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REQUIREMENTS OF MAN FOR PROTEIN

LONDON

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REPORTS ON PUBLIC HEALTH AND MEDICAL SUBJECTS

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PREFACE

In 1963 the World Health, and Food and Agriculture Organisations called together a group of experts on the various aspects of protein metabolism, in order that they might review the question of human requirements of protein, and revise the earlier report, F.A.O. Nutritional Studies No. 16 Protein Requirements.

Before this meeting, the two organisations asked for the views of experts in this country. A Working Party was set up under the chairmanship of Professor F. G. Young, F.R.S. to prepare a report for the Advisory Committee on Nutrition and Food Policy for ultimate submission to the World Health and Food and Agriculture Organisations of the United Nations.

The Advisory Committee approved and transmitted the report, but it was felt that the views and information it contained would be of value to many in this country, and should therefore be published.

The Working Party did not attempt to make precise estimates, because it was very conscious that there was a gap in knowledge bounded on the one hand by what can be legitimately deduced from, for example, balance experiments which aim in effect to determine the least amount of protein that can be consumed without actual harm, and on the other hand by conclusions drawn from for example, dietary surveys of communities considered to be free from malnutrition. Somewhere between the points arrived at by these two approaches lie the true requirements of proteins, with some margin for variation between different sections of the community, between individuals and for the same individual in different circumstances.

In Part II of the Working Party's Report, on Protein Metabolism and its Regulation, an indication is given of the ways in which variation can come about. Part III deals extensively with protein values, both in relation to the composition of the protein and of the energy content of the diet. In Part IV the human factors that are relevant are considered at some length, and Part V touches upon the effects of disease and injury upon requirements.

In years to come, improved techniques, both in the laboratory and in the study of populations at large, will narrow the gap in our knowledge. The Working

Party's Report will serve as a record of the limits to our knowledge as they are today.

I am glad to be able to pay tribute to the members of the Working Party—and especially to Professor Young, their Chairman. They voluntarily undertook a task of great complexity and have made a most valuable contribution to a major problem of nutrition.

G. E. GODBER,

Chief Medical Officer.

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REQUIREMENTS OF MAN FOR PROTEIN

Introduction

Terms of reference

In response to a request of the World Health Organisation and the Food and Agriculture Organisation for the concerted views of the experts of the United Kingdom on human requirements for protein, the Chief Medical Officer set up a Working Party with terms of reference—

'To consider the needs of man for protein in health, in disease and in trauma with particular but not exclusive reference to the dietary background in the United Kingdom, and in relation to the information which has accumulated since the report of the F.A.O. Committee on Protein Requirements 1957.'

The membership of the Working Party was as listed below:

Professor F. G. Young. Chairman	Professor of Biochemistry, The University of Cambridge.
Dr. W. T. C. Berry. Administrative Secretary	Senior Medical Officer, Nutrition, Ministry of Health.
Dr. Elsie M. Widdowson. Scientific Secretary	Assistant Director, Medical Research Council. Department of Experimental Medicine, Cambridge.
Dr. W. R. Aykroyd	Senior Lecturer, Department of Human Nutrition, London School of Hygiene and Tropical Medicine.
Dr. A. E. Bender	Head, Research Department, Farley's Infant Food, Colnbrook.
Dr. D. P. Cuthbertson	Director, The Rowett Research Institute, Bucksburn, Aberdeen.
Professor R. C. Garry	Professor of Physiology, The University, Glasgow.
Miss D. F. Hollingsworth	Head, Nutrition Branch, Food Science and Plant Health Division, Ministry of Agriculture, Fisheries and Food.
Dr. S. K. Kon	Deputy Director and Head Nutrition Department, National Institute for Research in Dairying, Shinfield, Reading.
Professor R. A. Morton	Professor of Biochemistry, The University, Liverpool.

Dr. H. N. Munro	Reader, Department of Biochemistry, The University, Glasgow.
Dr. R. Passmore	Reader in Clinical and Industrial Physiology, University of Edinburgh.
Professor B. S. Platt	Director, Human Nutrition Research Unit, National Institute for Medical Research.
Dr. A. M. Thomson	Senior Lecturer, Midwifery and Gynaecology Section, The University, Aberdeen.
Professor R. E. Tunbridge	Professor of Medicine and Clinical Medicine, The University, Leeds.
Professor J. C. Waterlow	Director, Medical Research Council, Tropical Metabolism Research Unit, The University of the West Indies, Jamaica.

In addition there were co-opted for advice on special aspects:

Dr. J. P. Bull	Director, Medical Research Council, Industrial Injuries and Burns Research Unit, Birmingham.
Dr. T. P. Eddy	Lecturer, Department of Human Nutrition, London School of Hygiene and Tropical Medicine.
Dr. S. M. Partridge	Senior Principal Scientific Officer, Department of Scientific and Industrial Research, Low Temperature Research Station, Cambridge.
Professor A. W. Wilkinson	Professor, Institute of Child Health, Hospital for Sick Children, London.

Plan of Study

The Working Party set up four Groups, each to consider different aspects of the problem of protein requirements:

Protein metabolism and its regulation.

Protein values of foods.

Requirements for protein in health.

Requirements in other circumstances.

Form and Content of Report

Part I of this report is compiled by the Working Party as a whole, and is a review of main issues raised by the groups in the course of their deliberations, and the Working Party's views on them. Parts II, III, IV and V are the four Group reports. Though these were the work of the members of each group, the Working Party as a whole is in general agreement with the views set out in them.

In what follows we have restricted ourselves primarily but not exclusively to a consideration of the results of investigations in the United Kingdom and in territories in close association with the United Kingdom. Only in this way could our deliberations be kept within bounds.

Throughout the text references have been made to, for example, "the earlier F.A.O. Report." This is Nutritional Study No. 16, Protein Requirements (F.A.O. 1957).

May, 1963.

PART I.

REVIEW OF REPORT OF WORKING PARTY

Quantitative Requirement for Protein in Health and Disease

We believe that our duty is to ascertain *physiological requirements*, which may be defined as those which are sufficient to cover all the varied needs of healthy individuals and which enable them to meet both the common and the rare stresses of daily life.

We decided to adopt the following approach to the problem of assessing physiological requirements: to attempt to fix a lower limit of requirement, which would be based as far as possible upon the results of laboratory investigations, and a higher limit based on observation of consumption of protein in situations where there was no reason to suppose a deficiency existed. Physiological requirements must lie somewhere between these two limits and attempts can be made to narrow the gap. In all considerations it is assumed that the calorie intake is neither deficient nor excessive.

Values for requirements have been expressed in terms of grams of protein per kilogram body weight per day. This is a rather crude method of expression but it is a practical one and was the one followed in the F.A.O. Report. It may be that requirements should ideally be related to lean body mass, but lean body mass cannot easily be determined. The values should be taken as relating to persons who are neither obese nor unusually lean. We have taken our protein to be the mixed protein of the British diet. The requirement of protein is of necessity initially worked out in terms of a protein of stated biological value, usually an ideal reference protein with a biological value of 100. Requirements may then be converted into minimal intakes of protein in practical diets by using the known biological values of such dietary protein to make the conversion. This is a procedure quite frequently adopted. We have taken the conversion factor for the British diet to be 80 but have borne in mind that no such average conversion factor can ever be precise. Biological value as defined in the Glossary to Part III of this report relates to the use made of the absorbed amino acids and does not allow for losses in digestion, which should in fact be taken into account. The essential amino acids in the proteins of mixed diets of adults are likely to be in excess of their requirements and in consequence a deficit, if any, will be in total protein.

(a) *The lower limit*

The term *minimum requirement* is used in this report to indicate the irreducible minimum.

Minimum requirements of adults. These have generally been assessed from the results of balance studies with a low protein intake. The assessment is beset with difficulties, among which are the following:—

(a) Balance experiments have been made on comparatively few individuals and there is clearly an important variation in protein requirement from

one individual to another. The F.A.O. Committee's figure of 0·35g. reference protein per kg. body weight per day for average minimum requirement of adults was based upon the results of balance studies, particularly those reviewed by Sherman (1920). Sherman himself stated how he thought the protein requirement for maintenance should be determined and concluded that "The recorded investigations which met all these conditions are not sufficiently numerous, nor do they cover a sufficient number of men or women, or a sufficient range of foods to form a satisfactory basis for any general deduction as to the amount of protein required in normal adult maintenance". These balance measurements were made on a mixed diet at low levels of protein intake and generally gave a minimum requirement of protein for equilibrium of the order of 0·5g. per kg. body weight per day. The F.A.O. Committee arrived at the figure of 0·35g. per kg. body weight per day of reference protein by applying a correction factor for biological value to Sherman's value and to the value obtained by other workers of 0·4g. per kg. body weight per day.

(b) The nitrogen excretion on a low protein diet is dependent on the previous nutritional history of the person. If the protein intake is reduced from a higher to a lower level, breakdown of body protein predominates over synthesis, "the labile body proteins" are depleted, but the person may eventually come into nitrogen equilibrium again. This does not necessarily mean that the lower intake of protein is sufficient for the person's needs, for his body now contains less protein than it did before. It is possible that the more labile components of tissue protein which he has lost are part of the functional cell cytoplasm and are essential for complete well-being.

Another method that has been used to arrive at the minimum protein requirement is based on the assumption that the minimum urinary nitrogen excretion is proportional to the basal metabolic rate, 2 mg. nitrogen being excreted per basal *kilo* calorie. On this basis the contribution of calories from protein to the basal metabolism would be of the order of 5 per cent. To this must be added an allowance for faecal loss. The same considerations as have been set out in the previous paragraph apply here. This method gives a figure close to one based on the data of the preceeding paragraph, but we have in fact not used it for adults.

Values for minimum requirements assessed in one or other of the two ways described above do not usually include any allowance for cutaneous loss of nitrogen. This is influenced by the amount of sweating, but even under conditions of minimal sweating about 500 mg. N a day may be lost which is equivalent to about 0·05g. protein per kg. body weight per day.

Values for minimum requirements for groups or for populations must be big enough to provide for normal individuals with high requirements. We think that, in order to make allowance for this, the mean minimum requirement should be raised by something rather more than twice the coefficient of variation that has been observed experimentally, that is to say by about 50 per cent.

If these upward adjustments are made to the figure of 0·35g. reference protein per kg. body weight per day the adult requirement becomes 0·6g. reference protein per kg. body weight per day. For a protein of biological value 80, which

corresponds to the value of the average British diet, the lower limit becomes 0·75g. per kg. body weight per day.*

Minimum requirements during growth

Here we must consider not only the amount of dietary protein needed for the maintenance of existing tissues but also that needed to provide for the tissue added during growth. The protein intake of a breast-fed infant is 2–2.5g. per kg. body weight per day; and this is accepted as being near to its physiological requirement. In the case of older children the only way of estimating the requirement for maintenance is to relate it to the basal calorie expenditure, and for growth by calculating the probable increments of protein in the body. From one year of age upwards the addition of protein to the body accounts for only a small fraction of the protein intake of children in the United Kingdom, and from the evidence available it seems likely that in well nourished children after the first year the requirement for maintenance is considerably greater than the requirement for growth. The growth spurt in adolescence involves such a quantitatively small increase in the protein in the body that the "hump" in the curve drawn by the F.A.O. Committee for requirements at adolescence is so small as to be negligible.

For the first year of life for practical purposes we accept a requirement of 2g. of reference protein per kg. body weight per day at 2 months tapering off to 1·5g. of reference protein per kg. body weight per day at 1 year. For older children it seems reasonable to diminish from 1·5g. of mixed protein per kg. body weight per day (= 1·2g. per kg. body weight per day of reference protein) at 5 years to 0·9g. per kg. body weight per day of mixed protein at 17 years.

Pregnancy

The latest estimates of additional protein laid down by the average normal pregnant woman represent a daily addition of 0·5, 3·0, 4·5 and 5·7g. protein during the successive quarters of pregnancy. The efficiency with which protein in the diet is utilized in forming new tissue protein during pregnancy is unknown, but if the figure of 50 per cent adopted on page 42 below is accepted, then the requirement for additional protein during the second half of pregnancy would be about 12·5g. mixed protein of the British diet per day.

Lactation

If following the F.A.O. Report the average protein in milk is taken to be about 10g. daily, and the efficiency is assumed to be 50 per cent then the average additional requirement would be 25g. daily of good mixed protein.

Senescence. Muscle mass decreases in old age, but it is not known whether this is physiological or pathological. In view of the paucity of knowledge we did not feel able to recommend special requirements for protein in senescence. The physiological requirement may be taken as the same as for the younger adult.

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*Objection may be raised to the introduction of this correction since it is possible that within this range of biological value the limiting factor for maintenance in adults is the total nitrogen and not the amount of pattern of the essential amino acids. If that is so the correction would not be valid. However, the original figures of Sherman obtained on a mixed diet were reduced by the FAO Committee to express requirements in terms of reference protein. The two corrections almost cancel one another and the validity of either does not affect the end result.

Serious zymotic disease and injury. The demand for extra protein as a result of disease and injury is threefold,

(a) for the regeneration, as far as is possible, of tissue lost, and for the healing of wounds;

(b) for the replacement of protein in body fluids lost either immediately or later;

(c) to make good protein lost as a result of the catabolic response to infection or injury.

The last is the most important.

Serious injury can impose a need to replace one kilogram or more of protein. A daily intake of 3g. or more of protein per kg. body weight is suitable for burned children. For adults an intake of 2 or 3g. per kg. body weight is appropriate.

The evidence for a possible relationship between protein intake and resistence to infection is contradictory and we have made no provision for an increase in protein requirement in this connexion. Infection can be properly regarded as one of the stresses of daily life.

(b) *The upper limit*

The calculations in the ensuing section are based on experience in the United Kingdom. Were similar evidence from other countries considered it is probable that the upper limit as defined on page 4 could be lowered.

Intakes during adult life. The average daily intake of protein by adults in the United Kingdom, excluding the elderly and the pregnant, is of the order of 90g. daily for men and 70g. for women, or 1·4 and 1·3g. per kg. body weight respectively. In that we know of no case of primary protein deficiency in either young or middle-aged adults it is probable that these mean figures are well above the average physiological requirement but it is impossible to state by how much.

Intake during growth. The average daily protein intake of children in the United Kingdom below 5 years of age is about 3g. per kg. body weight and by the age of 12 years it has fallen to 2g. per kg. body weight (Table I.). Though these values are high we are not in a position to say that they are unnecessarily high because there is in the United Kingdom a rough relationship between protein intake and growth rate when the averages are compared of children in different socioeconomic or other circumstances. This question is considered in more detail in Part IV of this report. There is of course no proof that the relationship is causal.

Intakes in Pregnancy. The average protein intake of pregnant women in the United Kingdom is 70–90g. per day. There is no indication that any significant proportion of pregnant women suffer from deficiency of protein, even after repeated pregnancies, and it may be assumed that an intake of 70–90g. is adequate.

Intakes in Senescence. The average intake of protein by the elderly is about 70g. per day for men and 60g. per day for women. Since there are no adequate

criteria by which the optimum state of nutrition in the elderly can be judged, these average figures cannot necessarily be used to indicate the higher limit referred to on page 4.

Miscellaneous factors which may influence requirements for protein

Other dietary constituents. Unavailable carbohydrate or other indigestible material reduces the apparent absorption of protein by causing an increase in the amount of intestinal secretions, in the weight of the stools and in the rate of passage of material through the gut. The amount of nitrogen lost in the faeces depends much more upon the amount of unavailable carbohydrate in the diet than upon the amount of protein. There is not enough unavailable carbohydrate in the average British diet to have any important effect on the absorption of protein.

Muscular activity. It is not generally accepted that urinary excretion of nitrogen is raised by muscular exercise *per se*, but the increase in muscular mass associated with athletic training and the maintenance of the increased musculature, will involve a slight increase in the physiological requirement for protein.

Climate. The dermal loss of nitrogen rises when there is much sweating, and this may be of practical importance in a population with a low protein intake in a hot climate.

Protein Metabolism and its Regulation

Before we discuss what amendment may be necessary to the figures set out above in order to allow for the different nutritive values of different dietary proteins, we wish to consider some relevant aspects of protein metabolism.

In Part II of this report we indicate that the nutritive value to man of dietary protein is influenced by many variables other than the nature and the amount of the protein in his food. The nutritive value is necessarily limited by the nature and the quantity of the amino acids available from the protein and it depends upon the completeness of digestion of the protein, and the effectiveness of absorption of the mixture of amino acids into the blood after a protein-containing meal. The completeness of digestion may be reduced by certain dietary factors, while the over-all absorption of amino acids is diminished by a high proportion of non-utilizable carbohydrate in food.

The needs of man may vary with age, not only in respect of the total dietary protein, but also with regard to the proportions of the essential amino acids relative to one another, and to the proportion of the total nitrogen in the protein contributed by the essential amino acids. Much more information is needed about the amino-acid composition of the proteins consumed by man, and the availability of these amino acids under many different conditions, before final conclusions can be drawn about the nature of these requirements in terms of protein consumed. Since the amino acids from one protein may supplement those from another simultaneously present in the food, the mixture of amino acids yielded by a meal as a whole, as opposed to that coming from the separate proteins it contains, is of importance. Since utilizable carbohydrate exerts a protein sparing action, and the total calorie intake must be adequate if avoidable protein catabolism is to be prevented, the task of determining in practice what

is the protein requirement of a group of people can be effectively undertaken only in relation to the diet as a whole.

Any assessment of the amount of protein required for well-being must depend on what functions, if any, are considered to be served by labile tissue protein, for it is difficult to state any protein intake of which it can be said that a rise will not temporarily cause retention of nitrogen in the body. The evidence about this problem is briefly reviewed in appendix 3 of this report. In the absence of firm evidence for and against, it seems desirable to regard labile body proteins as serving a useful function; but no decision is possible about the amount of dietary protein which is needed to ensure an adequate store of labile body protein.

The Value of Dietary Protein

We intend the term "Protein Value" to relate both to the quantity and quality of the protein in the food consumed by the human being under defined conditions.

The judgement of protein quality must ultimately be based on biological tests, preferably on Man. With a mixed diet the question is complicated by the mutual interaction of the proteins in the food, so that it is not possible to predict the biological value of a mixture from the known values of the constituent proteins. As a consequence each diet or mixture of foodstuffs has to be judged separately. This is a severe practical limitation on the use of biological tests for measuring protein quickly.

In principle the amino acid composition of a food in so far as it is known, should provide a measure of its protein quality. A score based on amino acid composition of the important foodstuffs provides a simple and widely used means of assessing the quality of a diet. The use of such a measure requires a standard of comparison. The concept of the reference protein developed by the F.A.O. Committee is an attempt to meet the need. The pattern was based on the knowledge available at that time of the requirements of human beings for nutritionally essential amino acids. Later evidence suggests that the problem of defining a pattern of amino acid requirements is extremely complicated, since the pattern may vary at different ages. Moreover, it cannot be taken for granted that the pattern of the unessential amino acids, to which the F.A.O. committee gave no attention, is unimportant.

An internationally accepted reference protein would undoubtedly be of great practical value. We have however at present no suggestions to make about amendments to the existing provisional pattern. In general, the amino acids most likely to be limiting are lysine, the sulphur-containing amino acids, and tryptophan. One or the other of these is limiting in most of the examples cited in Part III of this report. It may be enough to base a system of evaluation on these amino acids alone.

Chemical evaluation cannot in all circumstances be relied on for an accurate assessment of the value of protein for man since the amino-acids are not necessarily equally available to him. The depressive effect on the availability of amino-acids of heating food during normal preparation is slight, the main effect being on lysine and on the sulphur containing amino-acids. The loss can, however, become significant with excessive heating and also on prolonged storage

in the absence of refrigeration. When food has been treated in this way the chemical score with respect to lysine may become misleading with regard to the availability of this amino-acid to man.

Obviously however good the quality, the requirement will not be met if the quantity is insufficient. Therefore, since intake is dictated by calorie requirement the proportion of protein in the diet is important. Both aspects, quality and quantity, are combined in terms of utilizable protein calories per 100 total calories from the food. However, it remains to be seen whether this is preferable to expressing protein in terms of grams per day. Since it is not accepted that physical exertion *per se* leads to an appreciable increase in nitrogen loss, provided that the energy intake remains sufficient, some reservation must be extended towards any proposal that protein requirement should be related to the total calorie requirement.

Discussion

(1) *Requirements*

We have attempted to delineate as closely as possible a gap in our knowledge concerning protein requirements. This gap is bounded at the lower limit by what can be derived (albeit with much uncertainty) from the results of balance studies and similar information, and at the upper limit by information about the average dietary intake of protein in Britain. Somewhere between these limits which themselves are not precise, lie physiological requirements.

If we were more certain that labile body protein has a function and could assess the amount needed, and the protein intake required to maintain it, then we could raise the lower limit and so narrow the gap. As this is not the case, the only means of narrowing the gap is by lowering the upper limit.

The upper limit is based on the *average* protein intake of groups of people, and since obviously, about half the individuals must consume less than the mean and since primary protein malnutrition is virtually unknown in Britain, it may be inferred that this average is well above true requirement. But the following points need to be considered:—

(a) Protein intake might in some measure be based on instinct. Were this so, those with the lowest intake might be those with the least need. We know of no evidence of this.

(b) Those who eat least protein might be very small people. We have little evidence on the weights of those whose diets are surveyed (and obviously in future it would be useful to have it). But in this country protein intake is found to be related largely to calorie intake and the latter is not closely related to body size.

(c) The lower intakes recorded during the period of survey may be offset by higher intakes at other times. The dietary surveys lasted one week or less.

