	Normal diet	Low-protein diet	
	A	B	C
N entering pool, g/day:			
from food	15	5	5
from catabolism	45	45	10
total	60	50	15
N in urine g/day	12	3	3
as % of N entering pool	20	6	20

over, or is there some mechanism of economy which is extremely sensitive to changes in N intake?

Most authors who have attemped to measure total N turnover have used the method of San Pietro and Rittenberg (10), in which a single injection of a labelled amino acid is given and the turnover calculated from the peak specific activity of urinary urea, which occurs after 3-4 hrs. Since during this period all specific activities are changing very rapidly, the possibilities of experimental error are very large. The theory of the method depends upon a number of assumptions, of which the most important are that the free amino acid pool is homogeneous, and that transaminations are complete during the experimental period. The first of these assumption is unproven, the second probably invalid (17).

It seemed to us that a more satisfactory method would be to give a continuous intravenous infusion of a labelled amino acid, instead of a single injection. When this is done, the specific activity of the administered amino acid in the free amino acid pool should rise to a plateau. Fig. 2 shows that in the rat infused with lysine this plateau is reached in 3-4 hours, as judged by measurements of specific activity made on serum. The amino acid turnover can be very simply calculated from the plateau SA:

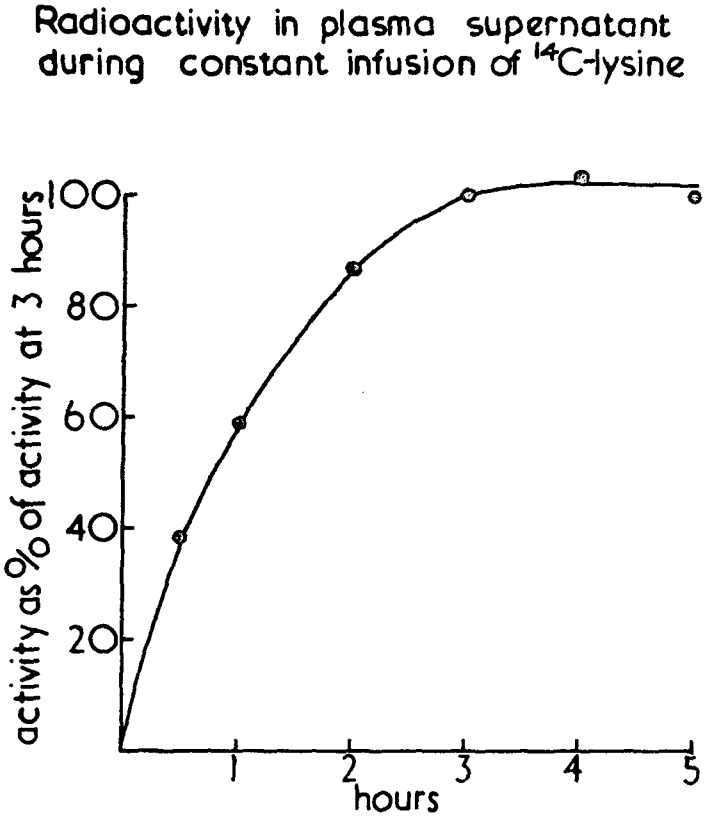

Fig. 2.—Radioactivity in non-protein fraction of rat plasma at different times after starting intravenous infusion of ^{14}C-L-lysine.

Q = turnover rate = rate of uptake of amino acid into protein (μM/hr).
q = rate of infusion of labelled amino acid (μM/hr).
S = SA of free amino acid at plateau.
s = SA of infused amino acid.

At the plateau, when the SA in the free amino acid pool is constant, the amount of radioactivity entering the pool (qs) must equal the amount of radioactivity leaving (QS).

$$\text{Therefore } Q = \frac{qs}{S}$$

This simple formulation assumes that there is no reentry of labelled amino acid from the breakdown of labelled protein. We have shown that over the first 7 hours this is in fact negligible (18). It also assumes that the free amino acid pool is homogeneous. Our results show that this is not quite true, but the error introduced by the assumption is not large (19).

