XXXIII. THE MINIMUM NITROGEN EXPENDITURE OF MAN AND THE BIOLOGICAL VALUE OF VARIOUS PROTEINS FOR HUMAN NUTRITION.

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HISTORICAL.

UNTIL comparatively recently, the search for the minimum protein requirements of the human body has been made on the assumption that protein is an entity, little regard being paid to whether the proteins were derived from meat, milk, cereals, etc.

The more important of the numerous investigations undertaken with this object are those of Hirschfeld [1887], Kumagawa [1889], Klemperer [1889], Peschel [1891], Lapicque and Marrette [1894], Sivén [1900] and Albu [1901][1].

In all of them the protein fed was derived from more than one source and often from several. The observations, which vary in precision, were made under conditions which were not uniform, particularly in regard to the total calories taken. Nevertheless, each experimenter succeeded in establishing that nitrogenous equilibrium could be maintained over short periods with one-third to two-thirds the standard laid down by Voit of 118 g. protein, equal to 0·39 g. N per kilo.

The minimum arrived at by the above experimenters varied between 0·08 g. and 0·18 g. N per kilo, most of the results being round about 0·1 g. The lowest value is that of Sivén, who considers that he ultimately attained nitrogenous equilibrium on a mixed diet containing 0·08 g. N per kilo, only 0·03 g. of which he regards as true protein, but as the only evidence that this small amount was sufficient is a positive balance of ·04 g. N on the last day of a four days experiment, decided negative balances occurring on the first three days, this conclusion would appear questionable. In another series in which the nitrogen intake was 37 % higher the evidence of nitrogenous equilibrium is satisfactory.

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[1] The earlier observations have been collected by Atwater and Langsworthy in their "Digest of Metabolism Experiments," *Bulletin* 45, U.S. Dept. of Agriculture, 1898. An excellent review of the work previous to his paper is given by Sivén. Most of the literature on the subject to date is referred to in Mendel's "Theorien des Eiweissstoffwechsels," *Ergebnisse der Physiologie*, 11 Jahrgang, 1911; Caspari's article "Eiweissstoffwechsel" in Oppenheimer's *Handbuch der Biochemie*, 1911, and Cathcart's *Physiology of Protein Metabolism* (1921). All of these contain good bibliographies.

The investigations of Neumann [1902] and Chittenden [1904] were undertaken with a somewhat different object, namely, to ascertain whether health and activity could be maintained over prolonged periods on a mixed diet of low content in protein. Neumann made three experiments on himself, each lasting four to ten months. The total calories of the diet amounted to 30–40 per kilo. Chittenden's observations were made upon 26 individuals and the duration varied between six and nine months in different cases. The total calories varied between 35 and 45 per kilo body weight. Nitrogenous equilibrium was obtained by Neumann on an intake of 0·15 g. N per kilo and in Chittenden's experiments with 0·1 g. to 0·17 g. per kilo in the different individuals. There is no reason to suppose that these figures represent minima. The conclusion drawn is that a nitrogen intake of one- to two-thirds of the amount laid down by Voit is sufficient to maintain health and efficiency over the periods during which the observations endured.

During the last decade of the 19th century, physiologists were becoming increasingly alive to the possible significance of differences discovered in the elementary composition and chemical properties of the proteins, and the uniform value hitherto attributed to them in nutrition was coming under suspicion. Rubner [1897] appears to have been the first to formulate the view that proteins had different biological values. He used this conception to interpret some early experiments of his on the utilisation of various foodstuffs [1879] in which he had observed that less nitrogen was excreted in the urine on potato diets than on bread diets although the adverse N-balance was smaller on the former. In the same article Rubner expressed the opinion that the old search for a protein minimum must be fruitless since there will be, not one, but many minima according to the nature of the foodstuff used. He does not appear to have attributed such variation to any difference in chemical constitution although doubtless this possibility was in his mind. At this date the chemical constitution of the proteins was obscure although a number of amino acids had been isolated from the decomposition products of proteins and differences in the amounts of these had been observed. A little later Kossel and Kutscher [1900] determined the histidine, arginine, lysine and ammonia derived from the hydrolysis of a number of proteins and Kossel [1901] as a result of his own and others' work came to the conclusion that the habit of regarding protein as a physiological unit was unsound and that, since proteins possess different chemical compositions, they will also have different values for the organism.

About the same time discoveries were being made in another direction. The researches of Cohnheim [1901, 1906], Kutscher and Seemann [1901], Abderhalden and his co-workers and others, proved that a much more exhaustive break up of the protein molecule than had previously been supposed takes place in the small intestine prior to absorption, and that what the body really receives is a mixture of amino acids and simple polypeptides. Loewi [1902], and later, Abderhalden and Rona [1904] showed by means of feeding

experiments with previously digested proteins that the abiuret products of such digestion were capable of maintaining animals in nitrogen equilibrium. Abderhalden believed at first that such amino acids were at once utilised for building up of blood and tissue proteins and in conjunction with Samuely [1905] attempted to ascertain whether the composition of the body proteins varied with the character of food proteins. A horse was fed for three days on gliadin containing 36·5 % of glutamic acid, but no increase in the very low content of this amino acid in the serum proteins could be detected. From these results Abderhalden and Rona [1906] drew the conclusion that in the renewal of body proteins a proportion of the amino acids arising from the food will be left over unless the body is capable of synthesising one amino acid from another. Since this proportion will depend on the relative composition of the food and body proteins the protein minimum must also be variable.

These discoveries provided a theoretical basis for Rubner's empirical conclusions and a stimulus for the further investigation of the subject.

Meanwhile the rôle played by protein in satisfying the energy requirements of the body, and the effect on the protein minimum of insufficient as against abundant provision for these needs from non-protein sources, was becoming more clearly realised.

The discovery of the variable composition of proteins and of the fact that certain of them are almost entirely deficient in one or more of the amino acids was followed by interesting researches to determine to what extent the animal body could, by the practice of economy or synthesis, dispense with the missing complexes. The deficiency of gelatin in tyrosine was ascertained early and the absence from it of cystine and tryptophan was discovered when these amino acids became known as protein constituents. The inability of gelatin to preserve the body in nitrogenous equilibrium has been shown by many investigations, but as this aspect of the subject has recently been dealt with by one of us in this journal [Robison, 1922, 1] it is unnecessary to review it here.

The discovery of tryptophan by Hopkins and Cole [1901] and of the deficiency of this amino acid in zein by Osborne and Harris [1903] was followed by an experimental enquiry by Willcock and Hopkins [1907] to ascertain whether zein would serve as an exclusive source of nitrogen for mice and if not whether the addition of tryptophan would enhance the nutritive value of this protein. Zein alone failed to maintain the animals but this was achieved by supplementing with tryptophan.

Following this pioneer work Osborne and Mendel [1911] planned a lengthy investigation of the biological value of different proteins in the light of the new knowledge of their chemical structure. Their earlier work was carried out before the importance of accessory factors was recognised but they became aware from their experiments with individual proteins that some factor other than the supply of protein, salts and energy was complicating their observa-

tions. In the continuation of their researches, the results of which have appeared in some 30 papers in the *Journal of Biological Chemistry* from 1912 up to the present, the error due to absence of vitamins was obviated. Osborne and Mendel [1912–1920] confirmed and extended the observations of Willcock and Hopkins and showed that the addition of 3 % tryptophan to the zein given was sufficient to maintain rats over a period of 182 days, but that they failed to grow. When 2 % of lysine, in which zein is also deficient, was added, growth occurred. The problem of maintainance is therefore distinct from that of growth. The observations of Osborne and Mendel are some of the most important contributions to our knowledge of nutrition. The choice of rats enabled great numbers of experiments to be carried out and the extension of the periods of observation to cover a large fraction of the normal life of the animal. They prove that rats cannot supply some of the missing amino acids and that the minimum requirements and relative nutritive value of any particular protein depend upon the proportion of essential amino acids it contains. Their work also shows how a knowledge of the composition of particular proteins may be used for the economical adjustment of the nitrogenous portion of an animal's dietary by arranging that one protein shall compensate for the deficiencies of another.

The supplementary value of proteins from different sources has also been investigated by McCollum, Simmonds and Pitz, and McCollum, Simmonds and Parsons [1917 to 1921], whose observations, like those of Osborne and Mendel, were carried out upon rats. They found that cereal proteins could be satisfactorily supplemented by the proteins of milk, meat, kidney and casein and gelatin. Proteins of various leguminous seeds also usefully supplemented cereal proteins, *e.g.* wheat together with navy beans or peas.

The ultimate test of the nutritive adequacy of a protein is its capacity to nourish a young animal and provide for its complete growth and development and this, as far as the rat is concerned, is the criterion of the American investigators to which we have briefly referred. It may be surmised that, broadly speaking, conclusions arrived at from experiments on rats will be applicable in general to human nutrition. On the other hand the human mechanism may differ in detail. It will, for obvious reasons, be long before information as to the complete adequacy of individual proteins and quantitative data as to their biological values is forthcoming for human nutrition. In the meantime the findings of Osborne and Mendel, and McCollum and his co-workers have been applied with advantage to the feeding of stock.

From this more general survey of the subject we will now return to consider observations upon the minimum requirements for equilibrium when nitrogen is supplied in different forms. We have already referred to the observations of Rubner which led him to the conclusion that a different nitrogen minimum would be discovered for different proteins. This surmise was subsequently investigated in his laboratory by Karl Thomas [1909] who introduced the term "biological value." The expression "physiological value"

had been previously suggested by Voit and Korkunoff [1895] for a similar conception. Karl Thomas defined biological value as the number of parts of body nitrogen replaceable by 100 parts of the nitrogen of the foodstuff. Thomas's definition is not concerned with the relative digestibility of the protein. The replacement of the "Wear and Tear" quota was recognised as the only proper basis for comparison and in order to determine this value he fed himself on a carbohydrate diet (starch, sucrose, lactose) of high calorie value for periods of several days, during which the daily output of nitrogen in faeces and urine was determined. The figure to which this output fell was taken as his minimum requirements for the time being. During succeeding periods varying from one to four days a similar carbohydrate diet supplemented by a certain amount of the foodstuff under examination was taken and the N-intake and output determined as before. The N-intake was not as a rule kept constant and sometimes varied considerably on the different days. In most cases a negative N-balance was obtained. From the results Thomas calculated his biological value by three formulae based on the above definition, but differing from one another according to the way in which the nitrogen of the faeces is dealt with.

Some of Thomas's experiments lasted four to five weeks though no individual foodstuff was taken for longer than four days at a time. A period of one or two days on nitrogen-free diet was usually interposed between the experiments. Sixteen foodstuffs were investigated and their biological values recorded. These varied from 100 % in the case of milk to 30 % in the case of maize. We shall have occasion to discuss some of his results after dealing with our own experiments.

Shortly before this work of Thomas appeared the results of experiments upon dogs with a similar object were published by Michaud [1909]. The output of nitrogen on diets of dog-flesh, sugar and fat was compared with that on diets of horse-flesh, caseinogen, gliadin, and edestin and on the carbohydrate and fat alone. Nitrogenous equilibrium was attained with an amount of nitrogen in the form of dog-flesh equivalent to the nitrogen output on the nitrogen-free diet. Negative balances were obtained with the other proteins, the greatest being in the case of gliadin and edestin.

Zisterer [1910] found differences between caseinogen, flesh and gluten. These were however, in his opinion, too small to have practical significance.

Observations upon pigs were made by McCollum [1911]. These animals lend themselves to metabolism experiments of this kind as they will consume sufficient of a diet free from nitrogen to obtain the necessary calories over a considerable period. Their minimum nitrogen expenditure can therefore be determined with reasonable accuracy.

After a period of a week upon a diet of starch alone, the animals were fed for several days with the same ration to which was added a small amount of gelatin, zein, caseinogen or other protein. This was followed by the starch ration for a further period of some days. An amount of nitrogen in the

form of gelatin equal to that of the urine upon the starch diet was found to cover 39 % and in the form of zein 73 % of the animal's expenditure. The same amount of nitrogen in the form of cereal protein did not cause any rise in the nitrogen of the urine and with caseinogen the rise was small. McCollum's experiments seem to avoid all the obvious pitfalls and his results indicate a much higher biological value for cereal proteins when fed to the pig than those arrived at by Thomas's experiments upon himself.

Hindhede [1913, 2] concludes that nitrogen equilibrium may be attained on a diet of potatoes and margarine containing only 20 g. of digestible protein. The figure is, however, arrived at by deducting the nitrogen of the faeces from the intake. This method of calculation is not in accordance with our knowledge of the origin of a considerable portion of the faecal nitrogen and will furnish a too favourable balance sheet.

Hindhede [1914] vigorously contests the findings of Rubner and Thomas and claims to have attained nitrogenous equilibrium on as small an amount of protein in the form of bread as of potatoes. He declares as a result of his lengthy experiments that the proteins of potatoes, bread and meat can replace those of the body gram for gram. With Hindhede's criticisms of some of Thomas' experiments and treatment of his data, we are, for the most part, in agreement but must at the same time admit the justice of a great part of Rubner's equally severe criticisms of Hindhede's evidence, in particular, as regards the justification for assuming that all the nitrogen of the faeces represents undigested food proteins.