For reasons such as these we have not departed from the average figures based on protein intake and have therefore not lowered the upper limit to the gap in our knowledge referred to above. It might well be rewarding in the future to study the characteristics of individuals, selected at random, who are found to be eating less protein than the average for their group.

Until the upper limit has been lowered by means such as this or on the basis of evidence about intake in other countries, or until a function for labile protein has been established and quantitatively expressed, it will be difficult to fix more precisely human needs for protein. Our findings are summarized in Table I.

TABLE 1

Requirements in terms of the mixed protein of the British diet

Summary of Working Party's calculations

Infancy	0—6 months	About 2g. per kg. body weight per day (of human milk protein)	
	6 months—1 year	About 2g. per kg. body weight per day (of protein of British diet)	
Children		*Lower Limit of knowledge*	*Upper Limit of knowledge*
	4 years	1·6g. per kg. of body weight per day	2·9g. per kg. of body weight per day
	12 years	1·2g. per kg. of body weight per day	2·0g. per kg. of body weight per day
Adults			
	Male	0·75g. per kg. of body weight per day	1·4g. per kg. of body weight per day
	Female	0·75g. per kg. of body weight per day	1·3g. per kg. of body weight per day
	Pregnancy (2nd half)	Add 12·5g. per day	
	Lactation	Add 25·0g. per day	

(2) *Recommended Allowances*

In setting targets of nutrient supply for countries there are certain hazards in the use of an appropriately weighted average of the requirements of different sections of the community. People eat foods, not nutrients, and the amount of protein consumed by adults is determined to a substantial degree by their calorie requirement. Unless as a result, for example, of education or legislation, foods specially rich in protein become the prerogative of the vulnerable groups, malnutrition must occur in any country where the average consumption is close to average requirement. It may occur also because of maldistribution resulting from social custom, and from economic and other related reasons.

It follows that some basis other than the weighted average of the requirements of different sections of the community is needed for the calculation of target figures for protein. One possibility is to arrive at the total energy requirement of a country, by means of an appropriately weighted average, and to stipulate that the proportion of protein in the diet shall be such that when it is consumed by children in amounts necessary to satisfy their calorie requirements, the quantity of protein the children obtain from it shall be sufficient for their needs.

Even this might not protect children whose energy expenditure was lower than average, but targets so high that even these children were protected might well prove beyond the resources of countries where protein supplies are low. It is unfortunate that in such countries education may not be at the state where food is likely to be distributed within the family in accord with the physiological need for protein. It may well be that the target for any country needs to be determined in the light of local conditions and customs including distribution of food within the family and the community. As a first step the target could be computed in the way we have suggested, in the hope that as that target is approached education and propaganda can bring about sufficient redistribution to make sure that all individuals receive enough for their physiological needs.

PART II.

PROTEIN METABOLISM AND ITS REGULATION

The subject of protein metabolism is a large one, and this report represents the area selected as being relevant to the assessment of protein requirements.

Protein Digestion, Absorption and Utilization

The action of the alimentary tract on dietary protein can be considered under three headings;

(a) *The sequential attack of the digestive enzymes on dietary protein*

Although the impressive array of enzymes which can attack bonds in the protein molecule suggests that few bonds can remain intact after traversing the alimentary tract, some stretches of peptide chain adjacent to sugar residues in mucoproteins have been found to be totally resistant to enzymic attack in vitro and consequently may not be well digested in vivo (Gottschalk and Fazekas de St. Groth, 1960; Rosevear and Smith, 1961; Johansen, Marshall and Neuberger, 1961). With normal mixed diets, such proteins are not likely to contribute more than a minor proportion of the total dietary protein and in consequence resistant peptides are unlikely to be nutritionally significant. Enzyme attack in the alimentary tract may also be inefficient because of inhibitors present in the diet, notably the soya bean trypsin inhibitor (Bondi and Birk, 1962). Other legumes also contain inhibitors. Some processes of heat-treatment of soya bean may not be adequate to inactivate this inhibitor, and the possible adverse effects of this should be borne in mind when recommending the use of soya bean preparations in diets. Finally, in a more general way, the factors determining digestibility of proteins require further exploration. In particular, poor digestibility seems to be a characteristic of proteins in many vegetables: legumes, cereals and even partially purified proteins such as leaf protein. In the crude material, it seems doubtful whether this can be attributed solely to the presence of fibre or the physical state of the nutrients. Exploration of this area may well be worth while promoting, since in areas where the intake of protein is marginal, the losses due to incomplete digestion may well be critical, particularly in view of the finding that in some countries (India, Puerto Rico) a large proportion of the population have an abnormal gut mucosa by Western standards (Baker, 1963). Since amino acids are not necessarily fully available, their availability should be assessed whenever possible by suitable biological, microbiological or chemical methods, as discussed in more detail in Part III of this report.

(b) *Endogenous secretion of protein into the alimentary tract*

The amount of protein secreted in the form of enzymes, mucin and as leakage of plasma proteins into the alimentary tract may amount in total to an appreciable amount of nitrogen per day. This raises the question of whether the amino acid pattern absorbed from the gut may not sometimes be an improvement on that presented by the dietary proteins, because of the contribution of the amino acids from endogenous sources (Nasset, 1956; Gitler, 1964).

(c) *The nature of the absorbed product and the mechanism of absorption*

The evidence is now heavily in favour of free amino acids as the ultimate products of digestion which are absorbed into the body. The possibility of minor quantities of peptides and even whole protein molecules being absorbed into the blood seems equally certain, but from a nutritional point of view these are not quantitatively important. The mechanism by which absorption of free amino acids takes place does not seem at the present time to have an important bearing on utilization of dietary protein. The same comment can be made on the fate of the amino acids once they are absorbed. As is well known, they can pursue anabolic or catabolic pathways. Within the area of protein biosynthesis, much exciting work is proceeding at the moment, but it does not appear to have immediate application to the study of the nutritional requirement for protein. However, as studies of protein metabolism progress, there must come a time when the overall picture of these metabolic activities provides a rational and detailed basis for assessing the nutritional needs for protein. Consequently, the professional nutritionist must remain alert to advances taking place in the field of protein metabolism.

Specific Dynamic Action of Proteins

The catabolism of amino acids is associated with an increment in the heat output of the body, the specific dynamic action. The S.D.A. after meals is a very variable phenomenon, and in consequence is difficult to study in the laboratory. At most, it amounts to an increment in energy expenditure of some 25 kcal per hour for a few hours of each day. Commonly, it is much less than this, and is always small in persons subsisting on diets which are not rich in protein. It is probably associated with a slight increase in the rate of protein utilization, and consequently in protein requirements, but the amounts involved are so small by comparison with the errors associated with assessing protein requirements as to be of no practical importance. In summary, S.D.A. is a phenomenon of considerable interest to the academic scientist; the public health worker may safely ignore it.

Hormonal Control of Protein Metabolism

While it is generally known that hormonal action on protein metabolism can result in either a net gain or a net loss of body protein, the linking of these effects to protein requirements is still very obscure. However, a number of endocrine glands show changes in their secretory rate with variations in the level of protein in the diet, the main participant being the anterior hypophyseal lobe, and it is possible that this aspect of hormonal function may become important to the nutritionist in the future. Endocrine disturbances have been described both clinically and experimentally in protein malnutrition (Leathem, 1963) and may be relevant to the development and the treatment of the condition.

Composition and Structure of Protein in Relation to Requirements

Several aspects of the structure of proteins have a bearing on their utilization by the body.

(a) *Protein structure and composition in relation to nutritional properties*

In view of the predominant absorption of dietary protein as free amino acids,

protein structure confers no special nutritional properties on individual proteins. This implies that the biological value of a dietary protein is determined by the concentrations of its essential amino acids. Consequently, the amino acid analysis of dietary protein sources represents an important adjunct in practical nutrition. With modern techniques of hydrolysis, this can now be carried out on natural foodstuffs, such as flour, potatoes, and vegetables, without interference from the carbohydrate present in the amino acid determinations (Dustin et al, 1953). The precision of analysis has improved to the point that errors will not normally exceed 3 per cent and the application of the new technical advances to nutritional problems is only limited by the availability of amino acid analysers. It is therefore recommended that this work be pressed ahead with more vigour, and that attention be paid not only to the average amino acid pattern in different foodstuffs, but also to the degree of variation of this pattern in foodstuffs obtained from different sources, such as wheat of several varieties.

(b) *The choice of amino acid or protein standards for assessment of biological value.*

In order to evaluate amino acid patterns of different foodstuffs some agreement must be reached on reference patterns. The current position, with several such reference standards in use, is an impediment to further progress in this field and some decision must soon be made on a definitive standard. This is a legitimate objective for F.A.O. The decision should also assess possible variations in such patterns with age, sex, physiological state, etc.

(c) *Supplementation between proteins.*

Defects in essential amino acid content of one dietary protein may be compensated by excess of the same amino acid in another dietary protein, thus giving supplementation. For this to take place, the two proteins must be consumed in the same meal, since the amino acids have to be presented simultaneously to the tissues for use in protein biosynthesis. However, many vegetable proteins are deficient in all essential amino acids to some degree, though deficiency is usually most pronounced for lysine and methionine. In consequence, supplementation is not likely to achieve dramatic effects unless the vegetable sources are chosen to be supplementary, in which INCAP have achieved success in blending vegetable crops of Central America.

(d) *Influence of other sources of assimilable nitrogen on protein requirements*

Some foods contain appreciable amounts of non-protein nitrogen. Thus much of the nitrogen of tubers like the potato consists of free amino acids and amides, and human milk also contains a significant amount of non-protein nitrogen (Fowden, 1960). In consequence, computation of the protein content of foodstuffs on the basis of total N multiplied by 6·25 may exaggerate the alleged protein present. Such areas of uncertainty will be clarified when detailed amino acid analyses become available for natural foodstuffs. It should also be recollected that not all amino acids present in plants are precursors of proteins; some 80 free amino acids other than the common 20 have been described in plant tissues. Some of these plants are used as human food, and in consequence can give rise in some instances to disease (e.g. lathyrism) and can lead the unwary investigator to suspect a metabolic abnormality when these unusual amino acids are excreted in the urine.

Protein Reserves

The evidence concerning protein reserves is given in more detail in appendix 2. It is generally conceded that the body contains some form or forms of readily available protein which is dissipated in emergencies, and that the concept of a reserve has some merit, provided that the term is used in a purely descriptive sense and does not imply an inert store. Reserve protein has been demonstrated in the liver, where gains and losses are accompanied by structural changes. Other sites which are readily depleted include the gastrointestinal tract, the pancreas and plasma albumin. Again, in the muscles of cattle, considerable variation in the diameter of myofibrils can be demonstrated in animals of differing nutritional status (Hammond and Appleton, 1932; Joubert, 1956; Goldspink 1962). Part of the nitrogen deposited in the body during experimental treatment with growth hormone is deposited in muscle in labile form and is released again rapidly when treatment ceases. However, attempts to define with precision the labile fraction of body protein reveal a spectrum of tissue stabilities, so that what is considered labile protein becomes a matter of definition. Allison (1964) considers that 20 per cent of body protein is dispensable in an emergency and regards this as the magnitude of protein reserves. The common criterion is to administer a diet low in protein and observe how much nitrogen is lost in the urine before the subject attains a more or less steady plateau again; this results in a temporary outpouring of nitrogen which, even if preceded by a very high protein intake, never exceeds 5 per cent of the total body protein and is commonly much less. The tissues which contribute protein to this nitrogen loss are mainly the liver and the gastrointestinal tract, and from these tissues the relative loss of nitrogen is much greater than the general figure of 5 per cent would suggest. The minor nature of the total change in body nitrogen content should therefore not obscure the possibility that individual tissues can undergo considerable depletion in a brief period of time. The basic question is whether the loss of cytoplasm from certain selected tissues is a matter of moment to the general well-being of the subject. This can be considered in relation to the general problem of protein requirements under two headings.

(a) *Assessment of minimum protein requirements*

The protein requirement of a species can be arrived at from the minimum output of N attained when a protein-free diet has been fed for a period, since replacement of this "endogenous" loss of body N is an obvious minimal need for any diet. In the human subject, allowing for faecal as well as urinary losses, this amounts to some 4·9g.N per day. If an amino acid source of high biological value, such as whole egg protein, is provided, the subject will come into N equilibrium on an intake as little as 4g. N per day. In practice, this has been achieved for human subjects receiving either whole protein or amino acid mixtures in its place. In such investigations, equilibrium was achieved at the low N intake by preliminary reduction of protein level in the diet prior to feeding the protein or amino acid mixture; consequently, these are the nitrogen needs of the body after depletion of its more labile protein components.

On the other hand, when one tries to legislate for some deposition of labile tissue components, there seems to be no level of protein intake at which it can be said that a further rise will not cause still greater retention of N in the body. Thus the mature rat has an endogenous urinary N output of some 23 mg. per

100g. body weight per day, and should thus attain N equilibrium on an intake of not more than 50 mg. N per day from a protein source of mediocre quality. When the intake of rats has been varied from 50 mg. N to 800 mg. N per 100g. body weight per day, deposition of protein in the liver continues to increase with each increment in nitrogen intake. It is thus a matter of opinion what constitutes an adequate level of protein intake to effect deposition of labile protein in the liver. It may be noted that the diets commonly consumed in different parts of the world provide considerably more protein than the computed minimum for equilibrium. If we accept 0·65g. per kg. body weight as adequate for equilibrium on a mixed diet (Hegsted, 1959), this represents 45g. of protein for a man of 70 kg., which provides 180 kcal. of his intake of 3200 kcal., or some 6 per cent of the total caloric value of the diet. The diet in many parts of the world provides some 10–14 per cent of its energy as protein. Consequently, the customary intake of protein allows for some deposition of labile protein.

(b) *Functional significance of labile body protein*

There are two aspects to this. First, does the functional capacity of an organ change when it is depleted of labile protein, and second, do the labile body proteins confer an advantage by acting as a source of amino acids under adverse conditions?

The liver of the protein-depleted animal has been shown to be less resistant to chloroform intoxication, to have less capacity to conjugate oestrogens, and to have a diminished clearance for bromsulphthalein. Furthermore, the capacity of the body to retain riboflavin and nicotinic acid is diminished, indicating that the loss of labile protein affects the capacity of the cell to retain other nutrients. These various observations suggest that the functional capacity of the liver cell and possibly of other cells is demonstrably changed by loss of labile cytoplasmic constituents. They do not, however, reveal whether such changes in functional activity are critical to the long-term health of the body as a whole.

The capacity of labile protein stores to confer benefit on the body by acting as a source of amino acids is disputed. The labile body protein is readily lost when the intake of protein is reduced, or when the individual receives an injury. Some experimental evidence suggests that a period of low protein feeding is better tolerated by animals previously given a high intake of protein, but equally negative experiments can be cited. There is at present no information to show whether the catabolic response to injury, which is most extensive in individuals previously on a high protein intake, is beneficial to recovery or not.

In face of these areas of indecision, it would seem desirable to consider that labile body proteins are important aspects of the metabolism of body protein which merit further exploration in their own right and that insufficient is known to make a decision on their nutritional significance. Since, however, the maintenance of abundant labile body protein involves consumption of a diet of extravagantly high protein content, exploration should be made of possible means of assessing the effect of dietary protein level on the function of different tissues, in relation to the amount of labile protein they contain. For example, at what level does the capacity of the liver cell to fabricate plasma proteins become impaired? In the last analysis, decisions on protein requirements will hinge on the question of the functional significance of labile body protein.

Individual and Genetic Variations

Genetic variation in requirements for individual amino acids merits exploration in deciding the amino acid needs of man. Such variation can arise from differences in rates of destruction of the essential amino acids by individual persons. This emerges more strongly when it is recollected that there is a surprising range of requirements revealed among different individuals for calcium, and that adaptation to low calcium intakes has been claimed for some races. A complete statement of the protein requirements of man demands an index of the range of variation which may be anticipated, so that safe upper limits may be set to include those persons whose needs are higher than the average.

Influence of Non-Nitrogenous Additions on Requirements for Protein

The main group of nutrients influencing protein utilization are the other energy sources, carbohydrate and fat. As energy sources they spare the utilization of dietary protein for energy-yielding purposes, but they also have some specific actions on body protein metabolism apart from their energy-yielding properties. Each aspect will be considered separately. A detailed discussion of the evidence is provided in appendix 2, p. 54.

(a) *Caloric intake and protein requirements*

The evidence of many investigations demonstrates that the N balance of the adult subsisting on an adequate protein intake is sensitive to changes in caloric intake. Increasing the energy intake beyond requirements leads to N retention, whereas reducing the energy value of the diet leads to N loss from the body. The influence of energy intake on protein metabolism may not be a direct one on utilization of dietary protein, for the response of N balance to the addition of a standard amount of protein is the same over a range of caloric intakes. Only when the energy content of the diet falls below a certain level does the response become impaired. Whatever the nature of the underlying biochemical mechanisms, it is true to say that both protein intake and energy intake can influence N balance and that severe restriction of energy intake can limit the utilization of dietary protein supplements, while conversely the effect of energy supplements may not be beneficial because of the low protein content of the diet.

This picture has practical implications—(i) an inadequate energy intake will of itself cause a loss of body protein, (ii) addition of protein to the diet of undernourished populations may not be fully effective if the energy intake is low, (iii) convalescence after injury and repletion of protein-depleted subjects is best assured by a high caloric intake, as well as by adequate dietary protein.

(b) *Interdependent actions of dietary carbohydrate and fat*

Dietary carbohydrate has an action distinct from its effect on protein metabolism as an energy source. Thus in the fasting subject, carbohydrate administration, but not the feeding of fat, will diminish output of nitrogen; exchange of fat for part or all of the carbohydrate of the diet on an isodynamic basis results in a temporary increment in urinary N

output, and this also occurs when the dietary carbohydrate is eaten at meals apart from the dietary protein. All these pieces of evidence suggest a specific action of carbohydrate on protein metabolism, involving the utilization of the incoming amino acids as well as the "endogenous" protein metabolism of the fasting subject. There is evidence that the basic mechanism may involve secretion of insulin and deposition of free amino acids in muscle, and that this may deprive other tissues temporarily of their full supply of amino acids. In normally nourished persons, this temporary reduction in plasma amino acid level may not be important, but in individuals whose intake of protein is marginal it may aggravate the poor supply of amino acids to key tissues such as the liver. The diets productive of protein malnutrition in infants are often notable for their high carbohydrate content as well as their low protein level. The excessive intake of carbohydrate may possibly be a factor in depriving the liver and other viscera of the limited supply of amino acids in circulation.

With regard to fat in the diet, work in the past has led to no very compelling evidence of a specific action, but some recent work on animals deficient in essential fatty acids suggests that this deficiency may lead to inefficient utilization of protein, possibly by interfering with energy metabolism.

Further research

Investigations needed are:—

(i) The efficiency of digestion of poor quality proteins.

(ii) The determination of the amino acid composition of food proteins.

(iii) Better definition of the reference pattern of amino acids and exploration of variations in this with change in physiological state.

(iv) Determination of the extent of the range of amino acid and protein requirements by individuals.

(v) Further study of the biological significance of protein reserves.

PART III

PROTEIN VALUES OF FOODS

It was recognized in the F.A.O. report on Protein Requirements that the nutritive value of the protein in food depends on the quantity of protein and its quality; the energy content of the food and therefore on the relative quantities of carbohydrate, lipids and alcohol; the caloric intake in relation to the consumer's needs; the nature and proportions in a diet of many essential accessory food factors; and the dietary regimen, e.g. the timing of meals and the distribution of protein between various meals during the day.

Measurement of Quantity of Protein

"Crude protein" [G]* is obtained from total nitrogen (N) by multiplication with the factor 6·25, the nitrogen usually being determined by the method of Kjeldahl. It is recognized that such values include, as well as the nitrogen of protein and amino acids, the amounts present in some of the vitamins and in non-nutrient substances such as purines and other nitrogenous substances which are contained in food.

The factor 6·25 approximates to the average nitrogen content of a variety of proteins; for example the factor 6·38 is used for milk protein and 5·7 for cereal proteins. These factors were determined on separated proteins but are sometimes applied to the whole foods from which they were obtained but which contain other sources of nitrogen; the use of any general factor for whole foods is likely to give results which are only approximately correct. Whether the measurement is of crude or total protein or nitrogen, it takes no account of the quality of the protein.

In measurements of the nutritive value of proteins, i.e. of protein values, errors due to the misuse of factors are minimized since it is usual to measure intake and excretion of nitrogen and to determine the percentage of ingested nitrogen that is retained in the body, or the weight increase associated with the retained nitrogen.

Methods of Determining Quality of Protein

The quality of protein can be estimated by biological, chemical or physical methods. The bases of these methods of estimation are:—

(i) determination of nitrogen balances; [G] these are of three types:

  (a) in which the term is used to denote the difference between nitrogen intake and output—as in determining the nitrogen balance index [G];

  (b) those involving the same measurements as in (a), with a correction for endogenous losses of nitrogen, as in the classical determination of the "biological value" (BV) [G] of proteins and net protein utilization (NPU) [G];

*G indicates that a definition is to be found in the Glossary.

(c) direct measurement of the nitrogen retained in the whole body, as in assays for NPU.

(ii) measurement of nitrogen accretion in or regeneration of tissues and body fluids, including proteins, e.g. enzymes;

(iii) changes in body weight, i.e., using "growth" as a criterion, notably measurement of protein efficiency ratio (PER) [G] and gross protein value [G];

(iv) microbiological techniques:

(a) growth of micro-organisms, as in the determination of relative nutritive value (RNV) [G];
(b) microbiological estimation of individual amino acids.