TABLE 4

TOTAL LYSINE TURNOVER IN RATS, MEASURED BY CONTINUOUS INTRAVENOUS INFUSION OF ^{14}C-LYSINE

	Turnover μM/100 g body weight/hr.	
Normal diet:		
Young rats, wt. < 160 g.	77	(8)
Old rats, wt. > 160 g.	61.5	(7)
Low-protein diet:		
Wt. < 160 g.	53.5	(6)

The results which we have obtained so far by this method are summarized in table 4. There was a significant negative correlation between rat weight (and hence age) and total turnover. It seems reasonable that metabolic processes should be slower in older animals. The average total turnover in control rats, in terms of protein, was 25 g per kg per day. This fits in with the suggestion of Drabkin (20) that metabolic processes in the rat occur 4-5 times faster than in man. In the depleted rats the turnover was reduced by about 30%. From other experiments we know that on this low protein diet the urinay N excretion is only about one-seventh of that of rats on the normal diet. The small fall in total turnover cannot account for this great reduction in urinary N.

Distribution of amino acid incorporation

Many authors have shown that when a labelled amino acid is administered, the proteins of liver become more highly

labelled, those of muscle less highly labelled, in animals on a low protein diet than in controls (21-25). Further information is needed, however, before deductions about synthesis rates can be made from simple measurements of the specific activity of proteins. First, it is necessary to know the size of the protein pools, because the greater the mass of protein, the greater the dilution of the label. A higher activity in liver could result simply from a reduction in liver size, the total incorporation being the same. We showed in experiments with ^{35}S-methionine, in which total incorporation was measured, that in protein-depleted rats a larger proportion of the radioactive amino acid was taken up by the viscera and a smaller proportion by the carcass, than in controls (26). This finding suggested that incorporation was occurring preferentially in the more vital organs independently of their size.

A second point which has to be considered is the specific activity of the free amino acid precursor. If one effect of a low protein diet were to reduce the concentration of free amino acids in the liver, then with a given dose of radioactivity the SA of the free amino acid would be increased. This in turn would result in a higher activity in the liver protein. This difficulty is overcome if the SA of the product —protein— is related to that of the precursor — free amino acid. The ratio: change in SA of protein/SA of free amino acid, sometimes called a flux, can be used as a measure of the rate of protein synthesis.

Spadoni and her co-workers in Italy (24) made measurements over a period of 2 hours after giving labelled lysine. They showed that in animals on a low protein diet the specific activity ratio in liver was increased above the control level, whereas in muscle it was decreased. In longer-term experiments, in which we gave a single injection of labelled lysine, and made measurements after 3 days, we found that in protein depleted rats there was reduced incorporation into muscle, which could not be explained by any change in the amount or SA of free lysine (8, 27).

The disadvantage of this type of experiment is that specific activities are changing, but the measurements are made at only one point in time, and therefore the calculations of flux are only approximate. This difficulty is avoided by the technique of continuous intravenous infusion (see above). Since

tne SA of the free amino acid precursor reaches a constant plateau, it is possible with certain assumptions to calculate the fractional rate of protein synthesis in any tissue from the simple relationship: change in protein SA per unit time/free amino acid SA at plateau (19).

TABLE 5

SYNTHESIS RATES OF TISSUE PROTEINS IN THE RAT, MEASURED BY CONTINUOUS INFUSION OF ^{14}C-LYSINE

	Fractional synthesis rate, %/day		
	Serum	Liver	Muscle
Normal diet:			
Young rats, < 160 g.	80	34	10.8
Old rats, > 160 g.	57	22	6.7
Low-protein diet:			
Wt. < 160 g.	51	26	4.5

The results of such experiments are shown in table 5. In older rats the rate of muscle protein synthesis or renewal was lower than in younger rats. As with total turnover, there was a significant negative correlation between the weight or age of the rat and the rate of muscle protein synthesis. In protein-depleted rats the synthesis rate was reduced in all the tissues studied, but the reduction was much greater for muscle protein than for the mixed proteins of liver or serum.

