SOME PHASES IN THE PHYSIOLOGY OF PROTEIN METABOLISM.

By

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"Saying, when will the new moon be gone....
that we may set forth wheat-making the epah small and
the shekel great and falsifying the balances by deceit?"

Amos, Chap.8, ver.5.

SECTION I.

PART I. HISTORICAL OUTLINE.

PART II. THE METHOD OF INVESTIGATION.

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Part I.

Historical Outline of the Development of Ideas on the Physiology of Protein Metabolism.

The study of the physiology of protein metabolism as distinct from that of fat or carbohydrate is one of comparatively recent date, scarcely older indeed than that of organic chemistry. The main reason for this was the want of an exact scientific classification of the different foodstuffs. The problem indeed could not be said to exist until in the first place a satisfactory chemical classification of the foodstuffs had been made and in the second place until it had been shown that the physiological response of the organism differed for each.

Aristotle nearly two thousand years ago, with the type of Greek intellect ever seeking unity, declared that there was only one kind of aliment which was extracted from the various foodstuffs ingested by the alimentary canal. Little work however was done on nutrition until the eighteenth century, and at the time of Lavoisier foodstuffs were classified either on the basis of their origin, e.g., farinaceous, or on their taste, e.g., sweet, sour, &c.

Magendie, after the discovery by Fourcrois and Vaquelin of the presence of nitrogen in organic matter, classified foods into the nitrogenous and the non-nitrogenous.

Fourcrois/

Fourcrois and Vaquelin followed up their work by proving the existence of nitrogen in the urine, and indeed actually prepared crystalsof urea nitrate. The path was thus opened for tracing the fate of the nitrogen-containing foodstuffs in the body. Fourcrois and Vaquelin failed, however, to appreciate the importance of their discovery, and it remained for Müller to speculate on the source of the urea of the urine. Müller explicitly questions whether the nitrogen in the urine was derived from the unutilised part of the food, or whether it came from the breaking-down of body tissue. Berzelius however had no doubts as to its origin and stated that urea was a product of the metamorphosis of living tissue.
It was Liebig however who first clearly appreciated the full significance of the urea in the urine and suggested it might be employed as an index of the transformation of nitrogenous foodstuffs in the body. Liebig accepted the classification of the foodstuffs into a nitrogenous and a non-nitrogenous part, and on this basis attempted to explain the rôle of each in the animal economy. The nitrogen-free foods he called "Respiratorische Stoffe" and the nitrogen-containing the "Plastische Stoffe." The function of the former was to become oxidised and supply heat to the organism, while the "Plastische Stoffe" went to build up organs or parts of organs which were being continually metamorphosed into dead materials

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so long as life existed. Liebig declared emphatically that nothing is more certain than that the carbon hydrogen and nitrogen excreted, although equal in amount to that ingested, do not proceed directly from the food. Liebig was an influential man in his time, and in order to appreciate the later development of ideas on protein metabolism one must understand his conceptions of metabolism as a whole. He accepted Lavoisier's teaching that oxygen caused the metabolism of the foodstuffs but differentiated between two important activities of the organism - namely muscle work and heat output. Muscle work was held to be associated with the transformation, or metamorphosis as he called it, of living tissue, i.e., nitrogenous material which once it was broken down could be oxidised to furnish heat. The non-nitrogenous materials on the other hand through their oxidation supplied heat and by combining with the inspired oxygen prevented the latter from attacking the living nitrogenous substance. He illustrates his theory by quoting the carnivorous panther which paces up and down its cage causing the breaking-down by its activity of nitrogenous materials in order to furnish sufficient carbon and hydrogen to act as a source of animal heat by oxidation. Bidder and Schmidt on the other hand declared that protein - a term first applied by Müller to the nitrogen-containing foods - before its oxidation did not become organised into morphological tissue. These workers held that only a small part of the protein ingested/

ingested went to replace tissue which was destroyed. Protein consumed in excess of this small quota was considered to be a "luxus consumption" and was oxidised in the blood without becoming built up into living tissue.

The actual amount of protein required for maintenance was deduced from the amount of urea in the urine during starvation - "Typische Albumenumsatz:" this represented the normal loss of tissue which must be continually replaced by the food protein. Bischoff in his book "Der Harnstoff als Maass des Stoffwechsels" again supported Liebig in regard to the origin of urea from broken-down tissue. He states that in the adult on a constant diet and when the body weight is constant intake and output tend to be equal in that the food replaces the effete materials of the tissues which have been broken down and eliminated. Bischoff appears to have had the incipient idea of metabolic equilibrium although he never succeeded in determining the existence of nitrogen equilibrium owing to the fact that only the urea nitrogen of the urine was estimated. He vigorously refuted Frerich's idea of the direct oxidation of the food protein in the blood by pointing out that the tissues would be imperfectly nourished if the blood was not first transformed into body tissue before being metabolised. His conception that the metabolism of protein took place in the tissues rather than in the blood was extremely important because from it he drew his main conclusions, namely, that the urea was a measure of/

of tissue metabolism. Voit, a pupil of Bischoff, now took up the problem. One of his earliest papers was to prove clearly and conclusively that no atmospheric nitrogen was utilised by the animal organism. He fed a dog for 58 days on flesh and showed that the total nitrogen of the urine and faeces was almost exactly equal to that ingested. He was thus the first to prove "Stickstoffegleichgewicht" (nitrogen equilibrium) in the living organism. In collaboration with Pettenkofer he built a calorimeter and proved the existence of both carbon and nitrogen equilibrium. Acting probably on a suggestion of Liebig's, they estimated both the CO_2 expired and the O_2 absorbed by the animal, and, with a knowledge of the chemical constitution of fat and carbohydrate, they were able to show what proportion of these two foodstuffs was being oxidised during the course of the experiment. The information derived from the respiratory exchange along with the nitrogen output in the urine enabled them to present a complete picture of the quantities of all three foodstuffs metabolised over a period of time. The elaboration of this method was of prime importance for the study of nutrition because a knowledge of the metabolism of protein can not be divorced from that of carbohydrate and fat; all three are intimately related, and although their separation is justified as a method of scientific investigation, it is probable that in the body the metabolism of the three should be considered as a whole. Voit during his life-time investigated a number of problems/

problems in metabolism, many of them the outcome of older theories. He showed in the first place that the nitrogen output in the urine was conditioned by the intake, by maintaining dogs on different levels of protein and supplementing the diet with carbohydrate and fat. Liebig's thesis that protein alone was the source of energy for muscle work likewise drew his attention, and as a result of experiments on a human subject he showed that the nitrogen output on a constant diet is scarcely at all influenced by muscular work. Voit, as a result of his labours, had now material to build a hypothesis of protein metabolism in general. To appreciate his ideas one must remember the state of contemporary knowledge of the chemistry of the foodstuffs. The classification of the foodstuffs into the three types had been established before the elementary composition and calorie value of each were known. The elucidation of the chemistry of the amino-acids and the structure of the protein molecule had however scarcely begun and the knowledge of the nature of the chemical processes of digestion was but vague. Nevertheless all proteins from different sources were not looked upon as of the same composition. This view arose from their different behaviour in nutrition rather than from any appreciation of difference in chemical composition. It had been recognised for example that gelatin and beef had not the same nutritive properties. Voit's theory however was not concerned with protein in vitro but/

but with the protein in vivo and he asserted that protein existed in the body in two forms:-

(1) Circulating protein;

(2) Organised protein.

The former was considered to be built up from the food protein and varied in amount according to the quantity of protein ingested: it differed from the organised proteins by the ease with which it broke down in starvation.

The main evidence for its lability was derived from the fact that a dog during the first few days of starvation excreted more or less nitrogen in the urine according as it had been previously fed a high or low protein diet. The reserve of circulating protein was maintained high if the protein intake was high: if now a fast supervened this circulating material was metabolised, the nitrogen output gradually falling until it reached a more or less constant value by about the fifth day. If the previous diet were low in protein, the nitrogen output during the first few days of a fast was correspondingly diminished but by the fifth day it fell to the same figure as on the fifth day of fasting after a high protein intake. From about the fifth day onwards the nitrogen output during a fast was relatively constant, independent of the nature of the previous feeding. Voit held that circulating protein was built up from the food ingested, varied in quantity according to the protein intake and was relatively easily broken down.

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The rate of metabolism of circulating protein was ultimately conditioned by the level of intake. Under certain conditions however circulating protein instead of being immediately metabolised was transformed into what he called organised or body protein - "Organeiweiss." Under normal conditions only a small fraction of the circulating protein was transformed into organised protein to replace losses due to destruction of red cells and epithelial debris, &c. If large quantities of fat were fed with the protein, the amount of circulating protein transformed into organised material was increased, thereby diminishing the quantity of circulating proteins. The main feature of the organised protein was its relative stability. It was held to be part and parcel of the cell structure and was drawn on comparatively slowly during a fast. Voit shows the contrast in the behaviour of the two different types of protein by pointing out that in the later days of a fast the quantity of nitrogen excreted daily amounts to only 1% of the total nitrogen content of the animal. This nitrogen comes from organised protein. On feeding the animal on beef, on the other hand, the nitrogen output can be increased 15-fold over that found in starvation. Under these circumstances the nitrogen is being derived largely from labile circulating protein.

Voit also maintained that organised protein must first of all be transformed into circulating protein before being/

being metabolised. A reciprocal balance was supposed to hold between these two types of protein. On high protein feeding following a fast there was a slow transformation of circulating protein into organ protein if fat were added to the diet or if the animal had a good reserve of fat on its body. On fasting the opposite process was supposed to take place. Concerning the cause of the chemical processes in the cell, Voit clearly realised that neither oxygen, as Lavoisier and Liebig thought, nor the foodstuffs themselves, were the cause of metabolism. He says the size of the cells and their power to metabolise, and the quantity and quality of foodstuffs determine their metabolism. The chief conclusion of Voit was the immediate derivation of urine nitrogen from food nitrogen. The food protein did not become living material before being metabolised. The organised protein, viz., the tissues themselves, were, except under certain conditions, considered to be stable and intact.

The rôle of the organised tissues was simply to metabolise the foodstuffs to their end products. One is justified in saying that Voit's views were clear cut and common sense in so far as they did not go beyond the experimental facts. His outlook, however, tended to view the organism - as static - as a mere metabolising agent. The same spirit guided him in his conception of the cause of metabolism; he did not philosophise or speculate, but wisely and empirically declared that the cause lay/

lay in the cells themselves. Liebig in a paper published just before his death expressed impatience with the new idea; he saw no justification for assuming two types of protein in the living organism, and his final opinion in 1870 may be quoted:- "Das Eiweiss wirkt nur durch die Dinge, die durchaus erzeugt werden, und so ist es mir so gut wie unmöglich mich in die moderne Begriffe von Organ-Eiweiss und circuliernden Eiweiss hineinzufinden die denn einerlei Ding sind." He still held firmly to his original view that all food protein became metamorphosed into living tissue before being broken down.

The criticism of Voit's views however was left to one of his contemporaries with a more philosophical turn of mind. Pflüger of Bonn possessed the faculty not only of destructive criticism but also that of constructive synthesis and his writings show him to be a man of very definite convictions. Pflüger's idea was that all the food protein became an integral part of the cell before being metabolised. He held that the food protein corresponding to Voit's circulating protein was stable and the living protein was the unstable. Food protein after absorption became plasma which entered the cell and was thus metabolised. In the change from food protein to living material the amide nitrogen group was supposed to become transformed into the reactive cyanogen radicle which then broke down with formation of ammonia and urea. In order to account for the low nitrogen output/

output in starvation he assumed only a partial break-down of the protein molecule, the nitrogenous moiety being resynthesised at the expense of nitrogen-free foodstuffs, carbohydrate and fat. This was considered to be a protective adaptation to preserve the integrity of the tissues during starvation or on a low protein intake.

A similar synthetic process took place during muscle work. Muscle work was thus associated with the nitrogenous turnover in the cells but not necessarily with an increased output of nitrogen in the urine. He vigorously criticised Voit's conception that it is circulating protein which is metabolised and not the protein in the cells. Voit's term "circulating" tended to imply that the material was metabolised in the blood or tissue spaces and if there was one thing about which Pflüger was convinced it was that oxidation and metabolism took place in the cells themselves. Pflüger's ideas were essentially a refinement of Liebig's of a continual metamorphosis of living tissue and its replacement by new material derived from the blood. Once again the idea arose that the living body is in a continual state of flux - an opinion expressed explicitly by Haller nearly two hundred years before. (It should be noted, however, that Haller thought rather of a mechanical wearing away followed by a replacement from the food and not the more modern idea of a chemical flux). Pflüger's dictum/

dictum however that all food must first become an integral part of the living cell before being metabolised might be open to various interpretations. It is known that cell life will not continue unless all the constituents are present from the smallest inorganic ion to the complex nucleins and it is difficult to give precedence to any constituent, or say whether it becomes an integral part of the cell or not. It must also be remembered that the cell exists only in a suitable environment -- in the case of the higher animals the blood. Under these circumstances it is undesirable to give precedence to any metabolite once ingested, whatever its nature, or whether it exists in the cell or in the blood. One can not consider any part of the organism - cell, nucleus - existing independently in the concrete, the unity of the organism must be kept in mind, living activity will not continue unless the whole organism is intact and in a suitable environment. Pflüger realised this point, however, and said that whether a metabolite actually becomes a part of the cell or not may ultimately prove to be. "Eine Stritte über den Kaiser's Bart führen."

Voit and Pflüger never met and the problem was waged between them for many years by publication. These two workers however laid down the fundamental issues of metabolism on which further work and speculation followed. An elaborate though suggestive hypothesis of the metabolism of all three foodstuffs was/

was put forward about the beginning of this century by Max Kassowitz and in its essentials it is a development of Pflüger's ideas. His fundamental thesis was that the food ingested was all built up not into protein but into protoplasm. He held that all three foodstuffs were built up into a labile complex protoplasm. The katabolism of protoplasm might now follow one or other of two pathways:-

(1) The active: (2) The inactive.

The active katabolism was associated with muscle work and the nitrogen-free part of the protoplasmic molecule - the sugar and fat moieties were thought to be completely oxidised to CO_2 and H_2O, while the nitrogenous moiety was re-synthesised by means of the carbohydrate and fat in the blood and liver into protoplasm again. The inactive metabolism on the other hand was characterised by the break-down of the molecule into glucose and fat which were stored in the various tissue depots, liver, &c. The nitrogenous part was then excreted in the tissue largely as urea. On this hypothesis he attempted to explain two of the fundamental facts of metabolism:-

(1) That given a sufficient calorie intake muscular work scarcely influences the nitrogen output in the urine;

(2) The nitrogen output rises and falls with the amount of protei ingested.

During work the active metabolism was involved with the resynthesis of the nitrogen moiety by means of the carbo-hydrate/

carbohydrate and fat of the diet. The rise in nitrogen output often observed on the day after work he explains as follows. The glycogen and fat reservoir is supposed to have been depleted by the demands of the muscles, consequently on the next day there is an inactive break-down of protoplasm, i.e., the formation of urea which is excreted and of glycogen and fat which are retained to replenish their respective depots. Under conditions without work the metabolism of the protoplasm is of the inactive type and varies according to the protein intake. Kassowitz's ideas are not entirely supported by clear-cut facts, but they bring to the fore the conception of the living organism as a continual flux and warn us from attempts to analyse the metabolism of the three foodstuffs independently of each other. According to him food, after ingestion, was built up into a unity - namely, protoplasm containing protein, fat and carbohydrate. The different chemical structure of the three main foodstuffs is a satisfactory basis for in vitro experiments, and indeed for the analysis of the physiology of digestion, but it is quite probable that in the living cell they are built up into some complex of protein, fat and carbohydrate of varying compositions. Under these circumstances it may prove more profitable to consider the metabolism of food as a whole or, in short, of protoplasm. Michael Foster, although he did not carry out much work in this field, nevertheless clarified ideas on certain aspects of metabolism. He points out that not/

not all protoplasm can be called living - part of it consists of material which is becoming alive and part of material which is becoming effete. He maintains that protoplasm may be considered to consist of units called somacules. These units in order to live must contain not only a portion of living substance but also of food for this substance in various stages from the raw initial food to the living stage and from this again down to the various waste products. From this conception two problems arise - (1) That of anabolism, and (2) That of katabolism.

The stressing of anabolism and katabolism as a working hypothesis is the most valuable part of his views. The conception however of protoplasm consisting of living units surrounded by anabolites and catabolites appears to savour too much of vitalism and hardly agrees with the modern conception of the unity of the cell. When Pflüger spoke of protein becoming an integral part of the cell his conception was identical with the protein becoming what Foster called a somacule. The importance and indispensability of a certain tension of the waste product CO_2 in the blood and hence in the cells for maintaining the normal physiological activity of the organism appears to me to give as much vitality to CO_2 as to any other cell constituent such as a somacule if such exists.

Rubner's book "Die Gesetze der Energieverbrauch bei Ernahrung/

Ernahrung" in 1902, with a statement of his isodynamic law, tended to reduce the interest in any one foodstuff and to stress the calorie. According to this law the different foodstuffs can replace each other in a dietary within limits provided equal calorie amounts are taken. The calorie then became the first concern which expressed, especially in the public mind, the value of a foodstuff, an idea that held until the appearance of vitamins on the scene.

In 1905 considerable interest was aroused by Folin's paper on the laws regulating the composition of the urine. Folin, as a result of a large series of experiments on human subjects on high and low protein diets respectively, held that there were two different types of protein metabolism. As a result of the analysis of the urine on high and low protein intakes he found that urea inorganic sulphates, and in a lesser degree uric acid and ammonia, varied according to the level of protein ingested. On the other hand, creatinine and neutral sulphur were practically constant regardless of the intake. The creatinine and neutral sulphur were held to be a product of a constant type of metabolism which he called the endogenous.

The other type of metabolism represented by the urea output was called the exogenous in that it varied according to the material ingested and was considered to be derived from it directly. This theory stimulated a considerable amount of work/

work in metabolism, particularly in regard to the origin of creatinine, but otherwise interest in the problem of protein metabolism has lagged.

The conception however of an endogenous nitrogen metabolism has been developed and a considerable amount of experimental evidence has been adduced in support of it. Rubner called this endogenous metabolism the "Abnutzungs-quote" - wear and tear quota, or the minimum endogenous nitrogen metabolism. This wear and tear quota is to be obtained by feeding a nitrogen-free diet containing a high percentage of carbohydrate for some days. For the human subject the nitrogen output finally reaches a figure of about ·03 grms N. for Kilo.Wt.

The proof by Abel in 1913 of the presence of amino-acids in the blood gave the study of protein metabolism a new outlook. Hitherto it had been a problem as to the immediate fate of amino-acids after absorption. Various suggestions had been made. Some thought blood serum was resynthesised in the wall of the intestine. Others thought that the liver built up and stored a protein-like material in much the same way as it stored glycogen. The leucocytosis after a protein meal suggested to others that they were responsible for carrying off the protein from the intestine and building it into new tissue. Abel's demonstration however tended to simplify unduly the problem

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of protein metabolism. By means of experiments with individual amino-acids and their derivatives on diabetic animals and on perfused organs a scheme of katabolism was worked out for most of the individual amino-acids. The metabolism of protein now became that of the amino-acids absorbed from the bowel. Superficially no link seemed to be missing, the food protein was hydrolysed to amino-acids in the intestine, these were absorbed into the blood, and each one catabolised in a definite way to CO_2 and urea. The possibility of an anabolic phase after absorption tended to be overlooked. It was implicitly assumed that the body would catabolise the amino-acids in the proportion to which they were offered to it. The attempts made by Hofmeister to prove a synthesis of food protein in the wall of the stomach and intestinal glands now appeared without significance in the light of investigations by van Slyke and Meyers who showed a rise in the amino-acid content of the blood after a protein meal.

An elaboration on quantitative lines of the fate of the amino-acids after absorption showed however that they were absorbed readily by the liver and muscles and some hours elapsed before they were diaminised with a consequent increase in blood urea. During this interval the amino-acids were not found in the muscles in the free state, and one must conclude that they were forming part of some higher complex before being metabolised. The fact however that when the protein intake/

intake is increased some three days elapse before nitrogen equilibrium is attained would tend to support the idea that some kind of labile material is temporarily built up in the tissues. Folin however, in an oral communication to the author, was inclined to hold that there was always a certain amount of amino-acid in transit diffused throughout the tissues. This is undoubtedly true, but Mitchell, Nivens and Kendall have shown that the non-protein nitrogen of the tissues of rats, whether they are fed a high protein or a protein-free diet, is remarkably constant.

A review of the various theories which have been suggested tends to show that they fall more or less into two groups which differ in the way the living organism is conceived. The school best represented by Voit looked upon the organism as more or less static. The foodstuffs were metabolised to maintain the vital functions and their waste products finally excreted. The other school represented by Pflüger conceived the organism as being in a state of flux - a dynamic state. The ingested food was not considered to be merely fuel to the cells and having no relation to the living organism, but was held to become an integral part of the living substance to be broken down in its turn. These statements, it must be noted, are exaggerated in order to bring out the bias of each. In many details Voit differed but little from Pflüger in that he allowed that owing to the continual wear and tear of tissues

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a small amount of food protein must become organised. The term static applied to Voit's conception of the living organism must not be taken literally; he realised that the cause of the metabolism depends on the cell and its capacity to metabolise, influenced also by the quantity and quality of the food. To put it shortly, Voit conceived the cell as an active agent transforming the foodstuffs, while Pflüger conceived it as an active agent transforming and being transformed by the foodstuffs. Either view has been productive of discoveries, and indeed will continue to be so. In all probability neither will turn out to be correct, and they are but the result of different temperaments and mental outlook applying themselves to a study of life for which no single formula has yet been formed.

Part II.

The Method of Investigation.