(v) chemical methods:

(a) those involving estimation of essential amino acids;
(b) estimation of "available" lysine (ALV) [G];
(c) estimation of total sulphur [G].

(vi) physical methods—usually involving a characteristic property of proteins which alters on deterioration e.g. absorption of orange G [G] and nitrogen solubility [G].

The methods may be used for two main purposes:—

(1) to assess the quality of protein-rich foods, concentrates and isolates, especially for detecting deterioration of quality during preparation, processing and storage;

(2) to determine the quality of protein in the estimation of the protein value of foods, diets or dietary regimens in relation to consumer needs.

The selection of methods for these two purposes and their validity will now be considered.

(1) Assessment of Quality of Foodstuffs of High Protein Content

All methods of assay of the quality of protein, except those that determine the individual amino acids, yield a result which is governed by the content of the limiting amino acid in the protein. Thus the results of assay accurately assess the quality of the protein of a foodstuff when it is fed alone, but they give little information as to its usefulness as a dietary supplement; for example, a protein rich in lysine or the sulphur-containing amino acids, yet poor in all the other essential amino acids and therefore having a low quality rating, might nevertheless be a valuable supplement to some diets. Further information as to its potential value as a dietary supplement could be obtained by a series of assays with added amino acids. The best method, however, of establishing the value of a foodstuff as a protein supplement to any diet is to assay mixtures of the diet and the foodstuff under investigation.

The effects of deterioration due to processing and storage can be measured with some precision (see discussion below on heat damage).

(a) *Selection of Methods*

Bearing in mind the above limitations, the method of choice is one that is

brief, convenient and as reproducible as possible. Of the methods listed in the Glossary, Protein Efficiency Ratio, NPU by body nitrogen and Biological Value by Mitchell's method are the most frequently used.

PER requires 4 weeks for the determination and involves no measurements other than weight gain and protein consumption of the animals. NPU by body analysis requires 10 days and involves nitrogen determination of the bodies (or the simplification introduced by calculating nitrogen from body water) and protein consumption. Biological Value or NPU by the full Mitchell procedure using 10 day collection periods requires 30 days for one protein or 50 days for three proteins and involves measurements of food intake and large numbers of nitrogen estimations on urine and faeces. The considerable amount of labour involved in the Mitchell procedure has led to its replacement, to some extent, by the NPU body analysis method, and there is good evidence that the two results agree reasonably well (although there is some unresolved evidence suggesting that the NPU method by analysis of the body yields lower results than the Mitchell method). It should be borne in mind, however, at this stage, that small differences in the quality of the protein are of little interest.

In view of the limitations discussed above it is clear that the most valuable results would be obtained from a determination of the available amino acids (G) in the protein or diet under consideration. Recent microbiological determinations of available amino acids show great promise in this direction but the methods have not been in use long enough to allow full assessment of them to be made.

(b) *Validity of Methods*

When the assessment is used to determine the characteristics of a protein, then the results can be applied directly to human nutrition (see below). For example, if processing or storage reduces the quality of a protein as determined in the laboratory, then the nutritive value of that material is also lowered for human subjects. Similarly, if assays reveal that a protein concentrate is rich (or poor) in lysine or methionine, then that material may be of correspondingly great (or less) value in human feeding.

The reproducibility of these methods of assay of protein quality is good. There appears to be reproducibility within about 10 per cent on NPU (body analysis), BV (Mitchell method) and PER determinations, although there is a wider spread with proteins of low nutritive value, i.e. for proteins with values of NPU standardized (G) below 40-50. The inclusion of a standard protein in the assay improves the reproducibility of the results.

NPU has the advantage over PER, that proteins that do not support growth cannot be assessed by PER, while all proteins can be measured by NPU down to a value of zero. While it is sometimes argued that proteins which do not permit growth can be of little value in the human diet, nevertheless it is often necessary to assay such proteins, and it is well known that proteins of low quality may have a surplus of one amino acid that would make the protein a valuable supplement to some diets.

(2) **Methods for Determining Quality of Protein of Foods as Customarily Eaten**

The measurement used in estimating the nutritive value of the protein in food as customarily eaten should be one that can be made in conditions which take

account of all the factors that may modify it. This can be partly achieved by an assay procedure in which the nitrogen retained in the body is measured when the food or mixture of foods is fed, i.e. by determining net protein utilization under the operative dietary conditions (NPU_{op}) [G]. The nutritive value of the protein of a diet, which is satisfactory when the diet is eaten in amounts supplying sufficient energy to ensure maximum nitrogen utilization, progressively falls as the caloric intake is reduced below this point. The reduction of protein value during under-feeding is due to combustion of protein consequent on the shortage of calories. Data are available from which it is possible to predict the effect on protein value of caloric restriction.

It has been known for many years that the biological value of protein falls as the proportion in a diet is increased above that required for maintenance; similarly the NPU falls with increasing concentration of protein above the maintenance level. For the determination of BV the protein is conventionally fed at 8 or 10 per cent concentration in the assay diet. This so-called "standardized" condition is thought, probably unjustifiably, to provide a basis for obtaining comparable data on different proteins. Standardized net protein utilization data (NPU_{st}) [G] are those obtained for the protein when it is present in a diet in an amount which just satisfies the requirements for maintenance.

In the FAO report on Protein Requirements the relationship between the chemical score [G] and BV as conventionally determined was examined. The proposal was made that the protein value of a diet could be assessed from the limiting amino acid content considered in relation to a tentative pattern of amino acid requirements. This procedure provided a measure of the degree of deficiency of the limiting essential amino acid. Improvements could also be proposed and their efficacy predicted. The conclusions reached, however, could provide only rough approximations, since no account was taken of the effect of protein concentration on the utilization of the amino acids, which appears to depend on the necessity during protein anabolism for some expenditure of energy derived from a fraction of the protein.

NPU, both operative and standardized values, can, however, be calculated from the chemical score, given the necessary data. Successful prediction of NPU standardized from chemical score depends upon a knowledge of representative values for the amino acids in foods and it is necessary to specify a reference pattern of amino-acid requirements. If possible, a correction should be made for the availability of the amino acids in the food, particularly when it is likely that it has been reduced through processing or storage. It may be noted that, in general, the digestibility of proteins in mixed diets is such that the protein value of the food determined by chemical scoring would be over-estimated by about 5 per cent. As will be seen below (page 28 and Table VI), predictions based upon the use of food composition tables for the average values for amino acid contents, and the provisional pattern of amino-acid requirements proposed by the 1957 FAO Committee, give good agreement with values determined by biological assay using rats. All but two of the diets described in Table VI were found to be limited by their content of methionine and cystine; thus the measure of agreement between calculated and observed values may be taken as an indication of the correctness of the figure for total sulphur-containing amino acids in the FAO provisional pattern.

TABLE II

Values of NPU Standardized of some proteins* measured on children by the balance sheet method and on growing rats by the body analysis method

Protein	Children	Growing rats
Whole egg	87	100
Human milk	95, 85, 95	100
Cow's milk	81, 79, 81	80
Sesame Flour	54, 53	54
Peanut flour	57, 53, 52	47
Cottonseed flour	51, 47	59

*From De Maeyer and Vanderborght (1961)

The same preparation was used for the assays on the children and the rats.

(a) *Equivalence of Values for BV and NPU both for Rat and Man*

In the report of the previous committee, comparative figures are given for the BV of several proteins, determined on growing rats and adult human beings. In view of the dependence of BV upon protein concentration, such comparisons for a protein are strictly valid only when the measurements are made at the same level of protein in the assay, or when the figures are corrected for differences in level, as in the calculation of NPU standardized. Table II shows values for NPU standardization for some proteins as measured on children by the nitrogen balance method, and on growing rats, using the analysis of the body method for NPU.

Table III shows values for NPU standardized of casein for four species calculated from figures given in the literature.

TABLE III

Values for NPU Standardized for casein for four species

Authors	Species	Method of assay	NPU_{st}
Allison (1955)	Rat	Balance slope	66*
Kon (1928)	Rat	Balance sheet	70
Miller and Payne (1963)	Rat	Body analysis	72
Allison (1955)	Dog	Balance slope	69*
Morgan *et al.* (1951)	Dog	Balance sheet	68
Blaxter and Wood (1952)	Calf	Balance sheet	80
Allison (1955)	Man	Balance slope	65*
Hawley *et al.* (1948)	Man	Balance sheet	68

With the exception of the values determined by the balance slope method, NPU_{st} has been calculated (by the method given by Miller and Payne, 1961a) from the figures for BV, digestibility, and protein concentration given by the various authors. For example, Blaxter and Wood give BV = 78·8, Digestibility = 87·3 and protein calories per cent total calories = 11·7%. Hence NPU_{op} = 68·9, and NPU_{st} = 80·0.

*Figures calculated from Nitrogen Balance Indices given by Allison, (1955), assuming a digestibility of 95 per cent. No correction has been made for protein concentration, as the measurements were made below maintenance.

Further evidence of equivalence of values for BV for rat and man is provided by the successful prediction of these values from the chemical scores, using the same reference pattern of amino acids. Such a comparison is made in the report of the FAO committee. It should be noted that, of the figures for chemical score listed, 55 per cent are derived from the total sulphur-containing amino acids of the protein, 25 per cent are from the lysine and 15 per cent from the tryptophan. This underlines the overwhelming importance in practical calculation of the levels in any chosen reference pattern of these three constituents.

(b) *Application—Net Dietary Protein Value* [G]

There are two ways of expressing the daily intake of protein. The simplest is to express it as grams of protein per day with due account of the variables which are implicit in Part IV of this Report. It can also be expressed in relation to the diet as a whole and if the latter course is followed there are alternative methods which can be adopted: (a) the weight of protein taken in each day can be related to the weight of the daily intake of food; (b) the daily intake of protein can be related to the daily calorie intake.

If reference protein [G] is defined as being a hypothetical protein containing 16 per cent N, which can be completely utilized in the body for anabolic purposes, then the use of NPU as the quality measurement has an important merit, in that, as will be shown later, requirements for protein and the quantitative expression of protein values of foods as eaten can be stated in the same terms; furthermore, if on evaluation, either by calculation or assay, a diet proves to be inadequate, measures to improve it to meet requirements can be predicted by calculation and their validity tested by assay.

Net dietary-protein value (ND_pV) is a term which has been introduced to express the protein value of food or a mixture of foods as customarily eaten. NPU is used as the measure of quality. For a given diet, the NPU_{op} is determined by assay or by calculation; using the following relationships:—

$$\text{NPU} = \frac{\text{retained N (g)} \times 100}{\text{intake N (g)}}$$

$$\text{and protein concentration} = \frac{\text{intake N (g)} \times 6{\cdot}25}{\text{food intake (g)}} \times 100$$

$$\text{Then } ND_pV = \text{protein concentration} \times \frac{\text{NPU}}{100}$$

$$= \frac{\text{retained N (g)} \times 6{\cdot}25}{\text{food intake (g.)}} \times 100$$

$$= \frac{\text{retained protein (g.)}}{\text{food intake (g.)}} \times 100$$

ND_pV is here expressed as grams of protein, equivalent to reference protein

as defined above, in 100g. of the diet. However, in view of the interdependence of the two dietary factors—protein and metabolizable energy—it is better to express ND_pV in terms of the calories which could be derived from the protein if it were all utilized for energy purposes, calculated as a percentage of the total metabolizable calories in the diet. This value is called Net Dietary-protein Calories per cent (ND_pCal %) [G]; it is

$$\frac{\text{retained protein (expressed in calories)}}{\text{total metabolizable energy in the food consumed}} \times 100$$

(c) *Comparison of Protein Values (ND_pCal %) in Relation to a Statement of Requirements*

ND_pCal % as defined above is equivalent to, and may be compared directly with, a statement of requirements in the following terms:—

$$\frac{\text{reference protein requirement (expressed in calories)}}{\text{total calorie requirement}} \times 100$$

This equivalence holds when the calorie value of the food is sufficient to meet the consumer's energy needs. The protein value to the consumer of a diet is reduced if it is fed in amounts insufficient to meet energy needs. However, the effect of caloric restriction on the protein value (ND_pCal %) to the consumer can be predicted (Miller and Payne, 1961c).

Scales of requirements for calories and for protein in terms of reference protein (as defined above) can be combined to give requirements in terms of ND_yCal %. Thus the concept of ND_pCal% as a measure of protein requirement is applicable to groups or populations. Individuals vary widely in their requirements for calories, and we do not know whether those who require most calories also require most protein. If they do, then the values applied to groups of individuals may be applicable to individuals also; but if the two requirements are not directly related then a value for ND_pCal % which represents a protein intake fully adequate for one person might represent an intake which is inadequate for another. However, in principle, if the requirements of an individual man or animal are known, a dietary regimen can be precisely specified in terms of ND_pCal %. In Table IV, values of ND_pCal % are given, calculated from the calorie and safe practical allowances for protein set out in the reports of the respective FAO committees. These values are typical values and hold only when food is given in amounts sufficient to meet the stated caloric requirements. The bases for determining the safe practical allowances are discussed in the FAO document and are clearly intended to provide for the needs of the great majority of individuals in any one group.

TABLE IV

Safe Practical Allowances †† for protein expressed in terms of $ND_pCal\%$

Subject	Calorie requirements †	Reference protein g/day ††	ND_pCal %
Infant	—	—	8·0*
Toddler	1230	24	7·8
Child	1970	29	5·9
Adolescent	2900	61	8·4
Adult	2960	34	4·6
Lactating mother	3200	76	9·5

*Value for human breast milk
†FAO (1957a)
††FAO (1957b)

The significance of these figures in relation to measurements made on practical human diets is shown by comparison of the requirements in Table IV with measurements by rat assay (Table V) of national dietaries compounded from food survey data and of individual traditional meals and dishes from several countries. It will be seen that the dietaries of tropical peoples consuming starchy roots, tubers or fruits as staple foods have protein values falling considerably short of the value of 8·0 ND_pCal % needed for young children. This is in accord with the prevalence in these areas of protein calorie deficiency amongst children in the age group 1 to 4 years. In Jamaica, Iran and Turkey, protein-calorie deficiency also occurs, yet the protein values of the diets assayed are high. There is evidence, however, that in these countries the calorie supply can be inadequate; protein-calorie deficiency in these areas is likely to be associated with the reduction of protein value consequent upon caloric restriction.

TABLE V

Protein values in $ND_pCal\%$ of diets and meals from various countries, measured by rat assay.

	Protein value	
Country	*Meals*	*Diets*
Gambia	6·5 (23)	
Ghana	6·2 (4)	5·2 (3)
Nigeria		5·9 (12)
Jamaica	6·2 (6)	7·9* (9)
India	6·3 (7)	
Iran		8·5* (8)
Iraq	5·8 (2)	6·1 (6)
Pakistan	7·3 (7)	6·9 (5)
Poland	9·2 (3)	
Turkey	7·0 (16)	8·8* (4)
U.K.	9·1 (23)	9·6 (38)

The figures in brackets are the numbers of different meals and different diets tested.

*The explanation of the occurrence of protein-calorie deficiency in these countries with diets of high protein value is given in the text.

Net dietary-protein values may be predicted by calculation from chemical data paying due regard to availability. The chemical score of the mixture of proteins in

a diet is calculated according to the method set out in the FAO report, and this value is taken as a prediction of NPU standardized; this, corrected for the level of protein in the diet, is expressed as protein-calories percentage of total calories by means of an equation derived from the values for NPU operative of three different proteins measured over a wide range of concentrations. Table VI shows a comparison of values calculated in this way with those obtained by rat assay technique.

TABLE VI

A comparison of the protein values of some human diets as determined by rat assay and as calculated from food tables.*

Origin	Staple[1]	Additional effective sources of protein[2]	Total no. of components	Observed values			Calculated values[4]		
				NPUop	Prot. Cal.	NDpCal[3]	Score	Prot. Cal.	NDpCal
				%	% Total Cal.	%	%	% Total Cal.	%
Gambia	Cassava	Pulses	4	45	2·8	1·3	53	2·1	1·1
Papua	Sago	Fish	3	75	3·5	2·6±0·2	74	4·1	3·0
Gambia	Cassava	Pulses	6	40	7·5	3·0±0·1	51	7·4	3·8
Jamaica	Sugar	Cornmeal	3	66	4·9	3·2±0·1	45	4·3	2·0
Gambia	Cassava	Fish	5	65	6·1	4·0±0·3	64	8·9	5·3
Britain	Potato	—	3	51	9·3	4·7±0·0	55	9·6	5·0
E. Pakistan	Rice	Pulses, milk	14	59	9·2	5·4±0·2	69	9·3	5·9
Jamaica	Maize	Fish	4	60	10·0	6·0±0·2	58	14·0	6·8
Britain	Wheat	Cheese	6	73	9·4	6·9±0·3	76	10·7	7·1
Nigeria	Sorghum	Pulses, fish	12	58	12·5	7·3±0·4	72	12·5	7·7
Gambia	Rice	Pulses, fish	3	63	11·2	7·1	68	12·2	7·2
Gambia	Maize	Pulses, fish	6	57	13·9	7·9	68	14·2	8·0
Britain	Wheat	Cheese	4	51	15·7	8·0±0·3	73	13·1	8·0
Britain	Wheat	Milk, meat, eggs	11	63	13·1	8·2±0·2	80	11·5	7·9
Persia	Wheat	Meat, eggs, milk	11	55	15·0	8·3±0·3	76	13·0	8·3
Nigeria	Sorghum	Milk, fish	13	63	14·0	8·8±0·3	76	15·1	9·0
Britain	Potato	Fish	2	47	18·7	8·8±0·0	70	18·7	9·6
Turkey	Wheat	Meat, pulses	11	59	15·7	9·2±0·2	71	15·3	8·7
Gambia	Rice	Pulses, fish	6	37	25·0	9·2	67	19·0	9·3
Britain	Potato	Meat	2	55	17·2	9·4±0·0	74	16·5	9·4
Britain	Potato	Eggs	2	72	14·0	10·1	100	14·0	11·2
Britain	Wheat	Fish, milk	5	47	23·0	10·8±0·2	80	23·0	11·5
Britain	Wheat	Milk	5	44	29·0	12·8±0·2	80	29·0	11·0

1 Chief source of calories.

2 Foods (excluding the staple) contributing more than 20 per cent of the protein in the diet.

3 The figures given are means, and the limits are standard errors.

4 Food composition tables used were Platt (1962); Orr and Watt (1957); and McCance and Widdowson (1960).

* From Miller and Payne (1961b).

Effect of Processing on Availability of amino acids

Nearly fifty years ago McCollum and Davis (1915) showed that excessive heat lowers the nutritional value of protein foods. These authors autoclaved casein (1 hr., 15 lb pressure) and Morgan and King (1926) showed a similar effect in toasted and puffed cereals.

It must be emphasized that treatment of this kind is extremely severe and beyond most of the usual domestic and commercial practices. Domestic cooking and commercial sterilization in cans do not appear to result in significant damage except in a few special cases such as the "explosion" of breakfast cereals and in roasting of meat. Even in roasting the excessive heat is applied to a very limited portion of the meat and although there is a detectable fall in nutritive value, it is small and some authors report no damage at all. For example, beef roasted 5 hr. at 300 °F, internal temperature 160 °F. showed no change in digestibility or BV (Mitchell et al., 1949). Similarly, roasting at 163 °C, internal temperature 80 °C, or canning or corning caused no fall in BV compared with raw beef (Mayfield and Hedrick, 1949). There are many other reports of no damage, even on frying bacon, but some authors (e.g. Wheeler and Morgan, 1958) observed a fall in PER in severely autoclaved pork. As these authors used very severe conditions, namely 120 °C for 120 min. the earlier generalization that normal domestic and commercial practices do not cause any damage still stands.

Baking can cause a fall in nutritive value. Thus Block et al, (1946) observed a fall in PER of a cake mix from 3·5 to 2·4 on baking and to 0·8 on toasting; the PER was restored to its original value on adding lysine. Rosenberg and Rohdenburg (1951) reported a loss of 2—16 per cent of the lysine of a loaf on baking, mostly at the crust, 5—10 per cent loss on toasting and 4—6 per cent loss on staling overnight in a stream of air.

The fall in nutritive value on baking is due to the Maillard reaction [G] between the lysine and reducing sugars present. Since lysine is already the limiting amino acid in the cereal, any reduction is at once apparent biologically; on the other hand, with moderately overheated dried milk, the loss of available lysine may not be detected by a single biological estimation since methionine is limiting and multiple biological estimations with added amino acids would be needed to reveal the effect on the lysine.

Addition of reducing sugars to cereal products before baking enhances the Maillard reaction, and there are several reports of the severe reduction of nutritive value in baked products which had been fortified with dried milk.

It has been shown repeatedly that most diets are limited by the sulphur amino acids so that the loss of lysine in baking need have no effect on the nutritive value of the whole diet. It can be concluded from the literature that there is little loss of nutritional value in the preparation of ordinary human foods other than that caused by baking.

The same is not true, however, of certain materials such as fish meal and oilseed cake which are sometimes used as human food though more frequently fed to animals. We must conclude that some of the less ordinary human foods are damaged by processing. Commercially available fish meals and oilseed cake

cover the whole range from complete lack of damage to very severe damage and, consequently, greatly vary in nutritional value, It is possible to process these foods without damage if sufficient care is taken, and much attention has been paid to this point in recent years.

Heat and storage can result in a loss of methionine and lysine of fish meal and certain of the oilseed cakes. It is not always possible to restore the nutritive value of such damaged foods by supplementation with methionine and lysine, and it is possible that other, as yet undetermined, amino acids are also damaged. Microbiological assay of available amino acids suggests that loss of all amino acids occurs on heat damage, and results of experiments with rats agree with this to some extent.