It seems therefore that in rats on a low-protein diet there is a well-marked change in the *distribution* of protein synthesis within the body. Synthesis is well maintained in liver, and no doubt in other visceral organs, but severely reduced in muscle. Our results also suggest that in the rat the skin probably behaves in much the same way as muscle.

Economy of amino acid utilization

The results quoted earlier suggest that in a rat on a low-protein diet the rate at which amino acids are liberated from body protein and appear in the free amino acid pool is not much reduced, compared with the rate in a normal rat. In spite of this, the urinary N output is very low. Obviously,

therefore, a smaller proportion of the available amino acids is converted to urea, and a larger proportion is re-incorporated in protein. In other words, on a low protein diet the net rate of loss of N from the body is reduced, and this is secured by an increased economy or re-utilization of amino acids for protein synthesis.

The over-all effect is well shown in experiments in which body proteins are labelled by a single dose of ^{75}seleno-methionine. This amino acid analogue appears to behave like natural methionine as far as protein metabolism is concerned*. It has the advantage over other amino acid tracers that ^{75}Se is a gamma-emitter, and therefore the amount of label in the body at any given time can be measured in a whole body counter. Fig. 3 shows the results obtained in 2 rats, one on a normal (Curve A), the other on a low protein intake (Curve B). For the first few days there is a rapid fall in radioactivity, but then the rate of loss becomes more or less linear, if the results are plotted semi-logarithmically. In the rat on a low protein diet this rate was only 1.85% per day, compared with 5.3% in the rat on the normal diet.

We thought it of interest to try to measure directly the extent to which amino acids liberated by catabolism of protein are re-utilized for synthesis — i. e. the extent of internal "recycling" of amino acids. To do this it is necessary to compare the behaviour of a label which is not re-utilized with one which is re-utilized. With blood proteins, such as plasma albumin, this can be done if the albumin is double labelled with a ^{14}C amino acid which is re-utilized, and with ^{131}I or ^{125}I, which is not. The rate of loss of ^{131}I or ^{125}I then gives the true half-life of the albumin; the rate of loss of ^{14}C is slower, because of re-utilization, and can be described by term 'apparent half-life'. The difference between the apparent and true half-lives is a measure of the extent of recycling.**

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* Since this paper was written we have seen the note by Holland and co-workers which suggests that although selenomethionine is a "physiologic trace amino acid", it does not behave entirely like methionine. (Holland, J. F., Peters, S., Bryant, B. & Blau, M., J. Clin. Invest. 45, 1024, 1966.)

** This comparison between true and apparent half lives can only be made if the re-utilized label is administered as an amino acid. When a labelled amino acid is given, about 5% of the dose is incorporated in plasma protein, and the remainder in other body proteins; it is the catabolism of these which provides the labelled amino acid to be re-utilized. The situation is quite different in experiments in which albumin, previously labelled by injection of ^{14}C amino acid in a donor animal, is then given to a recipient animal.