The general method of investigation employed in the following work was that of the balance experiment. The validity and limits of such a technique must however be kept in mind. Voit in one of his early experiments fed a dog over a period of 58 days on 29 Kilos. meat containing 986 grms. Nitrogen and recovered 982.8 grms. Nitrogen in the urine and faeces. In his later experiments he succeeded in obtaining equality of Nitrogen intake and output in 24 hour periods in dogs. Animals maintained in this condition he designated as being in "Stickstoff gleichgewicht" or Nitrogen equilibrium. This experimental condition is extremely arbitrary; it means no more than that over a period of time intake and output of Nitrogen are equal. Voit in his experiments was more concerned at that time in showing that there was essentially no other loss of Nitrogen from the body except by the urine and faeces. No hypothesis or speculation on the nature of Nitrogen equilibrium was made at the time, and indeed he was shrewd enough to call it what it was - nitrogen equilibrium, and not protein equilibrium. It must be remembered that the digestion and absorption of a meal requires but a few hours, while its metabolism, certainly as far as protein is concerned, may extend/

extend for three or even five days after. There is no equality between rate of absorption and rate of metabolism, and although nitrogen equilibrium may exist according to our experimental conditions, it gives no indication of what is taking place in the cell. Indeed it is highly probable that at no time is the cell in metabolic equilibrium. Further, the validity of assuming protein storage or loss from the nitrogen balance is not unconditionally true, particularly over short experimental periods, as Abderhalden and Bloch have shown. Waste products or other intermediary metabolites may accumulate or be flushed out of the tissues without there being any change in the protein content of the body. The rate or amount of the nitrogen excretion may after all be no index of the rate or extent of protein metabolism during that period as the nitrogen in the urine is only an end product and its rate of excretion represents only the rate of formation of the end product, assuming that the kidney responds quickly in its elimination. The evidence of Addis and Watanabe tends to show that although in short periods the response of the kidney to an increase in urea productions may vary considerably, these fluctuations are compensated over long periods. In general the nitrogen excretion in the urine can be taken as a measure of the nitrogenous waste products actually formed within the 24 hours. Since all proteins contain nitrogen yet differ considerably as regards the percentage of different amino-acids in their molecules/

molecules, the nitrogen output in the urine gives no indication as to the source or type of the protein from which it was derived. The presence of sulphur, however, in variable quantity in most proteins, in virtue of its excretion in the urine may give an additional clue as to what kind of material is being metabolised. By comparing the S:N ratio of the food ingested with that of the urine taken over a sufficiently long period of time a fairly reliable index of the quality or type of the material metabolised may be obtained. Several possibilities however have to be borne in mind in interpreting such results. The organism may metabolise protein as a homogeneous unit, i.e., all the amino-acids are metabolised with equal ease, the amount of each one metabolised depending simply on the percentage quantity in the protein ingested. If this be the case, the S:N ratio of the urine will be the same as that of the particular protein ingested, assuming that it has been metabolised. On the other hand, certain amino-acids in a protein may be selectively dealt with by the body in preference to others. Under these circumstances if cystine were metabolised relatively early the S:N ratio would be at first high and later on fall below that of the protein ingested. Provided however a sufficiently long period of time is taken the S:N ratio of intake and output would tend to be the same - assuming that the organism metabolises the protein ingested. Feder first noted a difference in the rate of excretion of

sulphur/

sulphur and nitrogen after a protein meal, but he drew no conclusions from it. Von Wendt declared that only by estimating both the sulphur and nitrogen of the urine could a true picture of protein metabolism be obtained. Within the last thirty years a number of investigators have employed both the sulphur and the nitrogen balance for investigating protein metabolism (Sherman and Hawk, Hawk, Hawk and Chamberlain, Falta, Ehrström, Hämalainen and Helme, Wolf and Osterberg, Bornstein, Gros, Cathcart and Green, Wolf and Osterberg, Lewis, Tsuji, Fay and Mendel, Denel, Sandiford, Sandiford and Boothby).

In the experiments to be described the sulphur and nitrogen balance have been largely employed in trying to elucidate the nature of protein metabolism. All the balance experiments were carried out on the author, a healthy made subject weighing 65 Kilo. and between 24 and 30 years of age, within the six years during which the work was done. A few experiments in regard to certain details of the problem were carried out on a dog. Laboratory methods employed. Total nitrogen, Kjeldahl. Total sulphur, Denis. Phosphates. Titration with uranium acetate. Uric acid, Hopkins' method. Urea and ammonia, Folin's method.

The investigations to be described fall under the following headings:-

(1) The retention of protein.

(2) The influence of muscle work on protein metabolism.

(3) The influence of water ingestion on protein metabolism.

(4) Experiments on the nature of the lability of the sulphur moiety in protein metabolism.

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SECTION II.

THE RETENTION OF PROTEIN.

The question of protein retention is one of considerable interest and practical importance; for, while storage of fat may be looked upon as a more or less inert reserve, the storage of protein brings other issues to the fore. The problem can be envisaged from two points of view, namely,

(1) The capacity of the organism to retain protein;

(2) The value of different proteins for promoting retention.

From the point of view of the organism one has to consider whether there is a capacity to retain protein in the form of active metabolic tissue or whether there exists an inert "deposit protein" - as it has been called by Lusk - capable of being drawn upon as glycogen or body fat. Another possibility is whether the retained material is influenced by the nature of the protein ingested or whether the body always stores a protein of the same composition as its tissues or of a particular tissue such as muscle. This question brings to the fore precisely how much Nature and Nurture are playing in the life of the individual as far as metabolism is concerned. Associated with the problem of retention is the larger issue of protein metabolism as a whole and the daily protein requirements for health. One may ask if, when a retention of protein has been effected, it is necessary to continue a high protein intake in order to maintain this reserve. It may be that the optimum protein condition is maintained only by a high average nitrogen intake and that the/

the mere fact of nitrogen equilibrium is no guarantee that the tissues are being maintained at their optimum nutritional level.

A series of experiments were therefore carried out in order to obtain information on the above-mentioned issues. In order to find out the influence of the body condition on its capacity to store protein three sets of basal diets were taken. Each diet was planned to keep the body at a different nutritional plane as regards the protein element.

Diet. Experiments I and II. N. free.

100 grms olive oil.
300 grms tapioca.
100 grms sugar.
1 apple.

2,500 Calories.

Diet. Experiments III and IV. Low N.

400 grms bread.
50 grms cheese.
125 grms butter.
250 grms jam.
1 apple.

2,600 Calories.

Diet. Experiments V. and VI. High N.

470 grms bread.
200 grms cheese.
100 grms butter.
100 grms jam.
1 apple.

2,860 Calories.

In order to find the influence of the type of protein on the nature of the material retained, gelatin and egg albumen were/

were superimposed separately for one day on each of the above basal diets. Gelatin is a sulphur poor protein, the S:N ratio of the samples employed in the different experiments varied between 1:26 and 1:35. Egg albumen is a sulphur rich protein with a S:N ratio of 1:8 to 1:9.9 in the different samples employed. It will be noted that these proteins are respectively poorer and richer in sulphur than muscle tissue with a S:N ratio of 1:15.0.

The method employed in these experiments for finding the capacity of the tissues to retain protein is known as the superimposition method, first applied so successfully by Falta. This consists in giving a basal diet, constant as regards quantity and quality until nitrogen equilibrium is attained, and then on one day superimposing the protein to be investigated and following the nitrogen and sulphur outputs daily until they have fallen to the values found on the pre-period, i.e., when the basal diet alone was ingested. The amount of the nitrogen and sulphur metabolised of the protein superimposed is calculated by subtracting the basal nitrogen and sulphur outputs from the total nitrogen and sulphur outputs in the urine on the days following the superimposition. The sum of the nitrogen and sulphur metabolised subtracted from the nitrogen and sulphur of the protein superimposed gives not only the quantity of each retained but also the quality in so far as the ratio of sulphur to nitrogen is concerned. Experiments 1 and 2, Table I.

TABLE I.

Date.	Diet.	T.N. grms.	T.S. grms.	S:N.	P_2O_5. grms.	Excess N over basal.	Excess S over basal.	S:N of Excess
14 April	Basal.(N.Free)	4.312	.3902	1:10.9	2.101			
15	" "	4.256	.2528	1:16.8	1.505			
16	" "	3.276	.1978	1:16.5	1.177			
17	" "	3.024	.2088	1:14.4	1.079			
18	" "	2.688	.2088	1:12.8	1.079			
19 Exp.1.	Gelatin.Super-imposed Exp.	4.564	.4506	1:10.1	.568	1.876	.2418	1:7.7
20	Basal.(N.Free)	4.340	.2528	1:17.1	.837	1.652	.044	1:37.5
21	" "	3.668	.2528	1:14.4	.894	.780	.044	1:17.7
22	" "	3.192	.2308	1:13.8	.695	.050	.022	1:2.2
23	" "	2.912	.2253	1:12.9	.624	.0224	.016	1:1.4
24 Exp.2.	Albumen.Super-imposed Exp.	3.696	.4506	1:8.2	.781	.784	.2253	1:3.4
25	Basal.(N.Free)	3.528	.5001	1:7.0	.454	.616	.2478	1:2.4
26	" "	3.836	.3792	1:10.1	.695	.924	.1539	1:6.0
27	" "	3.388	.3672	1:9.3	.569	.476	.1374	1:3.4
28	" "	3.276	.3462	1:9.4	.582	.364	.1209	1:3.6

TABLE I (Continued).

Date.	Diet.	T.N. grms.	T.S. grms.	S:N	P_2O_5. grms.	Excess N over basal.	Excess S over basal.	S:N of Excess.
23 Feb.	Basal Diet	5.460	.3737	1:14.6	1.491			
24	"	5.544	.3242	1:17.1	1.526			
25	"	5.768	.4396	1:11.9	1.526			
26	"	5.537	.4836	1:11.1	1.278			
27	"	6.552	.5056	1:12.9	1.278			
28 Exp.3.	Gelatin. Superimposed.	9.240	.7969	1:11.5	1.313	3.196	.3023	1:10.2
29	Basal Diet.	6.860	.5605	1:12.2	1.171	.816	.0659	1:12.3
1 Mar.	"	6.468	.4461	1:14.4	1.242	.424		
2	"	6.244	.4561	1:13.6	1.207	.20		
3	"	6.076	.4561	1:13.3	1.936			
4 Exp.4.	Albumen superimposed.	7.252	.7912	1:9.1	1.312	1.213	.2966	1:4.0
5	Basal Diet.	8.344	.7473	1:11.1	1.313	2.30	.2527	1:9.9
6	"	6.076	.4467	1:13.6	1.278	.032		
7	"	6.272	.4506	1:13.9	1.384	.023		

TABLE I (Continued).

Date.	Diet.	T.N. grms.	T.S. grms.	S:N	Excess N over basal.	Excess S over basal.	S:N of Excess
22 Mar.	Basal Diet.	11.116	.610	1:18.2			
23	"	10.506	.560	1:18.7			
24	"	10.416	.599	1:17.3			
25	"	11.284	.588	1:19.1			
26 Exp.5.	Gelatin super-imposed.	16.520	.901	1:18.3	5.236	.313	1:16.1
27	Basal Diet.	14.448	.626	1:23.0	3.164	.038	1:83.2
28	"	13.272	.670	1:19.7	1.988	.082	1:24.2
29	"	11.200	.615	1:18.1		.027	
30	"	11.680	.582	1:20.0			
31 Exp.6.	Albumen super-imposed.	13.883	1.198	1:11.6	2.604	.610	1:4.2
1 Apr.	Basal Diet.	15.232	1.187	1:12.8	3.944	.599	1:6.5
2	"	11.312	.736	1:15.3	.024	.148	1:0.16
3	"	11.704	.747	1:15.6	.416	.159	1:2.6

The basal diet in this series was nitrogen free and there was consequently no condition of nitrogen equilibrium. The diet under those conditions is ingested until the nitrogen output reaches a more or less constant figure which represents what Rubner called the "Abnutzungsquote" - or minimum wear and tear quota. This is considered by Folin to represent the endogenous protein metabolism of the tissues. The feature of the endogenous metabolism is that it can not be further reduced however long the nitrogen free diet is continued. The body under these conditions is in a relatively nitrogen-poor condition. This low level of nitrogen output is usually reached within 5 to 7 days or so according to the previous diet, but a continuation of the nitrogen free feeding will often bring it down a fraction. The endogenous nitrogen output usually amounts to about .04 grm. per Kilo body weight in the human subject.

In Experiment 1, Table I, 70 grms. gelatin containing 9.939 grms. N and .3705 grms. S with a S:N ratio of 1:26.5 were superimposed. The following figures show the balance.

Basal nitrogen output (preday) = 2.688 grms.

" sulphur " " = .2088 "

Nitrogen ingested..... = 9.939 grms.

Nitrogen excreted over basal = 4.759 "

Nitrogen retained ..... = 5.180 "

Sulphur ingested..... = .3705 grms.
Sulphur excreted over basal = .3683 "
Sulphur retained..... = .0022 "

The percentage retention of nitrogen was 52, while the sulphur retention was negligible. Apparently there was no selective storage of a material similar in composition to muscle tissue, nor did it correspond to that of the gelatin ingested. In Experiment 2, (Table I) egg albumen containing 9.9619 grms. N and .9995 grms. S with a S:N ratio of 1:9.9 was superimposed for one day. The following figures show the balance.

Basal N (pre day) = 2.912 grms.
Basal S " = .2253 "

Nitrogen ingested..... = 9.9619 grms.
Nitrogen excreted above basal = 2.800 "
Nitrogen retained..... = 7.1619 "

Sulphur ingested..... = .9995 grms.
Sulphur excreted above basal. = .9123 "
Sulphur retained..... = .0872 "

S:N of retained material = 1:82.3

Again the material retained corresponds neither to muscle tissue nor to the protein ingested.

The/

The retention of nitrogen is high however, 70% as opposed to 52%, in Experiment 1 with gelatin. The comparison is all the more valid because the total nitrogen ingested in both experiments was approximately the same. The interesting point to note, however, is the rate of metabolism of the ingested proteins. The maximum nitrogen and sulphur excretion when gelatin was superimposed was on the day of ingestion; it then gradually fell from day to day. On the other hand when albumen was superimposed the maximum sulphur excretion was on the day following and the maximum nitrogen excretion two days following the superimposition. The possible significance of this will be seen in comparison with the other experiments with those two proteins.

In Experiments 3 and 4, with a low protein intake as the basal diet, the same two proteins were superimposed.

In Experiment 3,70 grms. gelatin containing 9.936 grms. N and .3750 grms. S with a S:N ratio of 1:26.5 were superimposed.

Basal nitrogen (average of the two predays)	=	6.044 grms.
" sulphur " " "	=	.4946 "
Nitrogen ingested as gelatin.....	=	9.936 grms.
Nitrogen excreted over basal.....	=	4.634 "
Nitrogen retained.....	=	5.302 "

Sulphur ingested as gelatin = .3750 grms.
Sulphur excreted over basal = .3682 "
Sulphur retained..... = .0068 "

The experiment practically repeats the previous one on a nitrogen free diet; 53% of the nitrogen being retained and almost all the sulphur eliminated. Apparently the sulphur of the basal diet has not been utilised to supplement the gelatin in order to build up some definite body tissue such as muscle.

In Experiment 4 the same basal values were employed as in Experiment 3. Egg albumen containing 10.206 grms. N and 1.059 grms. S with a S:N ratio of 1:9.6 was superimposed. The following figures show the balance.

Nitrogen ingested as albumen = 10.206 grms.
Nitrogen excreted above basal = 3.768 "
Nitrogen retained..... = 6.438 "

Sulphur ingested as albumen = 1.059 grms.
Sulphur excreted above basal = .5495 "
Sulphur retained..... = .5095 "

The S:N ratio of the material retained is 1:14.69, a figure corresponding closely to that usually accepted for muscle - 1:14 - 1:15.

The percentage retention of the nitrogen is 72 - practically the same as in Experiment 2. The rates of metabolism of these/

these two proteins present similarities to those in the previous series on a nitrogen free diet. The albumen is again more slowly metabolised than the gelatin, but in contrast to Experiment 2 with albumen the maximum sulphur excretion is on the day of superimposition and the maximum nitrogen on the following day: in short the maxima are both one day earlier than in Experiment 2. This is of significance because the absolute amount of albumen nitrogen ingested in these two experiments is practically the same - 9.9 grms. in Experiment 2 and 10.2 grms in Experiment 4 - and the absolute amount eliminated is the same yet this latter quantity is metabolised more quickly. This would seem to indicate that the basal diet must have some influence on the capacity of the body to deal with the protein superimposed. In Experiments 5 and 6, Table I, the nitrogen content of the basal diet was about 14.0 grms., the urinary nitrogen did not however reach this figure as the average daily loss in the faeces was 2.2 grms. (Exps. 5 and 6, see Table I, p.29).

In Experiment 5, 70 grms. gelatin containing 9.936 grms. N and .3750 grms. S with a S:N ratio of 1:26.5 were superimposed on one day and the following balance obtained.

Basal nitrogen (N output on day before superimposition)	=	11.284 grms.
" sulphur " " "	=	.5880 "
Nitrogen ingested as gelatin	=	9.936 grms.
Nitrogen excreted over basal	=	10.386 "
Nitrogen lost.....	=	.450 "

Sulphur ingested as gelatin = .3750 grms.
Sulphur excreted over basal = .460 "
Sulphur lost..... .085 "

The S:N ratio of the material lost is 1:5.37. Little stress can be laid on it as the absolute figures are so small. Under the conditions of the experiment apparently none of the gelatin has been retained. The protein of the basal diet it seems has had no influence in supplementing the poor quality of the gelatin. It must be noted, however, that gelatin is a sulphur poor protein and that although the basal diet was relatively nitrogen rich the S:N ratio of the urine was about 1:18.0, i.e., below that of muscle tissue. Under these circumstances it is possible that the sulphur of the basal diet may have been required for the normal metabolic processes at the time, and could not be sacrificed to improve the quality of the gelatin for storage. It must also be noted that the main source of protein in the basal diet was the casein of the cheese. As casein is the prime protein for growth, it is only natural to suppose that any sacrifice of its amino-acids to supplement gelatin would mean a corresponding diminution in the biological value of casein itself. Mendel moreover has shown that in experiments on rats, fed an adequate dietary with casein as the sole source of protein, the cystine appears to be the limiting amino-acid. He found that cystine added to the/

the diet increased the rate of growth when the casein made up below 12% of the total food intake. Thomas found the biological value of casein to be only 66% as tested on the human subject. This finding, along with Mendel's experiments, would then tend to show that casein is unlikely to be capable of supplementing gelatin at least as far as its sulphur moiety is concerned.

In Experiment 6, Table I, egg albumen containing 9.80 grms. nitrogen and 1.204 grms. sulphur with a S:N ratio of 1:8.12 was superimposed.

Basal N (preday of Exp.5)	=	11.284 grms.
Basal S " "	=	.588 "
Nitrogen ingested as albumen	=	9.8 grms.
Nitrogen excreted above basal	=	7.00 "
Nitrogen retained.....	=	2.8 "
Sulphur ingested as albumen	=	1.204 grms.
Sulphur excreted above basal	=	1.5164 "
Sulphur lost.....	=	.3124 "

The same tendency is seen in this experiment as in the previous one, for the retention to be reduced. Only 28% of the nitrogen was retained, while there was a definite loss of sulphur. The albumen has apparently stimulated the catabolism of body or basal diet protein and effected the elimination of

.312 grms sulphur while the nitrogen associated with this sulphur has been retained. The tissues are thus relatively poorer in sulphur not only in virtue of the small amount of albumen nitrogen retained but also because of the loss of sulphur either from body tissue or the food protein in transit. The rate of metabolism of the two proteins in these experiments shows the same features as the previous ones. The maximum nitrogen and sulphur output was on the day of gelatin superimposition (Experiment 5), while in Experiment 6 the sulphur outputs were almost identical on the day of superimposition and the following day. The nitrogen output however did not reach its maximum until the day after superimposition as in Experiment 4 on the low nitrogen diet.

A review of those six experiments brings out several features in regard to the retention of nitrogenous material. In the first place there has been no definite tendency for the body to store passively a material of the same composition as that ingested. In the second place, under the conditions of these experiments, the type of material retained, with one exception, did not correspond to muscle tissue as far as one can judge from the S:N ratio. In Experiments 1 and 2, for instance, where sulphur poor and sulphur rich proteins were superimposed respectively, the material retained was nitrogen only. In Experiment 6 with albumen there was actually a loss of sulphur and a retention of nitrogen. Thirdly, these experiments/

experiments show that the nutritional plane of the body has influenced the amount of retention of the protein ingested. In Experiments 1 and 3 on a nitrogen free and low nitrogen diets respectively the retentions of gelatin nitrogen were 52%, 53%, while the retention of albumen nitrogen in Experiments 2 and 4 was 70%, 72%. It is probable that on both of those diets the body was below its optimum nutritive condition and retained the maximum percentage possible of the two proteins. In Experiments 5 and 6, however, with a larger nitrogen intake in the basal diet only 28% of the albumen nitrogen was retained, while all the gelatin was catabolised. Experiments 5 and 6 are instructive from two points of view. In the first place when the nitrogen content of the basal diet has been increased to 14.0 grms. there is less tendency to retain protein; the tissues were apparently at or near their optimum condition in regard to protein under the conditions of life of the experimental subject at the time. In the second place with the high nitrogen content of the basal diet, the body shows more discrimination in regard to the source of nitrogen selected for retention, 28% of the albumen nitrogen being retained, while the gelatin nitrogen was rejected. The fact that gelatin has been retained in some experiments and not in others merits some discussion as its rôle in nutrition has long been a subject of dispute. It is well known from the work of Voit who showed that gelatin fed over a period of

several/

several days could not prevent loss of body tissue. He stated that it was incapable of building up body tissue on organ protein according to his nomenclature. Gelatin, however, if supplemented with beef in the ratio of 1 part of gelatin to 4 parts of beef was capable of effecting nitrogen equilibrium. Krummacher showed that however much gelatin were fed a fasting animal the nitrogen loss due to starvation was only reduced to 60%. Abderhalden and Bloch pointed out that in short experiments with gelatin there may be a temporary retention followed by a loss. Their experiments were done on an alkaptonuric subject and it was observed that after a few days of the gelatin diet the homogentisic acid output increased without a concomitant increase in the nitrogen output until a day or two later. As gelatin is deficient in the precursor of homogentisic acid, the increased output of this acid must have arisen from the catabolism of body tissue to compensate for the deficiency of gelatin as a source of protein. The nitrogen balance itself, however, did not immediately show this loss of body tissue.