Abderhalden, Fodor and Röse [1915] carried out some experiments to determine the minimum requirement of nitrogen in the form of different kinds of bread and potatoes. The subject of the experiments was Hofrat Röse who possessed some peculiarly advantageous characteristics. Röse was accustomed to a monotonous diet, neither smoked nor drank alcohol and was in the habit of chewing his bolus 120 times before swallowing it. Experiments of three to eight days' duration were made on diets of potatoes, white wheaten bread, Swedish bread and kommiss brot, the last two being made from rye and containing bran. The experimental facts seem to us to warrant the conclusion that a gross intake of 4·5 g. of potato nitrogen, equal to 0·074 g. N per kilo, were adequate in the case of this Hofrat who chewed so long and so well. 9 g. N in the form of white wheaten bread was not quite sufficient and 10·8 g. N as supplied in the rye bread was only just enough to reach equilibrium. This is not, however, the interpretation placed upon the results by Abderhalden and his co-workers, who conclude that bread nitrogen is as good as potato nitrogen and that for both of them the minimum nitrogen requirement is round about 4 g. for a man of 60 kilos.

Rubner [1919] in the course of some studies of the capacity of certain vegetable nutriments to satisfy nitrogen needs, undertaken during the war, investigated different sorts of bread and the effect of milling to varying extent on the value of the product as a source of nitrogen. The paper covers a good

deal of ground and contains some particularly useful experiments with white wheat bread which can be compared with our own upon whole wheat. The bread was made, in one series, from white flour, 30 % milled, in the other of the same flour mixed with rye-bran to the extent of 30 %, so-called "Finkler brot." 10 g. of the N as contained in the fine flour and between 10 and 11 g. of that in the Finkler bread were adequate to maintain equilibrium.

Recently Sherman and his co-workers [1918, 1 and 2, 1919, 1920] have obtained results which are difficult to harmonise with those of Thomas. In experiments upon men and women, nitrogenous equilibrium was attained with an intake of 0·08 g. N per kilo, nine-tenths of this being supplied by cereal proteins and one-tenth by those of milk or apple. Wheat, maize and oats were found of equal value as a source of nitrogen and the view is taken that these cereal proteins possess a higher biological value than Thomas found. The effect of the supplementary action of the small quantity of milk may, in the light of the observations of Osborne and Mendel [1917] and of McCollum, Simmonds and Parsons [1921] be considerable.

Boruttau [1915] believes that the low value of cereals as a source of nitrogen is greatly improved when these are consumed without the removal of the bran, etc. The biological value of 145 % he obtained for the nitrogen of bran, is, in our opinion, an instance of the misuse of arithmetical formulae.

R. O. Neumann [1919] made an excellent experiment upon himself in 1917 in which he lived *exclusively* on rye bread, cane sugar and water for 40 days. Nitrogenous equilibrium was attained with 1000 g. bread and 300 g. cane sugar (= 9·9 g. N). The total calories of this diet amounted to 3630 or 63·8 per kilo. On raising the calorie value of the intake to 4434 (or 73 per kilo) the nitrogen excreted steadily fell to 7·3. This indicates that Neumann in a long continued experiment could maintain nitrogenous equilibrium on less than 7 g. of nitrogen in the form of rye-proteins if excess of calories were furnished by sugar. The experiment is also interesting as indicating the sensitiveness of the nitrogen balance to the addition of carbohydrate. This aspect of the experiment will be discussed later.

From a survey of the literature it is clear that certain of the proteins possess very different biological values both for growth and maintainance. There is, however, much uncertainty as to the degree to which the admixed proteins occurring in individual foodstuffs, where one protein to some extent complements the deficiencies of another, vary in value as a source of nitrogen.

The divergence of opinion is most marked when it is based upon metabolism experiments on man over limited periods.

OUR OWN OBSERVATIONS.

INTRODUCTORY.

We commenced our investigation lightheartedly with the comparatively modest object of re-determining the relative values of certain cereal proteins in human nutrition, in particular that of maize, in view of the significance given by Goldberger and others [1915, 1920] and Wilson [1921] to the low biological value of maize in the causation of pellagra. The difficulties in arriving at values which could justifiably be compared were soon, however, apparent and it became essential to investigate thoroughly the conditions under which valid results might be obtained. In so far as the problem can be solved by metabolism experiments on adult animals the one unexceptional way to determine the relative biological values of proteins would be to ascertain the minimum intake on which nitrogen equilibrium can be maintained in each case. This sounds simple but unfortunately a positive balance only tells one that the intake is sufficient but not how much it is in excess and a number of experiments have to be performed to ascertain the minimum quantity.

We were ourselves the subjects of the experiments. This is inconvenient but advantageous, for the experiments are exacting and necessitate constant supervision of one's actions if sources of error are to be avoided. The partial abandonment of the joys of life is to some extent compensated by interest in the results.

Nevertheless, the unnecessary multiplication of irksome experiments on one's self, each extending over many days, is a thing to be avoided and it would be very desirable if a couple of observations could be made and the minimum requirements calculated from these with sufficient accuracy. This is what Thomas attempted to do. But in adopting such a method an assumption is made, the truth of which is by no means self-evident, namely, that the value of any protein for biological purposes remains uniform whatever the amount taken. The assumption would be justified if the nitrogen were utilised in the first instance to form some complex, such as " Vorratseiweiss."

In this case the biological value, as pointed out by Abderhalden, would be determined by the ratio of the percentages, in food protein and body complex respectively, of that amino acid for which this ratio has the lowest value, unless the body has the capacity to synthesise that particular amino acid from others.

It might also be true if the nitrogen requirements are of varying nature so long as they are also indivisible, that is that no single requirement can be satisfied unless at the same time all the others are satisfied.

The former of these two conceptions would appear to have been accepted without question by Rubner and Thomas though the case of gelatin obviously could not be treated in this way. Gelatin was considered to be capable of sparing body protein to the extent of 30–40 % when fed in relatively small

amounts but unable to do more than this however much was taken. Its biological value, if calculated by any of Thomas's formulae would therefore appear quite appreciable when the intake was small but almost zero if the intake was very large. Yet, Boruttau [1919] has actually made use of these formulae to calculate the biological value of gelatin and has obtained a result of 58·2 %.

Another possible disturbing factor (which we have reason to suppose occurs) is the varying economy with which the body deals with the amino acids supplied to it, according to their abundance.

The various possibilities stated above may be made clearer by a diagram in which abscissae represent real nitrogen intake and ordinates the real nitrogen output.
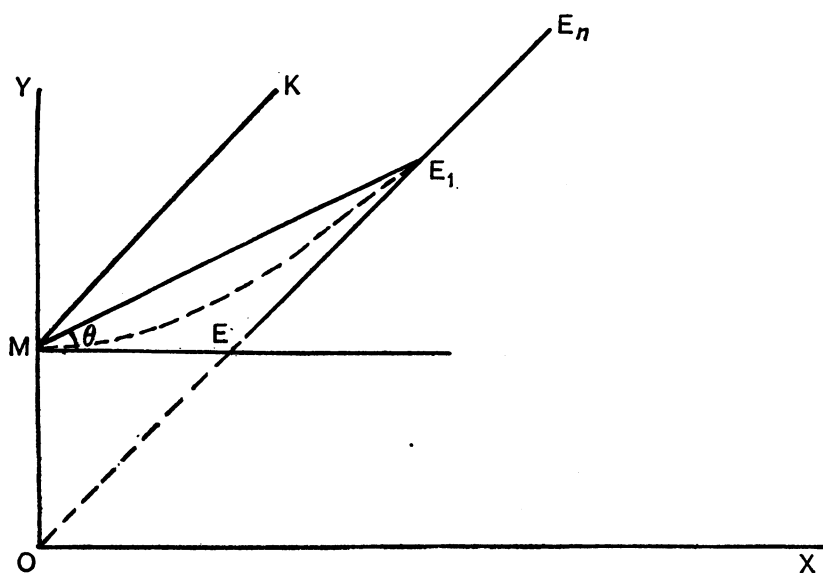
Fig. 1.

Let OM $(= m)$ be the output on a N-free diet of adequate fuel value. Then m is equal to the nitrogen minimum.

Suppose that an ideal protein (B.V. = 100) is fed in gradually increasing amounts and is utilised without waste. So long as the intake remains lower than m the output will remain constant and equal to m since the food protein saves an equal amount of body protein. The graph of intake and output will therefore be a line parallel to the x-axis and at E where $ME = MO$ the body will be in nitrogen equilibrium.

If now the intake be further increased, equilibrium will again result (unless the body is in a growing condition or has been previously starved of N) and the graph will now follow the line EE_n at an angle of 45° to the axis.

In the case of a protein of value less than that of the ideal protein just considered, equilibrium will not be attained on an intake equal to m but on

some greater amount e_1 (at the point E_1). On all amounts less than this, the output will exceed the intake and the graph will follow some line joining ME_1. Whether this line is straight or curved will depend on the conditions set out above, viz.:

(1) indivisibility of the nitrogen requirements of the body;

(2) uniform economy with varying nitrogen intake.

If these conditions are obtained the line ME_1 will be straight and its equation will be $y = m + x \tan \theta$ where y is the real output corresponding with any real intake x less than e_1.

For higher values of x the graph will follow the line E_1E_n.

Thomas's formulae can be very simply expressed in terms of θ; thus formula B

$$\text{B.V.} = 100 \, \frac{\text{Urine N in N-free diet} + \text{faeces N} + \text{balance}}{\text{N-intake}}$$

becomes

$$\text{B.V.} = 100 \, \frac{m + (x - y)}{x}$$

$$= 100 \, \frac{m + x - (m + x \tan \theta)}{x}$$

$$= 100 \, (1 - \tan \theta).$$

If the above conditions do not obtain, e.g. if a number of different amino acids are required for specific purposes, which are distinct and can be separately satisfied, the graph of a protein rich in certain of these acids but poor in others would be a curved line such as the dotted line joining M and E_1 in the diagram. This curvature would express the fact that a certain fraction of the body's needs could be satisfied by a smaller amount of this protein than would correspond with the amount required to obtain equilibrium. The angle θ and the biological value would then vary for different values of x.

The graph of a protein, unable by itself to satisfy any portion of the body's nitrogen requirements, would be a straight line MK parallel to OE_n, since the nitrogen output would always be equal to the intake $+ m$. For this line $\theta = 45°$ and the equation $y = m + x \tan \theta$ becomes $y = m + x$ while the biological value $= 100 \, (1 - \tan 45°) = 0$.

In our opinion there was very little reason for assuming that these graphs would necessarily prove to be straight lines. It is true that Thomas calculates his values from individual daily balances and takes the average of the results, but such daily balances are too variable and are subject to too great experimental errors to offer any satisfactory proof of the uniformity of the value. We therefore set out to obtain evidence on this question by determining as accurately as possible a number of points for the same protein but for different values of x. Our results will be considered later but we may here state that in the case of bread proteins the points do lie on, or close to, a straight line. In the experiments with nitrogen in the form of milk results were at first obtained indicating pronounced curvature of the line, and nitrogen equilibrium was not obtained until more than 11 g. of milk nitrogen was taken per day. By increasing the amount of carbohydrate however, so that the

fuel value of the diet was greatly in excess of requirements and the respiratory quotient greater than 1, equilibrium was finally reached with half this amount, and bearing in mind that when x, the intake, is very small, the physiological errors of experiment become relatively great, the observations could now perhaps be expressed by a straight line. As long as any doubt exists of the rectilinear character of the line ME it will obviously be prudent to place reliance only upon observations in which x and y are as large as possible short of equality.

FACTORS WHICH MUST BE CONSIDERED IF VALID RESULTS
ARE TO BE OBTAINED.

1. *The time required to reach a uniform N-output on a constant intake.*

The effect of the previous diet upon the N-output and the length of time required to reach a constant output on a constant intake which is either greater or less than that of the preceding period, was clearly demonstrated by the old experiments of C. Voit [1866, 1867]. The N-output of a dog during the first days of starvation varied with the amount of protein in the previous diet but fell gradually until a relatively constant figure was reached on the fifth or sixth day. When the dog was given a constant meat diet for some days and then a considerably greater (or less) amount daily during a further period a similar gradual increase (or decrease) in the N-output was observed during five or six days before equilibrium again set in at the new level. These observations have been repeatedly confirmed by Grubner [1901], Landergren [1903], Kinberg [1911] and many others.

The rapidity with which the nitrogen excretion diminishes obviously depends on the difference between the N-intake during the experimental and the preceding periods and will be greatest when a period of nitrogen starvation follows one of high protein intake or *vice versa*. There is no reason to suppose, however, that this gradual change is ever replaced by an immediate jump to the new level even when the difference between the two planes of N-intake is but small, though naturally the absolute amounts of the variations will be correspondingly less.

Whether the N-output is also influenced by the nature as well as the amount of the protein taken in the foregoing period is more difficult to decide. If part of the nitrogen of the previous diet is stored up in any form that can be utilised by the body (*e.g.* amino acids) and not merely in the form of unexcreted end products, we should expect the N-output during the first few days of the succeeding period to be influenced thereby—unless during both periods the body is in N-equilibrium. For example if the diet during the first period contains 10 g. of N from caseinogen, and during the second period a negative balance occurs on 10 g. of N from zein, the amount of this negative balance might very well be less during the first few days of the zein diet than on the latter days of the same period owing to the supplementary action of amino acids stored up during the caseinogen diet. The results of experiments [Robison, 1922] in which a diet containing gelatin as the sole protein followed

a diet of mixed proteins suggest that this does occur, and that therefore when the diet is changed in any way—either in amount or nature of the protein—the N-output cannot be considered to represent that of the second diet until some days have elapsed.