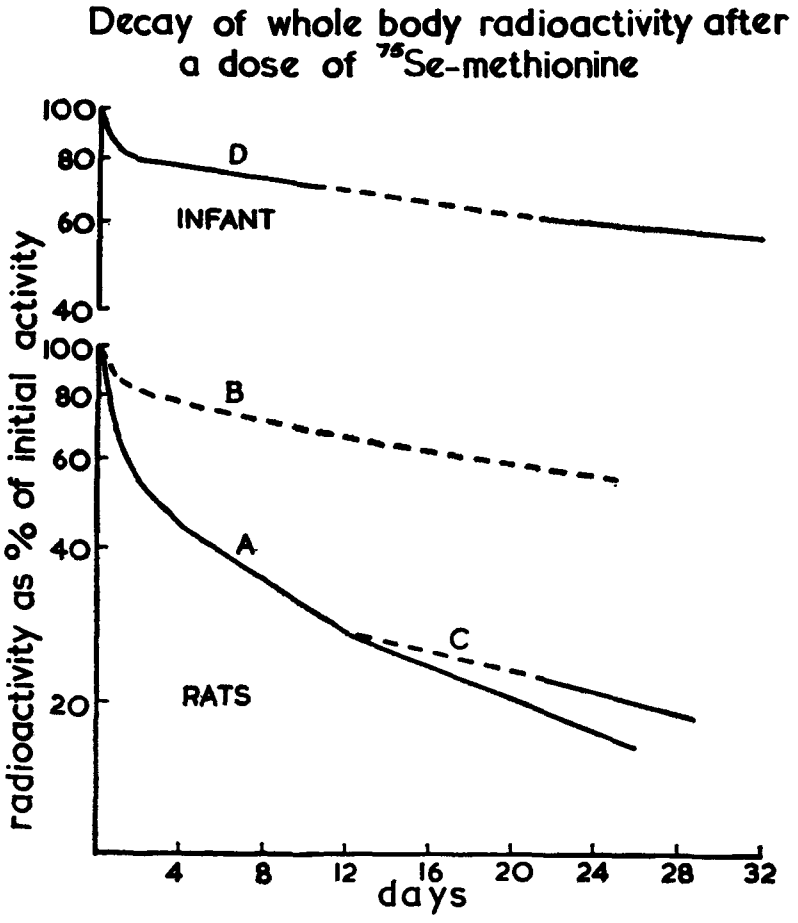

Fig. 3.—Decay of whole-body radioactivity after administration of ^{75}Se-methionine.

Curve A: rat, stock diet.

B: rat, 6% casein diet.

C: rat, stock diet initially; 6% casein between days 12 and 22.

D: human infant: high protein diet (3.6 g/kg/day) days 0-12:
high protein diet (3.6 g/kg/day) days 22-32.
low protein diet (0.75 g/kg/day) days 12-22:

This method cannot be applied to tissue proteins, because they cannot be removed, iodinated, and returned to the body. For work on the liver we therefore made use of the fact, first pointed out by Swick (28), that the guanidine carbon of arginine is not re-utilized. If a molecule of arginine, uniformly labelled with ^{14}C, appears in the liver free amino acid pool, it enters the urea cycle and exchanges its labelled 6-carbon atom for unlabelled carbon derived from the bicarbonate of the body fluids. If this amino acid is re-incorporated into protein, it will have lost the label from its 6-carbon atom but not from C atoms 1-5. We therefore injected uniformly labelled ^{14}C arginine, followed by $Na_2{}^{14}CO_3$ to raise the initial level of labelling of the 6-C of arginine. Liver and blood samples were taken at intervals, and the proteins hydrolysed. Arginine-1-C was liberated by treatment with arginine decarboxylase, and arginine-6-C by treatment with arginase and urease. Thus the specific activity of these two C atoms could be measured separately (29). Results of preliminary experiments are shown in table 6. The low protein diet caused very little change in the true half-life — i. e. the catabolic rate — of liver protein, whereas the apparent half-life was on the average almost doubled. It was calculated that even on a normal diet about

TABLE 6

TRUE AND APPARENT HALF-LIVES OF MIXED SERUM PROTEINS AND OF LIVER PROTEINS IN THE RAT

	DIET	
	Normal	Protein-free 10 days
Mixed serum proteins:		
True T½, days	2.0	3.4
Apparent T½, days	2.8	5.5
Mixed liver proteins:		
True T½, days	1.9	2.3
Apparent T½, days	5.5	9.2

Data from Stephen and Waterlow (1966), Nature, 211, 978.

60% of the arginine liberated by catabolism of liver protein was re-incorporated in protein. On a low protein diet the corresponding figure was 75%. Unfortunately no data are yet available for other tissues, except serum proteins.