Overheating can damage proteins, but it is equally well recognized that certain foods, in particular soya beans and legumes, improve in nutritive value on heating. Boiling, mild autoclaving or light toasting of soya bean increases its nutritive value (Osborne and Mendel, 1917), and the effect seems to be explained in part by the presence in the raw bean of a heat-labile trypsin inhibitor (Klose et al., 1946; Borchers et al., 1948). Excessive heat, however, such as autoclaving at a temperature above 120 °C, reduces the nutritive value (Klose et al., 1948). The nutritive value of processed soya bean must therefore be a balance between the increase due to the destruction of the trypsin inhibitor and the fall due to damage. The nutritive value of peas and beans is also improved by mild heat treatment.

Any of the biological methods of assay described earlier will reveal processing damage or improvement which result from processing, so long as it is the limiting amino acid that is involved. Chemical methods that involve acid hydrolysis are of no use when the Maillard reaction has occurred since acid liberates the combined amino acids which are not liberated by enzymic hydrolysis. When damage by heat or storage is significant, e.g., say a fall of 10—20 per cent or more in nutritive value, 'growth' methods, 'balance' methods or microbiological assays may all be of use. But of the biological methods only balance methods can be used with poor proteins that do not permit growth.

GLOSSARY OF TERMS

The following symbols have been used to indicate the degree of usefulness of the terms.

* currently used.

N new method.

L limited application.

* *Apparent Digestibility*

See Digestibility.

* *Available Amino Acids*

Amino acids in the food of an animal, which are available for protein anabolism. These may be measured directly by bioassay, or indirectly by chemical or microbiological methods (Carpenter, 1960); (Ford, 1962).

L *Available Lysine Value* (ALV)

A chemical determination (with dinitro-fluoro-benzene) of the free epsilon amino groups of the lysine radicals combined in the food protein (Carpenter, 1960)

* *Biological Value* (BV)

The proportion of nitrogen absorbed from the food that is retained in the body.

i.e. BV =

$$\frac{\text{food N– (faecal N–metabolic faecal N)–(urinary N–endogenous urinary N)}}{\text{food N–(faecal N–metabolic faecal N)}}$$

(Thomas (1909); Mitchell (1923)).

* *Chemical Score*

The content of each of the essential amino acids of a protein is expressed as a percentage of a standard; the lowest percentage, i.e. that corresponding to the limiting amino acid, is taken as the score (Block and Mitchell (1946)).

* *Crude Protein*

Total N $\times$ 6·25.

* *Digestibility* (True)

The percentage of food nitrogen that is absorbed,

i.e. $$\frac{\text{food N–(faecal N–metabolic faecal N)}}{\text{food N}} \times 100$$

If the correction for metabolic faecal N is not made the quantity is termed "apparent" digestibility.

* *Gross Protein Value* (GPV)

The difference in body weights of a group of chicks fed a basal 8 per cent cereal diet plus 3 per cent of the test proteins, and a control group fed the basal diet alone. Values are calculated per gram of test protein eaten and

referred to the value obtained using casein as a standard (Heiman, Carver and Cook (1939)).

* *Limiting Amino Acid*

The essential amino acid of a protein which is most deficient in comparison with a standard. See Chemical Score.

* *Maillard Reaction*

A reaction involving the combination of lysine with reducing sugars which occurs on storage in the presence of some moisture. The combined lysine is not available biologically but is released on acid hydrolysis.

* *Net Dietary Protein-Calories per cent* ($NDpCal$ %)

The utilizable protein content of a diet expressed as crude protein (q.v.) calories i.e., Protein calories per cent of total calories × NPU operative (q.v.) (Platt and Miller (1959)).

For earlier work on net protein requirement and energy content of diet (see Goettsch (1948)).

* *Net Dietary Protein Value*

The utilizable protein content of a diet, i.e. crude protein content × NPU operative (Platt and Miller (1959)).

* *Net Protein Utilization* (NPU)

The proportion of nitrogen in the food that is retained in the body, that is, the product of biological value and digestibility, i.e. NPU =

$$\frac{\text{food N–(faecal N–metabolic faecal N)–(urinary N–endogenous urinary N)}}{\text{food N}}$$

or

$$\frac{\text{body N of protein fed group-body N of non-protein fed group}}{\text{food N}}$$

If the measurement is made under dietary conditions which are just adequate to provide maintenance, the value is termed standardized (NPU standardised or NPU_{st}); measurements made under any other conditions have been termed operative (NPU operative or NPU_{op}).

Some workers indicate the percentage of protein in the test diet as a suffix e.g., NPU_{10}.

(Miller and Bender (1955); Miller and Payne (1961)).

For earlier use of expression—net utilization of protein see Goettsch (1951).

* *Nitrogen Balance*

The difference between intake and output i.e.,
intake N–faecal N–urinary N = apparent N retention.

* *Nitrogen Balance Index*

The slope of the line relating N balance to absorbed N values is equivalent to BV. If nitrogen intake is used in place of absorbed N, the values are equivalent to NPU (Allison (1955)).

L *Nitrogen Solubility*

An empirical test measuring the solubility of the protein in a series of solvents (Lyman, Chang and Couch (1953)).

L *Orange G Dye Absorption*

An empirical test measuring the absorption by protein of the dye orange G; applicable only to certain proteins (Fraenkel-Conrat and Cooper (1944)).

* *Protein Efficiency Ratio* (PER)

Weight gain per gram of protein eaten. Values are usually measured using rats. Originally maximum values with respect to dietary protein concentration were given. Some standardized procedures are available for example using diets contained 9·09 per cent protein (Osborne and Mendel (1917); A.O.A.C. (1960) Methods of analysis 9th edition).

* *Reference Protein*

A hypothetical protein containing 16 per cent nitrogen, which can be completely utilized for anabolic purposes in the body. Used by FAO (FAO Nutr. Stud. No. 16) for stating protein requirements.

N *Relative Nutritive Value* (RNV)

The value of the protein for promoting the growth of a micro-organism relative to that of a standard (usually casein = 100) (Fernell and Rosen (1956); Ford (1960)).

N *Total Sulphur*

The total sulphur content (S) of the food is determined, and the quantity $1000\ \frac{S}{N}$ is taken as a measure of chemical score (Miller and Donoso (1963)).

PART IV

REQUIREMENTS FOR PROTEIN IN HEALTH

In this section of the Report requirements are expressed in terms of g. protein per kg. body-weight. The values given are intended to relate to persons of average weight for height, and it is assumed that calorie intake is neither deficient nor excessive.

The concept of "optimal nutrition"

Consideration of this concept is an appropriate starting point. The Working Party agreed to base the definition of "optimal nutrition" on paragraphs 7 and 8 of the Report by the Panel* on Requirements of Protein, Calcium and Other Nutrients on "Present, and possible future, research on requirements of protein and calcium". These paragraphs are reproduced below, together with the definition of optimum requirements for protein and calories respectively given by the FAO Committee concerned.

"7. Satisfactory dietary conditions are those which lead to full development of physical and mental powers, together with resistance to sickness and degenerative changes, and which are conducive to a healthy old age. We do not include in such criteria those conditions which lead to the most rapid achievement of maximum body size, or those which induce the attainment of the greatest body weight or size. Such parameters can only have value if they contribute to the satisfaction of some or all the criteria set out above."

"8. We have not attempted to define in practical terms what is sometimes called the optimal intake of individual dietary constituents. On the basis of the general criteria proposed, and indeed on any others, the assessment of the full effect of nutritional practices cannot properly be attempted until the whole span of life can be reviewed. Substantial individual variations are to be expected and we do not see how we can devise useful detailed criteria by which to determine what could be properly called optimal nutrition at the present time."

"Optimum requirements are usually understood to represent the amounts of protein which will permit 'optimum function', a definition which though it may have a spurious clarity, is not too helpful. The term implies, no doubt, sufficiency of protein to enable some stores or reserves to be established, but here it should be pointed out that, while some stores of protein are needed to permit resistance to stress, the desirable size of such stores has not been established and must indeed depend on a variety of circumstances." (Food and Agriculture Organisation (1957 b).)

The FAO Calorie Requirements Committee (1957 a) has the following under

* Panel of the Ministry of Health's Committee on and Medical Nutritional Aspects of Food Policy.

the heading "Requirements for Health":

> "Estimates of calorie requirements at the physiological level must represent the needs of fully healthy individuals. They should reflect a food intake which enables people to lead an active life physically, mentally and socially, and to be highly productive in their occupational pursuits. No handicap through lack or excess of food should be involved".

These passages have much in common, but since they abound in ill-defined terms are not very helpful in practice. We doubt whether it is possible to devise a more precise short definition of "optimal requirements" or whether greater clarity would be achieved by more lengthy discussion of the concept. The duty of any expert committee on requirements should be to define physiological requirements, those which suffice to cover all the current needs of healthy persons, and enable them to meet both the common, and rare, stresses of daily life. If an individual's physiological requirements are met he is in an optimal state of nutrition. By contrast the term 'minimum requirement' is used in this report to indicate the irreducible minimum below which harm will result.

Physiological requirements: the approach to their quantitative delineation:

The FAO Committee on Protein Requirements (FAO, 1957 b) adopted a two-stage approach: "average minimum requirements" (meaning the minimum requirement as defined above for the group studied) were assessed in terms of "reference protein" (defined as a protein of "high nutritive value", such as those contained in milk, eggs and meat, and, later in the Report, furnished with a provisional amino acid pattern); these were then augmented to give "safe practical allowances" by making an adjustment to allow for individual variability, and to allow for the quality of the dietary protein. [The term "safe practical allowance" is not entirely satisfactory, because the allowances, though possibly satisfactory from a physiological point of view, are often considerably less than the amounts that adults inevitably obtain even on quite austere diets, in the process of satisfying their caloric needs.]

The FAO Report states that "the safe practical allowance must also make satisfactory provision for protein reserve", but in fact suggested no specific adjustment to cover this. We considered this problem at some length, together with the related one that the body is able to adapt to, and in the adult achieve N equilibrium at, a variety of levels of protein intake (Holmes, 1962; Jansen, 1962). The experimental estimation of the requirement for maintenance is bedevilled by the fact that the N excretion on a low protein diet is so dependent on the previous nutrition of the subject. The protein mass of the body is in dynamic equilibrium, with a rapid turnover rate. On a low protein diet breakdown predominates over synthesis, the protein mass gradually shrinks, and the N loss diminishes. "The longer the process is allowed to continue the lower will be the N output: but the size of that output will be related merely to the size to which the protein mass has shrunk, not to any normal requirement of the body . . . If such low levels are used to fix the protein 'requirement' there is obviously a risk that the organism will be pinned down, as it were, to a protein mass considerably lower than the optimum . . . which could have been reached on a more generous diet" (Holmes, 1962).

We preferred the term "labile body proteins" to a phrase such as "protein

reserves" (see page 16), and concluded that in spite of the scanty evidence available, labile protein should be taken into account in assessing protein needs.

Estimates of requirements based on the "irreducible minimum" have been challenged. It is possible to adduce rational arguments to adjust these to take account of, for example, cutaneous losses of N, and individual variability in requirements, but to make any additional quantitative assessment of the significance of labile body protein is not possible at the present state of knowledge.

If this argument is accepted the question must be faced: how are physiological requirements to be assessed, on which practical allowances may be based? A common solution has been to make a fairly arbitrary decision and to conclude, in the case of adults, for example, that there is no evidence to suggest that an intake of 1g. protein per kg. body-weight per day is undesirable or excessive (National Research Council, 1959; Keller & Kraut, 1962; Masek, 1962). However, we decided to adopt a two-way approach to the problem of requirements: to attempt to define the lower limit, which would correspond to the irreducible minimum required in a given situation, as evidenced by laboratory studies, and to attempt to set the higher limit, which would be based on observation of actual consumption of protein in situations where there was no reason to suppose a deficiency existed; it was legitimate to attempt to raise and lower these limits respectively, thus narrowing the "area of ignorance", in which an "estimated allowance" would have to be set.

The Lower Limit of the Area of Ignorance;

(a) *Maintenance in the Adult*

The FAO Committee's graph gives 'average minimum requirements', which are defined for an individual adult as 'the smallest amount of protein which will maintain nitrogen balance when the diet is adequate in other respects'. The Committee suggested 0·35g. per kg. body-weight per day as the average minimum requirement of adults for the reference protein. This value was based upon the results of balance studies, particularly those reviewed by Sherman (1920). Sherman stated how the protein requirement for maintenance should be determined. "An attempt to determine the protein requirement of maintenance, even for any one kind of protein, would ideally involve adherence to an otherwise uniform diet, with complete findings of nitrogen intake and output and with periodical increase or decrease of protein consumption, until it is shown that the body can maintain itself in nitrogen equilibrium on a certain amount of protein, of the kind or kinds which the diet in question furnishes, and not on any smaller amount. The recorded investigations which meet all these conditions are not sufficiently numerous, nor do they cover a sufficient number of men and women or a sufficient range of foods to form a satisfactory basis for any general deduction as to the amount of protein required in normal adult maintenance."

Sherman then took the results of 109 balance experiments, published by different workers in different countries before 1920, which came nearest to fulfilling these stated conditions, and set out the 'indicated protein requirement' for each. The mean for all these experiments was 44·4 with a range of 21–65g. per 70 kg. body weight or 0·635 (range 0·30—0·93) g. per kg. body weight per day. He then quoted some of his own experiments with subjects living largely on wheat or oatmeal, and came to the conclusion that 0·5g. per kg. body weight

was sufficient. Assuming a biological value of 60 for cereal protein, the requirement of "reference protein" with a biological value of 100 would be 0·3g. per kg. body weight per day. The FAO Committee stated without reference, that "average figures in the region of 0·4g. per kg. body weight have been reported by some other workers. Taking these and other facts into consideration the Committee suggests 0·35g. per kg. of body weight as the average minimum requirement of adults for the reference protein."

Leitch and Duckworth (1937) also went through the literature on N balance experiments which had been published up to that time, and they selected figures obtained in 21 studies on healthy people which fulfilled the conditions which they themselves considered important. These were that the subjects must not have been hospital patients, they must not have received drugs, and there must have been a preliminary period of at least three days at or near the experimental N intake before the balance period began. Eleven of these studies had been published before 1920 and most of the results were included by Sherman in his series. Leitch and Duckworth calculated regression lines for N balance against N intake, both for positive and negative balances, and the point where the chances of a positive and negative balance were equal corresponded to a mean protein intake of about 50g. a day.

From theoretical considerations involving the assumption that the minimum urinary N excretion is proportional to the basal metabolic rate (and using a figure of 2 mg. N per basal calorie) Hegsted (1957) arrived at a minimum daily requirement of 18·7g. of protein, which allowing for a faecal loss of 10 per cent he increased to 20g. per day of reference—or of 100 per cent biological value—protein. This minimal estimate he thought should in practice be increased by 30–50 per cent on the grounds that the efficiency of utilization of protein may fall as the dietary protein level is raised (Hegsted, 1959).

"Integumental replacement" (Mitchell and Edman, 1962), which includes cutaneous loss of N, is usually ignored in balance studies, and Sherman's (1920) figures do not include an allowance for it. The loss of N from the skin is influenced by the amount of sweating, but not by the N intake. Under conditions of minimal sweating, N lost in sweat, epithelial debris, hair and nails, may amount to about 500 mg. N per day (Darke, 1960; Kraut and Muller-Wecker, 1960; Mitchell and Edman, 1962). Dermal losses are increased when sweating is profuse, and they may be of particular significance in hot climates, especially where individuals consume a low protein diet (Consolazio et al, (1962))

Losses of N in the faeces are related to the amount of unavailable carbohydrate in the diet, but they, like cutaneous losses, are very little influenced by the amount of N in the food. Martin and Robison (1922), for example, found their mean faecal excretion of N to be 0·19g. per kg. per day on a "protein-free" diet, and 0·016, 0·020 and 0·024g. per kg. per day on diets similar in other respects but containing 0·33, 0·66 and 1·32g. of milk protein per kg. per day respectively. Unpublished observations of Durnin and Southgate on healthy young men and women eating mixed diets low in unavailable carbohydrate and providing 1·5g. protein per kg. per day also showed faecal excretions of 0·02 to 0·03g. N per kg. per day. There is not enough unavailable carbohydrate in the average British diet to have any important effect on the absorption of protein and it seems more logical to adopt a value of, say, 0·02g. N (equivalent to 0·125g.

protein per kg. per day) for faecal excretion, rather than to express the value as a percentage of the intake.

The following upward adjustments to the minimum requirement figure 0·35g. reference protein per kg. body weight per day would seem legitimate:—

(i) to allow for integumental losses: 0·05g. per kg.

(ii) to allow for individual variation in requirements. Rose (1949) found a very considerable variation in the requirement for essential amino acids for N equilibrium in adults (the requirement for lysine, leucine and valine by certain individuals was twice as great as by others), and the coefficient of variation of the 109 estimates of adult requirements collected by Sherman (1920) is 20·4 per cent. A reasonable adjustment would be to raise the minimum requirement by about 50 per cent, bringing it to about 0·6g. reference protein per kg. body weight per day. It is obvious that an average figure for minimum requirement implies that half of the group in question has a higher requirement. The effect of an adjustment of 50 per cent will be that the requirements of at least 95 per cent of the group will be covered. For a protein of biological value of 80, which corresponds to the chemical score of the average British diet (Hobson and Miller, unpublished results), the requirement would be about 0·75g. per kg. per day.

This is as far as it is legitimate to go by a procedure of this kind. No allowances for faecal losses have been made, as these must have already been considered in deriving the figure 0·35.

(b) *Childhood*

(i) *Infants*

The protein intake of a breast-fed infant is 2 to 2·5g. per kg. per day, which has been regarded by some as the ideal allowance—though it might be pointed out that in this respect "Nature" could be supplying a surplus. Formulae based on cow's milk commonly provide 3 to 4g. per kg., and there is much controversy, recently reviewed by Holt (1959) and Gordon and Ganzon (1959), as to which level is more satisfactory; these authors find it difficult to reach a conclusion, the former possibly favouring the lower, and the latter the higher level. Holt (1959) suggests that the most satisfactory approach would be to compare different regimens with the ability of the infant to withstand the challenges to which he is subject, both in infancy and in later life—an area in which, as he points out, knowledge is virtually non-existent.

In considering the lower limit to the area of ignorance, we decided to accept an average figure over the first year of life of 2g. reference protein per kg. per day as the minimum requirement of infants. In this connection it was noted that the Princeton Conference (FAO 1957 c) accepted the following figures for the protein requirement of the infant, in terms of human milk: 2·2g. per kg. at birth, falling gradually to 2·0g. per kg. at 2 months, and then to 1·5g. per kg. at 1 year.

(ii) *Children*

With regard to requirements for growth, the FAO Committee stated that "the concept of minimum requirements can also be applied in the

period of growth, except that here the criterion becomes the smallest quantity of protein which will support 'satisfactory growth'. This term calls for a definition of 'satisfactory growth', by no means a simple matter".

A possible working definition of 'satisfactory growth' is as follows: "The growth corresponding to that of healthy children in the community under consideration who belong to prosperous families and receive a diet sufficient in quantity and of good quality".

This definition, though somewhat circular in nature, allows for racial difference in the growth rate. But the existence of racial differences cannot be taken for granted. Thus, while healthy Japanese children are in general smaller at a given age than healthy American children, there has been a marked increase in the size of children of Japanese who have moved to the United States, and also in the size of children in Japan during the last decade (Mitchell, 1962). These changes are no doubt due to environmental factors, particularly diet.

(iii) *Protein Increments during Growth*

In default of satisfactory balance experiments, the only experimental evidence on minimum protein requirements for growth is from the increments of protein in the body during growth. These are shown in Table VII, based on the results of tissue and body analysis, together with figures representing the endogenous urinary loss, calculated on the basis of 2 mg. N per basal calorie. For comparison, mean figures of observed protein intake are also shown.

It will be appreciated how small the amounts of protein are that are added to the body after the first year. This point has been made both by Wallace (1959) and Hegsted (1959). From one year of age upwards, therefore, the increments of protein in the body only account for a very small proportion of the intake. We know little about the efficiency of conversion of food protein to body protein in children, but from the evidence available it seems likely that in children after the first year the requirement for maintenance is considerably greater than the requirement for growth.