It follows that metabolism experiments are subject to error from these causes and that the error diminishes as the duration of the experiment increases. As a compromise, we have exlcuded from calculation the figures for the first three or four days in arriving at the N-output. It is also advisable that the N-intake should not vary during the experiment. Most of Thomas's experiments are subject to both these sources of error.

2. *The time required for the elimination of errors due to the fluctuation in the N-output.*

The very considerable fluctuations that may occur in the amount of nitrogen excreted in the urine by men receiving an absolutely constant diet have been noted by Bornstein [1898], Atwater and Benedict [1902] and by Falta [1906], by all of whom the cause was considered to be psychical. Atwater and Benedict also noted the increased diuresis which often accompanied a high N-output and thought it probable that the diuresis was the direct result of the psychical stimulus and the cause of the increased N-elimination. Falta observed variations of 4–5 g. in the N-output on individual days although in equilibrium over the period as a whole.

Neumann [1899] studied the influence of variations of the urinary flow upon the daily output of nitrogen, the intake remaining constant. When diuresis was produced by increasing the water drunk from one to three litres, the N-output increased from 10·5 to 14·3 g. and did not reach the original level until the third day.

In almost all our experiments such fluctuations, amounting frequently to 25 % of the mean output, have occurred and not least in those experiments in which the most rigorous attention has been paid to constancy of diet and fluid intake, and to regularity in the mode of life.

At first we also were disposed to attribute these fluctuations to increased diuresis but the frequent lack of any correlation between the two compelled us to modify our opinion. Some factors (*e.g.* mental strain or excitement) may possibly affect both diuresis and N-output, but the latter does not always coincide with the increased volume of urine and may even vary in the opposite sense. In one experiment, for example, the minimum N-output corresponded with the maximum volume of urine.

Judging from the experiments of Voit and other workers on dogs and of McCollum on pigs, it would appear that in the case of these animals the fluctuations in N-output on a constant diet are usually less considerable than with man. It is clear that calculations based upon the nitrogen balance sheet for single days, a procedure frequently resorted to, are subject to very large errors and that these can only be eliminated by taking the average results over a number of days.

3. *The necessity for abundant energy supply.*

It has been universally recognised that proteins will be used as fuel unless an adequate supply of fat and carbohydrate is provided, but there have been different opinions as to what constitutes adequacy in this respect. In their search for the protein minimum some of the earlier workers considered it necessary to supply a diet of fuel value very greatly in excess of the energy requirements of the organism. Thus in Klemperer's [1889] experiments on two young men, a diet of 5020 calories, equal to about 75 calories per kilo was given. Sivén, however, was of the opinion that such excess was unnecessary and that the minimum could be reached without increasing the calorie value of the diet above the normal. The fuel value of Sivén's diets was equal to about 40 calories per kilo body weight.

Hindhede's [1913, 2] attitude is somewhat difficult to understand. He considers that an abundant calorie intake is necessary if the protein minimum is to be attained, but that this minimum will vary with the calorie value of the diet. He does not believe that a quiet old man, for whom a diet of 1500–2000 calories is sufficient, can have the same minimum as an active young man who requires a diet of 3000–5000 calories. From the results of his experiments, he calculates by simple proportion, the minimum for a standard diet of 3000 calories. As on a diet of 3900 calories F. Madsen's minimum was equal to 25 g. of digestible protein so, according to Hindhede, for 3000 calories the minimum would be 19 g. of digestible protein.

Rubner [1919] has criticised this procedure, and in our opinion justly, on the ground that it is unwarranted by the facts, and considers that the values so calculated to 3000 calories possess no scientific basis.

Rubner's own conclusions are that the N-minimum may sometimes be reached when no more than a third of the total energy requirements are satisfied, in other cases only when they are fully met, while in others a diet considerably in excess of these requirements will be necessary. These differences he considers are due to the varying nutritional condition of the body cells. The minimum is, however, most easily reached on an abundant carbohydrate diet.

In our own experiments on a diet nearly N-free we appeared to reach our N-minimum with an intake of 45–50 calories per kilo body weight of which about one-third was taken in the form of fat, whereas on a diet of milk (with additional carbohydrate), equilibrium was not readily obtained until the fuel value was increased to about 55 calories per kilo of which only 10 % was in the form of fat.

The effect of diets containing varying proportions of fat and carbohydrate on the protein minimum has been studied by Zeller [1914], who found that the mixed diet was just as efficacious in reducing the consumption of body protein as one of carbohydrate alone, so long as the proportion of carbohydrate to fat did not fall below 1 : 4.

Neumann's [1919] experiment upon himself, with a diet composed of bread and sugar, affords a striking demonstration of the effect of excess of carbohydrate in lowering the protein minimum. It is obvious that Prof. Neumann readily stores fat. Otherwise, he could not consume 73 calories per kilo over a period of three weeks, unless doing hard work. In his case, presumably, a greater excess of carbohydrate would be required to maintain the blood sugar at a high level than in our own, owing to the greater greed of his connective tissue cells.

It would seem, therefore, that the most certain way of determining the protein minimum would be to take a diet consisting mainly of carbohydrate and so much in excess of the energy requirements that the blood sugar is maintained high, the liver and muscles are kept well stocked with glycogen and the surplus is being stored as fat, as indicated by a respiratory quotient above unity. This was accomplished in the latter part of our experiment on milk. It is by no means easy for one of the meagre habit of the subject of the experiment (C.J.M.) as it increases the distaste for the sufficiently unappetising ration of starch and lactose and if persisted in too enthusiastically it produces unpleasant symptoms.

4. *Reduction of the nitrogen in the basal diet to a minimum.*

The carbohydrate (starch, lactose, etc.) and fat, etc. which form the basal diet for these experiments are nominally but not absolutely nitrogen-free. The amount of nitrogen taken in this form can of course be estimated but its biological value is unknown and this complicates the results. It is therefore important to reduce the nitrogen in the basal diet to a minimum by careful selection of the most suitable forms of such foods.

Most observers have neglected to take account of the nitrogen in the starch, etc. fed. As large quantities of such basal ration are consumed it may not be negligible in the case of experiments in which small quantities of some protein are being given.

5. *Accessory food factors and inorganic salts.*

The duration of metabolism experiments is limited by the difficulty of providing an adequate supply of the accessory food factors. The ill effects of long continued low-protein diets observed in some of the older animal experiments was no doubt sometimes due to the deficiency of one or other of these factors.

Fat soluble *A* can be introduced in the form of rendered butter fat or cod liver oil, and water soluble *C* in the form of lemon juice, the nitrogen content of which is very low, but we have found no method of introducing the water soluble *B* without an undue amount of possibly very valuable nitrogen.

When the diet is deficient in inorganic salts or is such as to afford an acid ash, adjustment by suitable amounts of a salt mixture is essential. McCollum and Hoagland [1913] found that the endogenous metabolism of the pig reached its lowest level when the animal was given an abundant carbohydrate diet together with a salt mixture of an alkaline character. When an acid salt mixture was given the urinary output rose, the increase occurring in the amount of ammonia. They concluded that this animal is not able to use the nitrogen of the urea fraction for the neutralisation of acid.

6. *The apportioning of the nitrogen in the faeces.*

The difficulty of correctly apportioning the nitrogen in the faeces to unabsorbed food nitrogen and excretion from the alimentary tract respectively, is the limiting factor in most experiments in which the total intake of nitrogen is small, and the way in which different observers treat the faecal nitrogen has given rise to much controversy and recrimination.

The daily nitrogen output in the faeces on a protein-free diet usually amounts to about 1 g. On other diets the amount may be considerably greater than this and the question arises—what is the significance of this excess? Does it represent unabsorbed food residues or increased loss of body nitrogen? This difficulty was recognised and discussed by Karl Thomas, whose three formulae for the calculation of the biological value of protein differ only in the assumptions that are made with regard to this point. In formula A the whole of the nitrogen of the faeces is assumed to represent unabsorbed food; in formula B it is assumed to arise entirely from the body, while in formula C 1 g. N (which is taken as the average output on protein-free diet) is assumed to be body nitrogen, any excess over this amount being ascribed to unabsorbed food.

Rubner [1915] has investigated this problem in connection with his researches on the digestibility of various foodstuffs. He has devised a method for estimating the amount of nitrogen in the faeces present in the form of undigested food residues (vegetable cell membranes) by making use of the insolubility of the latter in acid alcohol and in a concentrated solution of chloral hydrate in which he states bacteria, epithelial cells, etc. are dissolved. He considers that the rest of the nitrogen comes from the body and represents metabolic products, and he concludes that such body nitrogen forms a very considerable proportion of the increase in the total nitrogen of the faeces that commonly occurs when the diet consists largely of whole cereals, vegetables or fruit.

Rubner found that on a vegetable diet nearly half the total nitrogen of the faeces was soluble in acid alcohol whilst on a diet consisting chiefly of animal foodstuffs the proportion of soluble nitrogen was still greater. We have obtained similar results with faeces resulting from a mixed diet, but do not

know in what form the whole of this soluble nitrogen is present, and are there-
fore not able to·draw definite conclusions as to its origin.

So long as this uncertainty remains, the nitrogen of the faeces will be the
limiting factor for the accuracy of such experiments as those here described.
We have therefore followed Thomas's plan and have calculated our results
in two ways; the first, assuming that the whole of the nitrogen of the faeces
comes from the body and that this amount plus the urine N on N-free diet
represents the body's minimum requirements under the conditions of the
experiment; the second, assuming that the amount by which the nitrogen of
the faeces exceeds the average amount excreted on a N-free diet represents
unabsorbed food and is therefore to be subtracted from the total intake in
order to arrive at the true nett intake, *i.e.* the absorbed nitrogen. The truth
will probably lie somewhere between these two extremes.

EXPERIMENTAL.

Firstly as to general procedure. The N of all foodstuffs and beverages used
was determined. The food was weighed in the same condition as that in which
the N was determined and the total intake of N recorded in the tables can
be relied upon to plus or minus 0·01 g. The urinary excretion of each 24 hours
was collected in the presence of toluene, weighed, and duplicate determinations
of the N content made. Care was exercised to see that invalid sampling from
the deposition of either urates or ammonium magnesium phosphate did not
occur. The faeces, after collection each day, were mixed with some H_2SO_4 to
prevent decomposition and possible loss of NH_3 and great care was taken at
the end of each period to ensure the validity of the samples taken for analysis.
N determinations were made upon about 10 g. in duplicate, only closely con-
cordant results being accepted.

The evacuation of the intestines is not usually so regular and complete
that any great value can be placed on the figures for the nitrogen excretion
on individual days. The best that can be done is to determine the nitrogen
in the faeces for the whole experimental period and from this to calculate the
average daily output. The latter may vary within rather wide limits even
when the diet contains little or no nitrogen, and appears to depend to some
extent on the bulk and character of the faeces. In order that these might
be kept as uniform as possible agar-agar was taken when the diet consisted
wholly or largely of completely digestible foodstuffs.

Food was taken in approximately equal amounts three times a day at
the customary hours, and we led our usual life. In the experiments upon
milk we abandoned all attempts to make our basal ration of fat and carbo-
hydrate resemble a repast and drank a suspension of uncooked corn-starch
in a saturated solution of lactose. This was followed by an alkaline salt
mixture and 2 g. of agar-agar. In this way 600 calories were contained in
about a tumbler full. Microscopical examination of the faeces showed that the

starch was completely digested. When the starch-lactose mixture exceeded 250 g. at one meal some glycosuria occurred temporarily. On the N-minimum experiment the faeces were olive green as the bile pigment was unreduced. Little gas was formed.

The following are the essential data regarding the subjects of the experiments, ourselves:

> C.J.M. Age 56. Weight 61 kilo. Height 183 c. Very thin, stores fat with difficulty. Mode of life: laboratory work. Exercise: lawn tennis before breakfast for three-quarters of an hour and about three miles walk during the day.

> R.R. Age 37. Weight 59 kilo. Height 173·5 c. Spare, does not store fat readily. Usual mode of life consists chiefly in laboratory work. Very little regular exercise beyond daily walk of two to four miles.

Minimum nitrogen expenditure on carbohydrate-fat diet.

Our minimum nitrogen expenditure was determined in two experiments, during which our diet was as nearly as possible nitrogen-free. In the vain attempt to make this diet appetising much labour was expended in endeavouring to prepare the food in a varied and attractive manner. Biscuits made of corn starch with 20 % of fat proved quite palatable when taken in small quantities but nauseating in bulk. A biscuit, whose chief defect was its hardness, made from starch, dextrin and a little fat was finally adopted and formed, with butter and honey, the chief article of diet. A starch mould flavoured with lemon juice was also taken. Weak tea with lemon and sometimes black coffee and a little vermouth was drunk. After the first few days of this diet no desire was felt either for this or for any other food, nor did the sight of our first normal meal at the close of the experiment arouse any appetite. The drinking of a glass of hot milk, however, excited in a few minutes a very keen appetite and desire for food. The quantities of the individual constituents of the diet varied somewhat from day to day but were always accurately measured and noted. Those for a single typical day are set out in Table I while the total nitrogen intake and fuel value of the diet for each day are shown in Table II in which are also set out the daily output of nitrogen in the urine and faeces and the nitrogen balance. In this and in all other experiments the nitrogen in the tea and coffee has been assumed to consist chiefly of caffeine and to be excreted unchanged in the urine. It has therefore been subtracted in all cases from the total intake and from the urinary nitrogen output. Any error involved in this method of treatment must be of negligible dimensions.