The experiments described so far illustrate three aspects of the process by which the rat adapts to a reduced protein intake:

1. There is a moderate decrease in total protein turnover.
2. There is an alteration in the pattern of protein turnover. In some tissues, e. g. liver, the rate of protein synthesis is well maintained; in others, e. g. muscle, it is greatly reduced. It is probable that the fall in over-all protein turnover reflects mainly the reduced amino acid uptake by muscle.
3. Amino acids are more economically utilized; a larger proportion of the amino acids entering the metabolic pool are utilized for protein synthesis, and a smaller proportion excreted as urea. So far this effect has only been shown in the liver.

Before we begin to speculate on how these changes may be brought about, it may be useful to consider what information we have about similar changes in human subjects.

The effect of a low protein intake on albumin metabolism

Plasma albumin is the classical object for tracer studies of protein metabolism in man, for the simple reason that it is easily obtained and easily labelled. Some years ago it was shown that in malnourished infants the catabolic rate of plasma albumin was greatly reduced (30, 31). At that time it was not clear whether this was a response to a low protein intake *per se,* or to the state of malnutrition of the infant — two different, but related, things. More recently, Hoffenberg and co-workers (32) showed that in normal adults put on a low protein diet, the most notable change was a decrease in the quantity of extravascular albumin. There was also a small decrease in the rate of albumin catabolism. We have re-investigated this problem by studying albumin turnover in infants given a high, then a low and then again a high protein intake for successive periods of 7 days. A single injection of ^{131}I albumin was given at the beginning of the first period. The

data were analysed in terms of a 3-pool model (33). In view of the dietary changes it was not justifiable to assume a steady state. The equations were solved and the rate constants calculated with an IBM digital computer. Separate estimates could be made of the rates of synthesis, catabolism and transfer between extravascular and intravascular pools. Some results obtained so far are shown in table 7.

TABLE 7

THE PERCENTAGE CHANGES IN THE FRACTIONAL CATABOLIC RATE (F. C. R.) AND SYNTHETIC RATE (S. R.) OF ALBUMIN IN CHILDREN ON CHANGING TO A LOW AND THEN BACK TO A HIGH PROTEIN DIET

Subject		High to low protein diet	Low to high protein diet
M. M. 1	F. C. R.	— 7	+ 16
	S. R.	— 36	+ 57
P. W. 2	F. C. R.	— 24	— 1
	S. R.	— 53	+ 114
W. G. 2	F. C. R.	— 19	— 4
	S. R.	— 31	+ 66
D. S. 1	F. C. R.	— 27	— 7
	S. R.	— 10	+ 5

In all the cases there was a fall in the fractional catabolic rate of albumin when the protein intake was reduced. There was a tendency for this fall to persist, or even to become more marked, when the high protein intake was restored. The changes produced by the diet in the fractional synthesis rate were much greater than those in catabolic rate, and they were established more rapidly. This point is not brought out in the table, but is apparent from the computed curves. It seems,

therefore, that the initial response to a low protein intake is a reduction in the synthesis rate of albumin; this is not immediately balanced by a fall in catabolic rate, and therefore there is a decrease in the total albumin mass. It appears that the subsequent fall in catabolic rate must be a secondary response to the change in albumin mass, rather than a primary effect of the diet *per se.*

In the experiments the total intravascular albumin mass remained constant, and the depletion occurred at the expense of the extravascular pool, in agreement with the results of Hoffenberg *et al.* (32). The intravascular albumin concentration cannot therefore be expected to be a very sensitive index of depletion, although when measured in large groups of subjects it does show a significant relationship to the degree of malnutrition (34). It also seems clear that plasma albumin is not preferentially protected against the effects of a low protein intake, but it must not be assumed that this behaviour is typical of all body proteins. The experimental evidence quoted above suggests that liver protein in general is better protected than plasma proteins.