Had the investigators estimated the sulphur output it is extremely probable that a rise would have been observed along with the increase in homogentisic acid. Murlin carried out a series of experiments with gelatin on man. He showed that with a total nitrogen intake of 10% over the output in starvation and with a sufficient calorie intake 63% of the nitrogen could be replaced by gelatin if the remainder were in the form

of/

of beef without incurring a loss of body nitrogen. He also maintained that the capacity of the organism to utilise gelatin was greater the more impoverished the tissues were in protein, such as after a fast. This he considers a biological adaptation of importance and it is clearly illustrated from the experiments recorded in this paper. Umeda carried out a series of experiments on dogs fed an adequate carbohydrate rich diet containing 7 grms. nitrogen as protein. Gelatin was then superimposed for 8 days and a retention of 20% of the (presumably) gelatin nitrogen was noted. It is probable that the nitrogen and sulphur of the basal diet supplemented the gelatin in his experiments however. The increased nitrogen intake in the 8 days may have favoured retention by increasing the available metabolites in the tissues. Robison carried out a series of experiments on himself to discover the capacity of gelatin to cover the endogenous wear and tear metabolism.on a nitrogen free diet. Although the nitrogen ingested as gelatin was some five or six times the endogenous output in the urine, the sparing of body tissue or the reduction in the endogenous output was only 15%. Kauffmann found that gelatin fed as the sole source of protein to man and dogs was effective in maintaining equilibrium only if cystine tyrosin and tryptophan were added. Borutteau on the other hand found gelatin to have a biological value of 49% calculated as Thomas's formula. The basal diet however contained a small quantity of potate protein and this may have been responsible for the

favourable/

favourable outcome. Analysis of the results of the various workers along with those reported here tends to show that gelatin can fulfil a certain rôle in the metabolic processes. Robison's work showed that it was extremely inefficient for covering the wear and tear quota: as the endogenous wear and tear quota comes from body tissue with a S:N ratio of 1:15 it would appear that gelatin can not build up a material of this composition. The data in the present thesis showing a retention of nitrogen but not of sulphur would tend to confirm this, but it can not be overlooked that this retention of nitrogen on a low nitrogen and nitrogen free diet indicates that gelatin possibly can play some rôle in nutrition. The increasing value of gelatin to the organism the lower the nutritive plane is similar to Mitchell's figures for the biological value of protein fed at different levels in promoting growth in rats. He found that with 5% of milk protein in the food 93% was retained for growth, while with 10% in the food only 84% was retained. The general conclusion to be drawn from these experiments tends to show that the biological value of a protein to the organism can neither be obtained from an analysis of the protein nor can it be expressed in exact terms from experiments on animals. The commonly accepted standard of a protein is its capacity to promote growth,as in Mitchell's experiments, or to cover the wear and tear in metabolism, as in Thomas's. According to this standard gelatin has no value, yet the experiments recorded/

recorded here showing a retention of gelatin under certain circumstances and Umeda's experiments showing a retention of nitrogen when gelatin was added to a nitrogen containing diet show that too strict a standard should not be employed in assessing the biological value or perhaps better the nutritional value of a protein.

In all probability the mode of life of the organism, its environment in the broad sense and the ratio between the non-nitrogenous moiety of the diet (Umeda-Tsuji) markedly influence the value of a protein in nutrition. The point which must be kept in mind is that all experiments aiming at definite figures have been carried out - and indeed must be - under definite conditions which preclude their liberal application either to animals in nature or to the living community.

The question still remains however as to what kind of material has been retained in these different experiments. This query includes such problems as the site of retention and its function in nutrition. Voit who first studied this problem realised that the main issue was not the chemical composition of the retained material or the site of retention but the rôle it played in nutrition. His classification into organ protein and circulating protein was based on their different behaviour under certain circumstances which have been previously discussed. He had no facts at hand to indicate whether chemically these two types/

types were different. Rubner in 1911 employed his master's method in attempting to classify the different types of protein which might be retained and their function in nutrition. The following is his classification:-

(1) Protein retained during growth or convalescence: this was held to be a retention of organised or living material.

(2) Improvement quota (Meliorationseiweiss). The criterion for the existence of this was the fact that a normal adult on a high nitrogen diet may retain a certain amount of material which is not metabolised when the diet is changed to a nitrogen free one.

(3) Transitional protein (Ubergangseiweiss). This type of material was slowly metabolised over the first 18 days or so of a nitrogen free diet in contrast to the improvement protein which was more stable.

(4) Vorratseiweiss. (Voit's circulating protein). The quantity of this material retained at any one time is intimately associated with the protein content of the diet. A certain amount of this circulating protein is retained and lost within three days or so of increasing and decreasing respectively the protein intake.

The first two were classified as static and the last two as alimentary types of retention. The improvement quota was supposed to be retained in all the body cells regardless of their function and/

and thereby raising the nutritive condition of the tissue cells as a whole. The "Ubergangseiweiss" on the other hand was a temporary retention of material possibly concerned with the assimilation and metabolism of protein food. On a nitrogen free diet the necessity of this reserve is dispensed with and it is slowly metabolised. In this connection it is of interest to note that Seitz found the livers of ducks and hens fed a high protein diet to be increased in weight in greater proportion than the increase in body weight: an increase in 100% in liver weight with only a 15% increase in body weight was noted. Similarly (Mackay, Mackay and Addis and Mendel) observed a hypertrophy of the kidneys in rats fed a high protein diet. As both those organs are intimately concerned in the handling of protein in its course of metabolism it is not improbable that "Ubergangseiweiss" is a hypertrophy of liver and kidney and possibly some other tissues. Müller estimated the nitrogen to carbon ratio in glycogen and fat free muscles of dogs fed high and low protein diets and noted a slight increase in the N:C ratio in the animals fed the high nitrogen diet. This indicates some material slightly richer in nitrogen and possibly represents Rubner's improvement quota. Rubner holds however that the circulating (Vorratseiweiss) protein is simply a condition of a nitrogen equilibrium. If there were no temporary reservoir such as this, equilibrium would be impossible with an intermittent food intake. The main features

of/

of the "Ubergangseiweiss" and "Vorratseiweiss" is their relative lability. The latter is metabolised within a three days or so after the nitrogen intake has been reduced or stopped, while the former requires some twenty days (depending on the previous feeding) or so before it is completely metabolised. Grüber's experiment and analysis is probably the clearest on the question of circulating protein or "Vorratseiweiss;" and his figures are hence quoted here. It is an established fact that if the protein intake is suddenly increased equilibrium is not reached until some three or four days later, the nitrogen output gradually rising each day. Correspondingly, if the nitrogen intake is suddenly reduced a similar period elapses before the output is at the previous level. Grüber holds that if 100 grms. of protein are superimposed 80 grms. will be metabolised the first day, 13 grms. on the second, 5 grms. on the third, and 2 grms. on the fourth. Consequently if the protein has been superimposed daily, by the fourth day equilibrium will be established, the nitrogen in the urine on this day being derived in varying proportion from that ingested in the previous four days. This material which is retained when the nitrogen intake is increased and eliminated when it is decreased Grüber held to be protein and it illustrates the lability of what Voit called circulating protein. Thomas's observations on himself, on the other hand, show the difference between "Ubergangseiweiss" and circulating protein. A nitrogen free diet was ingested until the endogenous

wear/

wear and tear metabolism of 2.2 grms. per diem was reached. Four days of high protein feeding followed, during which 66 grms. nitrogen were retained; the nitrogen free diet was thus resumed. Thomas calculated that all the nitrogen eliminated above the endogenous level was coming from this retained material. The following figures are taken from his paper and show the trend of the nitrogen output on a nitrogen free diet.

	Grms.N.in Urine.	
Last day of high protein diet	77 grms.	
1st day N. prediet.	28.31)	) Derived mostly from
2nd "	10.70)	circulating protein.
3rd "	5.15)	)
4th "	5.16)	) Derived mostly from
5th "	4.72)	"Ubergangseiweiss."
8th "	3.46)	)
20th "	2.23	- Derived from body (organised) tissue.

It will be noted that the material metabolised from the 3rd day of the nitrogen free diet is relatively stable in that it is slowly metabolised from the 3rd to the 19th day of the N free diet: by the 20th day the endogenous wear and tear is being covered entirely by what is considered to be true body tissue. "Ubergangseiweiss" and circulating protein are then distinguished by a difference in their stability, the latter being/

TABLE II. Experiment 7.

Date.	Diet.	T.N. grms.	T.S. grms.	S:N.	N. above basal	S. above basal	S:N above basal	N. above upper basal	S. above upper basal	S:N above upper basal
15 Apr.	Basal	5.936	.494	1:12.01						
16	"	6.123	.527	1:11.61						
17	250 g. beef superimp.	8.196	.664	1:12.33	1.984	.137	1:14.48			
18 PERIOD I	"	9.301	.747	1:12.46	3.095	.220	1:14.06			
19	"	10.600	.791	1:12.71	4.394	.264	1:16.64			
20	"	9.772	.769	1:12.70	3.566	.242	1:14.73			
21	500 g. beef superimp.	13.212	1.120	1:11.79	7.006	.595	1:13.45	.982	.189	1:5.1
22	"	16.480	1.318	1:12.50	10.274	.791	1:12.98	4.250	.387	1:10.9
23 PERIOD II	"	18.424	1.352	1:13.62	12.218	.825	1:14.80	6.194	.421	1:14.7
24	"	19.824	1.406	1:14.09	13.618	.879	1:15.49	7.594	.473	1:15.9
25	"	19.420	1.384	1:14.30	13.214	.857	1:15.41	7.190	.453	1:15.8
26	250 g. beef superimp.	15.540	1.055	1:14.83	9.334	.528	1:17.67	3.310	.124	1:26.6
27	"	13.337	.939	1:14.20	7.131	.412	1:17.30	1.107	.008	1:138.3
28 PERIOD III	"	11.866	.923	1:12.55	5.660	.396	1:14.29			
29	"	12.600	.939	1:13.41	6.394	.412	1:15.27	.370	.008	1:46.2
30	Basal Diet alone.	9.819	.791	1:12.42	3.616	.264	1:13.69			
1 May	"	7.756	.532	1:14.57	1.553	.005	1:310.			
2	"	6.935	.560	1:12.38	.732	.033	1:22.1			
3 PERIOD IV	"	6.560	.560	1:11.71	.357	.033	1:10.81			

TABLE II (Continued).

Date.	Diet.	N. retained daily.	S. retained daily.	S:N of retention.	% N. retained each day	% S. retained each day.
17 Apr.	250 grms. beef.	4.040	.267	1:15.13	36.5	35.4
28	"	2.929	.184	1:15.91	26.4	24.9
19 PER. I	"	1.630	.140	1:11.6	14.7	18.5
20	"	2.458	.162	1:15.18	22.2	21.5
21	500 grms. beef.	6.410	.275	1:23.3	53.6	67.7
22	"	3.136	.077	1:40.4	26.2	18.9
23 PER. II	"	1.198	.043	1:27.86	10.0	10.5
24	"	-	-	-	-	-
25	"	.202	.011	1:18.3	1.6	2.6
Date.	**Diet.**	**N.lost daily.**	**S. lost daily.**	**S:N of loss.**	**% N.lost daily.**	**% S. lost daily.**
26	250 grms. beef.	3.310	.124	1:26.6	60.7	88.5
27	"	1.107	.008	1:138.3	23.1	5.7
28 PER. III		-	-	-	-	-
29	"	.370	.008	1:46.2	7.7	5.7
30	No beef.	3.616	.264	1:13.69	57.6	78.8
1 May	"	1.553	.005	1:310.6	24.8	14.9
2	"	.732	.033	1:22.1	10.0	9.5
3 PER. IV.	"	.357	.033	1:10.8	5.7	9.5

being metabolised relatively quickly when the nitrogen intake is reduced and the former more slowly.

In general it may be said that Voit and Rubner's ideas on the subject of retention are the most clear cut on which to base further work. A series of experiments were therefore planned on a slightly different basis from these just described in order to get more information about the different types of retention. The existence of a retention during growth and after convalescence must be held as an established fact. In general it is probable that the material retained is flesh although no balance experiments with nitrogen and sulphur estimations have yet been made in those conditions.

The only possible experiments which the writer could investigate on himself were obviously those concerned with Rubner's 2nd, 3rd, and 4th types of retention, namely, "Meliorationseiweiss" (improvement quota), "Ubergangseiweiss" (transitional protein) and "Vorratseiweiss" (circulating protein).

Experiment 7, Table II. A basal diet of the following make-up was ingested until nitrogen equilibrium was attained:-

470 grms. bread.
125 grms. butter.
200 grms. jam.
1 apple.

2,500 Calories.

250 grms. of beef were then superimposed daily for four days (Period I), then another 250 grms. beef were added

(500/

(500 grms. in all) daily for 5 days (Period II). In Period III the beef was reduced to 250 grms. again for 4 days and finally in Period IV basal diet was resumed. The results are tabulated in Table II.

Interesting information can be obtained in the first place by noting the total amount of nitrogen and sulphur excreted over the nitrogen and sulphur outputs of the basal diet. The figures employed as the basal nitrogen and sulphur output are the average of the 15th and 16th April and the 3rd May.

Basal Nitrogen = 6.206 grms.

" Sulphur = .527 "

The following shows the total nitrogen and sulphur and S:N ratio of the excess outputs in each of the four periods.

Period I, 4 days.	= 13.039 grms N.		
250 grms. beef daily	= .863 grms S.	S:N	1:15.10
Period II, 5 days.	= 56.331 grms N.		
500 grms. beef daily	= 3.947 grms S.	S:N	1:14.27
Period III, 4 days.	= 28.519 grms N.		
250 grms. beef daily	= 1.748 grms S.	S:N	1:16.31
Period IV, 4 days.	= 6.258 grms N.		
Basal diet alone	.335 grms S.	S:N	1:18.68

The average S:N ratio of the beef ingested was 1:15.65 and it will be seen that the material metabolised in each period is/

is not the same as judged by the S:N ratio.

The following figures show the total excess and S:N ratio for periods I and II when the nitrogen intake was being increased and for periods III and IV when it was being decreased.

Periods I and II excess.	69.370 grms N.	
	4.813 grms S.	
	S:N	1:14.41
Periods III and IV excess.	34.777 grms N.	
	2.083 grms S.	
	S:N	1:16.37
Total excess	104.047 grms N.	
	6.896 grms S.	
	S:N	1:15.09

In Period I the material metabolised has a S:N ratio of 1:15.1 which is slightly richer in sulphur than the beef. In Period II the ratio indicates that a material still richer in sulphur is being metabolised. In Periods III and IV the reverse phenomena are observed, the ratio falling first to 1:16.31 in Period III and then to 1:18.68 in Period IV. There appears to be a distinct regularity in the four periods which shows that the S:N ratio of the excess is richer in sulphur than that ingested when the nitrogen intake is rising (Periods I and II), while the reverse is the case when the intake is being reduced. The total excess of nitrogen and sulphur however

appears/

appears to have a S:N ratio closely approaching that of the beef ingested. The general trend of the S:N ratios shows that the higher the protein intake the richer in sulphur is the excess material eliminated. In Period I, for instance, with 250 grms. beef the S:N ratio is 1:15.10, while in Period II, with 500 grms. beef, the S:N ratio of the excess is 1:14.27. In order however to see the full significance of the results, the nitrogen and sulphur retention or loss in each period must be calculated. It is clear that in Periods I and II when beef was superimposed there was a retention for the first few days until equilibrium was attained; correspondingly in Periods III and IV when the protein intake was being reduced there was a loss until equilibrium at a lower level was reached. In order to calculate the retention a balance between intake and output is required. The faeces however were not analysed for nitrogen and sulphur as the estimation of the relatively small amount of sulphur in faeces is difficult and may not be absolutely valid in view of the probable loss of sulphur in the alimentary canal as H_2S. The following basis for calculation was therefore employed. It is assumed that when equilibrium is attained, the nitrogen and sulphur output in the urine represents the total nitrogen and sulphur absorbed from the alimentary canal. In Period I with 250 grms. beef the maximum nitrogen output was 10.6 grms. on the 3rd and 9.7 grms. on the 4th day of superimposition. In Period III with the same beef intake it was

11.8/

11.8 grms. and 12.6 grms. on the 3rd and 4th days respectively, and it is probable that the average of these is the more likely figure. The figures representing the nitrogen and sulphur absorbed in Periods I and III are therefore taken as the average of the 28th and 29th April. The figures employed are 12.233 grms N and .931 grms S. In Period I, Table II, it will be noted that there is a progressively decreasing retention each day until equilibrium is approached. The retention for each day is calculated by subtracting the nitrogen and sulphur output on that day from 12.233 grms. N and .931 grms S. respectively, these last two figures representing the intake or more strictly speaking the nitrogen and sulphur absorbed. In Period II the total absorption of nitrogen and sulphur is taken as the average of the last two days of that period, namely the 24th and 25th April - (19.622 grms. N, 1.395 grms. S), and the retention is calculated on the same basis as in Period I. In Period III as the nitrogen intake is reduced, the output for the first few days is greater than that absorbed; correspondingly the figures employed in Period I (12.233 grms. N, .931 grms S) are subtracted from the total nitrogen and sulphur output on each day on which they exceed this value. Period III represents a loss as the nitrogen and sulphur absorbed has been reduced from 19.622 grms. N and 1.395 grms. S to 12.237 grms N. and .931 grms S.

In Period IV when no beef was ingested the loss is calculated/

calculated by subtracting the original basal figures (6.206 grms. N and .527 grms. S) from the outputs on each day on which they exceed these figures.

The following figures show the retention or loss in each period.

Period I, 4 days. = 11.057 grms. N retained.
250 grms. beef. = .753 grms. S "
S:N 1:14.69.

Period II, 5 days. = 11.946 grms. N retained.
500 grms fat. = .406 grms S "
S:N 1:29.4

Period III, 4 days. = 4.787 grms N lost.
250 grms beef. = .146 grms S "
S:N 1:34.1

Period IV, 4 days. = 6.255 grms N lost.
250 grms beef = .335 grms S "
S:N 1:18.6

These figures show a definite symmetry as regards the S:N ratio. The material retained in Period I with 250 grms beef is similar in composition to the beef ingested, namely 1:14.69 as opposed to 1:15.65 (beef). In Period II with 500 grms beef the absolute retention due to the extra 250 grms beef is practically/

practically the same as in Period I, i.e., 11.0 grms. The quality of the material retained however at this higher level differs in that it is poor in sulphur, with a S:N ratio of 1:29.4 Correspondingly in Period III when the beef intake was reduced the S:N ratio of the loss is 1:34.1.

In short while the material lost in Period III is less than half the retention in Period II qualitatively this substance lost in Period III is approximately the same as that retained in the previous period (II) namely sulphur poor. In Period IV which is the counterpart of Period I the S:N ratio of the loss approache that of the retention in Period I. Again the loss in Period IV is less than the retention in Period I and as the same holds good for Periods II and III there is a retention all over. The following shows the balance.

Periods I and II. 23.003 grms N. retained.
1.159 grms S "
S:N =:19.84

Periods III and IV. 11.045 grms N lost.
.457 grms S lost.
S:N 1:23.25.

Total Retention 11.958 grms N.
.684 grms S.
S:N 1:17.48

The S:N ratio of what has been retained at the end of the experiment if compared with the S:N ratio of what has been retained by the end of Periods I and II shows that the body has been more conservative in the loss of sulphur than of nitrogen. In other words the S:N ratio of the 23 grms nitrogen retained by the middle of the experiment is 1:19.84 and the loss in Periods III and IV has raised the ratio of the retained material to 1:17.48 by eliminating relatively more nitrogen than sulphur.

This selective retention of sulphur is to be noted even in the individual periods.

For instance in Period I the S:N ratio of the retained material is 1:14.69 while in Period IV its counterpart the S:N ratio of the loss is 1:18.6: similarly in Period II the ratio of the retained product is 1:29.4 while in Period III its counterpart the ratio of the loss is 1:34.1. In general while the quality of the material retained at one level tends to be the same as that lost in a corresponding period of reduced intake the loss in nitrogen is slightly greater than that of sulphur. As regards the nature of the retained material, this experiment demonstrates the difference between circulating protein and the more stable "Meliorationseiweiss" or "Ubergangseiweiss." Which of these two proteins has been retained in this experiment can not yet be decided however. The loss in Periods III and IV represents circulating protein as Voit and Rubner understand it/

it and this retention appears to be conditioned entirely by the level of protein intake: once this falls a certain amount of circulating protein is lost according to the amount by which the intake is reduced. The characteristic of this circulating material lost in Periods III and IV is its poverty in sulphur, the S:N rate being 1:23.25. The complete retention however of the 11.9 grms. N and .684 grms S (S:N 1:17.48) can not be circulating protein as it has not been metabolised when the protein intake was reduced, and it is apparently much richer in sulphur than the circulating protein. It is important to note that its composition does not differ much from that of body tissue: its stability in the face of a falling nitrogen intake appears possibly to be associated with a sulphur content higher than that of the circulating protein. If then the assumption is correct that these two types of protein (circulating and Meliorationseiweiss or Ubergangseiweiss) have been built up in Periods I and II, it would appear that the "Meliorationseiweiss" or "Ubergangseiweiss" with a S:N ratio of 1:17.48 has been largely retained in Period I with 250 grms beef when the S:N ratio of the retention product was 1:14.69, while the unstable circulating protein has been retained in Period II where the S:N ratio of the stored material was low (1:29.4). It should however be kept in mind that these levels of intake are quite arbitrary and that in all probability in Period I the retention was largely though not entirely in the more stable form, while

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in Period II with 500 grms. beef the retention was predominantly in the form of circulating protein as judged by the S:N ratio. If this is true, it indicates that the lower the protein intake the more stable is the retention and presumably the more important is its rôle in metabolism. If this is true it illustrates an important biological economy. It need not follow however that the unstable circulating protein is of no importance; its rôle may possibly be to keep the metabolic activities at their optimum, while the more stable type of retention may be concerned with the integrity of the cell.