The nitrogen in the urine fell steadily until the last day of the experiment when a rather considerable rise occurred in the case of both C.J.M. and R.R. This is probably to be explained by the fact that, owing to the difficulty of consuming the food when all appetite was in abeyance, the fuel value of the diet was reduced during the last day or two.

Table I.

	N-minimum 2.		Diet R.R. 4. xii. 20		
Foodstuff	N per 100 g.	Calories per 100 g.	Weight g.	N g.	Calories
Corn starch	0·042	360	280	0·118	1008
Dextrin	0·065	360	50	0·033	180
Butter	0·080	775	90	0·072	698
Margarine (rendered)	0·010	900	15	0·002	135
Honey	0·023	327	55	0·013	180
Sucrose	—	395	25	—	99
Lactose	0·013	370	65	0·008	241
Lemon juice	0·067	40	30 cc.	0·020	12
Vermouth	0·005	140	25 ,,	0·001	35
Tea infusion	0·006	—	1200 ,,	0·072	—
Agar-agar	0·242	·—	15 g.	0·036	—
Salt mixture	—	—	5 ,,	—	—
			Total	0·375	2588
			Excluding tea N	0·303	45 per kilo

Table II.

N-minimum 1. Subject: R.R. Fluid Intake: 2000–2500 cc.

		Daily Intake			Output			
Date 1920	Body weight k	N (excluding tea and coffee) g.	N tea and coffee g.	Calories per kilo	N urine g.	N faeces g.	N total g.	Balance N g.
Nov. 15	58·5	0·30	0·16	56	8·64	—	—	—
,, 16		0·28	0·06	54	5·31	1·05	6·36	− 6·08
,, 17		0·30	0·15	55	3·63	,,	4·68	− 4·38
,, 18		0·25	0·10	51	2·66	,,	3·71	− 3·46
,, 19		0·22	0·06	43	2·25	,,	3·30	− 3·08
,, 20	57·7 (21. xi)	0·23	0·15	49	2·82	,,	3·87	− 3·64

N-minimum 2. Subject: R.R. Fluid Intake: 4000 cc.

,, 29	58·6	0·27	0·07	49	8·79	1·13	9·92	− 9·65
,, 30	(23. xi)	0·30	0·07	50	4·71	,,	5·84	− 5·54
Dec. 1		0·30	0·08	51	3·46	,,	4·59	− 4·29
,, 2		0·35	0·08	51	2·71	,,	3·84	− 3·49
,, 3		0·32	0·09	52	1·99	,,	3·12	− 2·80
,, 4		0·30	0·07	45	2·17	,,	3·30	. − 3·00
,, 5	57·7	0·27	0·06	44	2·01	,,	3·14	− 2·87
Average of last 3 days		0·30	0·07	—	2·06	1·13	3·19	− 2·89

N-minimum 1. Subject: C.J.M. Fluid Intake: 2000 cc.

Nov. 14	61·7	0·33	0·20	56	8·61	—	—	—
,, 15		0·30	0·20	47	4·89	1·24	6·13	− 5·83
,, 16		0·30	0·20	47	3·28	,,	4·52	− 4·22
,, 17		0·38	0·21	52	3·28	,,	4·52	− 4·14
,, 18		0·32	0·21	46	2·48	,,	3·72	− 3·40
,, 19		0·30	0·21	45	2·25	,,	3·49	− 3·19
,, 20	60·9 (21. xi)	0·21	0·18	35	2·49	,,	3·73	− 3·52

N-minimum 2. Subject: C.J.M. Fluid Intake: 4000 cc.

,, 29	61·9	0·34	0·31	59	8·89	—	—	—
,, 30		0·34	0·27	55	4·78	—	—	—
Dec. 1		0·34	0·33	55	3·40	1·17	4·57	− 4·23
,, 2		0·33	0·32	53	2·41	,,	3·58	− 3·25
,, 3		0·35	0·32	52	2·51	,,	3·68	− 3·33
,, 4		0·35	0·29	52	2·40	,,	3·57	− 3·22
,, 5	60·5	0·33	0·26	48	2·13	,,	3·30	− 2·97
Average of last 3 days		0·34	0·29	—	2·34	1·17	3·51	− 3·17

On plotting the amounts of urine nitrogen as ordinates against the time in days as abscissae it became apparent that all the points except the last would lie on, or close to a curve for which a simple logarithmic expression was found (Figs. 2 and 3). Among several possible interpretations of this curve is the simple one that the falling nitrogen output represents washing out of some metabolic products from the tissues, the amount washed out each day being proportional to that still present.

A second similar experiment was therefore carried out in order to confirm this result and also to discover whether the steepness of the curve could be altered by drinking large quantities of water and thus greatly increasing the volume of the urine. Apart from the volume of total fluid, which was doubled, the diet did not differ from that taken in the first experiment. The results are set out in Table II.

The regularity in the fall of the nitrogen output was again observed but the change in the rate of this fall was very small.

The average outputs in urine and faeces on the last three days of this experiment have been taken as representing the minimum nitrogen expenditure on such a diet. Whether this amount also represents the minimum expenditure on a diet that is absolutely nitrogen-free will depend on the biological value of the small amount of nitrogen in the food consumed during the above experiment. If this has a value of 100 %, *i.e.* if it can replace and therefore spare an equal amount of body nitrogen, the output thus determined will be equal to the real minimum expenditure. If the value of the food nitrogen is zero, *i.e.* if it is unable to satisfy any fraction of the body's requirements, it must be excreted in addition to the amount representing the latter and the minimum expenditure will therefore be equal to the observed output less the full amount of the nitrogen intake. Probably this nitrogen, which was present chiefly in the corn starch and butter, has a value intermediate between 100 and zero. The minimum nitrogen expenditure will therefore be some amount between those shown in the last two columns below.

Minimum nitrogen expenditure.

Subject	Urine g.	Faeces g.	Total output g.	Total output – intake g.
C.J.M.	2·34	1·17	3·51	3·17
R.R.	2·06	1·13	3·19	2·89

THE BIOLOGICAL VALUE OF THE PROTEINS OF WHOLE WHEAT.

A series of experiments was carried out with whole wheat flour with two objects in view:

(1) to determine the Biological Value of the wheat proteins from the minimum amount with which nitrogen equilibrium can be attained;

(2) to discover whether the Biological Value is uniform for varying amounts of wheat nitrogen.

The first of these has been investigated upon man by other workers, whose

results will be considered with our own. The great practical importance of this question and the astonishing divergence between the results of previous investigations were sufficient reasons for further study.

Method of experiment.

The large variations in the percentage of water, and consequently of nitrogen, in different parts of a loaf of bread and the difficulty of obtaining a satisfactory sample, render it impossible to estimate the total nitrogen content of the loaf with sufficient accuracy for these experiments. We decided therefore, to base our calculations on the flour and to bake the bread ourselves. By suitable manipulation it was found possible to prepare loaves from 500 g. of flour with a maximum loss of less than 0·1 %. The other materials used were butter (5 %), salt, baking powder (prepared from tartaric acid, sodium bicarbonate and corn starch) and water. The loaves were baked in the laboratory for about one hour at a temperature of 240°–250°, and were only very slightly browned so that no appreciable loss of nitrogen can have occurred during the baking. For the first period, a somewhat coarsely ground whole wheat flour containing 1·85 % N was used but on increasing the daily ration from 300 g. to 450 g., considerable discomfort was experienced from the large particles of bran, and the bread was poorly absorbed. For the second and remaining periods a very finely ground flour prepared from a mixture of English and foreign whole wheat was employed. We found it very palatable and well absorbed, as the figures for the nitrogen in the faeces indicate. Only when the daily consumption had been raised to 550 g. and the total calories to 63 per kilo did we experience any discomfort.

The experiment was carried out in duplicate on ourselves and commenced with a total nitrogen intake of nearly twice the amount of our minimum requirements. On this diet a considerable negative balance occurred and the amount of wheat nitrogen was therefore increased during successive periods until equilibrium was finally attained.

The daily ration of flour was kept constant during each separate period of the experiment, but some latitude was permitted in the amounts of the remaining constituents of the diet. The actual quantities taken were, however, measured and the variations in any one period were not such as to affect appreciably the total nitrogen intake or greatly alter the fuel value of the diet. The diet set out in Table III shows the average amounts consumed by R.R. from the 24th to the 31st of October but except for the quantity of flour it would with slight variations serve for the whole experiment.

The experimental results are set out in Tables IV and V.

As in the previous experiments it has been assumed that the nitrogen consumed in tea and coffee would be excreted unchanged and the amount has therefore been subtracted from both intake and output (urine N).

During certain periods indicated by the letter (A) in the first column of

Table III.

Foodstuff	Whole wheat. N per 100 g.	Whole wheat. Calories per 100 g.	R.R. Diet during period 5, 24–31 Oct. 1920 Weight g.	R.R. Diet during period 5, 24–31 Oct. 1920 N g.	R.R. Diet during period 5, 24–31 Oct. 1920 Calories
Flour, whole wheat	2·162	360	450	9·730	1620
Corn starch	0·042	360	36	0·015	130
Butter	0·080	775	56	0·045	434
Dripping	0·016	885	80	0·013	708
Honey	0·023	327	40	0·009	131
Marmalade	0·052	341	20	0·010	68
Cane sugar	—	395	56 ,	—	221
Lemon juice	0·067	40	20 cc.	0·013	8
Tea	0·006	—	960 ,,	0·058	—
Coffee	0·041	—	100 ,,	0·041	—
Vermouth	0·005	140	25 ,,	0·001	35

Total 9·935　3355

Excluding tea and coffee N 9·836　　58 per kilo

Table IV.

Diet: whole wheat. Subject: C.J.M.

Period	Date 1920	Body weight k	Daily Intake N bread g.	Daily Intake N total g.	Daily Intake Calories per kilo	Daily Output N urine g.	Daily Output N faeces g.	Daily Output N total g.	Balance N g.
1. (A)	Sept. 30–Oct. 2	61·80 (26. ix)	5·55	5·81	46	—	—	—	—
(O)	Oct. 3–5	61·50 (3. x)	,,	5·70	44	5·62	2·14	7·76	− 2·06
2. (A)	,, 10–14	60·45 (10. x)	8·32	8·63	56	—	—	—	—
	,, 15		,,	,,	,,	8·26	2·17	10·43	− 1·80
	,, 16		,,	,,	,,	7·62	,,	9·79	− 1·16
	,, 17	61·44	,,	,,	,,	6·98	,,	9·15	− 0·52
		Average	8·32	8·63	56	7·62	2·17	9·79	− 1·16
3. (A)	,, 18		8·65	8·97	57	7·86	2·35	10·21	− 1·24
	,, 19		,,	,,	,,	7·25	,,	9·60	− 0·63
	,, 20		,,	,,	,,	7·15	,,	9·50	− 0·53
		Average	8·65	8·97	57	7·42	2·35	9·77	− 0·80
4. (A)	,, 21	60·65	9·73	9·98	53	7·25	1·74	8·99	+ 0·99
	,, 22		,,	,,	,,	7·92	,,	9·66	+ 0·32
	,, 23	61·03	,,	,,	,,	7·30	,,	9·04	+ 0·94
		Average	9·73	9·98	53	7·49	1·74	9·23	+ 0·75
5. (O)	,, 24	60·95	9·73	9·85	60	8·65	1·94	10·59	− 0·74
	,, 25		,,	,,	,,	9·23	,,	11·17	− 1·32
	,, 26		,,	,,	,,	8·77	,,	10·71	− 0·86
	,, 27		,,	,,	,,	7·84	,,	9·78	+ 0·07
	,, 28		,,	,,	,,	8·93	,,	10·87	− 1·02
	,, 29		,,	,,	,,	8·63	,,	10·57	− 0·73
	,, 30		,,	,,	,,	7·68	,,	9·62	+ 0·23
	,, 31	61·60	,,	,,	,,	7·67	,,	9·61	+ 0·24
		Average	9·73	9·85	60	8·43	1·94	10·36	− 0·51
6. (O)	Nov. 1		11·89	12·00	61	9·13	2·53	11·66	+ 0·34
	,, 2		,,	,,	,,	8·33	,,	10·86	+ 1·14
	,, 3		,,	,,	,,	9·83	,,	12·36	− 0·36
	,, 4	61·40 (5. xi)	,,	,,	,,	9·63	,,	12·16	− 0·16
		Average	11·89	12·00	61	9·23	2·53	11·76	+ 0·2

the table, a quantity of stewed apples (225 g. raw fruit containing 0·11 g. N) was included in the diet. The letter (O) indicates that this fruit was omitted.

For several days prior to Sept. 30, 1920 (period 1) a bread diet containing about 7·3 g. N had been taken in order to eliminate the disturbing effect of the previous high protein dietary. For the same reason the first three days of this period and the first five days of period 2 have been excluded in calculating the average nitrogen balance.

Table V.

Diet: whole wheat bread. Subject: R.R.