Studies with ^{75}seleno-methionine

Fig. 3 shows that in the rat the rate of loss of isotope after a single injection of ^{75}Se-methionine depends upon the level of dietary protein. When this level is altered, there is an immediate change in the slope of the curve (fig. 3, curve C). The human infant appears to behave differently. A small dose (1 μc) of ^{75}Se-methionine was given by mouth, and the radioactivity remaining in the body followed by daily or twice-daily measurements in a whole body counter. The protein intake was altered from high to low, and then back to high. Each feeding period lasted 10-12 days. As curve D in Fig. 3 shows, altering the protein intake had little, if any, effect on the rate of isotope loss. During the low protein period, as would be expected, the urinary excretion of N and S was greatly reduced. Since the rate of isotope excretion remained the same, the 'specific activity' in the urine (counts per mM SO_4) was increased (table 8). There was also, during the period of low protein feeding, an increase in the ratio of S to N in the urine. This pattern has been found consistently in all children investigated so far. Similar changes in the urinary

TABLE 8

THE EFFECT OF THE PROTEIN CONTENT OF THE DIET ON THE URINARY EXCRETION OF SULPHATE AND RADIOACTIVITY IN AN INFANT GIVEN ^{75}SELENO-METHIONINE

	High protein I	Low protein	High protein II
Inorganic SO_4, mM/day	2.20	0.83	2.76
Urinary radioactivity, % of dose remaining excreted / day	0.52	0.48	0.49
Specific activity, % of dose / mM SO_4	0.23	0.52	0.18
mM SO_4 / mg N	1.52	2.32	1.57

S/N ratio in dogs fed different levels of protein were recorded by Bressani (35), but not commented upon.

It follows from these observations that during the period of low protein intake the N and S excreted in the urine must, on the average, have been derived from a source with a higher specific activity and a higher S/N ratio than during periods of high protein feeding. The analytical data of McCance and Widdowson (36) show that of all tissues liver contains the highest proportion of S to N. Moreover, in the human subject at 10 days after giving the isotope, liver proteins will probably still be more highly labelled than muscle proteins. Therefore, the most probable explanation of these urinary findings is that on the low protein intake there was a decrease in the turnover rate of poorly labelled proteins, such as muscle, while that of highly labelled proteins, e. g. liver, remained unchanged. This conclusion would fit in well with the experimental evidence that adaptation to a low protein intake involves alterations in the pattern of protein turnover.

The discrepancy between the results obtained with selenomethionine in the rat and in the human infant perhaps depends upon the time-scale of the experiments. In both cases the dietary periods lasted about 10 days, but this is a much longer proportion of the life-span of the rat than of man. In the rat 10 days after giving a labelled amino acid the greater part of the label is in the carcass proteins (26). Therefore a reduction in the turnover rate of these proteins might be expected to cause a fall in urinary isotope excretion. Although we have no certain evidence, it seems likely that in man the transfer of label from visceral to carcass proteins occurs more slowly than in the rat, so that 10 days after administration most of it will still be in tissues with a high rate of turnover.

Possible regulatory factors

Liver enzymes. In the past few years two groups of workers have made observations on enzyme changes in the liver, which are of great significance in the present context. Schimke (37) showed that in rats on a low protein diet the activities of most of the urea-cycle enzymes in the liver were reduced. On the other hand these enzymes were more active in rats which were starved, and therefore catabolizing protein to cover their energy needs. Spadoni and her co-workers in Italy (38) found that the activity of amino acid activating enzymes in the liver was increased in rats deprived of protein. Muscle behaved differently from liver, in that amino acid activation was not affected by the dietary protein intake.

The effect of these alterations in enzyme activity must presumably be that a free amino acid molecule entering the liver pool, whatever its origin, has a larger chance of being incorporated in protein, and a smaller chance of being degraded to urea. The end result would be exactly that which has been observed and described above — increased economy or reutilization of amino acids in the liver. Since urea is formed only in the liver, partial suppression of this pathway means that not only the liver, but the body as a whole, benefits from this more economical use of amino acids.

These experimental observations are so important that it seemed to us desirable to try to find out whether similar changes occur in man. To make these measurements on liver biopsy samples of only 5 mg is not easy, and undoubtedly the small scale of the work increases the variability of the results,

which in any case in human subjets must be larger than in rats on standardized diets. Some preliminary results are shown in table 9. They suggest that changes of the same order occur in malnourished infants as in rats on a low protein diet.