The experiment however can be calculated on another basis, partly in order to get more insight into the nature of retention and to validate the assumption which has been made in the method of calculation. The basis of the calculation, as has been mentioned, is that the nitrogen and sulphur outputs when equilibrium is attained represent the amounts of those elements absorbed. The possibility however must be kept in mind that on the one hand the beef ingested may have been partially digested and absorbed, the undigested moiety being eliminated in the faeces On this assumption the quality of the material absorbed would be identical with that of the beef. On the other hand there is the possibility that the beef was completely hydrolysed and that some amino-acids or peptides were more completely absorbed than others. On this assumption the quality of the material absorbed would not correspond to that of the beef. The calculations however

which/

which we record tend to indicate that what was absorbed was identical in composition to beef as far as one can judge from the S:N ratio. It will be remembered that in Period I, 12.233 grms N and .931grms S were taken as the figures representing the amount absorbed at that level. If the true basal figures of the nitrogen and sulphur output on the bread diet be subtracted from the above, we obtain figures which represent the nitrogen and sulphur absorbed from the beef ingested. The following figures show the absorption.

12.233 - 6.206 = 6.027 grms N.

.931 - .527 = .404 grms S.

S:N = 14.91.

This rate approximates that of the beef ingested (1:15.65). The nitrogen and sulphur contents of the beef ingested daily were 8.62 grms N and .550 grms S. The percentage absorption is therefore 69.8% N and 73.4% S.

A similar calculation may be made for Period II, using the same basal figures and taking as before 19.633 grms N. and 1.395 grms S as the amount absorbed at this level.

19.622 - 6.206 = 13.416 grms N.

1.395 - .527 = .868 grms S.

S:N = 15.45

This rate approximates still more closely to that

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of the beef ingested and represents an absorption of 77% N. and 78% S. of the beef intake which was double that of Period I. As the experimental error is least with the larger amount of beef, we may assume that what has been absorbed is beef digestion products and that no selective absorption of amino-acids has taken place. The assumption can be made however that all the beef was absorbed: one can then recalculate both the quantity and quality of the material retained. The data below show that quantitatively the figures for retention are much larger qualitatively however they are similar to the first method of calculation.

Period I. 250 grms. beef daily, 4 days.

N. actually ingested as beef	=	34.48 grms.
N. excreted over basal	=	13.039 "
N. retained	=	21.441 "
S. actually ingested as beef	=	2.20 grms.
S. excreted over basal	=	.863 "
S. retained	=	1.337 "

S:N = 1:16.03

Period II. 500 grms beef daily, 5 days.

N. actually ingested as beef	=	86.200 grms.
N. excreted over basal	=	56.331 "
N. retained	=	29.869
S. actually ingested	=	5.50 grms.
S. excreted over basal	=	3.947 "
S. retained	=	1.553

S:N = 1:19.23

The S:N ratios of the retained material, although their absolute values differ from those calculated by the first method, show the same trend, namely, the higher the nitrogen intake the poorer in sulphur is the retained material.

In Period III there should have been a loss as the nitrogen intake was reduced, but on the present assumption of complete absorption of the food protein the figures show a retention.

N. actually ingested as beef	=	34.48 grms.
N. excreted above basal	=	28.519 "
N. retained	=	5.961 "
S. actually ingested as beef	=	2.2 grms.
S. excreted above basal	=	1.748 "
S. retained	=	.452 "

S:N = 1:13.21

In Period IV the calculation is of course exactly similar to that by the previous method and represents the excess nitrogen and sulphur excreted above the basal figures of 6.203 grms N. and .527 grms S. The figures are repeated here for convenience.

N. excreted over basal = 6.255 grms.

S. = .355 "

S:N = 1:18.6

These results qualitatively show the same trend for each period (except III) as the figures based on the first method of calculation.

The total retention over the whole experiment shows however that the material is qualitatively similar to that which was ingested.

Total N. ingested as beef	=	155.160 grms.
Total N. excreted above basal	=	104.144 "
Total N. retained	=	51.016 "
Total S. ingested as beef	=	9.9 grms.
Total S. excreted above basal	=	6.893 "
		3.007

S:N = 1:16.97

On the first method of calculation 11.955 grms N. and .684 grms S. with a S:N ratio of 1:17.48 have been retained. These two methods of calculation in general therefore give

similar/

TABLE III.

Experiment 8.

Date.	Diet.	T.N. grms.	T.S. grms.	S:N	N.above basal.	S.above basal.	S:N above basal.
26 Feb.	Basal	6.566	.502	13.07			
27	Basal and 250 gms beef	8.204	.681	12.04	1.638	.179	9.15
28	"	8.943	.747	11.97	2.377	.245	9.70
29	"	9.204	.798	11.52	2.638	.295	8.94
1 Mar.	"	9.923	.824	12.04	3.357	.332	10.42
2	"	10.200	.824	12.37	3.634	.322	11.28
3	"	10.659	.860	12.39	4.093	.358	11.43
4	"	11.802	.890	13.26	5.236	.388	13.49
5	"	12.065	.936	12.88	5.499	.434	12.67

Date.	Diet.	N.retained daily.	S.retained daily.	S:N retention	% N. retained each day.	% S. retained each day.
26 Feb.	Basal	-	-	-	-	-
27	Basal and 250 g.beef	3.861	.255	1:15.14	24.8	26.9
28	"	3.122	.189	1:16.51	20.1	20.3
29	"	2.861	.139	1:20.58	18.4	14.9
1 Mar.	"	2.142	.112	1:19.12	13.7	12.0
2	"	1.865	.112	1:16.65	12.0	12.0
3	"	1.406	.076	1:18.5	9.0	8.1
4	"	.263	.046	1:5.71	1.6	4.9
5	-	-	-	-	-	-

similar results qualitatively. It would seem to the writer that the assumption made in the first calculation is the more valid, namely, that the nitrogen-sulphur output at equilibrium represents what is absorbed. Period III, by the 2nd method calculation showing a retention when the nitrogen output was reduced, indicates that the assumption of complete absorption must certainly be incorrect. Further, the figures giving the percentage absorption of nitrogen and sulphur indicate that it is beef digestion product which has been absorbed.

A similar experiment to the preceding was carried out which shows results tending to confirm the previous ones.

In Experiment 8, Table III, a basal diet similar to that in Experiment 7 was ingested and 250 grms. beef were superimposed daily until nitrogen equilibrium was attained. The time required to reach equilibrium was unexpectedly long as the body was storing nitrogen for some unknown reason. The beef was superimposed for 8 successive days until equilibrium was attained. The basal figures for the nitrogen and sulphur output were that of the preday 26th February.

Basal N output = 6.566 grms.

Basal S output = .502 "

The excess nitrogen and sulphur outputs and the S:N ratios are calculated for each day. It will be noted that the ratio of the excess in general tends to fall from 1:9.15 to 1:12.65. This means that relatively more sulphur than nitrogen

was/

was being eliminated at the beginning than at the end. The following is the total excess output for the 8 days.

(Table II, Exp. 7, See p.46)

Total excess N. over basal = 28.472 grms.

Total excess S. over basal = 2.543 grms.

S:N = 1:11.19

The S:N ratio of the excess (1:11.19) is definitely richer in sulphur than that of the beef ingested (S:N 1:15.65). The figure is also considerably higher than the ratio of the excess in the corresponding period in Experiment 7. In Period I, Experiment 7, the S:N ratio of the excess for the 4 days was 1:15.0. The retention of nitrogen and sulphur during those 8 days shows some points of interest. A priori, as the S:N ratio of the excess is higher than that of beef and higher than that of the excess S:N in Period I, Experiment 7, one would expect the S:N ratio of the retained material to be lower than the S:N of the retention in Period I, Experiment 7, and lower than that of the beef ingested. This is exactly what has been proved. The calculation is on a similar basis to that in Experiment 7, the total nitrogen and sulphur output on the 5th March being taken as the total daily absorption of these elements during the course of the experiment. It will be noted that the figures on the 5th March, 12.068 grms N. and .936 grms S., are almost identical with those employed as representing the maximum absorption in Experiment 7 (Periods I and III) on an exactly similar diet - (12.230 grms N., and .931 grms S.).

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The following retention is calculated.

Total nitrogen retained 15.520 grms.

Total sulphur retained .929 "

S:N 1:16.76.

The S:N ratio of the material retained in Period I, Experiment 7, Table II, was 1:14.69 - slightly higher than that of the beef ingested, while in this experiment as the S:N ratio of the excess was high - 1:11.19 - it is only to be anticipated that the ratio of the retained material should be 1:16.76, i.e., lower than that of the beef ingested (1:15.65).

As the S:N ratio of the total retention in Experiment 7 was 1:17.68, and as this was classified as "Meliorationseiweiss" or "Ubergangseiweiss" it would appear from the S:N of the retention product in Experiment 8 (1:16.76) that it also belongs to one of these categories. As however the nitrogen intake was not reduced after equilibrium was attained it is not possible to say how much circulating material might have been eliminated. An analysis however of the amount and S:N ratio of the daily retention in Experiment 8 has been made. In Table III the results are tabulated for each day. It will be noted that the S:N ratio of the retained material drops from 1:15.14 on the first day to 1:20.58, and 1:19.12 on the 3rd and 4th days; it then tends to rise rather irregularly for a day or two. This irregularity and the fact that equilibrium was attained only after 8 days indicates possibly that two types of retention

were/

were going on. It is the general experience that nitrogen equilibrium when the body is relatively well flushed with protein is attained within three or four days as in Grüber's experiments. Period II, Experiment 7, shows a striking example, the maximum nitrogen output being reached by the 3rd day. These three days represent the tissue required to fill the circulating protein depots. If for any reason some other type of protein is being retained, equilibrium or the filling of the circulating protein depôts will be delayed. It seems possible that in this experiment both processes have been going on. The tendency for the first two days has been to retain a material relatively sulphur rich and possibly in the light of the previous Experiment 7 "Meliorationseiweiss" or "Ubergangseiweiss:" on the 3rd and 4th days the ratio of the retained material falls, which might indicate a retention of circulating protein. From the fact that the ratio fluctuates for a day or two it appears that both processes are at work. It is indeed possible that an equilibrium between both types might exist. If this is true, then once so much stable protein is built up its quota of circulating material must be added to it. The point to note however is the tendency to retain the more stable sulphur rich material first which is analogous to the different types of material stored in Periods I and II respectively in Experiment 7. In Period I the sulphur rich material was retained, in Period II the sulphur poor.

The/

The general conclusions to be drawn from Experments 7 and 8 are that there is evidence for two types of retention (1) A material whose composition approaches that of muscle and which is retained when the nitrogen intake is reduced; (2) A material poor in sulphur and which is retained only so long as the nitrogen intake is kept up.

The former may be either "Meliorationseiweiss" or "Ubergangseiweiss," and its stability may be conditioned by its richness in sulphur. The latter appears to be circulating protein and its lability may be conditioned by its poverty in sulphur. The problem remains however as to whether this stable retention is "Meliorationseiweiss" or "Ubergangseiweiss" or indeed if such a distinction is justifiable. Rubner's and Thomas's sole criterion for the distinction was that the former was retained even if a nitrogen free diet were ingested, while the latter was slowly metabolised over a period of 20 days or so. As the differentiation between the two types was on the basis of their relative lability it is of interest to know whether they can be differentiated by means of their sulphur content.

Two experiments were therefore devised in order to gain some information on this question. A basal diet of the following nature was ingested for three days.

500 grms. beef.
3 eggs.
50 grms. cheese.
400 grms. bread.
50 grms butter.

TABLE IV.

Experiment 9.

Date.	Diet.	T.N. grms.	T.S. grms.	S:N
1928.				
22 Oct.	Basal (High N).	19.460	1.4179	1:13.72
23	"	24.948	1.5058	1:16.56
24	"	25.676	1.4619	1:17.56
25	Starvation.	13.608	.6924	1:19.65
26	"	17.056	.9123	1:18.69

Experiment 10.

1929.				
11 Apr.	Basal (High N).	17.184	1.4509	1:11.84
12	"	20.664	1.5498	1:13.33
13	"	20.748	1.5278	1:13.57
14	N. Free Diet.	10.437	.5276	1:19.78
15	"	5.572	.2967	1:18.07
16	"	4.592	.2693	1:17.05
17	"	4.454	.2910	1:15.29

The total nitrogen intake was approximately 26 grms. In Experiment 9, Table IV, there followed two days complete starvation except for water: in Experiment 10, on the other hand, a nitrogen free diet was ingested for four days. The nitrogen free diet was similar to that employed in Experiments 1 and 2. In Experiment 10 on the first day of the nitrogen free diet it will be noted that the nitrogen output dropped to be half while the sulphur output dropped to a third of their values on the previous day on the high nitrogen intake; correspondingly the S:N ratio fell from 1:13.5 to 1:19.7. On the 2nd day of the nitrogen free diet the nitrogen dropped 50%, while the sulphur dropped 40% of their values on the day before, the S:N ratio was still however high. On the 3rd and 4th days of the nitrogen free diet the sulphur and nitrogen output fell extremely little, while the S:N ratio tended to rise to a value of 1:15.29. It is this type of slow fall which Thomas observed on himself on a similar experiment and which is due to the gradual metabolising of what Rubner classified as "Ubergangs-eiweiss." A further analysis of the figures may however be made in two-day periods.

Nitrogen output on first two days of N.free diet 16.009 grms.

Sulphur " " " " .8243 "

S:N 1:19.42.

Nitrogen output on 3rd & 4th days of N.free diet 9.046 grms.
Sulphur " " " " .5603 "
S:N 1:16.14
Total Nitrogen output in the 4 days 25.055 grms.
" Sulphur " " 1.3846 "
S:N 1:18.09

It will be noted that the larger moiety excreted in the first two days is relatively sulphur poor while the smaller fraction eliminated on the last two days approaches in composition to body or muscle tissue. If the results of Experiment 7, Table II, are recalled, it will be seen that when the nitrogen intake was reduced, as in Periods III and IV, the material lost was sulphur poor: in Period III the ratio was 1:34, while in Period IV it was 1:18. This material lost in the last two periods of Experiment 7 was held to be circulating protein and one would be justified in assuming that what is excreted in the first two days of Experiment 10 is of the same nature. The S:N of the total retention in Experiment 7 was however 1:17.48 and the issue was whether to consider it as "Ubergangseiweiss" or "Meliorationseiweiss". In Experiment 10 the material excreted on the last two days of the nitrogen free diet had a S:N ratio of 1:16.14 and it may be questioned if this is "Ubergangseiweiss." If this assumption be true, then "Ubergangseiweiss" is characterised by its greater stability in that it was not metabolised in any amount on the first two days of the nitrogen free/

free diet. It can be asked, "Is this stability conditioned by its greater sulphur content?" "Is there any justification for assuming such a type of protein retention as "Ubergangseiweiss?" These two queries are associated in that body tissue has a S:N ratio of 1:14-1:15, while the S:N ratio of what has been eliminated in the last two days is 1:16.1. If organised or body tissue had been drawn upon on these two days, one would have expected a slightly higher S:N ratio. If the diet had been continued longer the nitrogen output would have fallen and the S:N ratio would probably have risen to a value of 1:14, indicating that the endogenous wear and tear quota is derived from body tissue. The figures in Experiment I, Table I, and others to be discussed confirm this. It then seems possible that there may be at least three types of protein retention, each one characterised by its stability and its sulphur content. The following is a tentative classification.

1. Circulating Protein. This material is unstable in that it is metabolised or eliminated in varying degree according to the drop in the nitrogen intake. Its steady retention is solely conditioned by the level of protein intake. It appears to be poor in sulphur.

2. Transitional Protein (Ubergangseiweiss). This type is relatively stable in that it is not lost from the body when the nitrogen intake is reduced but not cut out of the/

the diet. It is slowly metabolised however on a nitrogen free diet. This retention product appears to be richer in sulphur than circulating protein, but possibly slightly poorer in sulphur than body tissue.

3. Body Protein (Meliorationseiweiss, or organ protein). This is stable in that it is utilised most economically on a nitrogen free diet to cover the wear and tear output. Its sulphur content is that of body tissue.

If this classification is a relatively true picture of the nature of retention it would appear that possibly the sulphur content conditions the stability of each type. Further information on these retention products may be obtained by comparing Experiment 9 with Experiment 10, Table IV, where a two days' fast followed the high protein intake instead of a nitrogen free diet as in Experiment 10. In Table IV the figures for the two days' fast are tabulated. It will be noted that on the first day's fast the nitrogen and sulphur outputs are both half of that on the previous food day. This, as far as the nitrogen output is concerned, is similar to Experiment 10, while the sulphur on the other hand has not fallen so much relatively. In Experiment 10 the sulphur dropped from 1.5 grms. to .52 grms, while in Experiment 9 (fasting Experiment) it dropped from 1.46 grms. to .69 grms. The influence of a day's fast as opposed to a carbohydrate rich nitrogen free diet has extended in a small degree/

degree to the sulphur while the nitrogen output is uninfluenced. The S:N ratio on the first fast day is exactly the same as on the first day of the nitrogen free diet in Experiment 10. On the second day of the fast however the nitrogen and sulphur both rise to 17.056 grms and .912 grms respectively in contrast to the low figures of 5.572 grms and .2967 grms on the second day of the nitrogen free diet in Experiment 10. The S:N ratio is however the same on the corresponding day of each experiment. These figures show that the nitrogen free diet has exerted a very considerable sparing action on the breakdown of protein. The exact amount of the nitrogen and sulphur spared can be calculated by subtracting the output of these two substances on the 2nd day of a nitrogen free diet from the output on the 2nd day's fast. The following are the figures obtained.

N. output on 2nd fast day	17.056 grms.	Exp.9.
N. " " N.free day	5.572 "	Exp.10.
N. spared	11.484 "	
S. output on 2nd fast day	.9123 grms.	Exp.9.
S. " " N.free day	.2967 "	Exp.10.
	.6156	

S:N of spared material 1:18.65.

The carbohydrate of the nitrogen free diet has hence been responsible for sparing the breakdown of some 11 grms. nitrogen, and from the S:N ratio of this spared product, 1:18.65, it/

it might provisionally be called "Ubergangseiweiss" as its ratio is below that of body tissue. Definite evidence however in favour of the existence of such a type of retention product may be obtained by comparing Experiments 9 and 10 with Experiment 11, Table V, (p.74)to be discussed shortly. The question at issue is, is this 11.484 grms. N and .615 grms. S (which has been spared under the influence of carbohydrate) circulating protein, "Ubergangseiweiss," or true body protein? Circulating protein may be dismissed for the following reasons. Circulating protein is known to be eliminated as soon as the nitrogen intake is reduced: in general therefore on the first day of the N. free diet 80% of the circulating protein would be eliminated, on the 2nd day 15%, and approximately 5% on the 3rd day. On the second day of the N. free diet, Experiment 10, the T.N. output was 5.572 grms, and this figure would include any circulating protein in process of elimination: at most only a grm. or so could remain to be eliminated on the 3rd day of the nitrogen free diet. The extra 11.484 grms. nitrogen eliminated over and above this on the 2nd day's fast, Experiment 9, can therefore not be considered as circulating protein. It may then be assumed that this extra 11.484 grms. N and .6154 grms S, which have been eliminated on the 2nd fast day and spared catabolism in Experiment 10 must be derived either from body tissue "Meliorations-eiweiss" or "Ubergangseiweiss." A comparison with Experiment 11,

Table/

Table V, will give evidence that it is probably the latter.

In Experiment 11 a nitrogen free diet had been ingested until the daily nitrogen output in the urine was 2.996 grms. At this level it may be taken that all the circulating protein and most of the "Ubergangseiweiss" if such exists has been eliminated. Two days' fast followed (13th and 14th Jan.), the output of nitrogen being 2.4 grms. and 4.87 grms respectively. This means that on the 2nd day's fast the output of nitrogen is about 2.0 grms. above that of the pre-day, 12th Jan., on a nitrogen free diet. In Experiment 10 the nitrogen output is 11.48 grms. above what it would have been on a nitrogen free diet. It would then appear that in Experiment 10 some material was present in the tissues which was not circulating protein but was more labile than organised protein in that 11.48 grms. were metabolised on the 2nd day's fast in contrast to Experiment 11 where only some 2 grms. extra nitrogen were metabolised on the 2nd fast day. These figures would then lend some justification to the view that there is a retention product which is more stable than circulating protein in that its rate of catabolism can be spared by a carbohydrate rich nitrogen free diet: further, it appears to be more unstable than organised protein in that it is more easily broken down under fasting conditions. It is probably this slight instability which causes its slow but steady breakdown over a long period of nitrogen free feeding as Thomas observed. The S:N ratio of this 11.48 grms. nitrogen and .6154 grms./

grms. S (1:18.65) which may now be called "Ubergangseiweiss," would favour the hypothesis that its slight instability in contrast to body tissue is conditioned by a lower sulphur content.