Period	Date 1920	Body weight k	Daily Intake			Daily Output			Balance N g.
			N bread g.	N total g.	Calories per kilo	N urine g.	N faeces g.	N total g.	
1. (A)	Sept. 30–Oct. 2	58·65 (27. ix)	5·55	5·82	48	—	—	—	—
(O)	Oct. 3–5	57·70 (6. ix)	,,	5·71	49	5·62	1·47	7·09	− 1·38
2. (A)	,, 10–14	58·20 (13. x)	8·32	8·57	54	—	—	—	—
	,, 15		,,	,,	,,	7·21	1·98	9·19	− 0·62
	,, 16		,,	,,	,,	8·30	,,	10·28	− 1·71
	,, 17	58·13	,,	,,	,,	6·41	,,	8·39	+ 0·18
		Average	8·32	8·57	54	7·31	1·98	10·29	− 0·72
3. (A)	,, 18		8·65	8·91	56	8·06	1·25	9·31	− 0·40
	,, 19		,,	,,	,,	8·70	,,	9·95	− 1·04
	,, 20		,,	,,	,,	7·79	,,	9·04	− 0·13
		Average	8·65	8·91	56	8·18	1·25	9·43	− 0·52
4. (A)	,, 21–23	57·89 (21. x)	9·73	10·00	58	8·32	1·70	10·02	− 0·02
5. (O)	,, 24		9·73	9·84	58	8·41	1·81	10·22	− 0·38
	,, 25		,,	,,	,,	8·25	,,	10·06	− 0·22
	,, 26		,,	,,	,,	10·51	,,	12·32	− 2·48
	,, 27	57·75	,,	,,	,,	8·83	,,	10·64	− 0·80
	,, 28		,,	,,	,,	7·67	,,	9·48	+ 0·36
	,, 29		,,	,,	,,	9·74	,,	11·55	− 1·71
	,, 30		,,	,,	,,	9·50	,,	11·31	− 1·47
	,, 31	57·44	,,	,,	,,	8·54	,,	10·35	− 0·51
		Average	9·73	9·84	58	8·93	1·81	10·74	− 0·90
6. (O)	Nov. 1		11·89	11·98	63	9·30	2·68	11·98	0
	,, 2		,,	,,	,,	9·75	,,	12·43	− 0·45
	,, 3		,,	,,	,,	8·86	,,	11·54	+ 0·44
	,, 4	57·70 (5. xi)	,,	,,	,,	9·46	,,	12·14	− 0·16
		Average	11·89	11·98	63	9·34	2·68	12·02	− 0·04

A marked rise in the nitrogen output occurred with both C.J.M. and R.R. at the beginning of period 5 and the coincidence of the omission of the apples that had been included in the diet during the preceding period led us to consider whether there was here any relation of cause and effect. Two possibilities suggest themselves: (1) the small amount of apple protein (0·11 g. N) might possess high value to supplement the wheat protein; (2) the alkaline

ash of the fruit would partially neutralise the acid ash of the bread and this might affect the nitrogen expenditure. Neither of the above appears adequate to explain the facts and further, the results obtained during period 4 do not agree any better with those for periods 2 and 3, in which apples were included in the diet, than with those for period 5 in which they were omitted. The increased output must therefore, like the variations which occur from day to day, be left for the present unexplained.

From the results of these experiments we have calculated the biological value of the wheat proteins by two formulae, which differ only in the assumption made with regard to the nitrogen of the faeces. Both are based on Thomas's definition of this value as the number of parts of body nitrogen spared by 100 parts of the nitrogen of the food, and, when reduced to their simplest form, can be thus expressed

$$\text{B.V.} = 100 \frac{\text{Body N spared}}{\text{Food N absorbed}} = 100 \frac{\text{Balance }[P] - \text{Balance }[M]}{\text{Intake }[P] - \text{Intake }[M]}$$

where P signifies the experiment with the protein under investigation and M the nitrogen minimum experiment.

This correction for the small amount of nitrogen in the diet of the N-minimum experiment is strictly accurate only if certain provisos hold, viz. (1) that the same amount of nitrogen in the same form, or of the same biological value, enters also into the second diet (P); (2) that the value of this nitrogen is not increased by supplementary action with the other proteins of the second diet.

In our experiments with wheat and milk proteins the first proviso is partly but not entirely satisfied. Whether or not the second is also satisfied cannot be decided. The method, however, certainly involves a less error than if the N-intake in the N-minimum experiment is altogether ignored.

When the different assumptions as to the faeces are made the two formulae become:

$$\text{I. B.V.} = 100 \frac{\text{Balance}[P] - \{\text{Intake }[M] - (\text{Urine N }[M] + \text{Faeces N }[P])\}}{\text{Intake }[P] - \text{Intake }[M]}.$$

$$\text{II. B.V.} = 100 \frac{\text{Balance }[P] - \text{Balance }[M]}{\text{Intake }[P] - (\text{Faeces N }[P] - \text{Faeces N }[M]) - \text{Intake }[M]}.$$

In I the whole of the food nitrogen is assumed to have been absorbed so that the total intake is also the real intake. In calculating the minimum expenditure corresponding with the period in question the faeces N for this period is added to the urine N $[M]$ and the intake $[M]$ subtracted from the sum. This formula corresponds with Thomas's formula B.

In II when the nitrogen of the faeces is in excess of that occurring in the N-minimum experiment this excess has been assumed to represent unabsorbed food and has been subtracted from the total intake to obtain the real intake. The minimum expenditure has been taken as the actual balance on N-minimum diet, $i.e.$ Urine N $[M]$ + Faeces N $[M]$ − Intake $[M]$. This formula corresponds with Thomas's formula C except that in the latter an average

figure of 1 g. N, has been taken to represent the faeces N on a N-free diet. Both procedures have obvious disadvantages but there is very little difference in the results whichever is adopted.

A summary of the results for the separate periods with the biological values calculated from both the above formulae is given in Table VI.

<div align="center">Table VI.</div>

Diet: whole wheat bread. Subject: C.J.M.

		Intake		Balance	Biological value	
Period	Total N g.	Absorbed N g.	Calories per kilo	N g.	(1)	(2)
1. (O)	5·70	4·73	44	− 2·06	38·8	25·3
2. (A)	8·63	7·63	56	− 1·16	36·3	27·6
3. (A)	8·97	7·79	57	− 0·80	41·1	31·8
4. (A)	9·98	9·41	53	+ 0·75	41·0	43·2
5. (O)	9·85	9·08	60	− 0·51	36·0	30·4
6. (O)	12·00	10·64	61	+ 0·24	40·9	33·1
				Average	39·0	31·9

Diet: whole wheat bread. Subject: R.R.

1. (O)	5·71	5·37	49	− 1·38	34·2	29·8
2. (A)	8·57	7·72	54	− 0·72	36·8	29·3
3. (A)	8·91	8·79	56	− 0·52	28·7	27·9
4. (A)	10·00	9·43	58	− 0·02	35·5	31·4
5. (O)	9·84	9·16	58	− 0·90	28·0	22·5
6. (O)	11·98	10·40	63	− 0·04	37·7	28·1
				Average	33·5	28·2

THE BIOLOGICAL VALUE OF THE PROTEINS OF COW'S MILK.

The first experiment with nitrogen in the form of milk protein followed immediately after the second period on low nitrogen diet and was carried out in duplicate on C.J.M. and R.R. The total nitrogen intake was approximately equal to the minimum nitrogen expenditure and though equilibrium was not obtained the negative balance was not very large, from which we concluded that milk proteins would be found to possess a relatively high value. When the experiments were repeated with larger amounts of milk we were surprised to find the nitrogen balance still negative and equilibrium was only reached with diets containing over 11 g. of milk nitrogen. This result appeared so extraordinary as to lead us to suspect that it might be due to a deficiency in the energy value of the diet, although this was amply sufficient to cover our normal requirements. The unusually rapid loss in weight which occurred in some of these experiments pointed in the same direction, as did also the coincidence of the fall in the nitrogen output occurring in one period (C.J.M. 6–R.R. 3) with a pronounced rise in the temperature of the air.

A further series of experiments was therefore carried out on one of us (C.J.M. periods 7, 8, 9), the fuel value of the diet being increased to the maximum that could be consumed. In the first of these, nitrogen equilibrium

was practically attained with a diet containing 6·84 g. N and furnishing 57 calories per kilo. After an interval of one day, on which the basal ration together with a very little milk (3·0 g. N) was consumed, period 8 was begun. The diet contained 5·28 g. N and furnished 55 calories per kilo, but the effects of the continued excessive diet made themselves unpleasantly obvious in the form of a bilious attack, which threatened to terminate the experiment. By reducing the amount of carbohydrate consumed, so that the fuel value fell to 36 calories per kilo it was however just possible to carry on and after two days the condition was so far improved that the full diet was resumed. The nitrogen intake was not altered at all but the effect of the reduced diet was very marked in the increased nitrogen output, which persisted for several days after the calories were again increased. The period was extended for six days after the output had reached a fairly constant level and only the results for these days have been considered in calculating the average output. A decided though small negative balance occurred.

Table VII.

Milk. C.J.M.

Foodstuff	Nitrogen per 100 g.	Calories per 100 g.	Diet during period 9 20–25. viii. 21		
			Weight g.	Nitrogen g.	Calories
Milk	0·508	65	830	4·216	539
Starch	0·027	360	280	0·076	1008
Lactose	0·013	370	210	0·027	777
Honey	0·023	327	250	0·057	818
Margarine (rendered)	0·010	900	18	0·002	162
Sucrose	—	395	10	—	40
Tea	0·008	—	750 cc.	0·060	—
Agar-agar	0·242	—	6	0·014	—
Salts	—	—	4	—	—
			Total	4·452	3344
			Excluding tea N	4·392	54 per kilo

In period 9 the quantity of milk was again reduced but the high calorie value of the diet was maintained. The usual daily game of tennis was discontinued and no exercise was taken so that the excess of energy supplied was even greater than before. The weather also was very hot. The average of the last six days of this period showed a considerable negative balance and the biological value calculated from this agrees fairly well with that obtained from the results of periods 7 and 8 and also of period 2 (C.J.M.). This point is interesting because the calorie value of the diet in period 2 was lower than in any other, only 44 per kilo. The composition of the diet during period 9 (C.J.M.) is given in Table VII and the results of the experiments are set out in Tables VIII (R.R.) and IX (C.J.M.), while Table X is a summary of these showing the biological values calculated from the two formulae. It is obvious from the amounts of nitrogen in the faeces that the milk proteins were very completely absorbed so that formula 1 probably gives the closest approximation to the truth in this case.

Table VIII.

Diet: milk. Subject: R.R.

Period	Date 1920	Body weight k	N milk g.	N total g.	Calories per kilo	N urine g.	N faeces g.	N total g.	Balance N g.
								Daily Output	
"N-free"	Dec. 5	57·75	0	0·27	44	2·01	1·13	3·14	−2·87
1.	„ 6		2·73	2·98	48	2·49	0·66	3·15	−0·17
	„ 7		2·69	2·94	„	2·95	„	3·61	−0·67
	„ 8		2·76	3·09	„	3·22	„	3·88	−0·79
	„ 9		2·59	2·91	„	2·86	„	3·52	−0·61
	„ 10	57·50 (11. xii)	2·67	2·97	„	2·83	„	3·49	−0·52
	Average of last 4 days		2·68	2·98	48	2·97	0·66	3·63	−0·65
	1921								
2.	May 28	59·65	4·10	4·30	40	7·12	—	—	—
	„ 29		6·14	6·29	48	5·97	1·18	7·15	−0·86
	„ 30		„	„	„	5·91	„	7·09	−0·80
	„ 31		„	„	„	6·34	„	7·52	−1·23
	June 1		„	„	„	7·09	„	8·27	−1·98
	„ 2		„	„	„	6·55	„	7·73	−1·44
	„ 3	57·90 (4. vi)	„	„	„	6·21	„	7·39	−1·10
	Average of last 4 days		6·14	6·29	48	6·55	1·18	7·73	−1·44
	June 16		Mixed diet			12·29	—	—	—
3.	„ 17	59·60	13·61	13·69	47	13·48	1·37	14·85	−1·16
	„ 18		„	„	„	13·13	„	14·50	−0·81
	„ 19		„	„	„	12·29	„	13·66	+0·03
	„ 20		„	„	„	13·76	„	15·13	−1·44
	„ 21		„	„	„	12·39	„	13·76	−0·07
	„ 22		„	„	„	12·60	„	13·97	−0·28
	„ 23	58·50	„	„	„	11·10	„	12·47	+1·22
	„ 24		„	„	„	11·19	„	12·56	+1·13
	Average of last 5 days		13·61	13·69	47	12·21	1·37	13·58	+0·11

Table IX.

Diet: milk. Subject: C.J.M.