Following the argument used in discussing the maintenance requirements of adults, it is legitimate to augment the minimum figures reached in this way to make allowance for:

(a) integumental losses: these will be related more to surface area than to

TABLE VII

Protein intakes, increments and "requirements" of children (g. per kg. per day)

	Intake[1]	Increment in body[2]	Endogenous urinary loss[3]	Integumental and faecal loss	Minimum requirement of reference protein for growth and maintenance
Children					
1—2 years	3·0	0·1	0·66	0·17	0·93
2—3 years	2·9	0·08	0·64	0·17	0·89
3—4 years	2·9	0·07	0·61	0·17	0·85
4—5 years	2·8	0·06	0·59	0·17	0·82
Boys					
5—6 years	2·5	0·06	0·58	0·17	0·81
6—7 years	2·5	0·06	0·57	0·17	0·80
7—8 years	2·4	0·06	0·53	0·17	0·76
8—9 years	2·3	0·06	0·50	0·17	0·73
9—10 years	2·2	0·05	0·48	0·17	0·70
10—11 years	2·1	0·05	0·46	0·17	0·68
11—12 years	2·0	0·05	0·42	0·17	0·64
12—13 years	1·9	0·05	0·41	0·17	0·63
13—14 years	1·8	0·07	0·40	0·17	0·64
14—15 years	1·7	0·05	0·37	0·17	0·59
15—16 years	1·7	0·04	0·34	0·17	0·55
16—17 years	1·5	0·03	0·30	0·17	0·50
Girls					
5—6 years	2·5	0·06	0·55	0·17	0·78
6—7 years	2·5	0·06	0·53	0·17	0·76
7—8 years	2·4	0·06	0·52	0·17	0·75
8—9 years	2·2	0·06	0·49	0·17	0·72
9—10 years	2·1	0·06	0·46	0·17	0·69
10—11 years	2·0	0·06	0·44	0·17	0·67
11—12 years	1·7	0·07	0·41	0·17	0·65
12—13 years	1·7	0·06	0·39	0·17	0·62
13—14 years	1·6	0·03	0·37	0·17	0·57
14—15 years	1·5	0·02	0·33	0·17	0·52
15—16 years	1·5	0·01	0·31	0·17	0·49
16—17 years	1·3	0·006	0·30	0·17	0·48

1. From Widdowson (1947).
2. Calculated from values for body composition given by Widdowson and Spray (1951) and Widdowson and Dickerson (1963), and for body weights by Hathaway (1957).
3. Calculated on the basis of 2 mg.N/basal calorie (Hegsted, 1957). Basal metabolism values from Sargent (1961; 1962).

body weight, but in the absence of experimental data would probably not be overestimated by using the same figure as has been used for adults, 0·05g. protein per kg. per day.

(b) faecal losses: a figure of 0·12g. protein per kg. per day may be used as for adults.

The sum of all these values gives the mean minimum requirement of reference protein for growth and maintenance (Table VII).

An allowance must also be made for individual variation in requirements. If this is reckoned to be the same for children as for adults (page 38) then all the values in the last column of Table VII should be increased by 50 per cent.

If this procedure is adopted for two ages, say 4½ years and 12½ years of age, the lower limit of requirements becomes 1·23 and 0·94g. reference protein per kg. body weight respectively; or about 1·54 and 1·18g. 1 kg. of protein of biological value 80.

(iv) *Adolescence*

The growth spurt in adolescence is seen from Table VII to involve such a quantitatively small increase in the protein increment of the body that the adolescent "hump" in the FAO Curve of requirements is thought to be unnecessary.

(c) *Pregnancy and Lactation*

(i) *Pregnancy*

In a preliminary communication (Thomson and Hytten, 1961) it was estimated that a total of 800g. additional protein is laid down by an average normal pregnant woman. Subsequent investigation suggests that this may be a little low. The latest cumulative estimates calculated from published and unpublished information by Dr. F. E. Hytten are shown in Table VIII.

TABLE VIII

Protein laid down in the body during pregnancy

	Stage of gestation (weeks)			
	10	20	30	40
Additional Protein (g.) in:				
Product of conception	2	45	220	540
Uterus and mammary glandular tissue	30	130	180	200
Maternal blood	—	60	140	200
Totals (rounded)	30	230	550	950

These amounts, though not final, seem unlikely to be greatly modified in future. They represent an average addition of about 0·5, 3·0, 4·5 and 5·7g. protein daily, during the successive quarters of pregnancy.

No allowance has been made for storage of protein in sites other than the maternal blood and the organs of reproduction. The total store if there is one is unlikely to exceed the total amount of "labile" protein in man, some 300–400g. (Munro, personal communication). There is, indeed, no sound evidence for additional storage during human pregnancy. The theory that it occurs seems to rest entirely upon the results of balance experiments, now thought to be misleading (Thomson and Hytten, 1960), and upon analogy with laboratory animals, in which there may be some increase of carcass protein during pregnancy (Beaton, 1961). The present feeling of Thomson and Hytten is that there is no need to take account of hypothetical additional stores of reserve protein in making an estimate of the protein requirement specific to pregnancy, and that the totals given above need not be increased.

The efficiency with which reference protein in the diet is utilized in forming new tissue protein during pregnancy seems to be unknown. However, if a figure of 50 per cent is accepted, then the minimum requirement for additional protein during the final half of pregnancy would be of the order of 10g. reference protein per day as was suggested in the FAO Report.

(ii) *Lactation*

FAO (1957 b) based their recommendation on an average milk yield of 850 ml. daily, with a protein content of 1·2 g. per 100 ml. The protein output in milk is thus about 10 g. daily.

There is no new evidence available on the utilization of dietary protein for milk production. FAO assumed an efficiency of conversion of reference protein of 50 per cent: the origin of this figure is obscure, but appears to relate to the efficiency of utilization of dietary energy in milk production, which Hytten and Thomson (1961) have recalculated as 80 per cent. If it is assumed that protein also is used with 80 per cent efficiency the average requirement would be 12·5g. reference protein per day.

Alternatively, if the original assumption by FAO of 50 per cent efficiency is retained, the average additional requirement would be 20g. per day. In the absence of direct experimental evidence we felt that, in order to maintain consistency, the latter figure should be preferred.

(d) *Senescence*

The requirement of the human being for essential amino acids has been extensively explored in young subjects, but recently Swendseid and her colleagues have turned their attention to the older male (i.e. over fifty years of age) and have concluded that his needs for essential amino acids may be double those of the young adult. In the first series of studies (Tuttle et al, 1957) it was observed that a semisynthetic diet containing either 150g. of egg, or else a mixture of purified essential amino acids in the proportions and amounts found

in this quantity of whole egg, was inadequate to support N equilibrium, although these quantities were adequate for the young male. The older men achieved N equilibrium when the amounts were doubled. In subsequent studies it was established, with amino acid mixtures as the source of dietary N, that the absolute minimal amounts of methionine and of lysine needed for maintaining N equilibrium were twice those of the young adult. An account of these researches has been presented in summary form by Swendseid and Tuttle (1961).

Muscle mass and calorie requirements decrease in old age, but it is not known whether the extent of the N loss associated with old age is either physiological or desirable: osteoporosis, for example, may be related to a protein deficiency. The question of protein intakes of the elderly needs to be considered in relation to their efficiency in excreting the end-products of protein metabolism.

The FAO (1957 b) Report suggested the possibility that there may be an impairment of digestion in old age, but there is evidence to show that this does not necessarily occur (Widdowson, 1951; Durnin and Southgate, unpublished observations).

In view of the paucity of knowledge we did not feel able to recommend special requirements for protein in senescence. The figure for a minimum requirement can be taken as the same as that arrived at above for maintenance in the younger adult.

The Upper Limit of the Area of Ignorance

As already shown (pages 37 and 38) assessment of requirements from balances and similar studies involves guesses and approximations. But there is little if any other evidence, except the empirical one of identifying communities in which it is thought that protein deficiency to all intents and purposes does not occur, and determining their intakes of protein. The major weakness of this procedure is the impossibility of proving a negative; there are many conditions, such as impaired growth, which *might* be due to protein deficiency, but could equally well be due to other causes.

The entire argument of this Section is based on experience in this country; much of it is based on the paper "Protein Supplies in the United Kingdom", which appears on page 64. Were similar evidence considered from other countries it is probable that the "upper limit" could be delineated with greater precision, and the gap in our knowledge thereby reduced.

(a) *Maintenance in the adult*

Tables XIII and XIV (pages 67 and 68) give average figures for individual dietary records of adults in this country. Excluding the elderly and the pregnant, average figures are of the order of 90g. daily of protein for males and 70g. for females. Unless it is contended that up to half our adults are suffering from protein deficiency, these averages must be adequate. The figures of 1·4 and 1·3g. per kg. body-weight respectively are well above that reached at the lower limit of our area of ignorance. In that we know of *no* case of primary protein deficiency in adults, except perhaps in old age, it is probable that the mean figures given above are too high, but it is impossible to state where, below these average figures, the line should be drawn; it would be wrong to assume that even a minority of the community lives permanently on diets around the lower limits recorded in Tables XIII and XIV because one week's survey (or less than a week)

does not necessarily reflect the intake on other occasions. Moreover there is no evidence that intakes of protein vary closely in accord with physiological protein requirements. They do vary, however, with calorie intakes, and if there is no shortage of food, and no gain or loss of body-weight, with calorie requirements. However, as there is evidence that the consumption of calories is not related to body-weight (Thomson, Billewicz and Passmore, 1961), the consumption of protein is also probably independent of body-weight.

(b) *Childhood*

As Table XV (page 70) shows, the average figure for intake of protein rises, as would be expected, with age. If representative figures for body weight are applied (17½ kg. at 4½ years; 40 kg. at 12½ years of age; Scott, 1961) to representative average figures of intake (50g. at 4½; 80g. at 12½), this gives intakes of about 2·9g. per body weight at the lower, and 2g. per kg. at the upper age. These figures are close to those given in Table VII which refers to pre-war middle class children only.

These figures thus reached are quite out of step with any based on the theoretical calculations made in the preceding section (page 40) of this report. Yet, paradoxically, the mean figures cannot be used to define the upper limit of our area of ignorance with quite the same confidence as in the case of adults and pregnant women, because there is a rough relationship between growth rate and protein intake in this country when average figures for families of different size or of certain socioeconomic and other circumstances are compared. There is no evidence that the relationship is causal, merely that the area of ignorance cannot be defined at its upper limit if the criterion of adequacy of protein intake is to be that it shall support maximal rates of growth. The acceptability of this criterion is at least open to challenge. Moreover the ultimate differences in adult stature are likely to be very small, perhaps negligible. There is no other indication in British children that the average intake is anything but adequate.

(c) *Pregnancy*

The averages of the intakes of pregnant women (Table XIV) in the three surveys made since the war, are, respectively, 90, 91 and 72 or more g. daily. The first and the last of these figures are for primiparae only. There is no indication that any significant proportion of pregnant women suffer from deficiency of protein even after repeated pregnancies. It may be assumed that an intake of 90 g. daily is adequate, (an intake of 72g. may well be also, but the primiparae studied may have had physical reserves which might be used to cover a slight deficiency of intake.) On page 42 a figure of 10g. reference protein per day above minimum maintenance needs is found as necessary in pregnancy. This would be equivalent to a total minimum requirement of about 54g. protein of biological value 80 (for a woman of 55 kg. body weight). In that we know of no case of primary protein deficiency in pregnant women it might, as in the case of adults, be argued that the mean figure is too high; but again it is impossible to say at what point below the mean the line should be drawn, not only because the same objections apply as were instanced above, but also because there have been small fluctuations in stillbirth and neonatal death rate in the early part of the decade 1950–60, which happen to correspond roughly with fluctuations in the total supply of protein (Figure 2; see page 72). It must be stressed that this

relationship is unlikely (and certainly unproven) to be causal; it is merely included here as an additional objection to defining rigidly the upper limit of the area of our ignorance at any point below that of the average figure of 90g.

(d) *Senescence*

Average intakes of protein by the elderly and retired are of the order of 70g. per day for males (Table XIII) and 60g. per day for females (Table XIV). But since there are no adequate criteria by which the optimum state of nutrition of the elderly can be judged, these average figures cannot be asserted to indicate the upper limit of ignorance.

Other Variables

(a) *Climate*

Consolazio et al. (1962) discuss the excretion of N under a variety of conditions. At a high temperature (100 °F.), but with low activity, total dermal losses amounted to 4g. N per day. Acclimatization reduced the N losses of men doing a minimum of physical work in extreme heat after about five days, but even after acclimatization the increase in losses in sweat were not compensated for by decreased N losses from the kidneys and alimentary tract. It was found that N losses increased with an increase in physical activity and sweat rate, accounting for 13–14 per cent of the total N intake; these losses may be particularly significant in hot climates and of practical importance in populations with a low protein intake.

Issekutz, Rodahl and Birkhead (1962) found that the negative N balance induced in men by a severe cold stress persisted for several days after the ambient temperature increased from 8 °C to 22 °C in spite of an 18-fold increase in protein intake, and suggested that increased activity of the thyroid and/or the adrenal cortex was responsible.

(b) *Muscular activity*

It has long been accepted that protein metabolism is not raised by muscular exercise *per se*. No recent work throwing doubt on this conclusion is on record.

The increase in muscle mass associated with athletic training or with seasonal increase in muscular activity will involve a protein requirement above the normal while the increase is taking place, and maintenance of the increased musculature will require a slight increase in the physiological requirement of protein; on these grounds an increased intake of protein has been recommended for heavy workers (Keller and Kraut, 1962). Heavy sweating associated with muscular activity may lead to appreciable losses of N.

(c) *Other Dietary Constituents*

A summarized statement on "Calorie Intake and Protein Requirements" appears in Appendix 2 (page 53).

An additional point is that unavailable carbohydrate or fibre reduces the apparent absorption of protein by causing an increase in the amount of intestinal secretions, in the weight of the stools and in the rate of passage through the gut. The more unavailable carbohydrate a diet contains the more N will be

excreted in the faeces and the lower its apparent absorption. With a diet containing fairly large amounts of cellulose, the apparent absorption of protein may be only 85 per cent of the intake.

(d) *Harmful Effects of Excess of Protein*

Within the range of the protein content of diets customarily eaten by communities in different parts of the world, there is no evidence that high protein intakes have harmful effects. The highest expectation of life is found in countries with large per caput supplies of total, and particularly of animal, protein (Jansen, 1962). This does not exclude the possibility that very high protein intakes may contribute to the causation of certain degenerative diseases in adults. Such intakes are, however, associated with other dietary characteristics, e.g., a high fat intake, as well as with numerous non-dietary environmental factors.

A possible harmful effect of a high protein intake in the elderly because of their diminished renal function deserves mention.

Young babies are less able than adults to excrete the end-products of protein catabolism, and the danger of a high protein intake, particularly with too small an amount of fluid, must also be borne in mind.

Physiological Requirements

As stated above (page 10), these must be set within the area of ignorance whose upper limits have been discussed in the preceding sections, and which are summarized in Table IX and in Fig. 1 page 48). The precise level chosen must depend to a large extent on the nutritional significance attached to labile body protein. The important question here is whether or not the more labile components of tissue protein play a significant role in the well-being of the subject. On the answer hinges the decision as to how much protein the adult requires. If the labile body proteins are in fact part of the functional cell cytoplasm we have to decide whether cell function is impaired when they diminish, and if so, what level of protein in the diet will ensure optimal function, not merely in short-term studies but also for long-term survival and performance of the whole organism. It should be pointed out here that it is one thing for a small number of people to live apparently quite satisfactorily for a necessarily short experimental balance period on a low protein diet: it is quite another to conclude that a whole population could do so permanently. In the derivation of the upper set of figures shown in Table IX we have been concerned principally with the formulation of an approach. It may well be that if other countries adopted the same approach the area of ignorance could be narrowed considerably.

The proportion of energy in the diet that is provided by protein is shown for many regions of the world in Appendix 4 (page 63).

In considering the protein requirements of the age group 1 to 4, the fact-supported by abundant epidemiological and clinical evidence from all over the world—that the highest incidence of protein-calorie deficiency disease occurs in this age group, must not be over-looked.

TABLE IX

*Summary of Calculations**

(Biological value of protein: 80)

	Lower limit of knowledge	*Higher limit of knowledge*	*Difference between limits as % of: Lower limit*	*Higher limit*
	(as g. per day)			
Children c. 4 years	30	50	67	40
c. 12 years	50	80	60	38
Adults Male	50	90	80	44
Female	40	70	75	43
Pregnancy (last half)	55	90	67	40
Lactation	65	?	—	
(as g. per kg. body-weight per day)				
Children c. 4 years	1·54	2·9		
c. 12 years	1·18	2		
Adults Male	0·75	1·4		
Female	0·75	1·3		

* rounded figures

Further Research

We consider that further research on the following subjects is desirable:

(a) dietary and clinical surveys on the same individuals. This would go some way towards lowering the upper limit and so narrowing the area of ignorance;

(b) the assessment of the significance of labile body protein;

(c) the relationship of protein intakes and growth: this might be attempted by suitably controlled experiments recording the effect of supplements differing only in that one provided protein in much greater amounts than the other, though this begs the question as to the optimum rate of growth;

(d) the efficiency of conversion of dietary protein in growth, pregnancy and milk production;

(e) the possibility of harmful effects of excess protein, particularly with regard to renal function in the elderly;

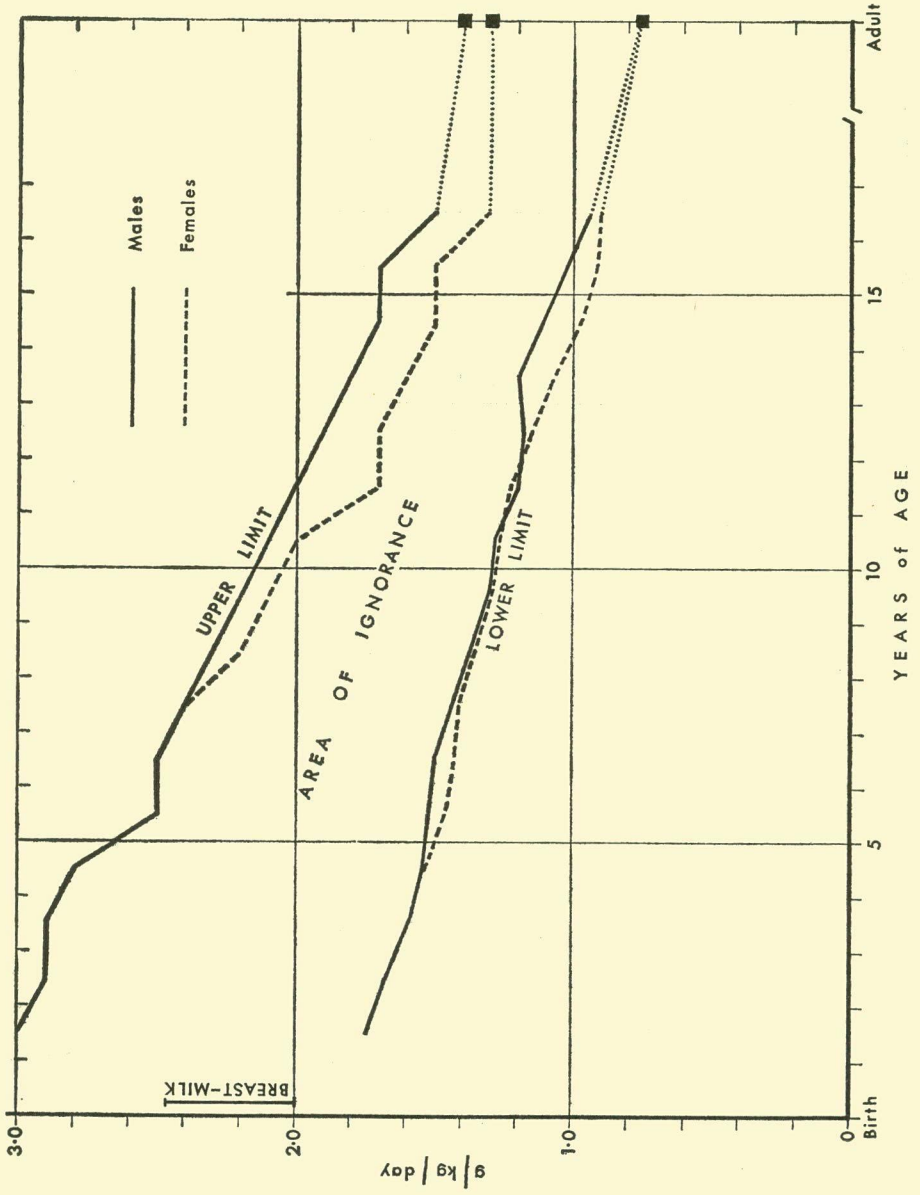
(f) whether amino acid requirements rise in old age;

(g) whether any of the degenerative diseases of old age (e.g. osteoporosis) can be linked to protein deficiency, either because of declining intake and/or because of increasing requirements.

FIG 1
HUMAN PROTEIN REQUIREMENTS

UPPER LIMIT : Observed Intakes, United Kingdom.
Data for ages 1 to 17 refer to 1935-39 (Widdowson, 1947)

LOWER LIMIT: Calculated from requirement for growth and maintenance (see Table VII), adjusted for individual variation (+50%), and for protein of biological value 80.



PART V

EFFECTS OF DISEASE AND INJURY ON THE REQUIREMENTS FOR PROTEIN

The prevalence of serious disease and injury

To judge whether protein loss due to disease or injury was likely to occur sufficiently frequently in the community to warrant special provision in assessing the needs of this country as a whole, the records were examined of the Ministry of Pensions and National Insurance relating to the incapacity rate of the working population. The assumptions made, and the detailed findings, are set out on page 76 and the conclusion reached is that the incidence of serious disease or injury is too low to warrant a place in computing this country's requirements. This is not to say that extra protein is not required, but rather that it is negligible in relation to the total needs of the country, and appropriately met by redistribution of supplies to the minority when it is needed.

The effect of serious zymotic disease and injury on the requirements of individuals

The demand for extra protein as a result of disease and injury is threefold.

(a) For the regeneration, as far as may be, of tissue lost and to heal wounds.

(b) To replace protein in body fluids lost either immediately or later, e.g. through haemorrhage (strictly speaking a tissue loss) and exudate.

(c) To make good protein lost as a result of the catabolic response to infection and to injury.

Whilst the metabolism of nitrogen after injury is extremely complex, and the overall nitrogen balance not entirely informative, some estimate has to be made of the order of magnitude of protein loss resulting from injuries, and this has been done for certain conditions in Table X. It will be seen that the major part of the loss arises commonly out of the catabolic response to injury, and the nature of this response has therefore been set out in detail on page 79. No attempt has been made to distinguish losses due to disuse atrophy from those of the catabolic response, and in fact liberation of protein for catabolism from labile protein, presumably largely in muscle, may determine the magnitude of atrophy in the early days after injury.