TABLE 9

ACTIVITY OF AMINO-ACID ACTIVATING ENZYMES AND ARGININO-SUCCINASE IN LIVERS OF MALNOURISHED AND RECOVERED INFANTS

	BIOPSY		
	Initial	Intermediate	Final
Amino-acid activating enzymes (μMP/mg protein/M)	1.59 (9)	1.32 (7)	1.06 (8)
Argininosuccinase (μM urea/mg protein/M)	1.07 (4)	1.57 (7)	1.78 (4)

Number of children in brackets.

Hormonal factors

Many of the features of adaptation to protein deficiency could be explained in terms of the action of two hormones — insulin and cortisol, which in some respects have reciprocal actions on protein metabolism. It is well known that in vitro (rat diaphragm) insulin both promotes the entry of amino acids into the muscle cell (39) and increases their uptake into muscle protein (40). These effects are not found with liver slices (41). We have some experimental evidence that in the whole animal insulin deficiency may mimic the effects of protein depletion. Rats made diabetic with alloxan were infused with ^{14}C-lysine; the incorporation in muscle was greatly reduced compared to that in liver, just as in rats on a low protein diet.

Cortisone, on the other hand, appears to promote protein synthesis in the liver, while causing a negative nitrogen balance in the body as a whole, so that nitrogen is gained by the liver at the expense of peripheral tissues such as muscle (3,

42). Growth hormone has been shown to decrease the activity of arginine succinate synthetase, the rate-limiting enzyme in the urea cycle (51). The concentration of growth-hormone in the plasma is increased in kwashiorkor (52).

It seems possible, therefore, that the alterations in the pattern of protein turnover which are found in the protein-depleted animal could be explained by a decrease in the activity of insulin with an increase in that of cortisol and growth hormone. Since corticosteroids in many instances seem to promote adaptive enzyme formation, this in turn could be a factor in producing the enzyme changes described in the previous section.

Evidence is accumulating that in protein malnutrition such changes do in fact occur. The following anormalities in carbohydrate metabolism have been demonstrated in children with protein malnutrition: fasting hypoglycemia; impaired glucose tolerance (43, 44); decreased plasma insulin activity (44); increased liver glycogen (45), and decreased glucose-6-phosphatase activity in the liver (46). Most of these features have been reproduced in pigs reared on low protein diets (47). Heard and Stewart (48) concluded that in the pig the picture was one of hypo-insulinism. The paradoxical combination of low insulin and hypo-rather than hyper-glycemia in the presence of a high carbohydrate intake is perhaps explained by the decrease in liver glucose-6-phosphatase (46).

Several workers (44, 49) have reported low levels of urinary steroid excretion in children with kwashiorkor, and have concluded that there is decreased adrenal activity. However, the urinary excretion gives little indication of the physiological state of the gland. Alleyne and Young (50) have shown that in malnourished children in Jamaica plasma cortisol levels are consistently high. The secretion rate appears to be normal, but the half-life in the plasma is prolonged. These results suggest impairment in the normal metabolism of the adrenal steroids. There was also, however, a negative correlation between plasma cortisol level and fasting blood sugar; it may well be that hypoglycemia inhibits the normal feed-back control by which high cortisol levels in plasma should cause a reduction in secretion rate. Whatever the mechanism, it seems reasonable to conclude from these results that the tissues are exposed to increased concentrations of glucocorticoids.

Practical implications

The results described in this paper suggest that adaptation to a low protein intake involves an alteration in the pattern of protein turnover in the body. So far we see only the outlines of this process; an immense amount of detail remains to be filled in. It is probable that the regulatory mechanisms are quite complex; both adaptive enzyme changes and alterations in hormone balance may play a part. How rapidly these changes are brought about we do not know, but it does not seem reasonable to expect them to be instantaneous.