In the foregoing discussion of these experiments it should be kept in mind that it is improbable that the body metabolises one type of protein exclusively until it is exhausted and then draws on the remaining reserves. It is much more likely that all types of protein, if they really exist, are drawn upon in variable degree during a fast or on a nitrogen free diet. In Experiment 10 on the nitrogen free diet it is likely that on the first day circulating protein was largely although not exclusively metabolised. On the 3rd day "Ubergangseiweiss" is the main source of protein, and by the 4th day body tissue is beginning to take an increasing part in the nitrogenous metabolism. It should be remembered that it is impossible to fix a S:N ratio for any of these types of protein with the exception of body tissue. A S:N ratio is a composite one and may simply express the average ratio of one, two or three different proteins which are being metabolised It is possible that instead of there being three distinct types of protein retention with varying sulphur contents, one type of variable and plastic composition is synthesised. If the food ingested ultimately becomes protoplasm as Kassowitz maintains, one can imagine the lability and composition of this protoplasm being conditioned/

conditioned by its sulphur content. Such a conception as the endogenous wear and tear quota would then take on an entirely different aspect. Rubner and Thomas assumed for instance that by the 4th and 5th day of a nitrogen free diet the "Ubergangs-eiweiss" was not capable of covering the minimum endogenous wear quota gram for gram. They assumed that all through the experiment the endogenous breakdown of 2.2 grms. daily is going on but requires a greater and variable quantity of "Ubergangseiweiss" to cover it. The writer has made a similar assumption by suggesting that its poverty in sulphur conditions its biological inefficiency for covering the wear and tear output gram for gram. It is however a pure assumption to assume a steady 2.2 grms. endogenous wear and tear output when the total nitrogen output is at some higher figure, whether the diet contains nitrogen or not. Let it however be assumed that the food is ultimately built up into - not a complex protein - but protoplasm of varying composition and stability. If this be presupposed, a nitrogen output of 4, 5 or 6 grms on a nitrogen free diet represents the steady wear and tear or disintegration of this protoplasm. The richer in sulphur this protoplasm becomes the more stable it is, and hence the output of nitrogen tends to fall to such a figure as 2 or 3 grms. per diem. Thus a nitrogen output of say 10 grms. in the urine is not to be looked on as made up of say 2.0 grms endogenous and 8 grms. from the disposal of food nitrogen. All food protein hence becomes a part of this protoplasm which may

be/

TABLE V.

Date.	Diet.	T.N. grms.	T.S. grms.	S:N	P_2O_5 grms.
1924.					
9 Jan.	Basal (N.Free).	4.788	.3022	1:15.8	1.789
10	"	4.382	.2748	1:15.9	1.831
11	"	3.052	.2528	1:12.0	1.420
12	"	2.996	.2418	1:12.3	1.164
13 Exp.11	Starving.	2.492	.2143	1:11.6	.752
14	"	4.872	.4286	1:11.3	1.348
15	Basal.	6.944	.4012	1:17.3	1.249
16	"	4.484	.2638	1:16.9	1.434
17	"	2.996	.2857	1:10.4	1.136
18 Exp.12	Gelatin superimposed.	5.901	.2473	1:23.8	.852
19	Starving.	3.984	.2693	1:11.0	1.064
20	"	6.804	.4836	1:14.0	1.462
21	Basal.	8.284	.5660	1:14.5	1.022
22	"	4.228	.2528	1:16.7	.908
23	"	3.332	.2253	1:14.7	.922
24 Exp.13	Albumen superimposed.	3.920	.4876	1:8.0	.795
25	Starving.	5.628	.5660	1:9.9	.780
26	"	7.112	.4506	1:15.7	1.448
27	Basal.	7.532	.5056	1:14.8	1.107
28	"	3.900	.2693	1:16.7	.866
29	"	2.996	.2583	1:11.5	.923

be considered to be the seat of the vital metabolic changes. Another possible explanation however of the variable amount of the endogenous wear and tear output may be the variable amount of this protoplasm. On a high protein diet a large amount of this protoplasm may be built up and if its rate of breakdown is a constant proportion of its quantity it is quite possible to explain the relatively high nitrogen output for some 8 days of a nitrogen free diet. Further information on this problem can however only be obtained by varying the experimental conditions.

A series of experiments were carried out in order to investigate the catabolic phase of metabolism in contrast to the preceding series which was concerned more with protein retention. The object of the experiment was to ascertain to what extent different proteins such as gelatin and egg albumen could spare the breakdown of body tissue in starvation. A basal nitrogen free diet similar to that employed in Experiments I and II was ingested until the nitrogen output had fallen to approximately the minimum endogenous wear and tear level. The protein to be investigated was then superimposed for one day, then followed two days' fast, after which the basal diet was again resumed. Experiment 11, Table V, was a control consisting of a two days' fast without the previous superimposition of a protein on the day before the fast, while gelatin and egg albumen were the proteins superimposed in Experiments 12 and 13 respectively. Various methods of calculation may be employed, but the main

issue/

issue is to attempt to find the sparing effect of these proteins on tissue loss in starvation. It must be noted that all these experiments show a negative balance in that the output of nitrogen and sulphur is greater than that ingested. This however does not necessarily preclude the possibility of a retention of part of the ingested material to replace in part the loss of body tissue. In Experiment 11 it will be noted that the nitrogen output does not fall to the basal value again until the 3rd day of the nitrogen free diet following the fast. It will also be noted that the nitrogen output rises above the basal value on the day of superimposition immediately preceding the fast in Experiments 12 and 13. Under these circumstances in calculating the nitrogen and sulphur output in the control it was necessary to include the preday, the two fast days and the first two post days, i.e., 5 days in all. In Experiments 12 and 13 the total nitrogen and sulphur output for the corresponding 5 days was calculated, beginning with the day on which the protein was superimposed. The following figures give the total nitrogen and sulphur output for the 5 days in the control. (Experiment 11)

Total N. output for 5 days 21.788 grms.

" S. " " 1.6097 "

S:N 1:13.53.

The S:N ratio is relatively high and would seem to indicate that the source of the nitrogen is muscle tissue. As will be discussed later, however, there is a lag in the nitrogen elimination/

elimination which probably accounts for the ratio being actually higher than that of muscle, namely 1:14-1:15. In Experiment 12, 70 grms. of gelatin containing 11.8 grms nitrogen and .3363 grms. sulphur with a S:N ratio of 1:35.0 were superimposed. The following figures give the total nitrogen and sulphur output over the 5 days, beginning on the day of superimposition, Jan.18.

Total N. output over 5 days 22.261 grms.

" S. " " 1.8190 "

S:N 1:16.08.

To obtain the loss of body tissue the nitrogen and sulphur of the gelatin ingested must be subtracted (this assumes that the gelatin has all been metabolised).

Nitrogen output	29.261 grms.	S. output	1.8190 grms.
Nitrogen ingested	11.8 "	S. ingested	.3363 "
Nitrogen lost	17.461	S. lost	1.4827 "

The total body loss is 17.461 grms N. and 1.4827 grms. S., in contrast to the control Experiment 11 where 21.785 grms. N and 1.6097 grms. sulphur were lost. The difference between these two sets of figures gives the amount of tissue spared.

21.789 - 17.461 = 4.328 grms N.

1.6097 - 1.4827 = .127 " S.

S:N = 1:34.07.

The body on this basis of calculation has been spared some 4 grms N. with a S:N ratio approximately the same as that

of/

of gelatin itself. This is unique and would indicate that in fasting the organism draws from different sources of body tissue: under certain circumstances it might appear that gelatin can spare the breakdown of a tissue of similar constitution. The tissues at the end of the gelatin experiment must hence be relatively sulphur poor. The fuller significance of the experiment will however be obtained when Experiment 13 with egg albumen has been discussed.

In Experiment 13, 495 grms. egg albumen containing 8.87 grms. N and .96 grms. S with a S:N ratio of 1:9.2 were superimposed on the 24th January. The following are the figures for the nitrogen and sulphur outputs over the 5 days.

Total N. over 5 days 28.092 grms.

" S " " 2.2791 " S:N 1:12.32.

If the same method of calculation is employed as in Experiment 12, and assuming that all the egg albumen has been metabolised, the loss of body tissue can be obtained.

Total N. output	28.092 grms.	
N. in albumen	8.82 "	
Body loss	19.272 "	
Total S. output	2.2791 grms.	
S. in albumen	.96 "	
Body loss	1.3191 "	S:N 1:14.72.

To obtain the amount of body tissue spared by the albumen/

albumen these figures must be subtracted from the total nitrogen and sulphur output in the control Experiment 11.

21.788 - 19.225 = 2.563 grms. N.

1.6097 - 1.3191 = .2963 grms S. S:N 1:8.65.

These figures would indicate that the body has been spared a sulphur rich material in contrast to Experiment 12 with gelatin where a sulphur poor material appeared to have been spared.

In short the body has been spared a material of the same composition as that ingested in each experiment if the assumption is correct that the proteins superimposed have been completely metabolised in each experimental period of 5 days. In general it would seem more likely that in each experiment the breakdown of body tissue has been the same and that instead of the gelatin and albumen being completely metabolised a certain moiety has been retained. A possible objection to this (see p.2 interpretation is the fact that in Experiments I and II, Table I,/ where the gelatin and albumen were superimposed under similar circumstances, without a subsequent fast, there was a retention of nitrogen only. In Experiment II, for instance, 70% of the ingested nitrogen of the albumen was retained while all the sulphur was eliminated. This fact at least indicates that all the albumen was absorbed.

The experiments under discussion however (Experiments

12/

12 and 13) differ in certain respects from those recorded at the beginning of the paper. In Experiments 12 and 13 the subject had already fasted two days (control Experiment 11) in addition to being on a nitrogen free diet from the 9th January. Under these conditions there would be a greater tendency to a retention of protein, particularly in the light of Murlin's work which indicated that the poorer the protein condition of the body such as after a fast the greater was the retention of the gelatin. Experiments 1 to 6, Table I, in this paper where those two proteins were superimposed on diets of varying protein content, give support to this idea not only for the gelatin but also for the albumen. Under these circumstances, it appears wiser to assume that the body loss has been the same in Experiments 11, 12 and 13 and hence calculate how much of the gelatin and egg albumen has been retained. The calculation will show that the nitrogen and sulphur of the gelatin retained is exactly the same in amount as the nitrogen and sulphur of the body tissue spared on the previous method of calculation. The calculation is however shown below in the case of gelatin (Experiment 12).

Total N. output for 5 days, Exp.12.	29.261 grms.
" " " Control.	21.788 "
	7.473 grms excess N. over control.
Total S. output in Exp.12.	1.8190 grms.
" " Control.	1.6097 "
	.2093 grms. excess S. over control.

There has been a loss of 7.473 grms nitrogen and .2093 grms sulphur over and above the control, but 11.8 grms. nitrogen and .3363 grms sulphur in the form of gelatin were ingested. The retention of gelatin is hence the difference between these two sets of figures.

11.8 - 7.473 = 4.327 grms N.

.3363 - .2093 = .1270 grms S. S:N 1:34.07.

The retention of the egg albumen can be calculated in the same way. The resulting figures are however exactly the same as those showing the amount of body tissue spared and the calculation is therefore omitted.

The following are the figures of the retention of egg albumen.

2.566 grms. N.

.2966 grms S. S:N 1:8.65.

In case of misconception it should be noted that the figures showing that the retentions of each protein are similar to those previously calculated on a different basis: this is simply an arithmetical result and depends on the fact that there are only three quantities to calculate with, namely, Total output in experiment in question, Total output in control, and Total intake in protein ingested. It is simply a question of assuming in the one case that all the ingested material has been metabolised and calculating the body tissue spared. In the other case, it is assumed that the body tissue broken down is the same in each

experiment/

experiment and calculating how much of the protein ingested has been retained.

If the S:N ratios of the material retained in each experiment are compared with the S:N ratios of the corresponding protein ingested the similarity is striking.

S:N gelatin	1:35.0.	S:N of retained material	1:34.0
S:N albumen	1:9.2	S:N of retained material	1:8.65.

In the face of those figures it appears more reasonable to assume a retention of the protein ingested rather than a sparing of body tissues of such divergent sulphur contents.

The interesting point to note however is that in spite of a fast so much of the ingested material has been retained. 36% of the gelatin nitrogen and 28% of the albumen nitrogen have actually been retained. Attention is also drawn to the fact that the metabolism of the protein was spread out over the 5 days after ingestion and not confined solely to the two fast days on which one would have expected any labile material to have been quickly utilised. Apparently the demand for energy is not the sole controlling factor in a fast. A calculation may be made of the actual amount of the nitrogen and sulphur of the gelatin metabolised on each of the 5 days of the experiment. This has been done by subtracting the nitrogen and sulphur outputs on each day of the control from the corresponding day of the gelatin experiment.

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The following figures show the amount of gelatin metabolised on each day. (Table V, see p.74).

Gelatin N. metabolised on day of ingestion			2.905 grms.	
"	"	1st fast day	1.492	"
"	"	2nd "	1.932	"
"	"	1st post day (diet resumed)	1.340	"
"	"	2nd	-	

Gelatin S. metabolised on day of ingestion		.0055 grs.	S:N	1:528.0	
"	"	1st fast day	.055	"	1:27.1
"	"	2nd "	.055	"	1:35.1
"	"	1st post day (diet resumed)	.165	"	1:8.12

Total N. of gelatin metabolised on two fast days	3.424	
" S " " "	.110	
S:N	1:31.12.	

It will be noted that a greater amount of gelatin has been metabolised on the day of ingestion that on either of the fast days. On the first post day 1.3 grms of gelatin nitrogen were metabolised. The sulphur excretion however offers a striking contrast: the amount of sulphur metabolised on the day of ingestion is almost negligible and the amount metabolised on each fast day is extremely small. On the first post day however the gelatin sulphur metabolised is relatively increased. It

will/

will thus be seen that the moiety of gelatin which has been metabolised has been distributed over 4 days, the amount eliminated each fast day being by no means inordinately large.

A comparison may be made with Experiment 1, Table I, where 70 grms. gelatin containing 9.939 grms N. and .3705 grms. S. with a S:N ratio of 1:26.5 were superimposed on a similar diet, but instead of a subsequent fast the nitrogen free diet was continued. The nitrogen and sulphur of the gelatin metabolised daily has been calculated for the 4 days following ingestion in Experiment I as follows:- (Table I, see p. 29).

N. output above basal (day of ingestion)	1.876 grms.			
" " 1st post day	1.652 "			
2nd "	.780 "			
3rd "	.504 "			
S. above basal (day of ingestion)	.2418 grms.	S:N	1:7.7	
" 1st post day	.044 "		1:37.5	
2nd "	.044 "		1:17.7	
3rd "	.022 "		1:22.9	

The question at issue is how is gelatin metabolised under fasting conditions on the one hand (Experiment 12) and when the energy needs of the body are being covered by a nitrogen free diet on the other (Experiment 1.) A comparison of the figures will show that the demand for energy has scarcely influenced the rate of metabolism of the ingested gelatin. It should be borne in/

in mind however that the calculation showing the amount of the gelatin metabolised on each fast day is based on the assumption that the breakdown of body tissue is the same on each fast day as in the control fast, Experiment 11, the excess above this being derived from the nitrogen and sulphur of the gelatin. It is possible that gelatin was actually metabolised to a greater extent and that the breakdown of body tissue was reduced. This is rendered unlikely by the fact that the S:N ratio of the excess is 1:31.12, a figure close to that of the ingested material, namely 1:35.

It will be remembered in the calculation of the total nitrogen and sulphur output in these experiments with gelatin and egg albumen one had either to assume a constant breakdown of body tissue, with a retention of a material of S:N ratio of 1:34 in the gelatin experiment, or assume that all the gelatin had been metabolised, and a certain amount of body tissue with a S:N ratio of 1:34.0 had been spared. The former assumption is obviously the more valid, but the corollaries bring some questions to the fore. As the breakdown of body tissue is the same in these three experiments whether gelatin or egg albumen were superimposed, and yet a certain retention of each had been effected, it would seem either that a retention of protein is quite independent of the breakdown of tissue in fasting or that food protein can be utilised only after a process of elaboration in the cells which may require several days. That the metabolism

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of ingested gelatin is independent of the breakdown of tissue in fasting has already been discussed. A comparison however of the nitrogen of the gelatin retained in Experiment 1 without fasting and Experiment 12 fasting will show that in all probability the same holds good for retention. In Experiment 1, 9.939 grms. gelatin N were ingested and 5.18 grms. retained, while in Experiment 12, 11.8 grms. gelatin N. were ingested and 4.327 grms. retained. The general inference is then that gelatin can not spare the breakdown of body tissue in fasting as previous workers have proved. Krummacher found a reduction in the loss of nitrogen on giving gelatin but as no sulphur estimations were made it is feasible that his results could be explained on the assumption of a retention of gelatin nitrogen while the loss of body tissue was uninfluenced.

Another more probable explanation for Krummacher's results is the sparing effect of the glucose derived from the gelatin ingested. The total nitrogen output after giving gelatin to a fasting animal would be increased but the glucose derived from the gelatin might be sufficient to effect a reduction in tissue breakdown and hence a balance might show that the loss to the body was less than when the animal was fasted without gelatin. The gelatin in his experiments was fed in large amounts and the yield of glucose would be quite considerable.

It has already been noted that the total nitrogen output in starvation is much higher after a fast following a nitrogen rich/

rich diet as in Experiment 9, Table IV, than one following a nitrogen free diet in Experiments 11 and 12. According to Rubner's classification the high nitrogen output in Experiment 9 would have been derived from circulating protein and "Uebergangseiweiss", while in Experiment 12 the body was completely depleted in these reserves, the nitrogen output having reached the endogenous wear and tear level. It has already been tentatively suggested that all food protein is built up into a protoplasmic material whose lability is conditioned largely by the quantity in transit and its sulphur content. The breakdown or catabolism of this material represents the endogenous and exogenous nitrogen of the urine. On a nitrogen free diet the slow fall in the output is due in the first place to a gradually diminishing quantity of this protoplasm and to an increasing stability of the moiety that remains - it approaches in sulphur content to that of muscle. The question may then be asked "Is this labile protoplasm - on a nitrogen free diet - finally diminished to such an extent that only a small fraction remains which is being replenished by a slow liquidation of tissue protein?" Voit held that after a few days' fast the nitrogen output was derived from organ protein after this organ protein had first been transformed into circulating protein. In short, organ protein could not be used directly. If on a nitrogen free diet only a small quantity of this protoplasmic material (or circulating protein) is being formed from organ protein,

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one would expect that the addition of sulphur poor gelatin would render it so unstable that the whole would be metabolised relatively quickly. The data available from the present experiments do not favour this conclusion as the gelatin was metabolised relatively economically over the experimental period of 4 or 5 days. If on the other hand there is a relatively large amount of this protoplasmic material kept in circulation, whether the subject is fasting or on a nitrogen free diet, the addition of 11 grms. gelatin nitrogen would not render it so unstable and hence the elimination of the gelatin nitrogen would be slower. The conception is that the gelatin is incorporated into this material which is being kept up for want of protein in the diet by the steady transformation of organ protein. Let it be supposed that there is a steady daily nitrogen output of 10 grms. and a steady reserve of circulating material in the tissues of only 10 grms N. It is clear that its quality would be considerably diluted by the addition of 11 grms. gelatin N., and consequently rendered so unstable as to be metabolised within a day or so. If however there was a reserve of say 100 grms. N with a daily output of 10 grms N., the incorporation of 10 grms. gelatin nitrogen would dilute it relatively little and hence the metabolism of the gelatin might be spread over 10 days or so. The figures available from Experiments 12 and 13, Table V, make it possible to estimate the amount of this material in circulation.

Its/

Its source under the conditions of the experiment must be the liquidation or transformation of body tissue. If on the other hand the diet contained protein, this and not body protein would be the source from which this protoplasmic material would be built up. This protoplasmic material will be provisionally called circulating protein. The following analogy is given in order to simplify the method of calculation. To a small vessel containing an unknown quantity of water let there be added 10 c.c. of alcohol: at the end of one hour 10 c.c. of fluid consisting of 2.5 c.c. of alcohol and 7.5 c.c. of water (i.e., alcohol to water 1:3) has run out. It then follows that there were originally 30 c.c. of water in the vessel before the alcohol was added, and at the end of one hour 30 c.c. of fluid remain, consisting of 7.5 c.c. alcohol and 22.5 c.c. of water. The 10 c.c. of alcohol represent the gelatin ingested, the 10 c.c. of fluid run out represent the nitrogen in the urine (say a 5 days' total) derived from the metabolism of body tissue and gelatin, while the 30 c.c. of fluid remaining represent body nitrogen (circulating material) plus gelatin nitrogen incorporated with it. The 30 c.c. of water found to have been originally present in the vessel represent the amount of circulating protein in the tissues at the time.

The following calculation shows the amount of circulating protein in transit in Experiment 12.

Total nitrogen output in 5 days	(Gelatin Exp.)	29.261 grms.
" " "	(Control Exp.)	21.788 "
Excess N		7.473 "
Total sulphur output in 5 days	(Gelatin Exp.)	1.8190 grms.
" " "	(Control Exp.)	1.6097 "
Excess S		.2093 "

S:N ratio of the excess 1:35.7

Of the total excretion of 29.261 grms. nitrogen 7.473 grms. have been derived from the gelatin and the remaining 21.788 grms. nitrogen from body tissue: therefore in the material metabolised gelatin nitrogen was present in the ratio of 7.473 grms. gelatin nitrogen to 21.788 grms. body nitrogen. Hence for 1 grm. of gelatin nitrogen metabolised there were $\frac{21.788}{7.473}$ = 2.91 grms of body nitrogen eliminated with it. Therefore in the circulating protein immediately after gelatin had been ingested the ratio of gelatin nitrogen to body (circulating) nitrogen was 1:2.91. 11.8 grms gelatin nitrogen were however ingested, therefore the amount of circulating protein originally present before gelatin was added was 11.8 x 2.91 = 34.358 grms. This 34.338 grms. circulating protein would have a S:N ratio of 1:15.0 as it was derived from body tissue. On the addition of gelatin its amount would be increased to (34.338 + 11.8) 46.138 grms. nitrogen with a S:N ratio compounded of that of gelatin (1:35.0) and that of

circulating/

circulating protein (1:15.0). Assuming the 34.338 grms. nitrogen of the circulating material had a S:N ratio of 1:15, the sulphur content would be 2.28 grms. The following shows the composition of the circulating protein after gelatin ingestion.

34.338 grms.	N (body protein)	2.28 grms	S (body protein).
11.8 "	N gelatin	.33 "	S gelatin.
46.138		2.61 "	S total.

S:N 1:17.7

Hence the addition of a sulphur poor material to a relatively larger amount of a circulating material of normal sulphur content would not reduce the average S:N ratio of the composite material to any great extent.

The slow rate of metabolism of the gelatin under these circumstances is therefore assumed to be due to its incorporation within a larger quantity of some material which was being slowly catabolised.

Experiment 13 with egg albumen can be calculated on a similar basis in order to see if there is any variation in the quantity of this circulating protein. The following are the figures obtained.