The evidence connecting nutrition and resistence to infection is very complex and it is impossible at this stage to make a firm statement.

TABLE X

Assessment of protein loss during first 10 days following severe injury or operation to a 70 kg man, also for comparison the protein loss in typhoid fever untreated by specific therapy

Source of loss	Simple fracture of femur or both bones of leg g.	Muscle wound equivalent to 4 hands in volume g.	35% burn involving in considerable part full thickness of skin g.	Gastrectomy g.	Typhoid Fever untreated by specific therapy g.
Loss of tissue	—	500—750	500	(20—180 Not replaced)	—
Haemorrhage or exudate	200g. due to initial haemorrhage around fracture.	150—400	150—400	20—100	—
Catabolic phase plus disuse atrophy	680 (may rise to 960)	750	750	625—750	675
Total protein lost	880—1,160	1,350—1,900	1,400—1,650	645—850	675

Protein equivalent to the nitrogen content of the body of a 70 kg. man is approximately 11·6 kg. (Thorpe, 1938: Davidson et al. 1959)

As Table X shows, serious injury can impose on the patient a need to replace protein in amounts of one kilogram or more and in the long run this may all have to be made good from the diet. The overriding consideration in computing the additional amount is the need to restore the patient to health as rapidly as possible. First blood or plasma loss should be made good. Most observers hold that the clinical and metabolic evidence indicates that during the first few days following serious injury in the previously adequately nourished person it may be unwise to push the intake of a well-balanced diet beyond appetite and this may fail during these first few days. For a day or two after the injury renal function may not have returned to normal. Thereafter, however, the patient should be encouraged to take as much as he can of a well-balanced diet relatively rich in protein. When appetite fails or the unassisted intake is inadequate there is a case for increasing the intake by fortified supplements and, if necessary, by tube feeding. It is generally accepted that it is possible to mask in considerable degree the catabolic loss by a large intake of a protein rich diet, but a loss during the first few days is not considered to be a matter for alarm. It must also be remembered that protein can only be used effectively for synthesis if the energy needs are covered. A high protein intake may be necessary for months after a large burn.

A daily input of 3 or more g. of protein per kg. body weight has been found suitable for burnt children. For adults an input of 2 or 3g per kg. body weight is judged appropriate. (Artz and Reiss, 1957; Bull, 1958).

Supposing the nett retention of protein to be 20g. per day it would take some two to three months to return to normal (if this is possible) in the case of injuries such as those specified in Table X. The rate of restoration gradually declines with time.

Other conditions

We have considered the possible importance, in the context of the requirements for protein of man, of conditions such as psychoneuroses, cancer and inborn errors of metabolism. We are unaware of good evidence that protein losses in psychoneurotic states arise out of causes other than the physical diseases and injuries that mental patients suffer, or from the wasting that may result from inadequate consumption of proper food. Whilst the cancer patient may stand to benefit if the drain of protein imposed by the neoplasm is made good, there seems no point in discussing here this problem. The problems that arise with inborn errors of metabolism, are so very rare as not to be relevant to the present enquiry.

APPENDICES TO REPORT

APPENDIX 1

The choice of Amino Acid patterns

by H. N. Munro

In order to evaluate amino acid patterns of different food proteins, some agreement must be reached on an acceptable reference pattern. Currently, several reference patterns are available and this is an impediment to using data on amino acid composition of dietary proteins.

Basically such a pattern must reflect the amino acid requirements of the consumer, and it is here that the main area of indecision lies. Estimates of human amino acid needs are largely derived from studies on small groups receiving amino acid mixtures over short periods. The criteria adopted for deciding amino acid needs are by no means uniform.

(a) The decision eventually rests on N balance measurements, but so far these have not included a quota for cutaneous, menstrual or other losses commonly neglected in N balance determinations. It is quite possible that the amino acid demands for these will differ in pattern from that of the body as a whole; thus cutaneous losses might increase the requirements for sulphur amino acids disproportionately.

(b) The use of N balance methods is much less precise than one would wish. Thus the end-point may be a "distinctly positive balance" (Rose, 1957) or "within 5 per cent of N equilibrium" (Leverton, 1956).

(c) A statistical approach to the assembled data has not been attempted. Some investigators have chosen as requirement the largest amount of an amino acid needed by any one individual in the group. This places the emphasis on the upper end of the range of requirements, and in effect means that the estimate will be decided by one individual in each series. From a statistical point of view, the use of the extreme values of a range is obviously hazardous; if the upper limit of needs of a population must be chosen, then it should be done by statistical means from all available data, and provided with an associated measure of error.

In view of these subjective decisions, it is no surprise that there are gross discrepancies in different patterns emerging from these studies. Thus the FAO reference mixture results in a ratio for leucine to tryptophan of 3·4, whereas whole egg protein, which has a biological value approximating to 100 and has thus been used as a reference standard, contains these amino acids in the ratio of 5·4 to one, some 60 per cent greater than in the FAO pattern. Indeed, Swendseid and Tuttle (1961) were able to reduce the tryptophan content of the FAO pattern by 20 per cent without impairing its capacity to maintain N balance in young adults. In consequence of these areas of indecision, refinement of amino acid analysis of foodstuffs to provide errors of less than 3 per cent in estimates for individual amino acids must now be matched by better knowledge of amino acid requirements.

This implies that exploration of amino acid patterns is an urgent objective. In expediting this, the following points might be considered.

(a) An initial study might be made of all data obtained so far with amino acid mixtures fed to human subjects, if possible by reference to the original data and not just the published papers. This material could be submitted to mathematical examination to determine what patterns could be substantiated on the basis of present information.

(b) From this survey, further trials of amino acid patterns could be planned This might be the occasion for international co-operation, which would have the further advantage that, once a pattern was established, individual investigators would be less likely to set up their own standards. Certainly, studies on amino acid requirements should be fostered in Britain.

(c) The following points seem desirable objectives for inclusion—

  (a) relationship of individual requirements of subjects to body weight, or a factor thereof:

  (b) effect of sex on subject's requirements:

  (c) relationship to age in adult life:

  (d) the proportion of total protein intake which must be in form of essential amino acids, and the effect of physiological status on this proportion.

APPENDIX 2

Caloric Intake and Protein Requirements

by H. N. Munro

The literature relevant to this subject now amounts to several hundred papers. Those appearing up to 1950 were reviewed by Munro (1951) and a revised review will appear in 1963.

Caloric intake and N balance

Does energy intake affect N balance?—All the experimental evidence conspires to show that the N balance of the adult subject subsisting on an adequate intake of protein is sensitive to change in energy intake, whether from carbohydrate, fat or ethanol, irrespective of whether the change is a large one or small. Thus increasing energy intake beyond requirements leads to N retention, whereas removal of energy from the diet result in N loss from the body. Experiments in which energy intake has been varied continuously from submaintenance to surfeit levels at a constant intake of protein have demonstrated that the relationship between energy intake and N balance is a continuous one throughout the range (e.g. Munro and Naismith, 1953). The magnitude of this action of energy intake on N balance is about 2g. N. for each 1,000 kcal. added or subtracted. It may be concluded that an inadequate intake of energy will by itself lead to loss of body protein.

Does energy intake affect the biological value of the dietary protein? This has been explored by Allison et al. (1946) using the nitrogen balance index as a measure of protein quality. Dogs were given diets providing different levels of energy intake and their N balance response to addition of protein to these diets was measured; the improvement caused by addition of a standard amount of protein was not affected by energy intake until the diet provided less than half of the animal's energy requirements. These observations receive confirmation from the studies of Campbell and Kosterlitz (1948) on the effect of diet on liver protein content of rats. These studies indicate that the capacity of the body to benefit from addition of protein to the diet is not impaired over a range of energy intakes, but that, below a certain critical level of caloric intake, this is no longer the case. The converse is also true, namely that addition of energy to the diet has a beneficial effect on N balance when the diet contains adequate amounts of protein, but at low levels of protein intake this N-retaining action becomes impaired. Calloway and Spector (1954) have made a survey of the published data obtained from human studies, and conclude: "To the general principle set forth—that on a fixed adequate protein intake, energy level is the deciding factor in nitrogen balance and that with a fixed adequate caloric intake, protein level is the determinant—may be added a corollary. That is, at each fixed inadequate protein intake there is an individual limiting energy level beyond which increasing calories without protein and protein without calories is without benefit". From these observations it may be concluded that addition of protein

to the diet of an undernourished population may not be fully effective if the energy intake is not adequate, and conversely that addition of energy yielding nutrients may fail to improve N balance if protein intake is limiting.

How does growth affect this interrelationship between caloric intake and body protein? The food intake of the growing animal, like that of the adult, is governed primarily by the energy needs of the body. In the case of the young animal, the demand for energy is increased under conditions favouring rapid growth. Consequently, the energy needs will vary with the effect of the diet on growth; subjects receiving a low protein intake will have less energy needs. It is thus possible that the low energy intake of the infant with protein malnutrition may partly arise from the reduced energy requirements of a child whose growth is impaired, and does not necessarily imply that caloric malnutrition accompanied the deficiency of protein in the diet. In measuring the incidence of protein-calorie malnutrition as opposed to protein malnutrition, it might be desirable to have some index of the caloric requirements of the human infant for maintenance as well as for growth. When the protein-deficient child receives treatment, it follows that energy requirements for maximal restoration of growth are considerable, an aspect which has been emphasized by Waterlow (1961).

Summary of points in the relationship of caloric intake to protein metabolism:

(a) An inadequate intake of energy will by itself cause a loss of protein from the body, and will consequently aggravate protein deficiency in the diet. Presumably populations subjected to chronic insufficiency of energy intake adapt so that the effect on body protein wears off, perhaps through reduced basal energy requirements.

(b) In undernourished populations, an increase in protein supply may not be fully effective if the energy intake is adequate.

(c) In convalescence after injury or disease, and in repletion after protein malnutrition, full utilization of dietary protein is best assured by a high caloric intake.

The action of dietary carbohydrate on protein metabolism

Dietary carbohydrate has an action distinct from its effect as an energy source, the evidence for which comes from three types of study:

(a) Administration of a dose of carbohydrate to a fasting human subject or animal induces a sharp reduction in urinary N output. Only part of this is associated with the fall in ammonia excretion consequent on the alleviation of ketosis. Fat does not have this action when given alone to the fasting subject.

(b) Exchange of fat for part or all of the carbohydrate of the diet on an isodynamic basis results in a temporary increase in urinary N output.

(c) Separation of the time of consumption of dietary carbohydrate and dietary protein so that they are eaten several hours apart causes a similar temporary increase in N output. Dissociation in the time of consumption of protein and fat does not have this action.

It has been suggested by Munro et al. (1959) that these all represent various forms of one basic effect of carbohydrate on protein metabolism, namely a

deposition of free amino acids in muscle through the action of insulin secreted in response to the ingested carbohydrate. In the normally nourished subject, the temporary fall in plasma amino acid level occasioned by this action of dietary carbohydrate is not important, but in the subject whose intake of protein is marginal, this sequestration of free amino acids in muscle for several hours after each meal may well aggravate the poor supply of amino acids to key tissues like liver. It will be noted that the diets productive of protein malnutrition in infants are notable for their high carbohydrate content as well as their low protein level. The excessive intake of carbohydrate may be a factor in the liver cell deterioration in such cases.

The action of dietary fat on protein metabolism

Work in the past in which beneficial effects of fat on protein metabolism have been examined has not provided impressive evidence of a positive action of dietary fat. Better growth on diets containing high proportions of fat can be attributed to increased food consumption, and experiments in which N balance has been studied under controlled food intake conditions has not yielded evidence of a specific role of fat in protein metabolism. However, Naismith (1962) has recently explored the utilization of dietary protein by rats in which essential fatty acid deficiency had been produced, and observed a less favourable N retention as compared with control animals receiving the same amounts of protein and calories. He suggests that the impairment in protein metabolism may result from a disturbance in energy utilization in the deficient animals, and that his observations may have relevance to the nutrition of human infants in under-nourished populations where fat consumption is negligible. This is obviously a factor which requires further consideration.

APPENDIX 3

The significance of Labile Reserves of Body Protein

By H. N. Munro

For more than a century, it has been recognized that tissues are differently affected by changes in nutrition. Some gain or lose substance with much greater rapidity than others, and this has led to the concept of a labile protein reserve in the body, available for rapid use in an emergency. Various investigators have referred to this material as storage protein, circulating protein, reserve protein or deposit protein. Each of these terms implies that the body contains a discrete type of protein which fulfils a reserve or storage role. Since such a storage form of protein remains unidentified, it is better to use a neutral term, such as labile body protein. An exhaustive review of literature on protein stores in the adult animal published before 1945 has been assembled by Kosterlitz and Campbell (1945).

The suggestion that the body of the well-nourished animal contains a store of readily-available protein originated with Voit. In 1866 he published studies which showed that the dog excretes considerably more N during the first few days of fasting than after some 5 to 6 days of the fast. Voit concluded that the extra N excreted during the initial period of the fast represents loss of labile body protein (Vorrathseiweiss, i.e., circulating or storage protein). Furthermore, he found that the excess N excreted during the first 6 days of a fast was directly related to the protein content of the preceding diet. From these data he concluded that the quantity of labile protein in the body is determined by the dietary level of protein and he computed from the extra urea excreted during the first 6 days of fasting that, even under the most favourable conditions for its accumulation, this labile body protein did not exceed 5 per cent of the total protein content of the body.

In subsequent studies, Voit (1867) demonstrated that, on changing from one level of protein to another, N output did not immediately become adjusted to the new level and he attributed this lag to variations in the amount of labile protein in the body. This observation has been repeatedly confirmed and is particularly evident when the subject is given a protein-free diet following a normal intake of protein. During the first few days of feeding a protein-free diet, there is a rapid decline in N output, followed by a slower fall until N output reaches a fairly steady minimum or "endogenous" level. The additional N above the endogenous level which is excreted during the first few days on the protein-free diet has been taken to represent loss of labile body protein and the magnitude of this extra N output has been used as a measure of the body's capacity to store such labile protein. Experiments on man (e.g., Thomas, 1911) and on rats (e.g., Campbell and Kosterlitz, 1948) suggest that this extra N output does not exceed 5 per cent of the total N content of the body at the time of instituting the protein-free diet.

It can thus be concluded that labile body protein has certain distinctive features:

(1) An increase in protein intake causes an immediate but limited deposition of labile body protein. Under the most favourable nutritional conditions,

the amount of labile protein deposited in the body does not exceed 5 per cent of the total body protein.

(2) Labile body protein gained as the result of a high protein intake is as rapidly lost again when protein intake is lowered. When the subject is fasted or fed a protein-free diet, labile protein disappears from the body within a few days.

There is evidence that changes in labile body protein occur as the result of factors other than alterations in protein intake. There is reason to conclude that *deposition* of labile protein occurs when carbohydrate is associated in the same meal as protein, and also as part of the action of anabolic hormones on protein metabolism. *Loss* of a labile protein component from the body seems to be source of the extra N in the catabolic response to operation (though it does not seem to account for the larger losses occurring in unplanned trauma), and is a source of material for plasma protein regeneration after plasmapheresis. These aspect of labile protein deposition and dissipation are discussed in detail elsewhere (Munro, 1964).

Nature and Sites of Labile Body Nitrogen

The exponential loss of labile N from the body would hold whether this represented loss of protein, of free amino acids or simply of end-products such as urea from the tissues and body fluids. The last-named was excluded by Martin and Robison (1922), since animals fed urea as the dietary source of N do not show the lag in N output characteristic of labile body N, when the urea is suddenly withdrawn from the diet.

The possibility of the labile N being in the form of free amino acids is also not in accord with the evidence. If some 5 per cent of body N is regarded as labile in the well-nourished animal, accumulation of this material as free amino N would demand an average concentration in the tissues of more than 120 mg. amino N per 100g. body weight. Since the tissues of the well-fed animal contain only about 20 to 40 mg. amino N per 100g. wet weight (Friedberg and Greenberg, 1947), it follows that even extensive reduction in the level of tissue free amino acid during protein depletion could not account for the excess N excreted in the urine during the first few days of depletion.

From these considerations it would appear most likely that the labile body N takes the form of protein, and all the subsequent evidence to be discussed is compatible with this view.

Effect of protein depletion on different tissues

The rate and extent to which tissues lose protein under adverse nutritional conditions is very variable. The extensive studies of Addis et al. (1936) showed that rats fasted for 7 days lose 40 per cent of their liver protein, 29 per cent of that of the prostate and seminal vesicles, 28 per cent of that of the alimentary tract in general, 20 per cent of that of the blood, 18 per cent from the carcass (muscle, skin, skeleton) and 5 per cent of that of the brain. Although these and much other data establish the special sensitivity of the liver to fasting and to protein depletion, they do not exclude the possibility that the protein content of some tissues not separately examined by Addis may be equally labile. In fact, Ju and Nassett (1959) have demonstrated that the pancreas and the small

intestine lose protein as quickly as does the liver during a few days of starvation. Another tissue whose protein content was not separately examined by Addis et al. (1936), was muscle, since this was included in the carcass analyses. Mendes and Waterlow (1958) report extensive losses of protein from muscle when young rats were subjected to prolonged protein deficiency; it would seem from examination of their data that the *rate* of response of muscle to a change in dietary protein level is inferior to that of liver. This is supported by the observation by Hagan and Scow (1957) that the thigh muscles of adult rats do not lose protein during the first three days of fasting, though there is an appreciable loss after 7 days of fasting.

It can be concluded that there is a spectrum of tissue response to conditions causing protein depletion, which varies from considerable lability to great stability. Furthermore, the tissues contributing to the N lost from the body during a period of starvation or of protein deficiency vary in their relative importance as depletion progresses. In short-term experiments, very labile organs such as liver, pancreas and small intestine are major contributors, whereas in more prolonged experiments, muscle becomes a major contributor because it is the largest tissue in the body (40 per cent of body weight). Waterlow et al. (1960) commented that "the so-called labile or reserve protein is that part of the cellular proteins which are most mobile and therefore most rapidly reduced in depletion". The relationship of the extra N lost in the urine during the early stages of protein depletion has been explored by Campbell and Kosterlitz (1948). These authors were able to account for slightly more than half of the extra urinary N output during the first few days of depletion as due to loss of liver N. Thus, while the liver is a convenient tissue on which to study the significance of labile tissue protein, it should not be forgotten that in other parts of the body there must exist a similar amount of N of equal lability.

Intracellular Changes Associated with Loss of Labile Protein

The cellular significance of labile body protein has been most extensively examined in the case of the liver cell. During the course of an investigation of glycogen storage in the liver, Pfluger (1903) proposed that the liver is also an organ for the storage of protein. There have been repeated attempts by chemical fractionation of the mixed liver proteins to identify a protein fraction which undergoes the gains and losses of such a store, but the evidence is essentially negative (e.g., Luck, 1936). A sensitive method for distinguishing between the effects of protein depletion on different cell proteins lies in the study of changes in enzyme activity. During protein depletion the majority of liver enzymes diminish either in proportion to the amount of protein lost from the liver, or to a greater extent. It has been argued (Rosenthal et al. 1950) that those enzymes whose concentration diminishes more extensively than does liver protein are situated in the labile part of the liver cell; examination of the published evidence (Munro, 1954) shows that there is no single intracellular fraction in which the more labile enzymes occur. Furthermore, the same enzyme in different tissues varies in its sensitivity to protein depletion, and it can consequently be concluded that the special lability of liver enzymes is a characteristic of protein metabolism in the liver and not of the enzymes. Thus Wainio et al. (1959) state that "the total protein and enzymes of liver are the most labile in protein depletion (cytochrome oxidase excepted), while the total protein and cholinesterase of

brain are very resistant. The total protein and enzymes of heart ventricle are more resistant than those of kidney, skeletal muscle and spleen, and almost as resistant as those of brain." This pattern of susceptibility reflects in general the readiness with which the proteins of these different tissues incorporate labelled amino acids fed in the diet (Schoenheimer, 1942).

Protein depletion affects the liver cell more extensively than by loss of protein. There is also a reduction of its ribonucleic acid and phospholipid (Kosterlitz, 1947) and further study of these changes has led to the conclusion that the machinery for protein synthesis in the liver cell is sensitive to fluctuations in the amino acid supply reaching the liver (Munro and Clark, 1960). In response to changes in protein intake there are rapid changes in the endoplasmic reticulum of the cytoplasm and in certain intranuclear constituents. Isotopic evidence suggests that these changes in cell architecture occur coincident with the period of absorption of amino acids from the gut, and that breakdown of the reticulum commences as soon as absorption is over.

The pancreatic cell also loses protein rapidly under unfavourable nutritional conditions; it resembles the liver cell in being essentially non-dividing and in having an abundant endoplasmic reticulum. Nevertheless, protein depletion induces loss of protein from the pancreatic cell without concomitant changes in intracellular structures similar to the changes induced in the liver (Munro, unpublished data.). Thus similarity in cell structure does not imply similarity in the mechanism of response to diet. Yet another type of response is exhibited by the mucosal cell of the small intestine, an organ which readily loses protein under adverse conditions. The mucosal cell is continuously renewed by rapid cell division (Leblond and Walker, 1956); protein depletion has no effect on the amount of protein and ribonucleic acid per cell, but retards the rate of cell division (Munro, unpublished data). Finally, muscle shows yet another pattern of response to protein depletion. In young animals on diets of inadequate protein content, the muscle cell proper loses protein but the connective tissue of the muscle continues to increase (Mendes and Waterlow, 1958). We thus have within a single tissue cell types with different sensitivities to diet.