If this picture is correct, then the net losses or gains of nitrogen which occur when the protein intake is altered represent merely a by-product of the lag in adaptation. This is quite a different concept from that of protein 'reserves', which are dissipated or built up when the intake is changed. The word 'reserve' automatically tends to imply something beneficial, so that high reserves are good, low reserves bad. According to our concept, there is no reason *a priori* why one pattern should be better or worse than another, provided that over-all equilibrium is maintained. An analogy might be that in a person going from sea-level to an altitude of 10,000 ft. there is a net loss of bicarbonate from the body to compensate for the low alveolar pCO_2. Provided that pH is maintained within physiological limits, there is no reason to suppose that the new equilibrium state is necessarily disadvantageous.

It would be difficult to explain on the conventional theory the results we have obtained in recent studies on adaptation in infants (2). It was found first, that adaptation occurs much more rapidly in the infant than in the adult. This is understandable if it depends on metabolic reactions which in general tend to be more rapid in younger subjects. Secondly, the net loss of N per kg body wt. was only about 25% of that which occurs in adults. It would seem to us surprising that a well-nourished child should have only one quarter of the 'protein reserves' of an adult. Thirdly, the loss of N was of the same magnitude in malnourished as in well-nourished children. This loss is supposed to represent labile or reserve protein, but it is a distortion of language to apply the term 'reserve' to a quantity which is not altered by the state of nutrition.

Our argument therefore leads to the deduction that the concept of labile or reserve protein is an illusion. The WHO/

FAO Expert Group on Protein Requirements were therefore quite justified in basing their estimates of requirements on the intake needed to maintain N balance, and in rejecting the view that there is a higher 'optimum' intake needed to maintain well-filled protein stores (4).

RESUMEN

Se describen experimentos diseñados para dilucidar el mecanismo por el cual los animales y el hombre se adaptan a una ingesta baja de proteína.

Estudios por infusión constante de aminoácido marcado han demostrado que en la rata depauperada de proteína existe una pequeña reducción en el recambio de proteína total: sin embargo, esto no es suficiente para explicar la gran reducción en la excreción de nitrógeno urinario.

Experimentos de infusión constante o de inyección única indican que en ratas en una dieta baja en proteína existe un cambio en el patrón de recambio proteico: la síntesis de la proteína del carcás (músculo y piel) se encuentra reducida, mientras que la proteína del hígado se mantiene.

La preservación de la síntesis en el hígado parece depender parcialmente del aumento de la reutilización de los aminoácidos liberados por el catabolismo de la proteína del tejido. Esta economía podría provenir de cambios de adaptación enzimática —disminución de la actividad de las enzimas del ciclo ureico y aumento de la actividad de las enzimas activadoras del aminoácido en el hígado. Estos cambios, previamente descritos por otros investigadores en la rata, han sido demostrados también en el hígado humano.

Estudios en niños con metionina marcada con selenio 75 confirman, hasta cierto punto, el concepto de que cuando la ingesta proteica es limitada, el recambio en el hígado se mantiene con preferencia. Sin embargo, no todas las proteínas producidas en el hígado actúan en la misma forma; estudios de la dinámica de la albúmina en niños muestran que cuando se altera la ingesta proteica existe un cambio rápido en la velocidad de la sêntesis de la albúmina, acompañada de una redistribución de albúmina entre los espacios intra y extravasculares. Subsecuentemente y con mayor lentitud ocurre un cambio posiblemente compensatorio en la velocidad del catabolismo de la albúmina.

Los cambios hormonales pueden jugar cierto papel en estos ajustes. El aumento de la cortisona y la disminución de la actividad de la insulina podrían tener el efecto de promover la toma de aminoácido por el hígado a costa del músculo.

Puede concluirse que la pérdida neta de nitrógeno que se verifica cuando se reduce la ingesta proteica resulta simplemente del retraso de tiempo con que el mecanismo de adaptación entra en juego y, en consecuencia, no podría lógicamente considerarse coom la pérdida de reserva proteica. Se discuten las implicaciones prácticas de este concepto.

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