Nitrogen output for 5 days (egg albumen Exp.)	28.092 grms.
Nitrogen " " (control Exp.)	21.788 "
Nitrogen excess	6.304
Sulphur output for 5 days (albumen Exp.)	2.2791 grms.
Sulphur " " (Control Exp.)	1.6095 "
Sulphur Excess	.6696 "

S:N excess 1:9.11.

Of the total 28.092 grms. N metabolised 6.304 grms. N. were derived from the albumen and the remainder from the body tissue or circulating protein.

∴ 6.304 grms. N of albumen were associated with 21.788 grms body N

∴ 1 grm. albumen N was associated with $\frac{21.788}{6.304}$ = 3.4 grms. body N.

8.87 grms. albumen nitrogen were ingested

∴ 8.87 x 3.4 = 30.15 grms. circulating protein was originally present before the albumen was ingested. This figure agrees approximately with the 34.338 grms. calculated by means of the data of Experiment 12.

As the figure obtained in Experiment 13 is approximately the same as that obtained 6 days earlier in Experiment 12, during which period the body had lost some 20 grms. of nitrogen presumably from this circulating protein, it must hence be assumed that a transformation of organ tissue into circulating protein has been going on continually in order to keep it at this level of about 30 grms. N. It should be noted that the calculation can never be absolutely correct as the gelatin is being continually and progressively diluted by the steady addition from the tissues of so much new circulating protein. The figures may however be taken to indicate that a quantity of labile circulating material is always present and that only a certain moiety is metabolised daily. The fact that the calculations with both of these experi-

ments/

experiments show approximately equal amounts of this protein would appear to indicate that the body always endeavours to keep it at a more or less constant level, at least when on a nitrogen free diet. The general conclusion drawn from the experiments carried out so far may be stated shortly as follows.

A circulating material (labile protoplasm or circulating protein) is always present in the tissue. This protein is the immediate source of the nitrogenous turnover of the body. During a fast it is kept up by the transformation of body tissue.

On a nitrogen free diet the breakdown of this circulating protein is less than on a pure fast, due probably to a resynthesis of the nitrogenous moiety or to there being no necessity for its breakdown to supply energy as in a complete fast. On a nitrogen containing diet, the food protein supplies the material with which this circulating material is built up. The amount built up will depend on the protein intake; if this is eliminated from the diet, there is a compensatory liquidation of organ protein in order to keep a certain amount of circulating material always in transit. The rate at which this protein breaks down is conditioned by (1) The amount in circulation, being higher the greater the nitrogen intake, and (2) By its stability; this is conditioned probably by its sulphur content.

The conception as to whether the cells metabolise this protein in the proportion in which it is present in the tissues or/

TABLE VI.

Experiment 14. Control.

Date.	Diet.	T.N. grms.	T.S. grms.	S:N.	P_2O_5 grms.	P_2O_5:N
1925.						
23 Feb.	Basal N.Free	5.096	.357	1:14.2	1.405	1:3.62
24	"	4.458	.324	1:13.7	1.391	1:3.20
25	"	4.144	.346	1:11.9	1.278	1:3.24
26	Starvation.	4.816	.527	1:9.1	.951	1:5.06
27	"	11.008	.774	1:14.3	2.442	1:4.50
28	"	13.196	.802	1:16.4	3.436	1:3.84
1 Mar.	"	11.872	.819	1:14.4	3.138	1:3.78
2	Basal N.Free	8.120	.484	1:16.7	1.058	1:7.67
3	"	4.500	.423	1:10.6	.951	1:4.73
4	"	3.752	.357	1:10.5	1.192	1:3.14
	Experiment 15.					
1924.						
4 Feb.	Basal N.Free	4.900	.341	1:14.3	1.564	1:3.13
5	"	3.808	.253	1:15.5	1.150	1:3.30
6	"	3.528	.231	1:15.2	.923	1:3.82
7	Starvation.	3.248	.247	1:13.1	.979	1:3.31
8	70 g. gelatin alone.	7.364	.407	1:18.1	.781	1:9.42
9	Starvation.	8.176	.390	1:20.9	1.547	1:5.28
10	"	9.324	.555	1:16.8	2.059	1:4.52
11	Basal N.Free	6.636	.357	1:18.3	.682	1:9.73
12	"	3.920	.253	1:15.5	.866	1:4.52
13	"	3.360	.231	1:14.5	.823	1:4.08

or whether this protein, or, as it should be called, protoplasm, actively disintegrates of itself in virtue of its being vitalised, as Pflüger would have it, is one which will be discussed later. A further series of fasting experiments on a slightly different basis were therefore carried out in order to get more information as to the nature of the circulating material. The two experiments to be described consisted of a 4 days' fast preceded and concluded by a nitrogen free diet as in Experiments 11, 12 and 13. Experiment 14, Table VI, served as a control, while in Experiment 15 70 grms. of gelatin were ingested alone on the 2nd day of the fast. The adoption of the usual nitrogen free diet preceding both fasts is of course to eliminate the influence of any previous food protein. The basis of calculation employed consisted in estimating the total nitrogen and sulphur output for the 4 fast days + the 2 post days on the nitrogen free diet. The following are the figures obtained for Experiment 14(Control), Table VI.

T.N. output over 4 fast days + 2 post days = 53.512 grms.

T.S. " " " = 3.829 "

S:N = 1:13.94

In experiment 15, 70 grms. gelatin containing 11.8 grms. N and .3367 grms S with a S:N ratio of 1:35.0 were ingested on the 2nd fast day. The following gives the balance.

T.N. output on 4 fast days + 2 post days (gelatin ingested). = 38.668 grms.

T.S. " " " = 2.209 "

S:N = 1:17.5

The first point to note is that the total nitrogen and sulphur output in the control Experiment 14 without gelatin is actually higher than Experiment 15 with gelatin. This brings out very markedly the nutritive condition of the body on the metabolism during a fast.

Experiment 15 with gelatin was carried out a few days after Experiments 11, 12 and 13 just described: these three experiments were done without a break and involved a 22 days' period with 3 separate 2 day fasts. The control Experiment 14 on the other hand was done a year later and no metabolic experiment had been carried out for some months previous to it. The high nitrogen output in the control appears strongly to indicate that some labile reserve of material was drawn on which was wanting in Experiment 15. If the figures are examined it will be noted in Experiment 14 that the nitrogen output rises to a maximum on the 3rd fast day and then drops to 11 grms. on the 4th fast day. A nitrogen output of 11 grms. per day is much higher than would have been observed if the fast had continued. Cathcart's subject Beauté excreted between 8 and 10 grms. nitrogen daily during his 14 days' fast. The nitrogen output of Benedict's subject Levanzin rose to a maximum within the first few days and then fell. Howe, Hawk and Matthil observed in a dog that the nitrogen output in the second of two long fasts was much less than on the first even although a period of realimentation intervened. This rise in the nitrogen output on the/

the first few days of a fast in Experiment 14 would then appear to indicate that some labile material is eliminated in the early days of starvation. Howe, Hawk and Matthil's results would also favour the idea that apart from some labile reserve some protective mechanism can be brought into play to limit the loss of body nitrogen. In the relatively short periods however of the fasts described in this paper in comparison to those recorded by Howe, Hawk and Matthil's dog, it is more probable that the high nitrogen loss in the control Experiment 14 as compared to Experiment 15 was due to some reserve material. A comparison of the total nitrogen and sulphur output in these two experiments can therefore give little information as to influence of gelatin on the nitrogen metabolism. The phosphorus outputs were therefore employed in order by indirect means to obtain some indication of the fate of the ingested gelatin. The assumption made is that when a body tissue is broken down there is a definite ratio of phosphorus to nitrogen. The ratio P_2O_5 to N in muscle is about 1:6.6; Cathcart found a maximum P_2O_5:N ratio of 1:6.22 on the 14th day in his fasting subject, while Benedict's subject reached a maximum of 1:5.96 on the 12th day. The average fasting value of the P_2O_5:N ratio appears then to be about 1:6.0. The daily figures recorded in Experiment 14, Table VI, show however considerable variation, and in general the ratio tends to be higher than those found by previous workers in longer fasts. As a basis for calculation therefore the P_2O_5:N ratio of the total nitrogen/

nitrogen and P_2O_5 output over the 4 fast and 2 post days in Experiment 14 was calculated: the figure obtained was 1:4.46. A similar calculation was made in order to obtain the S:P_2O_5 ratio in Experiment 14, which amounts to 1:3.12. If it is assumed that these ratios hold good in Experiment 15 with gelatin, the nitrogen and sulphur derived from body tissue can then be calculated from the phosphorous output. Any excess above the figures obtained can then be assumed to be derived from the metabolism of the superimposed gelatin. It should be noted that the gelatin was phosphorus free.

The following figures show the balance.

Total P_2O_5 excreted in 6 days (Exp.15 with gelatin) = 6.914 grms

The theoretical nitrogen output derived from body tissue is hence

$$6.914 \times 4.46 = 30.036 \text{ grms.}$$

The theoretical sulphur output $= \frac{6.914}{2.12} = 2.216$ grms.

The actual N. output in 6 days of Exp.15	=	38.668 grms.
Theoretical body loss	=	30.036 "
Excess derived from gelatin	=	8.632 "

N. ingested as gelatin	=	11.8 grms.
Gelatin N. metabolised	=	8.632 "
Gelatin N. retained	=	3.168

Actual sulphur excreted	=	2.209 grms.
Theoretical body sulphur loss		2.216 "
Excess derived from gelatin		---

Sulphur ingested	=	.3367 grms.
Gelatin sulphur metabolised	=	--
Gelatin sulphur retained	=	.3367 "

These figures indicate that 3.168 grms. nitrogen and .3367 grms. sulphur of the gelatin have been retained. The S:N ratio of this retained material is 1:9.43. These figures appear to indicate a preferential retention of sulphur under the condition of the experiment. Too much stress should not however be put upon the absolute value of these figures owing to the indirect method of calculation employed. The general conclusion nevertheless that sulphur is selectively retained can be considerably substantiated by a comparison of this Experiment with Experiments 11 and 12. If the total sulphur output on the 2nd day's fast, 14th January, in Experiment 11, Table V,and on 20th January, Experiment 12, be compared with the output on the 2nd day's fast, Experiment 15, 8th February (the day of superimposition) it will be noted that it is actually lowest on this particular day of Experiment 15. The sulphur output on the 2nd fast day of Experiment 15 has hence been uninfluenced by the ingestion of the gelatin and supports the contention of the preferential retention of sulphur. This preferential retention of sulphur has been noted by Lewis in dogs under similar conditions and its significance in regard to the rôle of sulphur in protein metabolism will be discussed later. The rate at which the gelatin has been metabolised is

of some interest. If the S:N ratio on the day gelatin was ingested, 8th February, until the end of the Experiment on the 13th February, be compared with the S:N ratio of the corresponding days in Experiment 14, the control, it will be noted that the former are distinctly higher even until the last day of each experiment. In Experiment 15 with gelatin the S:N ratio rises to a maximum of 1:14.5 on the last day in comparison to 1:10.5 on the last day (4th March) of Experiment 14, the control. Apparently the gelatin is still being metabolised 6 days after its ingestion, 3 of which were fast days. This would appear to indicate either that gelatin is retained with great tenacity or, in the light of the previous discussions, it has been incorporated with some relatively larger reserve of protein and the whole then slowly metabolised. This again supports the results obtained in the experiment recorded at the beginning of the paper that the more impoverished the nitrogen condition of the tissues the greater the retention of the gelatin. The results would further indicate that even in fasting the energy needs are not the only factor since gelatin which was rejected on a high nitrogen diet, Experiment 5, Table I, was economically utilised under the conditions of Experiment 15.

The Retention of Beef Extractives.

The influence of extractives from beef merits some consideration, particularly in view of the popular belief in extracts and/

and beef juice as part of a complete dietary. Liebig was convinced that flesh extractives had a function of a higher order to perform than that of protein. He held that protein could not replace extractives in a dietary as they belonged to a totally different category. Experiments of Bischoff and Voit are quoted as illustrating the enhanced value of bread protein when fed along with beef extract and on the basis of these Liebig concluded that extracts of beef improve the food value of vegetable proteins. It appears however from his paper that Liebig's conclusion was influenced more by his conviction that all the constituents of muscle are involved in muscular activity than by the few experiments carried out on this question. It can probably be said with truth that Liebig was largely responsible for the popular belief in extractives which is exploited even to the present day by various commercial firms. Experiments carried out within the last 30 years or so present however rather conflicting results. Burgi fed meat extractives to starving dogs and found that they exerted no sparing action on the nitrogen output and in addition was mostly excreted unchanged, 5% only being unaccounted for. Rubner came to the same conclusion, but qualified it by saying that if the tissues were poor in certain constituents present in the extract, a retention might occur. He further showed that the carbon poor extractives were excreted before the carbon rich. Thomson stated that extractives improve the digestibility of the food taken with them and promote nitrogen

TABLE VII.

Experiment 16.

Date.	Diet.	T.N. grms.	T.S. grms.	S:N	N.above basal.	S.above basal.	S:N of excess.
1924.							
25 May	N.Free Diet.	5.152	.522	1:9.8			
26	"	3.948	.341	1:11.5			
27	"	3.388	.258	1:13.1			
28	"	2.744	.247	1:11.2			
29	Extract superimposed.	3.416	.313	1:10.9	.761	.066	1:11.53
30	N.Free Diet.	2.917	.231	1:12.2	.262		
31	"	2.576	.247	1:10.4			

Experiment 17.

1927.							
15 Nov.	Basal Diet.	8.316	.500	1:16.62.			
16	"	7.971	.505	1:15.78			
17	"	8.699	.522	1:16.66			
18	Extract superimposed.	10.958	.622	1:17.61	2.630	.113	1:23.27
19	Basal Diet.	9.186	.566	1:16.41	.858	.057	1:15.05
20	"	9.203	.549	1:16.76	.875	.040	1:21.87
21	"	8.330	.538	1:15.48	.002	.027	

retention. Voltz and Beaudrexel on the other hand found that they did not influence the absorption of any of the three foodstuffs and neither was a retention to any extent appreciable. Thomas in his experiments on the biological value of proteins found that beef was over 100% efficient in covering the minimum endogenous wear and tear output. As only some 90% of the nitrogen in the beef existed as protein, the remainder being extractives, he concluded that the latter have a definite rôle in nutrition and can not be ignored as part of a dietary.

The experiments recorded here were primarily concerned in finding out to what extent, if any, a retention of extractive was possible. Two different basal diets were employed, one a nitrogen free diet and the other a nitrogen containing diet of bread and cheese. The extract was superimposed for one day and the excess nitrogen and sulphur estimated for each day in the usual way.

In Experiment 16, Table VII, a nitrogen free diet was ingested until the nitrogen output had fallen to 2.744 grms. per diem. A solution of a commercial extract ---- containing 2.689 grms. N and .1236 grms. S with a S:N ratio of 1:19.7 was superimposed on the 29th May. The basal values for calculating the excess outputs of nitrogen and sulphur were taken as the average of the preday, 28th May, and the last post day, 31st May. The following shows the balance obtained.

N ingested	=	2.689 grms.
Total excess N above basal	=	1.023 "
N retained	=	1.666 "
S ingested	=	.1236 grms.
Total excess S above basal	=	.066 "
S retained	=	.0576 "

S:N retained = 1:28.9 grms.

It will be noted that there is quite an appreciable retention, namely 61% of the nitrogen and 46% of the sulphur. The S:N ratio of the retained material is however low: this is in keeping with most retention experiments on a nitrogen free diet. It will be recalled that in Experiments 1 and 2, Table I, the nitrogen only of the gelatin and albumen respectively was retained. This tendency to a holding on of nitrogen only is to be observed by noting the S:N ratio of the urine in this Experiment 16. The ratio varies between 1:9 to 1:13.0, indicating an early metabolism of sulphur and a delay or relative retention of the nitrogen.

Experiment 17 was planned to test the retention of extracts superimposed on a dietary containing protein in the form of bread and cheese which may be considered to be extractive free. The basal values were calculated on the average nitrogen and sulphur output of the three predays, 15th, 16th and 17th November. On the 18th November a solution of the same extract containing/

containing 4.356 grms. N and .219 grms. S with a S:N ratio of 1:19.84 was superimposed and the following balance obtained.

N ingested	=	4.356 grms.
N excreted above basal	=	4.365 "
N lost	=	.009 "
S ingested	=	.219 "
S excreted above basal	=	.237 "
S lost	=	.018

Although nearly twice the amount of extract was taken in this experiment as in the previous one, no retention could be noted. It would thus appear that extractives have a nutritive value only under certain circumstances, when, for instance, the tissues are in a nitrogen poor condition as in a protein free diet. It is to be noted that Rubner stated that a retention of extractives might occur under certain circumstances such as after a fast. These experiments again show the precautions necessary in assessing the biological value of a protein. It has already been pointed out in the beginning of this paper that the capacity of the body to retain different proteins depends not only on the protein ingested but also on the nutritive condition of the organism at the time. These experiments recorded here would hence tend to justify the value of extractives in debilitated or convalescents recovering from wasting diseases. It should be pointed out however that the value of an extract

can/

can not be assessed merely on the result of a balance experiment. The indirect benefit obtained from its flavour in stimulating the appetite and hence increasing the food consumption in such subjects can not be ignored.

The question however arises as to what form is the material retained in. The ratio indicates that it is certainly not muscle protein. It is here that Kassowitz's conception of the food ingested being built up into protoplasm may provide a scheme into which the facts may be provisionally fitted. The experiments recorded in this paper all go to show that a retention of material of varying sulphur content and of varying stability may be retained in the body. As was clearly appreciated by Liebig, all the constituents of muscle are concerned in normal activity and hence it might be more justifiable to think in terms of the protoplasmic requirements of the living organism rather than of the narrower term protein.

According to the evidence brought forward in the previous experiments of a circulating protein or protoplasm in the tissues, the extract would be incorporated into it and plays its rôle as one of the constituents of living matter.

The Significance of the Output of Nitrogen and Sulphur in the Metabolism of Protein.

In all the experiments discussed so far it will have been noted that the nitrogen and sulphur outputs do not always run pari passu. The clearest indication of a variation in the output of the one element relative to the other may be gathered from the S:N ratio in the urine for each day or series of days as the case may be. The variation in the ratio may be due either to an uneven rate of metabolism of these elements or to the fact that the body is metabolising a material containing more or less sulphur in relation to nitrogen than that of the basal diet at the time. It has already been noted that the S:N ratio of the different proteins employed varied between 1:8 and 1:35, while muscle tissue has a value of 1:14.0 and 1:15.0 approximately. Under these circumstances considerable variations in the S:N ratio may be obtained if these proteins are metabolised as homogeneous units without assuming a different rate of metabolism of sulphur relative to nitrogen. It should also be noted that the experimental time period is an important factor in investigations of this kind. The S:N ratio of the urine in a three-day period after a protein superimposition might agree with that of the protein ingested while the daily or hourly ratio might show considerable variation. A considerable number of investigators - Feder, Falta, Von Wendt, Cathcart/

Cathcart and Green - found that the sulphur moiety of ingested protein tended to be metabolised and eliminated more quickly than the nitrogen. Hawk and Chamberlain on the other hand find in short periods that the elimination of sulphur follows that of the nitrogen after beef ingestion. Fay and Mendel observed the S:N of the urine in dogs to correspond to that of the food if the animal were in a normal nutritive condition; if however the feeding followed a fast the ratio in the urine tended to be lower than that of the food ingested, indicating a preferential metabolism of nitrogen or a selective retention of sulphur. Lewis made the same observation on feeding beef to fasting dogs. Hawk however in experiments on the human subject found that the S:N ratio of the urine tended to follow that of the food ingested. Falta was probably the first to make a definite statement on the basis of his own experiments. He employed the superimposition method with three different proteins, namely egg white, casein and veal, and the excess nitrogen and sulphur outputs were calculated for the subsequent days.

With all three proteins the sulphur moiety was found to be metabolised more rapidly than the nitrogen. It was further found that the times required for the different proteins to be metabolised varied considerably. Six days elapsed before the nitrogen and sulphur of egg albumen were metabolised, while veal required but three: with casein on the other hand the sulphur was all metabolised in two days while four days elapsed before

all/

all the nitrogen was eliminated. Falta's conclusion was that in the catabolism of protein the sulphur moiety is the first to be attacked. The results which will be discussed here will tend to confirm his conclusions so far as they go. The scope however of following the nitrogen and sulphur output in relation to the intake has not been sufficiently appreciated. Little work has yet been done on the retention of nitrogen and sulphur under various conditions. The data in this paper however provide material for obtaining a further insight into the nature of protein metabolism as a whole. The generally accepted view of considering nutrition and metabolism as consisting of two processes - the anabolic or building up and the catabolic or breaking down - has tended to be lost sight of, particularly in the interpretation of experimental results. This holds good particularly for the anabolic phase which on account of the difficulty of investigation has been relatively neglected. It should be borne in mind that a real picture of protein metabolism can only be obtained if something be known of both aspects. The metabolic processes in the organism probably consist in an unstable equilibrium between these two activities and it may be taken as a good theoretical basis to assume that the anabolic phase is as important as the catabolic.

The earlier workers hence interpreted all their results on catabolic lines and even they could not come to any definite opinion/

opinion as to whether the sulphur or the nitrogen was metabolised first. The main cause for the divergence of opinion is largely due to the different experimental conditions employed and to a too narrow basis of interpretation. An analysis however of the nitrogen and sulphur output in the experiments so far recorded will tend to show that sulphur does play a definite rôle in both phases of protein metabolism.

In Experiments 1, 2, 3, 4, 5, 6, Table I, the daily S:N ratios have been calculated and in addition the daily excess nitrogen and sulphur and S:N ratios are given.

The experiments in which gelatin was superimposed on different diets will be discussed first - Experiments 1, 3 and 5. It will be noted in all three experiments that, of the nitrogen and sulphur metabolised each day, the greatest amount is on the day of superimposition, the excess nitrogen and sulphur falling gradually each subsequent day. The S:N ratio of the daily excess however bears no relation to that of the material ingested. The S:N ratio of the excess on the day of superimposition in each experiment (Experiment 1, 15th April, Experiment 3, 28th February, Experiment 5, 26th March) are 1:7.7, 1:10.2 and 1:16.1 respectively, while that of the gelatin ingested was 1:35. On the day following the superimposition there was always a corresponding fall in the ratio of the excess nitrogen and sulphur.