Thus labile tissue protein has different implications in different tissues of the body. However, in each case examined, it means much more than a change in level of an inert protein store and probably represents the overt evidence for a complex readjustment of protein synthesis within the cell to a change in amino acid supply. In the case of the liver cell, this readjustment appears to be made after every meal containing protein and the labile cell constituents can be regarded as highly unstable. We do not know what advantage this instability confers on the cell, and until we do, it is difficult to arrive at a conclusion about the optimum level at which the amino acid supply to the cell should be maintained. Although the total amount of body protein which is engaged in such labile gains and losses is only 5 per cent of the total, it must by its very lability undergo fairly rapid exchange with the free amino acid pool and may therefore play a disproportionate role in the metabolism of dietary amino acids. Because of the much smaller overall protein metabolism of large animals like man than of small animals like the rat, labile proteins probably assume more importance in the economy of protein metabolism in the human subject (see Munro, 1964, for details).

The Nutritional Significance of Labile Body Protein

The important question here is whether or not the more labile components of tissue protein play a significant role in the well-being of the subject. On the answer hinges the decision as to how much protein the adult should consume in order to achieve lasting health. If the labile body proteins are in fact part of the functional cell cytoplasm, we have to decide whether cell function is impaired when they diminish and if so, what level of protein in the diet will ensure optimal function, not merely in short-term studies but also for long-term survival and performance of the whole organism.

Labile proteins and the assessment of minimal protein needs

As pointed out by Hegsted (1959), the protein requirement of a species can be arrived at from the minimum output of N when a protein-free diet is fed for a period, since replacement of this " endogenous " loss of body N must be a minimal demand on any diet. In the human subject, allowing for faecal as well as urinary losses, this minimal need amounts to some 4g. N daily. If an amino acid source of high biological value, such as whole egg protein, is provided the subject will come into N equilibrium on as little as 4g. N intake per day. In fact, this has been attained with human subjects receiving mixtures of amino acids providing as little as 3·5g. N per day (Rose and Wixom, 1955; Tuttle et al., 1959.) In such investigations, equilibrium level was achieved at the low N intake by preliminary reduction of protein level in the diet prior to feeding the protein or amino acid mixture; consequently, these are the nitrogen needs of the body after depletion of its more labile components.

On the other hand, when one tries to plan for some deposition of labile tissue protein, there seems to be no level of protein intake at which it can be said that a further rise will not cause still greater retention of N in the body. For example, Henry et al (1953) report experiments on adult rats receiving protein intakes varying from 50 mg. N to 800 mg. N per 100g. body weight per day. Protein deposition in the liver continued to rise throughout the whole range of intakes; calculation shows that rats on the lowest intake of 50 mg. N were likely to be already in N equilibrium. It is thus a matter of opinion what constitutes an adequate level of protein intake to effect deposition of labile protein in the liver. It may, however, be observed that the diets commonly consumed in different parts of the world provide considerably more protein than the computed minimum for equilibrium. If we accept 0·65g. protein per day per kg. body weight as adequate for equilibrium on a mixed diet (Hegsted, 1959), this would mean 42g. of protein for the FAO reference male subject weighing 65 kg. This amount of protein will contribute about 170 kcal towards the FAO reference man's total daily energy requirements, assessed at 3200 kcal for subjects undertaking light work. In other words, only 5 per cent of his energy intake need be in the form of protein in order to attain N equilibrium. In many parts of the world the diet eaten provides 10 to 14 per cent of the calories as protein, and in consequence allows for some deposition of labile protein. It seems a dangerous assumption that, because man can achieve N equilibrium over short periods at less than half this level, his customary level of intake is unduly high.

The Functional Significance of Labile Body Protein

Two aspects have to be considered here. First, does the functional capacity of an organ change when it is depleted of labile protein, and, second, does the presence of labile protein in the body confer an advantage by acting as a source of amino acids under adverse conditions?

In the case of the liver, it has been shown (Wang et al., 1949) that the capacity to clear bromsulphthalein declines in parallel with loss of liver protein. Other functions of the liver are also sensitive to dietary protein level; thus the capacity to inactivate oestrone is impaired in rats receiving protein-free diets (Vasington et al., 1958). It has also been found that chloroform administration causes less liver damage in dogs whose livers had not been depleted of protein (Miller and Whipple, 1940; Miller et al. 1940). A less obvious effect of protein depletion is the loss of other important nutrients. Co-enzymes and minerals may be lost in significant amounts, and it has been amply demonstrated that the capacity of the body to retain riboflavin and nicotinic acid is impaired on low protein intakes (Bro-Rasmussen, 1958). Whether such changes in functional activity are critical to the long-term health of the body as a whole is not known.

The capacity of labile protein stores to confer benefit on the body by acting as a source of amino acids is disputed. Holt et al. (1962) found that a previous high intake of protein conferred no benefit on rats receiving a protein-free diet for several months; weight loss and survival on the protein-free diet were unaffected by previous protein intake. With fasting rats, Samuels et al. (1948) observed that those previously on a high intake of protein succumbed more rapidly. On the other hand, Shapiro and Fisher (1962) consider that reserve protein deposited in the chick by feeding a high intake of protein can induce better growth during a subsequent period of low protein intake. Labile protein stores are also dissipated in the catabolic response to injury, which is most extensive in subjects previously on a high intake of protein, but there is no decisive evidence to show that healing is specifically benefited by the amino acids released from labile protein deposits as the result of the catabolic response.

Conclusion

It would seem desirable to regard labile body proteins as important in the metabolism of body protein and deserving of exploration in their own right as examples of tissue adaptation to amino acid supply. The evidence regarding their nutritional importance is difficult to assess without a knowledge of their metabolic significance. It is certainly unjustifiable to assume on the scanty evidence available that they can be safely ignored in assessing protein needs.

APPENDIX 4

The Contribution of Protein to Calories in National Food Supplies

by D. P. Cuthbertson

(Calculated from "Second World Food Survey," FAO., Rome, 1952)

Region and Subregion	*% of Calories from Protein*		*Animal Protein as % Total Protein*	
	Range	Average	Range	Average
FAR EAST				
South Asia	7·9–10·3	9·5	13·6–21·2	16·7
East Asia	9·2–12·4	11·0	9·5–11·0	10·2
North-East Asia	—	10·2	—	15·1
Pacific Islands and Malay Peninsula	—	9·0	9·5–22·8	16·1
NEAR EAST	9·0–13·7	12·3	13·3–36·0	20·1
AFRICA				
Northern Africa	—	13·6	—	24·6
Central and Tropical Africa	8·7–11·4	10·2	11·9–24·2	17·1
Southern Africa	8·3–11·8	10·8	19·5–40·0	30·0
LATIN AMERICA				
River Plate Countries	12·8–14·5	13·4	55·2–67·4	60·5
Rest of Latin America	9·8–12·2	10·8	27·0–46·5	36·1
EUROPE				
Western Europe	11·6–14·2	12·2	40·5–51·5	48·6
Northern Europe	11·9–14·0	12·8	46·4–66·5	56·8
Southern Europe	12·5–12·8	12·7	22·5–33·3	27·7
Germany and Austria	10·8–11·9	11·5	27·5–38·4	34·0
Eastern Europe	10·7–12·6	11·7	23·2–30·5	27·6
UNION OF SOVIET SOCIALIST REPUBLICS	—	12·9	—	25·8
NORTH AMERICA AND OCEANIA	11·5–12·0	11·8	62·0–68·5	66·7

The data indicate the range in the proportion of energy in the diet contributed by dietary protein that is encountered in various countries throughout the world, and may be contrasted with the relative constancy of this proportion in British diets, as shown in Tables XII, XIII, XIV and XV, (pages 66—69).

APPENDIX 5

Protein Supplies in the United Kingdom

by J. P. Greaves and D. F. Hollingsworth

At the national level

The total protein available at the retail level in 1961 is given in Table XI (page 65) as g. per head per day, and as a percentage of the allowances, weighted for the entire U.K. population, based on the recommendations of the BMA, NRC, and FAO.

The trend in protein supplies since before the war, together with the percentage animal protein, and protein calories, is shown in Fig. 2.

At the household level (National Food Survey)

The mean amounts of protein, for 1959, 1960 and 1961, in the diets of all households and households of different composition, that is, mean "consumption", are shown as g. per head per day in Table XII (page 66). In the same table are given the consumption for these groups in the same years, separately, expressed as a percentage of the recommended allowances of the BMA, NRC, and FAO.

It will be seen that the changes occurring over the 3 years in any household group are insignificant. In order to illustrate the effect of the presence of children and adolescents in lowering consumption, Fig. 3 shows the mean figures of consumption (expressed as a percentage of recommendations) for certain selected types of household. Fig. 3 also brings out the fact that the three systems of expressing requirements (BMA, NRC, and FAO) produce in practice results which very closely parallel each other.

At the individual level

Data of individual intakes, obtained from published British sources, are summarized in Tables XIII, XIV and XV (pages 67—70). The tables show the mean daily intake of all the subjects, with its standard deviation and coefficient of variation; the minimum and maximum mean intakes over the period in question shown by any subject; and the proportion of animal protein and of total calories supplied by protein in the daily mean.

It is seen that the proportion of total protein derived from animal sources was usually greater than 50 per cent and always greater than 40 per cent; that in general the protein calories are very constant, around 11 to 13 per cent; and that the coefficient of variation tends to be large, of the order of 20 per cent, except in those cases (elderly people living in institutions; army cadets) in which the opportunity of varying the diet was small.

TABLE XI.

Protein Supplies in the United Kingdom

I. At the National Level

Total protein available at the retail level in the United Kingdom in 1961 — 85·7 g/head/day
Proportion of total protein accounted for as *animal protein:* — 59·9%
Proportion of total *calories* provided by protein: — 10·85%
} (a)

(For figures in previous years see accompanying graph—Fig. 2)

Distribution of population, June 1961 (*b*)				*Recommended protein allowances* (g/head/day)		
			%	BMA	NRC	FAO
Children	1 year		1·7	28	24	25
	1–3		4·9	46	40	25
	4–6		4·4	56	50	26
	7–9		4·3	68	60	35
	10–12		4·5	86	70	48
Boys	13–15		2·6	110	85	69
	16–20		3·4	119	100	62
Girls	13–15		2·4	96	80	55
	16–20		3·4	88	100	38
Men	21–64	(c)	27·6	82	70	42
	65	(d)	4·5	62	70	42
Women	pregnant	(e)	0·8	96	78	54
	21–59	(c)	25·3	69	58	36
	60	(d)	10·2	55	58	36
Recommended total protein allowance—weighted for whole population				73·4	64·0	39·7
Allowing 15% for wastage:				86·4	75·4	46·7
Total protein as % of recommendation:				*99*	*114*	*183*

(a) From Board of Trade Journal—31 August, 1962.

(b) From Registrars General's Annual Estimates of the Population, England and Wales, Scotland, and Northern Ireland, 1961.

(c) Assumed moderately active.

(d) Assumed sedentary.

(e) Assumed as $\frac{5}{12}$ of the infants under 1 year of age, increased by 10 per cent to allow for infant mortality (the last 5 months of pregnancy being the relevant ones).

TABLE XII

PROTEIN SUPPLIES IN THE UNITED KINGDOM

II. AT THE HOUSEHOLD LEVEL (National Food Survey)*

Household Composition	Mean consumption 1959–1961. *Protein* g/head/day	Animal protein %	Protein calories %	Consumption of Protein expressed as a Percentage of the B.M.A., N.R.C. and F.A.O. Recommendations 1959 B.M.A.	1959 N.R.C.	1959 F.A.O.	1960 B.M.A.	1960 N.R.C.	1960 F.A.O.	1961 B.M.A.	1961 N.R.C.	1961 F.A.O.
All Households	75	59	11·5	99	105	169	101	106	171	102	107	171
Households with one man, one woman and												
No other (one or both 55 or over)	85	62	11·6	119	120	197	122	123	201	123	123	201
No other (both under 55)...	91	61	11·5	120	126	206	122	126	206	124	129	211
One child	79	60	11·5	106	113	180	107	113	179	110	115	183
Two children	68	59	11·5	96	102	165	99	104	168	100	104	166
Three children	63	58	11·3	90	97	156	92	97	156	92	97	156
Four or more children ...	59	54	11·2	82	89	142	85	90	145	88	94	150
Adolescents only	83	59	11·4	94	102	167	96	103	170	98	103	169
Adolescents and children	71	56	11·2	83	91	144	85	92	145	86	91	144
Other households with												
Adults only	80	61	11·6	113	115	188	116	115	188	117	116	190
Adolescents but no children	82	58	11·5	94	101	168	97	103	171	97	103	170
Children with or without adolescents	69	56	11·5	91	98	156	92	98	156	95	100	159

*"Domestic Food Consumption and Expenditure" (Relevant years).

Annual Reports of the National Food Survey Committee, H.M.S.O., London.

TABLE XIII
VARIATIONS IN PROTEIN INTAKE: ADULT MALES

Source	Number of subjects	Period of survey (days)	Information about subjects	Mean daily value for all subjects (g)	Minimum daily mean for any subject over the period (g)	Maximum daily mean for any subject over the period (g)	Standard deviation of subject means (g)	Coefficient of variation of subject means (%)	Animal protein as proportion of mean total protein intake for all subjects %	Protein calories as proportion of mean total calorie intake for all subjects %
Adam, Best, Edholm, Fletcher, Lewis & Woolf (1958)	57	21	Army recruits	94·3			6·1*	6·5		9·9
Adam, Best, Edholm, Goldsmith, Gordon, Lewis & Woolf (1959)	6	18	Army recruits, age 17–20	108·6	92·6	122·8	10·78	9·9		10·6
Edholm, Fletcher, Widdowson & McCance (1955)	12	14	Cadets, average age 19	99	84	111	10·5	10·6		11·5
Widdowson, Edholm & McCance (1954)	77	7	Cadets	104						11·2
Kitchin, Passmore, Pyke & Warnock (1949)	61	7	Students, at home	101	74	163	19	18·8	45	13·3
	47		Students, in lodgings, average age 19	102	77	137	15	14·7	46	14·1
	19		Students, in hostels	97	81	109	11	11·4	40	13·1
Widdowson (1936)	63	7	Middle class, age 18–89	97·6	53	167	23·8	24·4	68·5	13·1
Bransby, Daubney & King (1948b)	15	3	Various (-by calculation)	76·8	65	98	8·4	11·0		13·3
			(-by analysis)	84·2	70	109	11·6	13·8		15·1
Heady (1961)	118	7	Bank officials, age 40–55	83	42	122	15·2	18·3		11·7
Bransby (1954)	152	7	Industrial workers	109			19·4	17·8	50	12·3
Garry, Passmore, Warnock & Durnin (1955)	19	7	Miners	121	94	173	20*	16	49·7	12·0
	10		Clerks	96	78	142	16*	17	53·0	12·6
Durnin, Blake, Allan, Shaw, Wilson, Blair & Yuill (1961)	9	7	Elderly industrial workers: Heavy	113·3	85·5	139·2	17·6	15·5	60	14·1
	24		Elderly industrial workers: Medium	90·2	70·6	109·2	11·2	12·4	60	12·4
Bransby & Osborne (1953) ...	125	7	Elderly, at home, age 67	71					55	13·6
	8	7	Elderly, at residential home, age 74	70					49	11·1
Pyke, Harrison, Holmes & Chamberlain (1947)	12	7	Elderly, active, in small institution, age 60–85	74·1	70·8	77·6	2·1	2·9	45·1	13·7
	12	7	Elderly, infirm, in large institution, age 61–85	75·0	67·7	79·6	3·6	4·7	50·6	14·5
Blake, Durnin, Aitken, Caves & Yuill (In preparation)	9	7	Elderly, retired	72·2	59·7	89·8	10·8	14·9	60·6	14·3

*Estimate only.

TABLE XIV

VARIATIONS IN PROTEIN INTAKE: ADULT FEMALES

Source	Number of subjects	Period of survey (days)	Information about subjects	Mean daily value for all subjects (g)	Minimum daily mean for any subject over the period (g)	Maximum daily mean for any subject over the period (g)	Standard deviation of subject means (g)	Coefficient of variation of subject means (%)	Animal protein as proportion of mean total protein intake for all subjects %	Protein calories as proportion of mean total calorie intake for all subjects %
Andross (1936)	109	3–5	Students, age 21 years	76·1			16·8	22·0	58·5	15·0
Kitchin, Passmore, Pyke & Warnock, (1949)	71	7	Students, at home	76	51	118	13	17·1	51	14·0
	74		Students, in lodgings	78	49	102	13	16·7	49	13·7
	26		Students, in hostels	79	56	99	12	15·2	48	13·6
Yudkin (1951)	5	28	Students	72·8	57	84	11·4	15·7	57·2	14·5
Bransby, Daubney & King (1948b)	18	3	Various (-by calculation)	61·4	45	87	11·4	18·6		12·9
			(-by analysis)	69·4	51	91	12·9	18·6		15·0
Widdowson & McCance (1936)...	63	7	Middle class, age 18–65	67·3	28	90	12·4	18·4	68·4	12·8
Widdowson, & Alington (1941)	57	7	Middle class	64			12	18·8	52·8	12·2
Durnin, Blake & Brockway (1957)	12	7	Middle-aged housewives	66·2	48	83	9*	13	60	12·6
	12	7	their adult daughters	69·5	56	82	7*	9	60	12·3
Bransby & Osborne (1953) ...	178	7	Elderly, at home, age 62	57					56	13·1
	8	7	Elderly, at residential home, age 69	60					55	11·2
Durnin, Blake, Brockway & Drury (1961)	17	7	Elderly, living alone, age 60–69	62·4	29·2	85·0	12·0	19·2	60·*	13·2
Durnin, Blake, Allan, Shaw & Blair (1961)	21	7	Elderly, at home, age 60	61·7	41·7	87·3	12·3	19·9	40	12·7
Pyke, Harrison, Holmes & Chamberlain (1947)	9	7	Elderly, at home	51·9	34·8	73·7	11·4	21·9	53·6	14·7
	18	7	Elderly, in almshouse, age 65–85	45·4	32·7	58·8	6·7	14·7	52·0	12·7
	12		Elderly, infirm, in large institution, age 71	60·4	53·9	66·1	4·3	7·1	50·7	15·3
			Pregnant Women							
McCance, Widdowson & Verdon-Roe (1938)	53	7	Higher Income group	80	59	111	9*	11	66	13·0
	58	7	Lower income group	64	26	103	13*	20	53	11·9
Roscoe & McKay (1946)	35	7	Various	91	44	133	15*	16	53·8	14·3
Hobson (1948)	111	7	First pregnancies, 96 between 3 and 6 months	90	55	120	13·8	15·3	56	15·0
Thomson (1958)			First pregnancies, majority in 7 months:—							
	101	7	Husbands in white collar occupations	80·4			17·2	21·4	58·1	12·2
	109	7	Husbands in skilled manual occupations	78·4			15·1	19·3	56·5	12·5
	279	7	Husbands in semi and unskilled manual occupations	71·8			15·7	21·8	56·2	12·3

*Estimate only

TABLE XV

VARIATIONS IN PROTEIN INTAKE: CHILDREN AND ADOLESCENTS

Source	Number of subjects	Period of survey (days)	Sex	Age	Mean daily value for all subjects (g)	Minimum daily mean for any subject over the period (g)	Maximum daily mean for any subject over the period (g)	Standard deviation of subject means (g)	Coefficient of variation of subject means (%)	Animal protein as proportion of mean total protein intake for all subjects (%)	Protein calories as proportion of mean total calorie intake for all subjects (%)
Widdowson (1947)	20	7	Boys	1—2	37	28	61	7·7	20·8	68	13·2
	23			2—3	41	29	57	8·2	20·0	71	12·0
	20			3—4	49	29	80	10·9	22·2	65	11·9
	23			4—5	52	34	82	11·5	22·1	67	11·8
	28			5—6	50	36	64	7·4	14·7	66	11·9
	24			6—7	55	26	71	11·0	20·0	65	11·6
	21			7—8	63	46	80	8·3	13·2	65	12·0
	23			8—9	60	39	86	11·4	19·0	62	11·2
	21			9—10	68	53	94	10·3	15·1	63	11·3
	25			10—11	73	47	94	13·3	18·2	63	12·2
	27			11—12	72	43	112	14·6	20·2	64	11·7
	22			12—13	76	57	117	14·5	19·0	62	11·9
	21			13—14	79	53	117	15·7	19·8	61	11·8
	36			14—15	89	56	132	20·5	23·0	61	11·8
	22			15—16	100	78	132	15·5	15·5	62	12·1
	27			16—17	94	72	138	17·1	18·2	62	12·5
	32			17—18	95	71	142	18·4	19·3	62	12·1
	20			18—19	97	67	115	13·3	13·7	62	11·8
	23	7	Girls	1—2	38	27	51	7·4	19·4	76	13·7
	21			2—3	42	33	64	8·0	19·1	64	12·0
	21			3—4	46	28	72	9·4	20·4	65	12·2
	20			4—5	50	22	65	9·9	19·8	64	11·9
	20			5—6	49	38	70	8·5	17·3	67	11·7
	25			6—7	59	41	80	9·6	16·3	66	12·3
	22			7—8	60	47	79	9·5	15·8	67	12·4
	20			8—9	59	46	73	7·3	12·4	66	11·7
	20			9—10	62	36	87	12·1	19·5	64	11·7
	27			10—11	67	50	89	11·3	16·9	63	11·8
	30			11—12	64	26	111	14·8	23·1	61	11·5
	31			12—13	69	44	99	12·4	17·9	56	11·8
	34			13—14	72	43	128	16·4	22·7	58	11·6
	38			14—15	77	47	103	13·5	17·5	60	11·9
	41			15—16	78	46	113	15·6	20·0	62	12·4

	36			16—17	71	49	98	11·9	16·7	63	12·3
	21			17—18	73	50	101	13·2	18·0	59	12·0
	21			18—19	76	55	97	12·0	15·7	66	12·5
Bransby & Fothergill (1954) ...	461	7	Boys and girls	6—12 m.	38			8·6	22·6	74	14·1
				1	41			11·4	27·8	61	12·3
				2	46			11·3	24·6	59	11·9
				3	47			11·4	24·3	57	11·8
				4	51			10·6	20·8	55	11·8
Bransby, Daubney & King (1948a)	49	3	Boys and girls	10—15							
			By weighing		83	49	124	18	21·7		12·5
			By using homely measures		89			16	18·0		12·9
			By questioning		81			18	22·2		12·4
			By chemical analysis		89	51	124	16	18·0		15·0
	32		Boys	12							
			By weighing		75						10·8
			By questioning—with no aids		84						10·9
			By questioning—with memory aids		85						11·1
Cook, Davidson, Keay & McIntosh (1944)	39	7	Boys	14—15	76·6	54	147	16·4	21·4	44	14·0
Roscoe & McKay (1946) ...	37	7	Boys and girls, school-children	7—12	83	57	127	12*	14	48·2	12·6
										mean intake as g protein/ Kg body weight	
Durnin (preliminary unpublished (results)	43†	3 or 7	Boys and girls	0—6 m.	30·7	16	55	7*	23	5·0	15·4
	21			7—12 m.	39·9	24	54	5*	13	4·1	15·5
	61			1—2 yrs.	45·7	21	69	8*	18	3·8	14·0

* Estimate only. † 3 subjects only were breast fed during the study: the amount of breast milk obtained was not estimated.