Period	Date 1920	Body weight k	N milk g.	N total g.	Calories per kilo	N urine g.	N faeces g.	N total g.	Balance N g.
"N-free"	Dec. 5	60·45	0	0·33	48	2·13	1·17	3·30	−2·97
1.	„ 6		3·15	3·37	49	2·42	1·29	3·71	−0·34
	„ 7		„	3·36	„	2·57	„	3·86	−0·50
	„ 8		„	3·42	„	3·01	„	4·30	−0·88
	„ 9		„	3·40	„	3·12	„	4·41	−1·01
	„ 10	59·30 (11. xii)	„	3·45	„	3·22	„	4·51	−1·06
	Average of last 3 days		3·15	3·42	49	3·12	1·29	4·41	−0·99
	1921								
2.	April 30	62·60	4·02	4·17	44	4·74	1·22	5·97	−1·80
	May 1		„	„	„	4·58	„	5·80	−1·63
	„ 2		„	„	„	4·09	„	5·32	−1·15
	„ 3		„	„	„	4·25	„	5·47	−1·30
	„ 4	62·20	„	„	„	4·53	„	5·75	−1·58
	Average of last 4 days		4·02	4·17	44	4·36	1·22	5·58	−1·41
	May 10		Mixed diet			8·00	—	—	—
3.	„ 11	62·20	6·20	6·35	48	6·13	1·18	7·31	−0·96
	„ 12		„	„	„	5·71	„	6·89	−0·54
	„ 13		„	„	„	5·26	„	6·44	−0·09
	„ 14		„	„	„	5·80	„	6·98	−0·63
	„ 15		„	„	„	6·08	„	7·26	−0·91
	„ 16		„	„	„	6·07	„	7·25	−0·90
	„ 17	62·15 (18. v)	„	„	„	6·21	„	7·39	−1·04
	Average of last 4 days		6·20	6·35	48	6·04	1·18	7·22	−0·87

Table IX (*continued*)

Diet: milk. Subject: C.J.M.

| | | | Daily Intake | | | Daily Output | | | |
Period	Date 1921	Body weight k	N milk g.	N total g.	Calories per kilo	N urine g.	N faeces g.	N total g.	Balance N g.
"N-free"	May 27		—	—	—	8·38	—	—	—
4.	,, 28	62·40	7·63	7·78	47	7·05	0·96	8·01	−0·23
	,, 29		,,	,,	,,	7·11	,,	8·07	−0·29
	,, 30		,,	,,	,,	7·55	,,	8·51	−0·73
5.	,, 31		8·62	8·77	47	7·48	0·98	8·46	+0·31
	June 1		,,	,,	,,	8·58	,,	9·56	−0·79
	,, 2	61·30	,,	,,	,,	8·41	,,	9·39	−0·62
	,, 3		,,	,,	,,	8·67	,,	9·65	−0·88
	Average of last 3 days		8·62	8·77	47	8·55	0·98	9·53	−0·76
	June 16			Mixed diet		10·12	—	—	—
6.	,, 17	61·80	11·32	11·44	46	9·71	1·45	11·16	+0·28
	,, 18		,,	,,	,,	10·46	,,	11·91	−0·47
	,, 19		,,	,,	,,	10·21	,,	11·66	−0·22
	,, 20		,,	,,	,,	10·19	,,	11·64	−0·20
	,, 21		,,	,,	,,	10·82	,,	12·27	−0·83
	,, 22		,,	,,	,,	8·09	· ,,	9·54	+1·90
	,, 23		,,	,,	,,	9·60	,,	11·05	+0·39
	,, 24		,,	,,	,,	9·71	,,	11·16	+0·28
	,, 25	61·70 (26. vi)	,,	,,	,,	10·28	,,	11·73	−0·29
	Average of last 6 days		11·32	11·44	46	9·78	1·45	11·23	+0·21
	July 2			Mixed diet		6·33	—	—	—
7.	,, 3	61·10	6·65	6·84	57	5·66	1·28	6·94	−0·10
	,, 4		,,	,,	,,	5·55	,,	6·83	+0·01
	,, 5		,,	,,	,,	5·35	,,	6·63	+0·21
	,, 6		,,	,,	,,	5·70	,,	6·98	−0·14
	,, 7		,,	,,	,,	6·35	,,	7·63	−0·79
	,, 8	61·80	,,	,,	,,	5·04	,,	6·32	+0·52
	Average of last 4 days		6·65	6·84	57	5·61	1·28	6·89	−0·05
	July 9	62·10	—	3·00	54	5·16	—	—	—
8.	,, 10		5·16	5·28	55	4·54	1·09	5·63	−0·35
	,, 11		,,	,,	36	3·99	,,	5·08	+0·20
	,, 12	61·00	,,	,,	36	5·57	,,	6·66	−1·38
	,, 13	60·80	,,	,,	53	5·28	,,	6·37	−1·09
	,, 14	61·10	,,	,,	57	5·29	,,	6·38	−1·10
	,, 15	61·30	,,	,,	55	4·71	,,	5·80	−0·52
	,, 16	61·60	,,	,,	54	4·63	,,	5·72	−0·44
	,, 17	61·90	,,	,,	53	4·83	,,	5·92	−0·64
	,, 18	61·85	,,	,,	52	4·06	,,	5·15	+0·13
	,, 19	61·85	5·04	5·18	52	4·65	,,	5·74	−0·56
	,, 20	62·00	,,	,,	52	4·24	,,	5·33	−0·15
	Average of last 6 days		5·12	5·25	53	4·52	1·09	5·61	−0·36
9.	Aug. 16	61·60	4·24	4·58	53	15·79	—	—	—
	,, 17	61·85	,,	,,	,,	10·78	—	—	—
	,, 18	61·85	,,	,,	,,	6·46	—	—	—
	,, 19	61·90	,,	,,	,,	5·83	—	—	—
	,, 20		,,	4·39	,,	4·69	1·00	5·69	−1·30
	,, 21	61·80	,,	,,	,,	4·00	,,	5·00	−0·61
	,, 22	61·80	,,	,,	,,	4·10	,,	5·10	−0·71
	,, 23	61·60	,,	,,	,,	5·04	,, ·	6·04	−1·65
	,, 24	61·60	,,	,,	58	4·78	,,	5·78	−1·39
	,, 25	61·75	,,	,,	55	4·72	,,	5·72	−1·33
	Average of last 6 days		4·24	4·39	55	4·56	1·00	5·56	−1·17

Table X.

Diet: milk. Subject: C.J.M.

Period	Intake			Balance N g.	Biological value	
	Total N g.	Absorbed N g.	Calories per kilo		(1)	(2)
1.	3·42	3·30	49	− 0·99	74·7	73·6
2.	4·17	4·12	44	− 1·41	47·3	46·6
3.	6·35	6·34	48	− 0·87	38·4	38·3
5.	8·77	8·77	47	− 0·76	26·3	28·6
6.	11·44	11·16	46	+ 0·21	33·0	31·2
7.	6·84	6·73	57	− 0·05	49·7	48·8
8.	5·25	5·25	53	− 0·36	55·6	57·2
9.	4·39	4·39	55	− 1·17	45·2	49·4
			Average for last 3 periods		50·2	51·8

Diet: milk. Subject: R.R.

Period	Total N g.	Absorbed N g.	Calories per kilo	Balance N g.	(1)	(2)
1.	2·98	2·98	48	− 0·65	66·0	83·6
2.	6·29	6·24	48	− 1·44	25·0	24·4
3.	13·69	13·45	47	+ 0·11	24·2	22·9

Table XI. *Basal metabolism of C.J.M. on normal diet and on carbohydrate and fat (= 55 cals. per kilo) with 4·4 g. milk N.*

Date	Diet during previous 24 hrs.	Oxygen consumed per min. cc.	R.Q.	Calories per 24 hrs.
Aug. 10	Normal	225·6	0·711	1557
,, 12	,,	236·3	0·928	1699
,, 13	,,	227·1	0·684	1566
,, 14	,,	220·9	0·820	1557
			Average	1595
,, 20	Milk	211·5	0·974	1529
,, 21	(4·39 g. N, 55 cals.	178·0	1·159	1359
,, 22	per kilo)	197·3	1·093	1478
,, 23	,,	185·7	1·152	1415
,, 24	,,	195·0	1·044	1440
,, 25	,,	196·1	1·055	1453
,, 26	,,	210·4	1·017	1540
			Average	1459
,, 27	Normal	221·9	0·915	1590
,, 28	,,	216·8	0·889	1550
,, 31	,,	228·0	0·811	1604
			Average	1581

During period 9 the subject's basal metabolism was determined on waking and the results were compared with similar determinations carried out during previous and succeeding periods when the diet was normal. These results are set out in Table XI and show a decrease of about 8 % in the basal metabolism on the milk diet.

DISCUSSION OF RESULTS.

THE MINIMUM NITROGEN EXPENDITURE.

During the second experiment to determine our nitrogen minimum the output of nitrogen in the urine fell to an amount equal to 0·035 g. per kilo body weight in the case of C.J.M. and 0·034 g. per kilo for R.R.˙ The average amounts for the last three days of this period were slightly above the minima, being 0·038 g. and 0·035 g. respectively. If the nitrogen of the faeces is included the average output for the same period was 0·057 g. per kilo for C.J.M., and 0·055 g. for R.R. The nitrogen intake amounted to 0·005 g. per kilo.

These figures are somewhat lower than most of those recorded by other workers [Landergren 1903; Folin, 1905; Kinberg, 1911; Graham and Poulton, 1912], but this may be explained by the fact that their diets unavoidably contained more nitrogen than ours. Karl Thomas [1910] determined his minimum expenditure on a purely carbohydrate diet in seven experiments carried out over a period of two and a half years and found that this minimum fell from experiment to experiment, the final amount for the output in the urine alone being 2·2 g. or 0·029 g. per kilo, while that for urine and faeces combined was 2·9 g. or 0·039 g. per kilo. At this period Thomas weighed 75 kilos and had put on a good deal of body fat. In some of his earlier experiments, however, it seems probable that the minimum output was never reached as the diet was continued for too short a period.

In McCollum's [1911] experiments on pigs, which were fed on a diet of starch, a salt mixture and water, the minimum nitrogen output fell to a level corresponding closely with that reached by us. Thus a pig weighing 68·4 kilos excreted 0·039 g. N per kilo in the urine. For smaller animals the output per kilo was somewhat greater.

The determination of the nitrogen in urine and faeces does not, of course, give a complete account of the loss of nitrogen from the body. To these must be added the loss through hair, beard and nails, through loss of epidermis and in sweat. Except for the last named these losses are all very small in amount but the loss through the sweat may be considerable. Benedict [1906] has shown that a resting man may excrete 0·071 g. N per day in this way while with moderate work the loss may amount to 0·13 g. N per hour. McCollum considers that the nitrogen of the faeces should also be classed with these as representing losses that may be termed accidental in character and that the nitrogen of the urine alone is to be taken as representing the essential tissue metabolism of the body.

The amount of nitrogen excreted in the urine on the successive days of the experiments in which the diet was nearly free from nitrogen, is plotted in Figs. 2, 3, and 5. The amount diminishes in a fairly regular manner, at first quickly and then more slowly until the minimum is reached. The points lie on or near the graph of a simple logarithmic equation
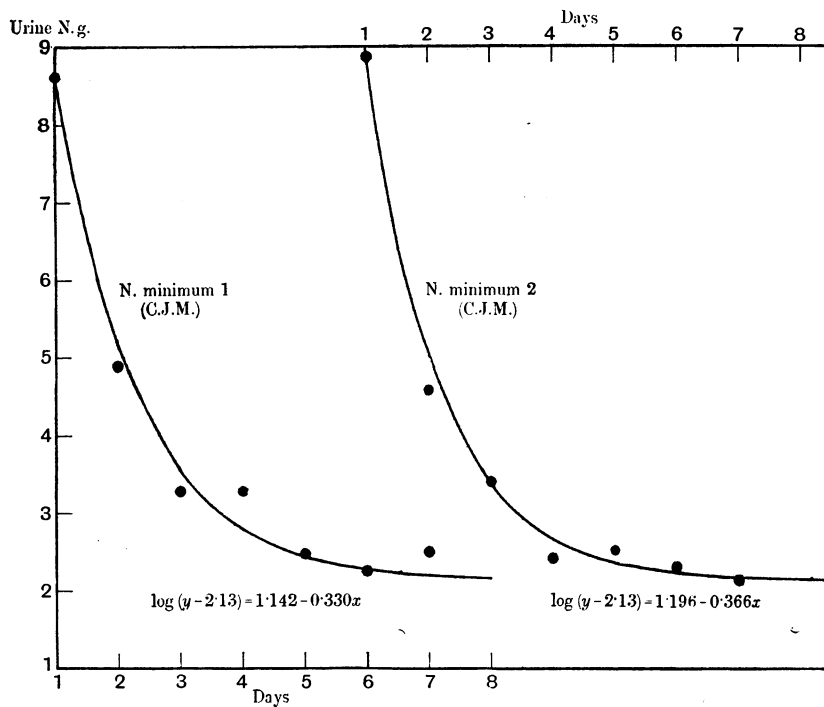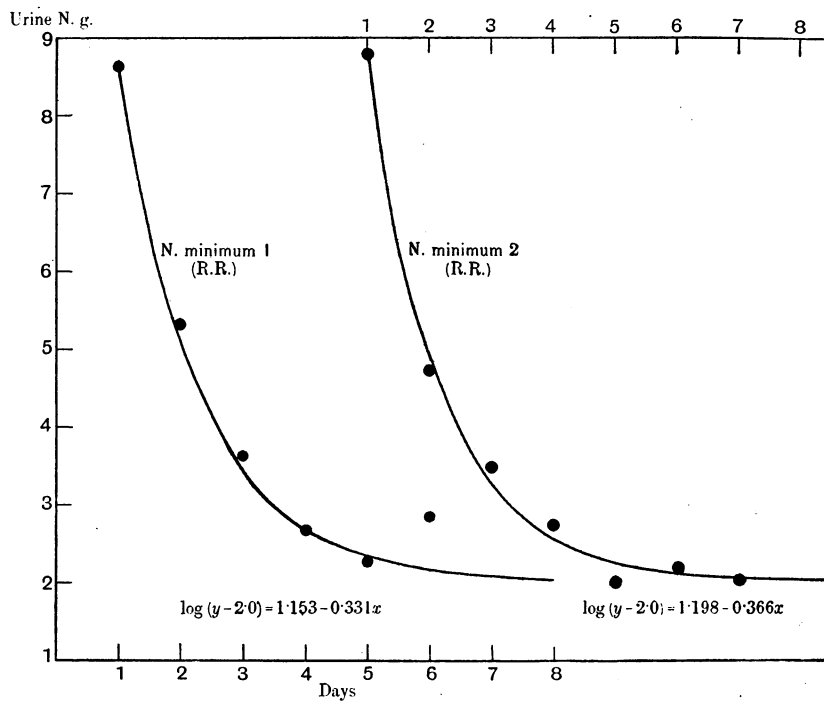
$$\log (y - \lambda) = a - kx$$

Fig. 2.