If then the excess is derived from the gelatin ingested, the conclusion seems warranted that under these conditions the sulphur/

sulphur fraction has been metabolised in advance of the nitrogen. A further point to note is the influence of the basal diet on the S:N ratio of the excess excreted on the day of superimposition in each experiment. It will be remembered that the nitrogen content of the basal diet was increased in each experiment: Experiment 1, N.free, Experiment 3, low N,and Experiment 5, high N. diet. The S:N ratio of the excess on the day of superimposition falls as the nitrogen content of the basal diet increases: in Experiment 1 the ratio is 1:7.7 while in Experiment 3 it is 1:16.1. It is also to be noted that the absolute amount of the excess nitrogen and sulphur on the day of superimposition increases from Experiment 1 to Experiment 3, although the intake was the same. This increase is more marked in the case of the nitrogen than in that of the sulphur, hence the fall in the S:N rate of the excess the higher the protein intake of the basal diet. The conclusion to be drawn is that the catabolism of the gelatin has been accelerated by superimposing it on a N containing diet: the higher the N content of the basal diet the greater the acceleration of the catabolism. If this acceleration affected the N and S moieties in the same degree the S:N ratio of the excess on the day of superimposition would be the same in each experiment. As has been mentioned above, this is not so as the ratio falls the higher the nitrogen content of the basal diet. It appears then that although the sulphur is catabolised in all three experiments in advance of the

nitrogen/

nitrogen, the influence of a N containing basal diet has been to accelerate the catabolism of the sulphur and nitrogen, but the latter in greater degree than the former. The nitrogen output would appear to catch up on the sulphur.

In Experiments 2, 3, and 6, Table I, with egg albumen the figures for the excess nitrogen and sulphur confirm the conclusion that the sulphur is metabolised in advance of the nitrogen. The general trend of the nitrogen and sulphur outputs differs however from those observed in the experiments with gelatin.

In Experiment 2 it will be noted that the maximum excess sulphur output is not on the day of superimposition, 26th April, but on the following day, while the maximum nitrogen output is yet a day later. It has already been shown that the nitrogen retention in this experiment amounted to 70%. The 30% which has been metabolised however is not dealt with immediately, but appears to be temporarily retained for a day or two, possibly in some unstable form. The interesting point to note however is that the delay in sulphur excretion has held back that of the nitrogen. If egg albumen were catabolised simply as a mixture of amino-acids the delay of one day in reaching the maximum sulphur output and two days in the case of the nitrogen is not easily understood. It seems possible then that ingested protein is built up into some complex and then metabolised in a definite order. The figures for Experiment 2, Table I, indicate also that the egg albumen/

albumen is not completely metabolised even five days after ingestion as the S:N ratio is still high and the nitrogen and sulphur outputs are not quite at their basal values. This slow rate of catabolism repeats what Falta had observed on superimposing egg albumen. Experiments 4 and 6 show the same delay in the excretion of nitrogen and sulphur except that the maximum outputs of each are one day earlier than in Experiment 2.

This fact, in conjunction with the decrease in the S:N ratio of the excess on the days of superimposition in Experiments 2, 4 and 6, confirms what has been observed in the gelatin experiments, namely the higher the nitrogen content of the basal diet the more quickly is the superimposed protein metabolised, the influence being more marked on the nitrogen than on the sulphur moiety. In short, the nitrogen output tends to catch up on the sulphur. A glance at the absolute amount of the excess nitrogen and sulphur and its S:N ratio on the day of superimposition (24th April, Experiment 2; 4th February, Experiment 4; and 31st March, Experiment 6) will show the excess on that particular day to increase and the S:N ratio to decrease as the nitrogen content of the basal diet increases. The question may be asked how if some protein complex is built up from the ingested material or if it is incorporated with some other circulating material, how its metabolism is delayed in the case of albumen. It has been shown previously, Experiment 7, that the poorer in sulphur the retained material is the more labile it

becomes. In these three experiments with egg albumen, a highly sulphur rich material must be stored in the tissues at the end of the first day only. After four days, however, it was found in Experiments 2 and 4 that all the sulphur and 30% of the nitrogen had been eliminated.

It is possible that any retained material which varies in its sulphur content either above or below that of the tissues tends to become unstable. If this be true, it would indicate that the organism tends to regulate the composition of its circulating material so as to approach that of muscle.

Experiments 7 and 8 illustrate the advantage of a detailed analysis not only of the daily excess nitrogen and sulphur, but also of the daily retention of these elements. The figures in Table II will show the possibility of gaining an insight into the anabolic side of protein metabolism.

Experiment 7 has already been discussed in regard to the gross retention in each period. In Table II are given the daily excess outputs over the basal figure (beef free diet) for each experimental period. In addition the excess outputs of sulphur and nitrogen in Periods II and III have been calculated, using the figures of Period III as a basal. It will be remembered that in Periods I and III, 250 grms. of beef were superimposed: the basal nitrogen output for these two periods was taken as the average of the two last days of Period III - 28th/

28th and 29th April. The daily nitrogen and sulphur retention and its S:N ratio are tabulated along with the percentage of nitrogen and sulphur retained each day. The percentage is calculated by taking the proportion of nitrogen and sulphur retained each day in relation to the total amount of these elements retained in that particular experimental period. For instance in Period I, 11.057 grms. nitrogen and .753 grms. S. were retained and of this 4.040 grms. N and .267 grms. S were retained on the first day, i.e., 36.5% and 35.4% respectively. Similarly in Periods III and IV the daily nitrogen and sulphur loss is given and the percentage of each element lost daily in relation to each period separately. It will be remembered that the retention is calculated not on the balance between intake and output but on that between the output and the absorbed. The amount absorbed was taken as the figures for the nitrogen and sulphur in the urine when equilibrium was attained.

In Period I (250 grms. beef superimposed) the S:N ratio of the daily retained material is extremely constant except for the 3rd day on which it rises to 1:11.6, and curiously enough there is a rise next day in the absolute amounts retained. Correspondingly the percentage retention of nitrogen and sulphur is remarkably constant each day except for the third.

In Period II, 500 grms. beef, the S:N ratio of the material retained daily, though low as compared to beef, is higher on the first day (1:23.3) than on the second (1:40.4). This would appear/

appear to indicate that during retention the sulphur is retained in advance of the nitrogen, in spite of the fact that the S:N of the total retention in this period is low - 1:29.4. It is particularly instructive to compare the daily excess nitrogen and sulphur output for this Period II with the daily retention. As has been mentioned the excess output is given on two bases, namely (1) excess over the basal beef free diet, and (2) excess over the 250 grms. beef diet. The S:N ratio of the excess may be seen to fall from 1:13.45 on the first day to 1:15.41 on the last day of Period II on the first basis of calculation, while on the second basis it falls from 1:5.1 to 1:15.8. These figures indicate clearly that the sulphur is catabolised in advance of the nitrogen, while the figures for the retention indicate that the sulphur is retained in advance. The S:N ratios of the excess and of the retained material for each of the five days of Period II are given below in order to show how the anabolic and catabolic phase run concurrently: the excess calculated on the second basis has been quoted.

	S:N excess or catabolised.	S:N retained or anabolised.
21 April	1:5.1	1:23.3
22	1:10.9	1:40.4
23	1:14.7	1:27.8
24	1:15.9	--
25	1:15.8	1:18.3

These figures show the fallacy of estimating the S:N ratio of the excess output alone: in this period (II) for instance the sulphur content of the excess is higher than that of muscle, and a hasty conclusion would be drawn - correct in so far as it goes - that sulphur is catabolised in advance of nitrogen. The material retained on the other hand is relatively poor in sulphur yet the sulphur has preceded the nitrogen in the process of retention. In Periods III and IV as the nitrogen intake is being successively reduced, there is a diminishing loss until equilibrium is attained at a lower level. In Period III the S:N ratio of the loss on the first day is 1:26.6 and on the 2nd day 1:138.3, while the figures giving the percentage of nitrogen and sulphur lost on each day in relation to the loss in the whole period show that 88% of the sulphur is lost on the first day and only 60.7% of the nitrogen. This again confirms the previous finding that although the material lost is sulphur poor the sulphur precedes the nitrogen in catabolism. Period IV shows the same features in regard to the early catabolism of the sulphur moiety. It may be asked how this phenomenon is not observed in the analysis of the nitrogen and sulphur outputs and retention in Period I. It is extremely probable that as the S:N ratio of the total material retained approaches that of muscle this selective retention may have been masked. It is possible that the early retention of sulphur might be obscured in a 24 hour period and yet show up in an 8 or 12 hour period.

For/

For instance in Period II if the percentage of nitrogen and sulphur retained in the first two days had been calculated, the preferential retention of sulphur would not be noted. The per cent retentions on the first day of Period II are 67% S and 53.6% N, on the 2nd day 18.9% S and 26.2% N; the per cent retention over the two days is however 76.6% S and 80.8% N: the retention of nitrogen appears to be greater in a two day period although the difference is not very great. It must be kept in mind that it is impossible to say how many days a retention process requires. It is possible that if a protein is superimposed for several days a certain fraction of the sulphur is retained on the first day, while it requires perhaps two days for the corresponding nitrogen moiety to be retained. Under these circumstances the preferential retention of sulphur would be easily noted. In practice, however, on the 2nd day of superimposition an additional fraction of sulphur is probably retained and the retention of its nitrogen quota is not completed until the 3rd day. Similarly on the 3rd and 4th days a diminishing fraction of sulphur is retained until equilibrium is reached and retention ceases. It is only to be expected under these conditions that in following the retention of nitrogen and sulphur over a period of days a steady state is reached and the preferential retention of sulphur obscured.

Experiment 8, Table III, illustrates this steady state

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in the process of retention. In Experiment 8, Table III, 250 grms. beef were superimposed on a basal diet similar to that employed in Experiment 7. Eight days elapsed before equilibrium was attained and consequently it will be noted that there was a steady but decreasing retention during the first seven days. The S:N ratio of the total material retained over the seven days is 1:16.76, while the S:N ratio of the total excess is 1:11.19. It will be noted that the S:N ratio of the daily excess tends to drop steadily from 1:9.15 to 1:12.67. The S:N ratio of the retained material is highest on the first day, 27th February, and from then it tends to drop until the 3rd and 4th day. The tendency however for the retention of a material relatively sulphur rich at the beginning and correspondingly sulphur poor later on is seen by comparing the S:N ratio of the total retained material (1:16.76) with the S:N ratio of the material stored on the first day (1:15.14) which is slightly higher and with the S:N ratio of the 7th day's retention (1:18.5) which is correspondingly lower than 1:16.76. The early retention of sulphur is also observed though in a small degree in this experiment by noting the percentage of sulphur and nitrogen retained on the first day, namely 26.9% sulphur and 24.5% nitrogen.

On the subsequent days the percentage daily retention of each element is approximately the same. This experiment thus tends to confirm the contention that the sulphur is the

mobile unit in that it takes the lead not only in the catabolic but also in the anabolic phase. The results are not quite so marked in Experiment 8 as in Period II of Experiment 7, but it is quite possible that the process is obscured under certain conditions. An analogy with what has been observed in the catabolism of superimposed protein in Experiments 1 to 6, Table I, may however provide an explanation. It was noted in these experiments that the higher the nitrogen content of the basal diet the more quickly the superimposed protein was metabolised and that this acceleration affected the nitrogen more than the sulphur, or, as it was expressed, the nitrogen tends to catch up on the sulphur in the catabolic phase. Is it not possible then that under certain circumstances the nitrogen may tend to catch up on the sulphur in the process of retention and so obscure the mobile rôle of the latter? It seems that only here and there hints are given of the mobile rôle of sulphur in anabolism and that experimental conditions, particularly in regard to the minimum length of time during which collections and estimations of urine are made, determine whether an analysis of the phenomenon is possible.

The S:N ratios in Experiment 11, 12 and 13, Table V, show the same consistency in the behaviour of sulphur in protein metabolism. The ratio in the control experiment 11 rises progressively from the first day of the nitrogen free diet on the 9th January from 1:15.8 to 1:12.3 on the day before fasting: it/

it rises still further until the 2nd fast day when it reaches 1:11.3. On the first post day (nitrogen free diet) it falls suddenly to 1:17.3 and from then until the 17th January it rises to 1:10.7. The progressive rise in the ratio in the prefast and fast days is almost certainly due to a lag in the nitrogen output behind the sulphur. It seems therefore that even although it is body tissue which is being catabolised the sulphur output precedes that of the nitrogen as has been observed with ingested protein. In this particular case it appears as if the nitrogen output has been delayed until the first day after the fast, when it is suddenly metabolised and thereby lowering the ratio to 1:17.3. That this is the likely explanation seems probable when the S:N ratio of the total nitrogen and sulphur outputs over the five days - preday, two fast days and two post days - is recalled: the figure obtained was 1:13.53, which would appear to indicate that the ultimate source of the material catabolised over these days was probably body tissue.

The increased nitrogen output on the first post day, 15th January, in Experiment 11, Table V, appears to contradict the theory of the sparing action of carbohydrate on the breakdown of protein in starvation. The nature of the phenomenon must however be considered. Two explanations of the sparing action of carbohydrate on tissue catabolism are possible. The organism may catabolise the ingested carbohydrate and hence

obviate/

obviate the necessity of drawing on body protein for its energy supply. According to Cathcart however the breakdown of protein may be the same whether carbohydrate is given or not, but in the former condition the nitrogen is resynthesised again by means of the food carbohydrate in much the same way as lactic acid is rebuilt into its precursor in the muscles. In support of this hypothesis Cathcart instances the appearance of creatine in the urine of his fasting subject and its disappearance when a nitrogen free carbohydrate rich diet is given. Fat however appears to be incapable of reducing the tissue loss in starvation and Landergren and Ringer conclude that the increased nitrogen breakdown in fasting in contrast to that on a carbohydrate rich diet is to supply the necessary quota of sugar to the organism. This difference in the influence of fat and carbohydrate would therefore tend to confirm what has been previously suggested, namely, that the energy needs in fasting are not the only factor as qualitatively these two nitrogen free foods are not isodynamic in their capacity to spare body tissue. If Cathcart's assumption be true, the nitrogen and sulphur output in the urine is no index of the turnover of protein in the tissues. It has already been pointed out that the nitrogen output on the first post day rises instead of falls under the influence of the nitrogen free diet. This nitrogen was considered to be derived from

body/

body protein, the sulphur of which had been eliminated a day or two earlier: the carbohydrate of the nitrogen free diet appears then to be incapable of resynthesising this delayed nitrogen, probably because the sulphur nucleus is already eliminated. If, however, the sulphur output on the first post day, 15th January, be compared with that on the last fast day, 14th January, it will be noted that in contrast to the nitrogen it is reduced slightly from .4286 grms. to .4012 grms. This would then tend to confirm the theory that sulphur is the mobile unit since, if carbohydrate spares the breakdown of the protein molecule or effects its resynthesis, its influence should be first directed to the sulphur moiety. A similar phenomenon is observed in Cathcart's fasting subject Beauté who was given a starch cream diet for three days following his fast. The following figures show the nitrogen and sulphur output on the last fast day and the three days on a nitrogen free diet.

	T.N.	T.S.	S:N.
14th day of fast.	7.78	.536	1:14.5
1st day of starch cream	7.43	.476	1:15.6
2nd " "	3.58	.275	1:13.0
3rd " "	2.84	.285	1:9.9

It will be seen from the S:N ratios of the last fast day and first post day that the sulphur output has been reduced relatively/

relatively more than that of the nitrogen. The S:N ratio tends to rise progressively to 1:9.9, exactly as in the pre-period and the post period of Experiment 11, indicating a lag in the excretion of nitrogen. The influence of the nitrogen free diet in Experiment 11 does not however give any indication as to whether the breakdown of body protein is spared or whether a process of resynthesis takes place. It is indeed possible that both processes are involved and that the total nitrogen turnover is much larger than what is actually excreted in the urine. Experiment 12 again shows the sulphur moiety to play the mobile part in metabolism. On the day gelatin was superimposed, 18th January, it will be noted that although the nitrogen output rose the sulphur output was actually lower than that of the previous day. A comparison of the sulphur output on the preday of Experiment 11, 12th January, with that on the day gelatin was superimposed in Experiment 12 will show the figures to be almost identical. This illustrates the preferential retention of sulphur in the anabolic process and agrees with Lewis's results on a fasting dog. A fasting dog was given beef for one day and a marked fall in the S:N ratio was noted, indicating a selective retention of sulphur. In another experiment the beef was superimposed for several days and the S:N ratio though low at first tended to rise gradually as the retention diminished. The animal then continued its fast/

fast and the ratio was found to rise, indicating according to the author a breakdown of the sulphur rich material previously retained from the beef ingested. This selective retention of sulphur is always most marked when the condition of the tissues is low, as after a fast. In Experiment 12 it will be seen that no nitrogenous food had been ingested for the previous nine days, two of which were complete fast days. It should be noted however that this retention of sulphur was temporary as the total balance in Experiment 12 showed that 80% of the nitrogen and sulphur of the gelatin had been metabolised within the five days after superimposition. It will be recalled that the gelatin was metabolised over a period of five days, as a comparison of the corresponding figures of Experiments 11 and 12 will show . The rise in the nitrogen output on the first post day, 21st January, in Experiment 12, repeats what was observed in Experiment 11; the sulphur output however shows a rise. This rise might indicate that the carbohydrate has not been effecting its sparing action, but it is possible that it exerted its usual sparing action on the breakdown of body tissue or some other transitional protein while the gelatin continued to be metabolised as being possibly incapable of permanent retention. It will be recalled in this connection that in Experiment 1, where gelatin was superimposed on a similar diet but without a subsequent fast, all the sulphur of the gelatin was eliminated and 50% of the nitrogen retained.

It should again be emphasised that this slow rate of metabolism of the ingested gelatin even under fasting conditions speaks strongly for a mediate rather than an immediate metabolism of the ingested foodstuffs. Experiment 13 with egg albumen tends in general to be similar to Experiment 12 with gelatin, the nitrogen and sulphur outputs being only a little higher on the first post day. The fact that the total nitrogen has not risen so much on the first post day, 27th January, as compared to the rise observed on the corresponding days of Experiments 11 and 12, may possibly be due to there being less lag in the excretion of nitrogen: this tends to be confirmed by the figures for the total nitrogen output on the two fast days which are distinctly higher than the corresponding figures for the two fast days in Experiment 12 with gelatin. The selective retention of the sulphur tends to be obscured by the fact that the albumen has such a high sulphur content. The sulphur output on the day the albumen was superimposed rose about .26 grm. while the S ingested in the albumen amounted to 1.319 grms; hence there was a considerable retention of the ingested material on the day of superimposition.

Experiments 14 and 15, Table VI, it will be recalled, consisted of 4 day fasts preceded and concluded by a nitrogen free diet. Experiment 14 served as a control, while in Experiment 15, 70 grms. gelatin were superimposed alone on the 2nd fast day. If the total nitrogen and sulphur output on the day

gelatin/

gelatin was superimposed, 8th February, 1924, Experiment 15, be compared with the output of nitrogen and sulphur on the corresponding day of the control, 27th February, 1925 (2nd fast day), Experiment 14, it will be seen that a retention of both has occurred. As has been previously discussed, however, this control is not strictly comparable as it was done after some months of normal feeding, while Experiment 15 followed within a few days of the three fast experiments - 11, 12, and 13. It is considered advisable, therefore, to compare the nitrogen and sulphur output on the 2nd day's fast in Experiment 15 with the output of these elements on the 2nd day's fast in Experiment 11. In Experiment 11, Table V, the nitrogen output on the 2nd fast day is 4.872 grms., while the sulphur output is .4286 grms.: on the day gelatin was superimposed in Experiment 15 (2nd fast day) the nitrogen output is 7.364 grms., while the sulphur output is .407 grms. These figures clearly show that all the sulphur and a considerable part of the nitrogen of the ingested gelatin have been retained: in spite of the fasting condition the anabolic phase is by no means in abeyance. The material retained on this day is, as can be seen from the S:N ratio, slowly metabolised over the next few days. These facts would appear to favour the idea that under all circumstances ingested protein is first built up into some complex or anabolised before being broken down and the end products eliminated. The sparing action of the carbohydrate of the basal diet is shown strikingly

in both Experiment 14 and 15. The influence is again most marked on the sulphur output, which is reduced in proportion more than the nitrogen as the S:N ratio drops on the first post day as compared to the last post day in each experiment: in Experiment 14 it drops from 1:14.4 to 1:16.7, while in Experiment 15 it drops from 1:16.8 to 1:18.3. The ratio then tends to rise progressively until the end of each experiment.

The general inferences to be drawn from an analysis of the nitrogen and sulphur outputs in conjunction with the intake has led one to formulate the following conceptions. Biologists and physiologists alike are agreed that the metabolic processes taking place in the body cells can best be described as consisting of a building up process or anabolism and a breaking down process or catabolism. It is noteworthy how little attention has been given to the anabolic phase by students of nutrition. This is partly due to the fact that while the end products of catabolism are easily obtained and measured, the end products of anabolism are not except as an increase in weight or as a positive balance of some substance such as protein, fat, or glycogen. Further, the different stages of catabolism of many foodstuffs can often be obtained by various experimental techniques. This difficulty in investigating the anabolic side has tended to make workers in this field forget its existence in practice although in theory they may readily assume its existence.

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The general inference which one draws from scientific literature is that the organism tends to be looked upon merely as a catabolic machine for consuming the foodstuffs. The writer is firmly convinced that a complete picture not only of protein metabolism but also of nutrition in general will only be achieved when the nature of both processes has been elucidated. It is hoped the analysis just made has served to show that there is an order not only in the catabolism, but also in the anabolism of protein. It would appear that in these two phases sulphur or a sulphur containing moiety is the mobile or one of the mobile units in the process.