PROTEIN SUPPLIES AT THE RETAIL LEVEL IN THE U.K. FROM PRE-WAR TO 1961

Board of Trade Journal – 31 Aug. 1962

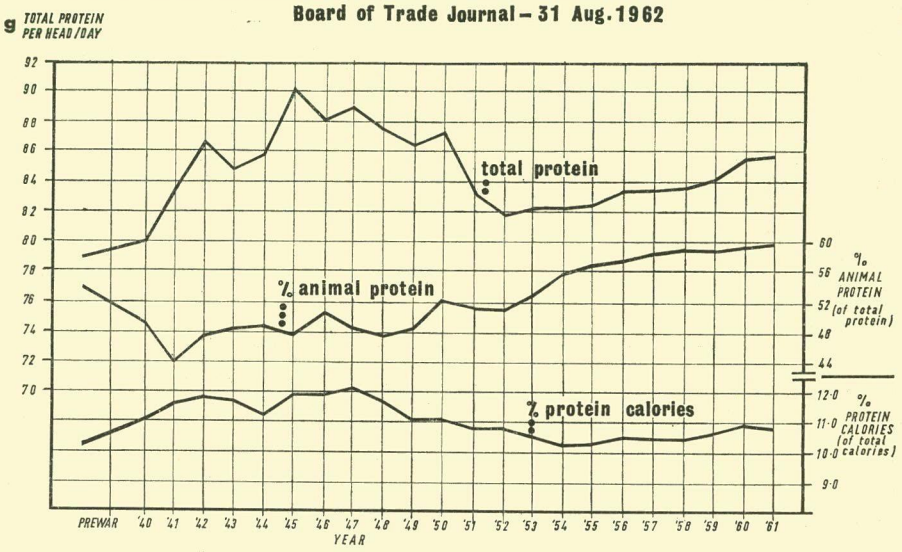


FIG. 2

N.F.S. Consumption of Protein as a Percentage of Recommended Allowances, showing the Influence of Successive Numbers of Children in the Household. (mean figures for 1959, 1960 and 1961)

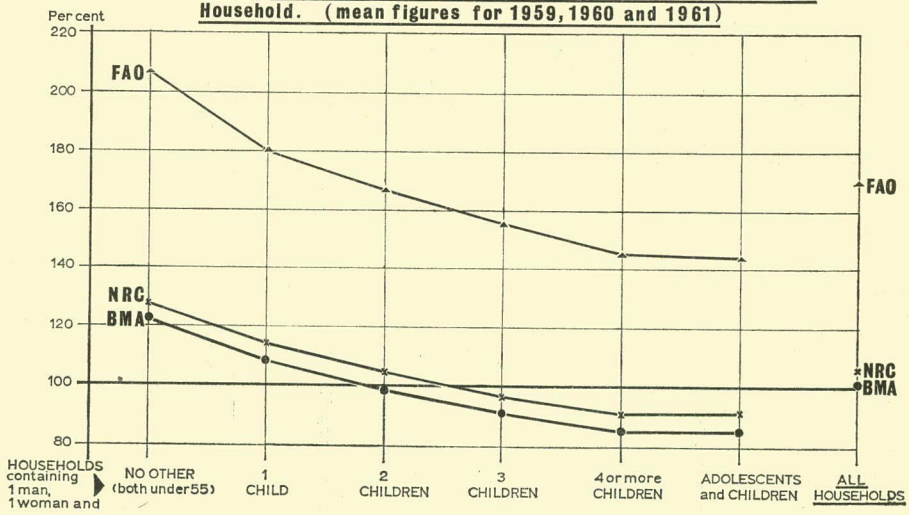


FIG. 3

APPENDIX 6

Perinatal Mortalities and Growth Rates in England and Wales

by W. T. C. Berry

The National Food Survey records show (appendix 5, Fig 3) that no matter what recommended allowances are used as a criterion of adequacy, the larger the number of children in the family the lower the consumption of protein when compared with "requirements" based on these allowances. In addition the National Food Survey shows, albeit in less degree, a tendency for the protein content of the diet to fall in relation to the reported income of the head of household, even when count is taken of the different compositions of families within each income group (Berry and Hollingsworth, 1963).

When the stillbirth and neonatal death rates of this country are corrected for parity and age of mother, there still emerges a higher mortality in the less favoured social groups (Heady and Heasman, 1959).

The growth of children in large families is less, age for age, than in small families (Yudkin, 1944; Scott, 1961). The same applied to children of different social classes in a study made by the Ministry of Health in 1950–51 (Berry and Cowin, 1954).

The stillbirth and neonatal death rates, which were falling before the war, fell substantially during the war. Duncan, Baird, and Thomson (1952) in examining the relevant records for Scotland, felt that improved nutrition (not necessarily related to protein intake) played an important part. The growth rate of children has been increasing for several decades, including the war period. Some increase in dietary protein, more particularly in the less favoured socio-economic groups, occurred during and/or after the war (Berry and Hollingsworth, 1963).

The intake of many other nutrients, and in addition several other non-dietary factors, may be presumed to have fluctuated more or less hand in hand. There is no evidence that these loose associations between protein intake, perinatal mortality, and growth rate, are causal.

Figure 2 in Appendix 5 shows the fluctuations that have occurred since the war in the total protein supplies available for consumption by the whole population, in the proportion of animal protein and in the percentage of calories derived from protein. These data suggest that the diet in the first half of the past decade contained rather less protein than in the second half. In the absence of information on the distribution of food within the family it is difficult to relate these trends to changes in the health of particular sections of the community.

If throughout this period, there had been a steady improvement in perinatal mortality rate and a progressive increase in growth rates of children, it might be inferred that the diet contained sufficient protein (and other nutrients) for all sections of the community.

Table XVI shows the stillbirth and neonatal death rates of England and Wales (from Registrar-Generals returns) over the relevant period. Table XVII (from Ministry of Education records) gives the unweighted mean of heights of children in 14 areas of England.

In neither case was there an uninterrupted record of "improvement", and where "adverse" fluctuations occur these tend to be rather more in the first than the second half of the recent decade.

It should be re-emphasized that in no case is there any evidence that the small fluctuations that are recorded are causally related. The aim of this Appendix is rather to set out in detail the basis of the reservations that have been expressed on page 44, when attempting to define the upper limit to the area of our ignorance in relation to the protein requirements of children and pregnant women.

TABLE XVI

Stillbirths and neonatal deaths in England and Wales
(1945 to 1960)

(x) Stillbirths per 1,000 total births.

(y) Early neonatal death rates (under 1 week) per 1,000 live births

	(x)	(y)
1945	27·6	18·0
6	27·2	17·8
7	24·1	16·5
8	23·2	15·6
9	22·7	15·6
50	22·6	15·2
51	23·0	15·5
2	22·7	15·2
3	22·4	14·8
4	23·5	14·9
5	23·2	14·6
6	22·9	14·2
7	22·5	14·1
8	21·5	13·8
9	20·8	13·6
60	19·8	13·3

TABLE XVII

Average heights of children in 14 *areas of England*
(1949 to 1960)

	Aged 5 years		Aged 14 years	
	(Inches)			
	Boys	Girls	Boys	Girls
1949	43 ·1	42 ·6	61 ·6	61 ·7
1950	43 ·1	42 ·8	62 ·0	61 ·5
1951	43 ·0	42 ·6	61 ·7	61 ·3
1952	43 ·1	42 ·4	61 ·9	61 ·3
1953	43 ·3	42 ·8	62 ·1	61 ·5
1954	43 ·0	42 ·7	62 ·1	61 ·5
1955	43 ·3	42 ·9	62 ·2	61 ·3
1956	43 ·2	42 ·7	62 ·0	61 ·3
1957	43 ·2	42 ·8	62 ·4	61 ·6
1958	43 ·1	42 ·8	62 ·5	61 ·8
1959	43 ·3	42 ·8	62 ·8	61 ·6
1960	43 ·2	42 ·9	62 ·9	61 ·8

APPENDIX 7

Prevalence of Serious Disease and Injury

by W. T. C. Berry

The magnitude of protein losses in certain serious conditions is set out in the table of Part V of the report. Some idea is needed of their prevalence. Without it no opinion can be formed as to what, if any, allowance should be made in computing the protein requirements of a country.

Below, it is assumed that—

(a) No provision is needed for minor diseases of adults because they automatically consume a sufficient excess of protein.

(b) That minor diseases in children on marginal intakes might have to be made good from reserves; depletion of reserves would be followed by a check to growth; any calculation of the physiological requirement for growth which is based on actual records applies to children who unavoidably suffer minor diseases. Therefore no estimate need be made here for the prevalence of minor diseases.

The records of the Ministry of Pensions and National Insurance, for the years 1957/8, show how many insured claimants were incapacitated from work at any given time, and for how long. The population involved is in effect the working population of the United Kingdom.

It has been arbitrarily assumed here that any disease condition with a median duration of less than 14 days incapacity could be excluded as being "minor". It was assumed that the diseases listed in Table XVIII could be excluded as not causing a significant loss of protein though they incapacitated for more than 14 days.

All diseases with an incapacity rate of less than 0·05 persons per thousand at a given time were excluded.

This left diseases which might or might not cause some protein loss (Table XIX) and diseases which very probably did, albeit often of a magnitude less than that considered on pages 49—51. These are listed in Table XX.

On May 31st, 1958, the numbers of persons claiming incapacity from diseases listed in Table XX were 14·2 per thousand males and 14·8 per thousand females. The corresponding numbers in the "doubtful" diseases listed in Table XIX were 7·9 and 11·3.

The data are subject to the drawbacks that commonly arise when records are used for purposes other than those for which they are intended. For example cancer is rarely recorded on the certificate; seasonal epidemics are rare in May; and so on. But conversely some diseases may incapacitate for a very long time of which only a small part was associated with an increased need for protein. It is probable that the inaccuracies of the analysis tend to cancel one another out.

Insured persons are not identical with other groups in respect of liability to stressing diseases. Logan and Cushion (1958), indicate that the total incidence of the diseases listed in Table XX (excepting bronchitis) is about half as high in those under 15, and about twice as high in those aged 65 and over. Data from general practice are not necessarily comparable with those of industry (particularly in younger and older groups) but it is reasonable to infer that even though stressing diseases may be much commoner among the old, the overall incidence in the population is not such as to warrant a special computation, in assessing the requirements of a country, for the effects of stressing diseases.

TABLE XVIII

Disease causing 14 or more days disability of a sort thought unlikely to increase protein needs.

"Other diseases" of eye
Hypertensive disease
Varicose veins (other than phlebitis and thrombophlebitis)
Unspecified diseases of arteries
Ankylosis and acquired musculoskeletal deformities
Congenital malformations

TABLE XIX

The number of claimants incapacitated for work per thousand insured persons, on May 31st, 1958, (M.P.N.I. Records) suffering from a disease of which doubt was felt whether or not an increase could be caused in protein requirements:—

	Male	*Female*
Disease of thyroid gland	0·1	0·5
Psychoneurotic states	3·5	6·4
Multiple sclerosis	0·3	0·4
"Other diseases" of C.N.S.	0·2	0·2
Arteriosclerotic degenerative heart disease	2·1	1·2
"Other diseases" of heart	0·7	0·8
Chronic ulcer of skin	0·1	0·1
Arthritis	1·4	3·0
"Other diseases" of bone and organs of movement	0·2	0·2
Total	8·6	12·8

TABLE XX

The number of claimants incapacitated for work, per thousand insured persons, on May 31st 1958, (M.P.N.I. records) and suffering from diseases and accidents likely to cause more than 14 days absenteeism and likely to increase protein requirements:—

	Male	*Female*
Tuberculosis,		
(a) pulmonary	1·8	2·6
(b) other	0·1	0·2
Scarlet fever and similar diseases	0·1	0·1
Malignant neoplasm	0·1	0·1
Benign neoplasm	0·2	0·3
Anaemias	0·2	1·2
Vascular lesions of C.N.S.	0·5	0·3
Cerebral paralysis	0·5	0·3
Haemorrhoids	0·2	0·1
Phlebitis and Thrombophlebitis	0·1	0·3
Pulmonary embolus and infarct	0·1	0·1
Pneumonia	0·4	0·2
Bronchitis	4·3	2·2
Silicosis etc.	0·5	0·1
Pleurisy	0·2	0·1
Ulcer of stomach	0·5	0·3
Ulcer of duodenum	0·4	0·2
Appendicitis	0·3	0·5
Hernia; abdominal	0·5	0·1
Diseases of gallbladder	0·1	0·2
Other diseases of intestine and peritoneum	0·2	0·3
Other diseases liver and pancreas	0·1	0
Nephritis and nephrosis	0·1	0·1
Infections of kidney	0·0	0·2
Hyperplasia of prostate	0·1	—
Orchitis and epididymitis	0·1	—
Diseases of breast	0	0·2
Other diseases of genitalia	0·1	0·8
Complications of pregnancy	0	1·3
Abortion	—	0·1
Osteomyelitis and periostitis	0·1	0·0
Internal derangement kneejoint	0·1	0·0
Displacement intervertebral disc.	0·4	0·3
Surgical treatment and operation	0·7	1·2
Fracture of skull, spine, trunk	0·2	0·1
Fracture of upper limbs	0·2	0·2
Fracture of lower limbs	0·6	0·4
Total	14·2	14·8

APPENDIX 8

The Catabolic Response to Physical Trauma and Surgical Operation

by D. P. Cuthbertson

Physical injury results in damage to, or loss of, body protein, both at the site of injury, and when sufficiently severe, as part of a general reaction to the trauma. In order to initiate repair of the damaged tissues, the injury is followed locally by an inflammatory reaction in which the participants are fundamentally structures and fluids composed of protein. The most prominent feature of the general response to injury is the increased output of nitrogen, sulphur, phosphorus, and potassium in the urine and a rise in body temperature preceding it slightly in time (Cuthbertson, 1930, 1932, 1942, 1960). Many other observers have confirmed this loss of nitrogen in burns (Browne, 1943; Moore *et al.*, 1950; Bull, 1958). These changes may or may not be preceded by a state of traumatic shock. The local and general responses to injury overlap and cannot be considered as independent aspects.

The inflammatory reaction can be caused by pressure, friction, heat, cold, corrosion or infection (Hunter, 1794). It is an adaptive defensive mechanism which tends to restore the injured part to its normal function. Inflammation is a process not a state: the affected area undergoes continuous changes (Florey, 1962).

The early reactions of tissues to injury are dominated by vascular changes, primarily exudation from the small blood vessels which bring both soluble and cellular anti-microbial factors from the blood into the tissues. With the passage of protein into the tissue fluids the osmotic balance is disturbed and is exaggerated as the plasma proteins may break down by proteolysis and so increase the number of molecules that can exert osmotic pressure. The increased blood flow has a parallel in increased lymph flow. The lymphatics undergo changes which eventually facilitate the removal of the excess fluid poured into the tissues from the blood vessels.

Mechanical agents may damage cell membranes and disturb the spatial arrangement of enzymes and their substrates. Blockage of the blood supply to a portion of the tissues causes an infarct in which the cells are deprived of oxygen and other substances that are necessary for the balance of synthesis and breakdown. Chemical substances may cause widespread damage to cell structures, or merely inhibit the function of an enzyme; or, like radiation, injure chromosomes. Such damage may also produce striking changes in the plasma and cells of the blood.

Apart from the initial vaso-vagal disturbances that sometimes follow almost immediately on receipt of even trivial injuries in the conscious subject—one of the effects of sympathetic amines—the next systemic effect in severe injuries is generally a diminution of metabolic activity with circulatory deficiency due to loss of circulating blood volume as the central feature—the period of traumatic

or surgical shock. In non-fatal injuries it is generally followed within some 12 hours by a period of increased metabolism in which some degree of "traumatic fever" can be detected (Cuthbertson, 1932). It would seem to correspond to the period of local inflammatory defence and demolition in the surviving organism and is generally accompanied over the next few days by evidences of a marked increase in protein catabolism, characterized by increased excretion of nitrogen (mainly as urea) and sulphur (mainly as sulphate), phosphate and potassium in the urine (Cuthbertson, 1942). The faecal excretion of nitrogen remains relatively unchanged. The loss of nitrogen and sulphur following an injury, such as the accidental fracture of a long bone of the leg, can be quite considerable and, reaching a peak within the first week following the injury, may on occasion exceed the whole of the nitrogen content of the liver within the ten days following receipt of the injury (Cuthbertson, 1932). Similar findings have been reported in untransfused cases such as fractures (Flear and Clark, 1955). Its extent is in part a reflection of the previous nutritive state of the patient. Previous protein depletion diminishes its extent (Munro and Cuthbertson, 1943; Munro and Chalmers, 1945; Browne, 1944).

There is initially a diminished urinary flow lasting a day or two (Cuthbertson, 1932) but if kidney damage occurs there will be a rise in the urea content of the blood and in crush injuries this may be severe and accompanied by myoglobinuria. If the liver suffers too greatly from the effects of anoxia the amino acid content of the blood may rise in proportion to the damage.

While in man the excessive urinary loss may reach a maximum about the third to eighth day following injury, there may still be a loss of nitrogen even six weeks afterwards (Cuthbertson, 1932, 1942). Muscle is suspected as being one of the main sources of this catabolized protein (Cuthbertson, 1930, 1932; Levenson *et al.*, 1959; Born, 1962).

Davies *et al.* (1962) have reported a marked increase in the catabolism of labelled human albumin in patients with severe burns and other forms of injury. The peak occurring between the third and sixth days coincided with the peak of urinary nitrogen excretion. Substantial amounts of albumin were lost from the burned area as exudate. The breakdown of this albumin could only have contributed a small fraction to the total nitrogen.

In disuse atrophy there is also a loss of nitrogen and sulphur in the urine (Cuthbertson, 1929; Sc∅hnheyder *et al.*, 1954), and where there is excessive pain on movement the loss will be greater than that observed experimentally on immobilized, but otherwise normal subjects.

In Table X (page 50) an attempt has been made to set out the extent of these losses (compounded from the observations of Bull (unpublished); Grant and Reeve (1951); Wilkinson (unpublished); and Cuthbertson (1932, 1964), together with data on typhoid fever derived from Müller (1884)). The general deterioration in severely burned patients produced by pain, pyrexia, anaemia and wound seepage results in anorexia and further complicates the nutritional deficiency (Artz and Reiss, 1957).

It has been suggested that this elevation in protein catabolism with resultant fever and increased oxygen consumption—this "traumatic fever"— is a reaction of the body to enhance the healing process by accelerating the reactive processes

which involve both inflammation and repair (Cuthbertson, 1932, 1942; Cope *et al.*, 1953: Cairnie *et al.*, 1957). It is possibly akin to the higher temperature found during early growth. Poor healing has been reported in debilitated patients who fail to respond to burns by increased nitrogen excretion.

This catabolic reaction to injury is self-terminating. The total protein loss may amount to one kilogram or more in the space of ten days. The overriding consideration is to restore as rapidly as possible the patient to health and to that degree of functional activity as the nature of the injury will permit. The first need is to make good blood or plasma loss; the rest has to be made good through the diet and will take months in severe injuries.

During the first few days following serious injury in the previously adequately nourished subject it may be unwise to push the intake of a well-balanced diet beyond appetite and this may fail during these first few days. Thereafter, the patient should be encouraged to take as much as he can of a well-balanced diet relatively rich in protein, but where appetite fails or the unassisted intake is inadequate, there is, as has been stated, in the body of Part V, a case for increasing the intake by fortified supplements and, if necessary, by tube feeding. If the nitrogen needs are thus looked after those of sulphur, phosphorus and potassium will also generally be included.

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