Fig. 3.

in which x is the number of days, y is the nitrogen output in the urine, and λ the minimum value of y. A closer agreement is obtained if λ is given a value slightly lower than the minimum actually reached.

Thus in Fig. 3 the curve R.R. 1 is drawn from the equation
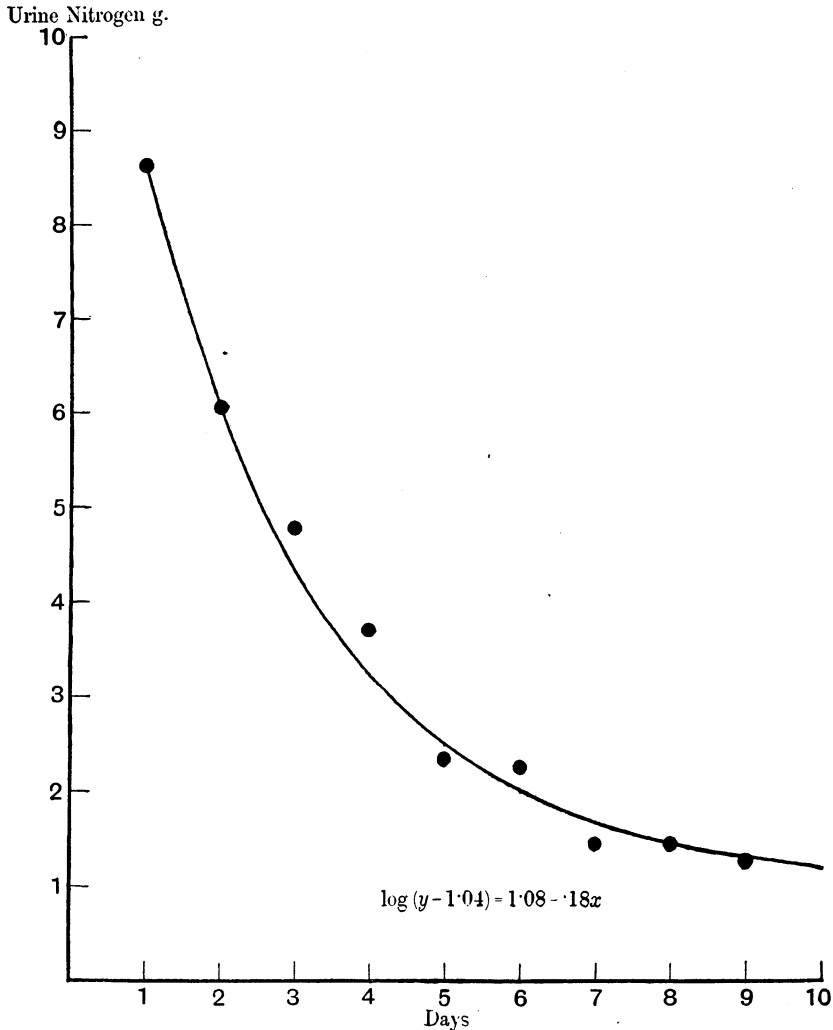
$$\log (y - 2\cdot0) = 1\cdot153 - 0\cdot331x$$

Fig. 4. E. V. McCollum's experiment on a pig. Daily output of nitrogen in urine on starch diet after ingestion of zein.

and fits the points reasonably well, but a still closer approximation is obtained with the equation

$$\log (y - 1\cdot73) = 1\cdot1209 - 0\cdot2832x$$

the agreement between the observed and calculated values of y being extraordinarily good for all points except the last.

Day of experiment	y calculated from equation $\log (y-1\cdot73)=1\cdot1209-0\cdot2832x$	y obtained
1	8·61	8·64
2	5·31	5·31
3	3·60	3·63
4	2·70	2·66
5	2·24	2·25
6	1·99	2·82
20	1·73	—

A question is thus raised: Does this amount 1·73 g. represent the real minimum expenditure, which, from some cause was not realised in either experiment? At present this must be left unanswered, but further experiments may give some information on the point.
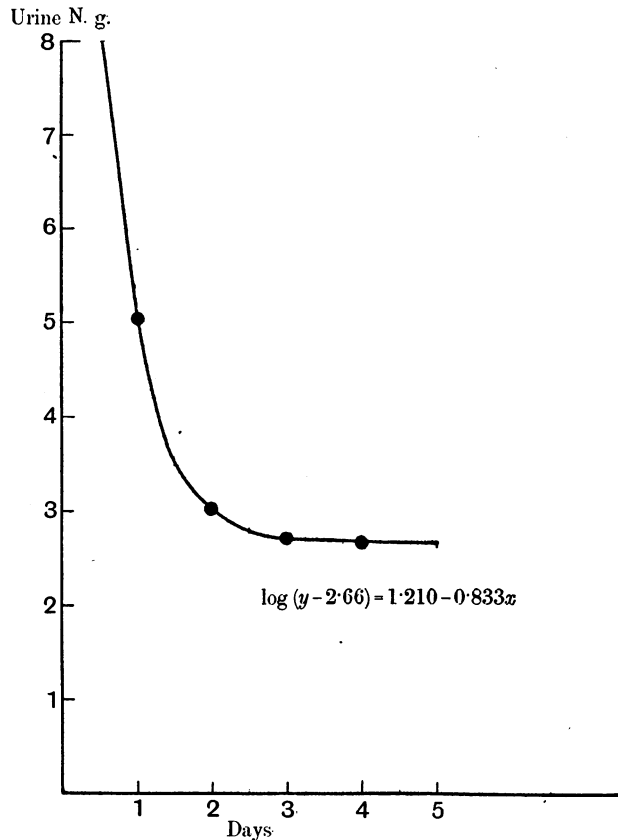
Fig. 5. E. V. McCollum's experiment on a pig. Daily output of nitrogen in urine on starch diet after ingestion of urea.

The difference between experiments 1 and 2 lies only in the amount of total fluid taken which was about 2000 cc. daily in the first and 4000 cc. in the second. This makes but slight difference in the value of k, that is in the rate at which the nitrogen output falls. Kinberg [1911] has previously drawn attention to this regularity but has not attempted to deduce any mathematical

expression from his results. Thomas [1910] also recognised that on a protein-free diet the nitrogen of the "Vorratseiweiss" leaves the body with varying rapidity according to the amount present, but was unable to find any exact relationship either in his own results or in those of Landergren. He calculated the amount of "Vorratseiweiss" excreted, by subtracting the minimum output ("Abnutzungsquota") from the urine nitrogen, but the value of this minimum was taken from experiments of four days' duration and was probably too high. Landergren's results show a fair agreement with the graph of an equation of the type given above if λ is taken as 2·5 instead of 3·0. Thomas's results are more irregular. In considering the agreement of the results of such experiments
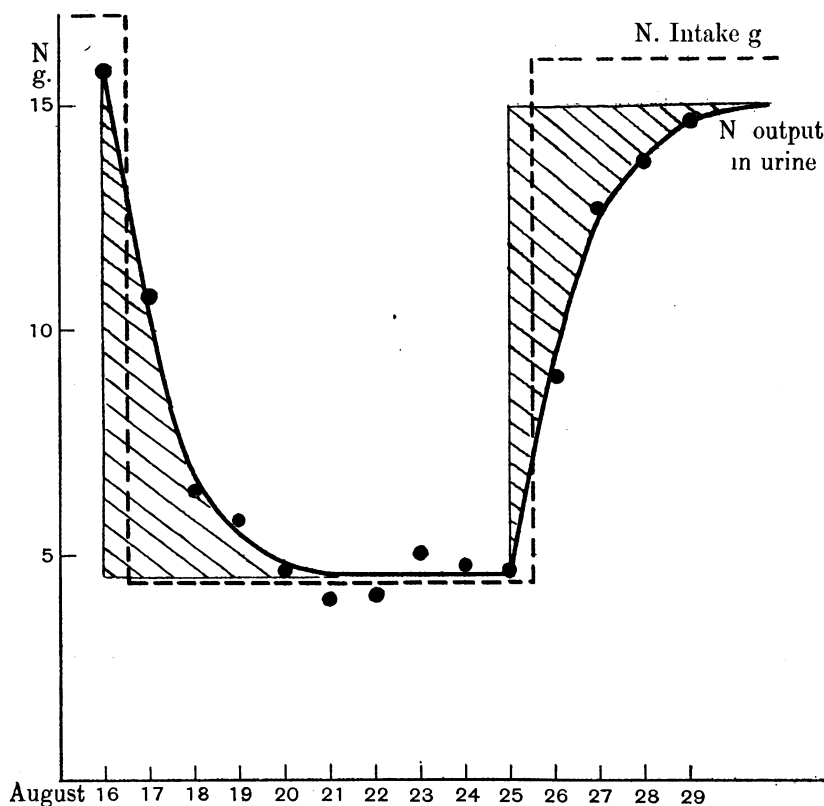
Fig. 6.

with those calculated from these equations, the tendency of the nitrogen output to oscillate even when the nitrogen intake is constant must be borne in mind. Such oscillations are present in most of the experiments considered, but do not alter the general character of the curve. In McCollum's experiments on pigs a similar regularity also appears as may be seen from Figs. 4 and 5. Fig. 4 shows the nitrogen output on a starch diet after a diet containing zein and Fig. 5 shows the output after the ingestion of urea. The curves are of the same type but "k," i.e. the rate at which the output falls, is very much

greater after urea than after zein. This point is of interest as it seems to indicate that the store of nitrogen which exists in the body after a protein diet, and which is rapidly given up on a diet free from nitrogen is not present entirely in the form of urea. As to the form in which this nitrogen occurs, very little can be definitely stated. It may be as resynthesised protein (Vorratseiweiss), or as amino acids adsorbed in the tissues, or compounds of intermediate complexity. It is certainly present partly as urea and other end products of metabolism. If our conclusions as to the rate at which this storage nitrogen is excreted are correct the interpretation is, that the amount removed from the body on any day is proportional to the amount still present. This might hold whether the reaction involved was the hydrolysis of protein, the deaminisation of amino acids or simply the washing out from the tissues of the end products of nitrogen metabolism.

On reversing the process, and after a minimal N-intake for ten days, suddenly increasing the nitrogenous food consumed, nitrogen at first remains in the body and equilibrium between intake and output does not occur for several days. Fig. 6 is a graph of the results of an experiment designed to show this. The broken line represents intake of N, the solid line output. The shaded area on the left hand represents the stored N gradually removed on dropping the intake from 17 g. to 4·4 g. and the right-hand area the amount again stored on resuming a diet containing 16 g. N. The curves are reciprocal and the two shaded areas approximately correspond.

THE NATURE OF THE MINIMUM NITROGEN REQUIREMENTS OF THE BODY.

The low level to which the nitrogen output falls on a protein free diet is evidence of the smallness of the body's daily requirements in this respect. We know from Folin's [1905] researches that the reduction in the nitrogen on such a diet occurs mainly at the expense of the urea fraction, the ammonia and uric acid being reduced to a relatively much smaller extent while the creatinine remains constant. These facts led Folin to conclude that protein metabolism is of two types, (1) "tissue" or "endogenous," which tends to be constant, and is represented largely by such products as creatinine, neutral sulphur, and to a less extent by uric acid, (2) "exogenous" which varies with the amount of protein consumed and is represented chiefly by urea. The nitrogen required for processes of the first type is alone essential, but Folin recognised that equilibrium at this low level may not be possible since a certain amount of protein may always fall prey to the exogenous metabolism. The distribution of the nitrogenous constituents of the urine was determined during our second minimum experiment and the milk diet immediately succeeding this.

The results [Robison, 1922, 2] are similar to those of Folin. The minimum nitrogen output was lower than any recorded by him, and the percentage of urea was correspondingly reduced, the minimum figure being 37·2 %, while the sum of the urea and ammonia amounted to 54·8 % of the total nitrogen.

The multifarious transactions involved in endogenous metabolism are not likely to be conducted with perfect economy. When much protein is hydrolysed and the products mobilised and used for the synthesis of proteins of another composition such as those of the blood or for the manufacture of thyroxin or adrenaline it is unlikely that the whole balance of unwanted amino acids escapes deaminisation and conversion. Leakage of this kind may account for no inconsiderable fraction of the minimum nitrogen expenditure and it is perhaps in this direction that, by adaptation, the body may effect some saving. The experiments of Thomas and Hindhede would appear to show that this does in fact occur.

The amount of carbohydrate eaten may also influence the degree of this waste since the process of deaminisation is reversible and will be affected by the rapidity with which the non-nitrogenous products, hydroxy or ketonic acids, are removed by oxidation or conversion into carbohydrate. It is probable that an excess of carbohydrate in the blood would retard either action and in consequence deaminisation.

THE QUESTION OF UNIFORMITY OF THE BIOLOGICAL VALUE.

We have seen that the validity of the method adopted by Thomas for the determination of the biological values of proteins depends in the first place on the uniformity of this value when varying amounts of the same protein are consumed. The investigation of this question was one of the objects of our experiments and the results must now be considered from this point of view. The three proteins so far studied were chosen on account of the wide difference in the values attributed to them. The biological values of milk and wheat proteins as given by Thomas are 99·71 and 39·56 respectively, while gelatin has long been known to be deficient in several essential amino acids, so that no amount, however great, can completely satisfy the body's nitrogen requirements.

The results of the experiments described in this paper together with those on gelatin, previously recorded by one of us [Robison, 1922, 1] are plotted in Figs. 7 and 8, in which the values of the nitrogen intake for different periods are the abscissae and the corresponding amounts for the total output in urine and faeces are the ordinates. For this purpose the nitrogen of the faeces in excess of the amount on the "protein-free" diet has been taken as representing unabsorbed food and has been subtracted from both intake and output.

In the case of whole wheat proteins, the results are strikingly similar with both individuals and the points obviously lie on or close to a straight line passing through the points representing the minimum expenditure on the low nitrogen diet. The agreement is remarkably good considering the errors inseparable from such experiments. The results for periods 4 and 5 show some discrepancy but these periods were continuous and should probably be considered as a whole, the only difference in the diets being the inclusion of
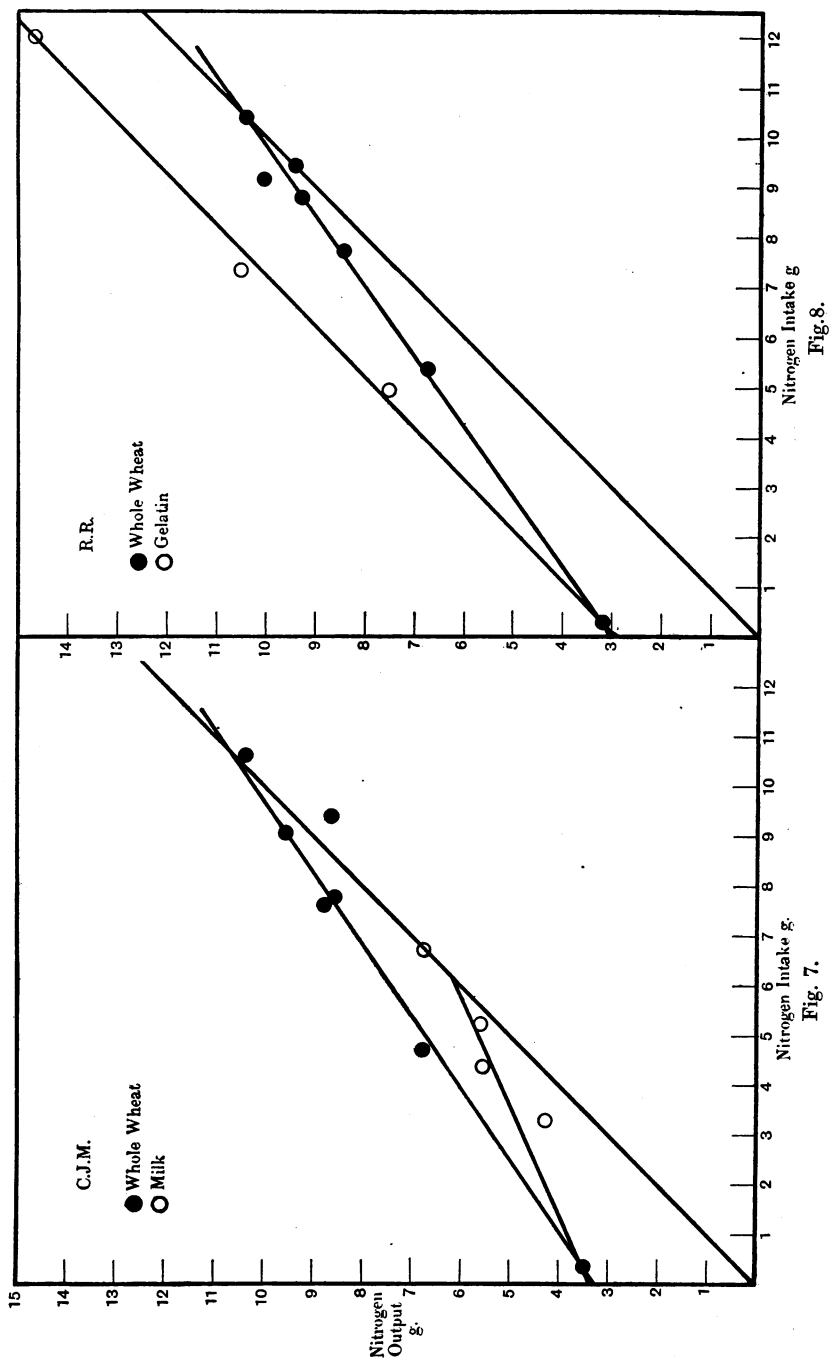
C.J.M.

Whole Wheat
Milk

Nitrogen Intake g.

Fig. 7.

R.R.

Whole Wheat
Gelatin

Nitrogen Intake g

Fig. 8.

a small quantity of apples in the former and its omission from the latter. The average for the two periods gives a point lying close to the line. The uniformity of the biological value is therefore satisfactorily proved for the proteins of whole wheat.

In the case of gelatin also the points fit a straight line reasonably well, but if this line passes through the point representing the nitrogen minimum (as drawn in the figure), it will at some distant point intersect the "equilibrium line" (at 45° to the axis), *i.e.* nitrogenous equilibrium will be attained at this point, which we believe to be impossible. On the other hand if it is drawn parallel to the "equilibrium line" it will cut the axis of y at a point slightly below the observed minimum. We must conclude, therefore, either that the line is slightly curved near the minimum or that the observed value of the latter is somewhat higher than the real one. If the capacity of the gelatin is limited to the reduction of "leakage" these two alternatives have practically the same significance.

Milk presents a more difficult case. Only the results for those periods in which a diet of abundant fuel value was taken (C.J.M. 1, 7, 8, 9) have been plotted but even these do not agree well with any straight line or other regular curve. The most that can be said is that in view of the possible errors in such experiments where the intake is small, the results are not inconsistent with the uniformity of the biological value of this protein.

From the consideration of these three cases we may conclude that the general assumption of this principle made by Thomas, occasioned no serious errors in his conclusions. Caution must be used in extending this principle to all cases without investigation and reliance should not be placed upon results of experiments in which the negative balance is large.

THE BIOLOGICAL VALUES OF THE PROTEINS OF WHOLE WHEAT AND MILK.

Whole Wheat.

The biological values calculated from the different periods of our experiments with whole wheat proteins agree very well amongst themselves and give an average of 35 for those obtained on C.J.M. and 31 for those obtained on R.R. Thomas's value is somewhat higher, 39·56. The latter figure was based on the result of two experiments each of three days' duration and one of four days. During none of these experiments was the nitrogen intake constant; in one, the amounts for the separate days were 4·0 g., 7·3 g., 9·0 g. respectively. The minimum requirements corresponding with these three periods were taken as 4·63 g., 3·991 g. and 3·316 g. N (urine only), these amounts being determined in experiments of four and three days' duration. In the first of these the nitrogen output on the four days was 18·32 g., 10·17 g., 7·39 g., 4·63 g. but there is no evidence that the last figure represented Thomas's minimum requirements. The biological value of wheat proteins was calculated from each separate

day's balance and out of these widely varying results those for certain days were selected in a somewhat arbitrary fashion. The experimental results of his third period, in which the intake was nearly constant, indicate a similar biological value for wheat proteins to that found by ourselves.

The result Hindhede [1913, 1] obtained upon F. Madsen with white bread, namely a positive balance of about 1 g. per day over a 28 day experiment on 13·73 g. N was certainly not a minimum quantity and the amount necessary for N equilibrium may be considerably less. His further experiments [1914] were made with rye bread ("Schwarzbrot") and the diets contained considerable amounts of fruit or vegetables, which accounted for 0·5 g. to 1·9 g. N. These experiments were carried out in duplicate on F. and H. Madsen and were continued for four months so that they possess great value. In the final period of six days the fruit was omitted and positive balances were obtained on 13·52 g. (F.M.) and 10·68 g. (H.M.) bread nitrogen respectively. By subtracting the whole of the nitrogen in the faeces Hindhede calculates that the amounts absorbed were 8·49 g. and 6·28 g. respectively. We do not agree with this method of treating the faecal nitrogen and cannot accept his conclusion that bread proteins possess equal value with those of potatoes, of meat and of the body; but there is no doubt that in his experiments, equilibrium was obtained on a lower intake of nitrogen in the form of rye bread (and still lower when supplemented by fruit) than our minimum for whole wheat. This is confirmed by the experiments of Neumann [1919], who, by long continued diet of very high calorie value, was ultimately able to retain nearly 3 g. N daily with an intake of 9·9 g. N in the form of rye proteins. Neumann's experiment was upon himself, and was in every way unexceptionable, but it may perhaps be significant that it was preceded by a prolonged period of semistarvation during which he was investigating the German civilian ration of 1916–17. This is a further indication that, apart from the influence of loss of body weight, the organism can gradually accommodate itself to a lower ration of nitrogen, perhaps by the exercise of greater economy.

Abderhalden's [1915] observations on Röse, although as pointed out earlier in this paper, not susceptible of the interpretation he places upon them, do indicate that the latter could get into equilibrium with about a gram less N in the form of white bread than we could with bread made from the whole grain. Röse's nitrogen expenditure on a nitrogen-free diet was not ascertained so we cannot estimate the biological value for this diet.

The recent observations of Rubner [1919] upon the proteins of white flour are interesting in relation to our own, because they show, we think, that from the point of view of biological value, the proteins of the endosperm are equal, if not superior to, those of the whole seed.

In most of Sherman's experiments on the value of cereal proteins these are supplemented by a certain amount of milk and the results are not directly comparable with ours, but in some experiments on white bread [1920] the diet consumed would not appear to be greatly different from that taken by

us and we are therefore the less able to explain the difference between his results and ours. Sherman's subject, a man weighing 80 kilos, attained equilibrium on a diet containing 6·0 g. N over 95 % of which was derived from white bread and the remainder from apples and butter. The energy value was only 34 calories per kilo. The bread was purchased from a bakery and probably contained a small amount of milk but how much was not known.

Milk.

The results of our experiments with milk proteins do not agree so closely as those for the whole wheat. The very low values calculated from the results of periods 3, 5, 6 (C.J.M.) and 2, 3, (R.R.) are not easy to explain. That they are in some way due to the lower calorie value of the diet seems clear but this was in no case below that of our normal diet and amply covered our energy requirements. The high values obtained with both subjects in period 1 are perhaps connected with the previous nitrogen starvation and a consequent increase in the economy with which the body may deal with the protein supplied to it.

If we consider only periods 7, 8, 9 (C.J.M.) in which the conditions were the same and the energy supply abundant the biological value for milk proteins is equal to 51 %. This value is only half of that found by Thomas, but the criticisms we have made in discussing his experiments with bread apply with still greater force in this case. His value (100 %) was calculated from the nitrogen balance on a single day of an experiment lasting only two days on which the intake was 6·24 g. and 7·28 g. respectively. The minimum requirements were taken as 3·99 g. which is probably much too high. If the value of milk protein were as high as Thomas makes out it is difficult to see how he could explain the large negative balances occurring in his experiment with "Frauenmilch" (cow's milk with extra sugar and cream). During the first two days of this experiment the fuel value of his diet was obviously too low, but in the last three days it was equal to 40–45 calories per kilo, the N-intake being 15·3 g.–17·3 g. yet the negative balance was never less than 1·0 g. The results of this experiment appear to agree with our experience, and to suggest that a high intake of milk nitrogen tends to result in increased expenditure of body nitrogen, unless the fuel value of the diet is raised very much above the normal. So far as we are aware the value of milk protein has not been the subject of any other investigation on man.

Conclusions.

From our observations upon ourselves we conclude:

(1) That our minimum nitrogen expenditure by the urine is somewhat less than 0·038 g. and 0·035 g. per kilo in C.J.M. and R.R. respectively.

(2) That on taking a diet of carbohydrate and fat of adequate calorie-value the nitrogen excreted in the urine falls in a regular and orderly manner,

capable of simple mathematical expression, approaching a minimum in five to seven days. On resuming an ordinary nitrogenous diet the reciprocal phenomenon occurs.

(3) Bearing in mind the considerable experimental errors, the ratio $\dfrac{\text{Body N saved}}{\text{Food N absorbed}}$ appears to remain constant. whatever amount of nitrogen is taken in the form of whole wheat bread, until equilibrium is reached.

(4) In the case of milk the experimental errors are proportionately greater and the most we can say is that this ratio may remain constant.

(5) In the case of gelatin the ratio certainly does not remain constant and there is no indication that the amount of body nitrogen saved increases beyond that effected by the smallest quantity of gelatin fed.

(6) The application of Thomas's method of determining biological values is justified in the case of bread, doubtful with milk and impossible with gelatin.

(7) Until Thomas's procedure has been ascertained to be justifiable for the particular proteins concerned, the ratio $\dfrac{\text{Body N saved}}{\text{Food N absorbed}}$ should be determined close to, but below, the point of equilibrium.

(8) The mean biological value of the nitrogen contained in the whole wheat grain as determined by six experiments on each of two adults was 35 % (C.J.M.) and 31 % (R.R.).

(9) The mean biological value of the nitrogen in cow's milk, derived from three experiments upon C.J.M. in which an excess of calories (55 per kilo) was taken, was 51 %.

(10) Biological values arrived at from experiments of comparatively short duration, however well justified, have a limited significance.

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