Evidence has been adduced that possibly under all circumstances ingested protein is first of all built up into some complex before being metabolised. The results would appear to indicate that ingested protein is metabolised mediately after it has been incorporated with some pre-existing protein or possibly protoplasm: the ingested material thus becomes an integral part of a living structure which in turn is slowly metabolised.

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SECTION III.

THE INFLUENCE OF MUSCLE WORK ON PROTEIN METABOLISM.

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It may be said with truth that muscle work as a problem of scientific interest owed its inception to Lavoisier some 150 years ago. He was the first to prove that work was associated with an increased consumption of oxygen. To the lay mind, however, the question of work is intimately associated with food consumption and it was to this aspect that Liebig at the beginning of the nineteenth century directed his attention. Liebig with his classification of the types of food into nitrogenous and non-nitrogenous first raised the problem - not fully answered yet - as to which foodstuff furnishes the energy for muscle activity. Voit with the present day classification of the foodstuffs into protein, fat and carbohydrate added still further to the problem, since these three energy yielding foods were theoretically capable of furnishing fuel to the muscles. Since Voit's time each of the three foodstuffs and all three together have been postulated as fuel for the muscles. According to Liebig it was the nitrogenous food alone that was consumed in the performance of work by the muscles. It appears he was impressed by the similarity in the chemical constitution between muscle tissue and animal protein and could conceive of no food other than the nitrogenous as capable of supplying energy to muscle. Voit and Pettenkofer with the technique available for determining the proportion of the three foods undergoing oxidation at any one time, carried out experiments to test Liebig's hypothesis. They logically/

logically concluded that if protein is the source of power for muscle work, there should be an increase in nitrogen in the urine of a subject doing work. Their experiments were negative in this respect and they concluded that the nitrogen free foodstuffs and fat in particular were oxidised by the working muscle. One of the classical experiments however was that of Fick and Wislicenus not only because of its apparent conclusiveness but also on account of the criticism to which Liebig subjected it. Fick and Wislicenus climbed the Faulhorn some 2,000 metres high and calculated in Kilogrammeters the total amount of work performed by each. The theoretical work done in raising their bodies to the summit was 106,250 Kgmts. (Fick) and 105,825 Kgmts.(Wislicenus). They assumed however that muscle was 50% efficient and added an arbitrary figure for the work of the heart and respiration, making the total theoretical energy consumption by the body 319,274 Kgmts. work (Fick) and 368,574 Kgmts.(Wislicenus). From the nitrogen output in the urine it was calculated that not more than 106,250 Kgmts. work could have been furnished from protein in the case of Fick and 105,825 Kgmts. work in the case of Wislicenus. The authors concluded then that work is performed at the expense of carbohydrate and fat. They compared muscle to a steam engine which consumed coal but did not utilise its own structure. The experiment without proving that muscle work might not be performed at the expense of protein appeared at least to show that nitrogen free foods could function as well.

Liebig/

Liebig, in a vigorous criticism shortly before his death, did not even allow the possibility of this conclusion. In the first place he stated that potential energy in the form of protein might be stored in the body and utilised during work without the nitrogen appearing in the urine. He affirmed that urea nitrogen was not necessarily the end product of muscle activity as muscle tissue by the methods of his time had been shown to contain very little urea. In the second place he held that the combustion of the foodstuffs in a bomb and in the body are not the same thing and quoted the case of the transformation of sugar to alcohol by yeast. He stated that the heat produced in the change sugar to alcohol plus the heat of combustion of the alcohol was greater than that of the original sugar. He quotes as an analogy the difference in the heats of combustion of diamond and carbon and the 43% difference in the heats of combustion of the two isomers of cyanic acid. His criticism must have appeared rather weak in the face of the commonsense conclusions of Fick and Wislicenus, but he was unable to accept the mechanical conception of metabolism proposed by those investigators. The result of his attack, possibly on account of his reputation, kept the problem alive among scientists as not finally settled. The sustained interest however on the question of the relation between work and protein or food intake in general has been maintained partly by its economic and hygienic importance in regard to manual labour and partly by its application/

application to athletics. Hence in a discussion and indeed scientific investigation on this subject one can not ignore the habits as regards food of those who do manual labour or indulge in sport. The evidence however from such sources is not altogether clear cut. Frankland recorded that the diets selected by labourers carrying out excavation work contained large quantities of fat. Fick and Wislicenus pointed out that the chamois hunters in Switzerland take provisions consisting largely of the non-nitrogenous foods such as fat and sugar.

Weston, the long-distance walker, is said by Blyth and Pavy to have partaken of a diet containing 223 grms. of protein, 60 grms. of fat, and 500 grms. of carbohydrate, 4700 calories in all, of which carbohydrate furnished nearly a half. On the other hand there is the general public opinion that those doing heavy labour, particularly athletes, desire a diet rich in protein and preferably beef. Sundström noted that Swedish acrobats consumed approximately 180 grms. of protein daily and the standard diets of most of the armies during the war all contained liberal quantities of protein. The general results from observations of this kind do not appear to mark out any one foodstuff as being the one *par excellence* chosen by those doing muscular work of any kind. It should be remembered however that the calorie intake of all subjects doing strenuous work may be double that of individuals in a sedentary occupation: under those circumstances the general tendency/

tendency may be to make up the necessary calories by an increase in all three foodstuffs. It should further be considered however that there may be a difference on the one hand between hard and fatiguing work carried out over a period of days or weeks and on the other hand a short bout of strenuous exertion lasting but an hour or two. There seems to be a general tendency for young active children and even adults after a period of exercise to have a craving for carbohydrates, in all probability because they are quickly available for the tissues. The outcome of all these considerations and conflicting views has been a considerable amount of investigation by different workers, each one trying to prove his own contention. The extreme views of Liebig that protein is the source of power for muscle work has become however considerably modified. Pflüger it is true made an absolutely lean dog do 100,000 Kgmts. work on a diet which consisted of fat free beef only. The animal remained in good condition and kept its weight throughout the experiment. Pflüger's dictum from this investigation was "Keine Muskelarbeit ohne Eiweisszersetzung." This experiment certainly proved that protein could cover the cost of muscle work, in a dog at least, but not that it necessarily must do so. Pflüger explained the fact that work could be performed on a medium nitrogen diet on the assumption that the nitrogen in the urine was all derived from the working muscles, while the other tissues of the body consumed/

consumed fat instead. He also invoked the conception that the protein molecule broken down by muscle work might be re-synthesised again by means of the nitrogen free food of the diet. The general conviction at the back of his mind was that the nitrogen turnover in a working muscle was the same regardless of the quantity or quality of the food. If the protein intake was high it replaced that broken down in the muscles, while if it was low, the nitrogenous moiety of the muscle protein was resynthesised by means of the nitrogen free foods. It was this consideration or possibly an intuition that prompted him to express so dogmatically "Keine Muskelarbeit ohne Eiweisszersetzung." It is now generally recognised however that the nitrogen free foodstuffs are a source of power for the muscles.

Most workers since Voit and Pflüger have confined themselves to investigations as to which of the nitrogen free foodstuffs is the main fuel for muscle work, while others have been concerned as to whether work has any influence whatsoever on the output of nitrogen in the urine, and if so what is its significance. Smith in experiments on himself and prisoners working on a treadmill found that there tended to be an increase in the nitrogen output on the day following work. He considered that the vital transformations in the active muscle were associated with an increased turnover in nitrogenous material without of necessity there being an increase of urea in the urine. Parkes, investigating the urinary nitrogen output/

output in two men doing 200,000 Kgmts. work for one day on a constant diet, noted a rise in the nitrogen output in the urine of about 1 grm. on the day following. Flint noted the same rise in nitrogen output after prolonged exercise and interpreted his results along the same lines as Pflüger. He also observed that the increase in nitrogen output after work was in proportion to the level of nitrogen intake. He thence concluded that the food is not consumed directly but must first be built up into the muscle tissue before utilisation. Smith estimated the urinary nitrogen of a horse during work and rest respectively and found an increased output during the work period: the food intake was however not strictly controlled, but the increase in nitrogen output if it came from protein was quite insufficient to cover the cost of the work.

Hirschfeld observed that work had no influence on the nitrogen in the urine regardless of the level of nitrogen intake. He concluded that previous workers had given insufficient nitrogen free calories to cover the cost of the work and hence there was an increase in protein catabolism. Speck in a discussion of the influence of work on metabolism states that there may be during work an increase in nitrogen output which is greater the higher the nitrogen intake. He considers that the cells, if a demand is put on them, consume the material offered, hence if the protein intake is high the increased nitrogen output in the urine will be proportionately greater. He thought

that there was no evidence of a greater turnover of nitrogen in the muscles during work. Dunlop, Paton, Stockman and Maccadam, in experiments on human subjects, noted an increase in the nitrogen and phosphorous output on the day after work. These workers observed that training appeared to exert some influence, as the uric acid and phosphorous output was increased only in those subjects in poor condition. Their general conclusion was that work increases the catabolism of protein. Garratt working in short experimental periods noted a fall in the nitrogen output during the work period and a rise in the six hours following. Campbell and Webster observed an increase in total nitrogen, neutral sulphur and creatinine in the night urine after doing 67,000 Kgmts. work during the day.

Schafer attempted to find out whether rest in bed in contrast to ordinary routine work had any influence on the nitrogen output. He detected however no difference in the nitrogen and sulphur outputs or their partition in the urine under the conditions imposed. Cuthbertson on the other hand showed that enforced rest in bed with a limb in splints caused an increased output of sulphur,nitrogen, phosphorus, and calcium in that order of priority. He holds on the basis of those experiments (carried out in part on normal uninjured subjects) that when the level of functional activity is altered, either by an increase or a decrease, some time elapses before equilibrium is again attained. In the case of diminished muscular activity

there is a loss of nitrogen until the catabolism of functional tissues is equal to the diminished anabolism. In the case of muscle work on the other hand the anabolic response takes some time to equal or exceed the increased catabolism of the muscle: hence when muscle activity is altered in either direction there is a preliminary loss of tissue for the first few days. Bornstein showed in the human subject that muscle work carried out over a period tended to promote retention of nitrogen probably in the muscles. Caspari working with dogs observed an increased nitrogen output during work in some experiments, while in others there was a tendency to retention. Krummacher showed in the human subject that work caused an increased output of nitrogen which was less the more nitrogen free foodstuffs were added to the diet. He thought that the increase in nitrogen output was simply a result of a temporary starvation in the cells as the increase in nitrogen bore no relation to the work done. Frentzel working with dogs noted an increased nitrogen output which was greater if the animal was fasted than if a protein fat diet was given. Chambers and Milhorat also noted an increase in the nitrogen output of fasting dogs both during the work period of 3 hours and in a similar after period. If carbohydrates were given the increased nitrogen output was diminished or absent. They maintained that the nitrogen was derived from some reserve which was gradually exhausted as

the/

the fast extended, since no rise in the nitrogen output following work could be noted in the later days of starvation. Further, since the increased urinary nitrogen following work was reduced by carbohydrate ingestion, they considered that the increase in protein breakdown was to supply glucose to help to cover the cost of the work. Chauveau showed in dogs that muscular work had no influence on the two-hourly output of urinary nitrogen after a protein meal either of beef or gelatin, and stated that protein is not immediately concerned in covering the energy needs involved in muscular activity. Atkinson confirmed this observation and conclusion on a dog doing work after 750 grms. beef. Chauveau also showed that the increased energy output after protein ingestion can not be used to cover the cost of muscle work: he maintained that glucose is the fuel for muscle work and that fat must first be transformed into glucose before it can be utilised by the muscle. According to this conception fat would be less economical a source of energy for muscle than carbohydrate. Anderson and Lusk also noted that the increased metabolism associated with the specific dynamic action of protein could not be used to cover the energy needs of the active muscles, while the specific dynamic action of glucose on the other hand could. Zuntz however showed that all three foodstuffs were as efficient as sources of power for muscle work, which would tend to disprove Chauveau's contention that glucose is the only fuel.

Frentzel/

Frentzel and Reach on the other hand found a rise in the R.Q. during work whether the subject was on a carbohydrate or fat diet: the cost of work was however the same in either case. Heinemann, on the other hand, found that work was accomplished more economically at the expense of fat than of carbohydrate and refuted Chauveau's conclusion that fat must first be transformed into carbohydrate with a loss of 20% of its heat value before being available as fuel for muscle. Benedict and Cathcart working on a human subject found a tendency for the quotient to rise during work above the basal value and fall below it in the after period. They concluded that there was a preferential utilisation of carbohydrate during work while the lower quotient in the after period was due to an exhaustion of the glycogen reserves, fat being consumed in greater amount than in the pre-period. Their experiments on diets rich in fat and carbohydrate respectively gave no indication of the economical superiority of one foodstuff over the other. Krogh and Lindhard found on the other hand that the cost of work on a fat rich diet was on an average 11% greater than on a carbohydrate rich one. No urinary analysis was however made in Benedict and Cathcart's and Krogh and Lindhard's experiments. An extended series of experiments both on the respiratory exchange and the urinary output were carried out by Cathcart and Burnet on diets varying in their proportion of protein, fat and carbohydrate. The routine in their experiments consisted/

consisted of 4 predays, 6 work days and 4 post days. The amount of work done daily was 25,000 Kgmts. in a one-hour period. As regards the urinary output it was noted that there was a small rise in the nitrogen and sulphur excretion which lasted often into the post (rest) period. The sulphur output however was relatively less than that of the nitrogen, for while the total S:N ratio of the urine is varied between 1:11 and 1:15 in the different experiments, the S:N ratio of the excess was 1:28 except in one case (beef diet) when it approached that of the urine. The excess nitrogen output in general tended to be greater the higher the protein intake. Mitchell and Krüger criticised their results adversely by stating that the increase in nitrogen output might possibly be due to an insufficient calorie intake. They held that the problem could only be solved by finding out whether the endogenous nitrogen metabolism was increased by work. In their experiments on rats a nitrogen free diet was fed and the animals were put to work on a treadmill: the total nitrogen output by faeces and urine did not show any increase and they concluded that muscle work has no influence on protein metabolism. In an investigation of this kind however rats hardly seem suitable animals, particularly in regard to their comparison to the human subject. Thomas is so far the only investigator who has carried out an experiment on the human subject to show the influence of work on the urinary output on a nitrogen free diet. The diet was ingested until the urinary

nitrogen/

nitrogen had fallen to 2.85 grms. per diem and then followed three days' work on an ergometer and three post days without work.

The average nitrogen output on the four predays was 2.96 grms, then followed three work days on which 97,000, 105,000 and 136,000 Kgmts. work were done on an ergometer: the average daily output for those three work days was 2.41 grms. N. A three days' post period followed with an average daily output in the urine of 2.78 grms. N. Thomas takes as the true wear and tear output the average of the last two post days (2.27 grms. N.) and compares this with the average of the last two work days (2.94 grms. N.), while the average work done was $\frac{105,000 + 136,000}{2}$ = 120,000 Kgmts. 120,000 Kgmts. work has hence caused the loss of .67 grm. N. from the tissues. His conclusion was that work had very little influence on the nitrogen metabolism as on the assumption of 25% efficiency for muscle there was only 41 mgs. extra nitrogen for 100 Kalories muscle work done. Rubner in a paper on the endogenous wear and tear output elaborates somewhat Thomas's results. Rubner assumed that of the 2.2 grms. endogenous output about .95 grm. came from the muscles on the basis that the muscular system makes about 40% of the body weight. If the excess nitrogen output of .67 grm. comes from the increased endogenous metabolism of the muscles the proportional increase is greater, namely .95 grm to/

to 1.62 grms (.95 + .67). Rubner also points out that in all probability all the muscles do not increase their endogenous metabolism as only a certain unknown amount of the body musculature is involved in the work. This would make the percentage increase in the endogenous metabolism of the muscle still greater. Rubner drew no further conclusions as to the significance of this increase but the experiment is striking in regard to the small effect of such an amount of work on the nitrogen output. It must be remembered, however, as Cathcart pointed out in his experiments, that the increased nitrogen excretion does not reach its maximum until the 3rd or 4th day of work, and that while Thomas observed a negligible increase on a nitrogen free diet, Cathcart and Burnet observed the increase to be greater the higher the protein intake. The general results of most of these investigators appear to be that if there is any increase in nitrogen output it bears no relation to the work done. Within recent years, largely owing to the work of Hill and Meyerhof, the problem has tended to become unduly simplified, while the main issue has been lost sight of. Those investigators have been concerned for the most part with excised muscle and the general trend of their work shows that carbohydrates are the main source of fuel. The possibility that protein has a rôle to play in the process has never been considered by them. It seems that there are two entirely separate problems to be solved. The work of Hill and Meyerhof has/

has been concerned in elucidating the phenomena involved in the contraction and restitution phase of muscle as an isolated organ. Such work is extremely valuable so far as it goes and it is indeed possible that in the actual contractile and restitution phase of muscle activity glucose is indispensable. This however by no means solves the larger question as to what is the relation of muscle activity in the intact organism to the nutritive processes as a whole. The physiologist must endeavour to look upon the organism as a unit and remember that in the intact organism muscular activity is associated with a series of concomitant changes in the activity of the other organs. Similarly the student of nutrition must keep in mind that the energy output in the resting organism is coming from all the tissues of the body in varying degree and it is legitimate to ask what is the metabolic response to an increased energy consumption by the muscles. It might be said that muscle work simply draws away one of the nitrogen free foodstuffs to supply energy and leaves the nitrogenous turnover uninfluenced. This however leaves unexplained many of the consequences of muscle activity. It is a common experience that the muscles hypertrophy after a period of prolonged work. It is also a fact that the muscle must be kept in normal functional activity since excised muscle within a few days loses its power of perform ing work.

It will be appreciated that one of the main issues for the physiologist is not whether protein, fat, or carbohydrate, or all three, are the sole source of fuel, but what is the metabolic response as a result of muscle activity in the intact organism. It is then from the nutritional standpoint that the experiments recorded here have been carried out, and in view of this several possibilities have to be borne in mind. It may with certainty be said that protein is not, at least directly, the source of power for muscle work: the increase in nitrogen output observed by different workers has always been totally inadequate to account for the work performed. Indeed apart from the increase in nitrogen output the total nitrogen intake has usually been inadequate to cover the cost of the work. In this respect the experiment and conclusions of Fick and Wislicenus still stand. If, however, muscle be compared to a machine as those workers did, it may be asked if there is not a wear and tear associated with its activity, which a priori should be greater the more the work done. Pushing however the comparison of muscle or a living cell to a machine should be done with caution. The wear and tear of a machine is entirely due to frictional forces between materials which have no relation to the fuel of such a machine. In the living cell on the other hand structure and fuel are essentially similar constituents and as the cell contents are of a fluid nature there is no justification for assuming a frictional or mechanical disintegration/

disintegration to be associated with activity of the muscle. It must also be remembered that each cell unit is extremely small where only chemical and molecular forces come into play on a small scale, consequently if a wear and tear or disintegration of tissue does exist it must have an entirely different significance from that implied in the mechanical sense. To some it may seem strange that since Liebig's time there has never been wanting some protagonist for the idea that the nitrogenous material does play some rôle in muscle activity. Further, this idea has persisted in face of the fact that any observed increase in nitrogen excretion after work is minimal. Such investigators in all probability felt convinced that muscle work was in some way associated with an increased mobilisation and turnover of nitrogenous material. Further sanction is given to the study of the nitrogenous turnover in muscle by the number of extractives present in that tissue. Those who have investigated the excised muscle as a contractile organ have scarcely inquired as to the function of the various extractives or found reason to relate them in any way to the processes involved in contraction or relaxation. Recently Fiske and Subbarow have shown however that muscle creatine is associated with phosphorus in muscle as a labile compound, and as phosphorus seems to be associated with the contractile phase of muscle, it is possible that his discovery may initiate

a/

a new and broader conception of the problem. The discovery by Hopkins of glutathione in all tissues may also lead to its playing a rôle in the oxidation processes associated with the restitution phase. It may be unwise to prophesy but it is possible that the problem in the future may be as to how there is so little increase in the nitrogen output after work.

Experiments on Muscle Work.

The following experiments were carried out with diets which varied in the source of their protein. The work done varied between 20,000 and 35,000 Kgmts. daily in the different experiments and was carried out on a hand or bicycle ergometer according to the experiment. The day was divided for experimental purposes into the usual 24-hour period as far as the collection of urine was concerned, while the work was carried out in a 1-hour period. The work done was never exhausting and no ill-effects were ever observed on any occasion. As in Cathcart and Burnet's observations, the experiments were divided into three periods, namely a preperiod, a work period, which varied from 4 to 18 days in the different experiments, and a post period.

In Experiment 18 the basal diet consisted of the following:-

100 grms. cheese.
125 grms. butter.
150 grms. jam.
470 grms. bread.
1 apple. 2870 Calories.

TABLE VIII. Experiment 18.

Date.	T.N. grms.	T.S. grms	S:N	Kgmts. work.	Average N. 3-day period.	Average S. 3-day period.
1926.						
10 Jan.	7.980	.610	1:13.08			
11	8.848	.643	1:13.76		8.614	.627
12	9.016	.630	1:14.30		S:N 1:13.73	
13	9.156	.654	1:14.00	20,129		
14	8.848	.621	1:14.24	20,000	9.174	.641
15	9.520	.648	1:14.69	20,120	S:N 1:14.31	
16	9.688	.692	1:14.0	20,000		
17	9.940	.648	1:15.33	20,835	9.809	.651
18	9.800	.615	1:16.26	21,750	S:N 1:15.09	
19	9.912	.670	1:14.79	20,166		
20	9.940	.648	1:15.33	20,068	10.320	.681
21	10.108	.725	1:13.94	21,088	S:N 1:15.16	
22	9.912	.747	1:13.26	21,081		
23	11.368	.851	1:13.35	21,825	10.397	.752
24	9.912	.659	1:15.04	22,110	S:N 1:13.82	
25	9.324	.637	1:14.63	22,132		
26	9.352	.648	1:14.34	21,310	9.324	.644
27	9.296	.648	1:14.34	21,711	S:N 1:14.47	
28	8.848	.615	1:14.37	22,418		
29	8.932	.615	1:14.52	21,911	8.792	.618
30	8.596	.626	1:14.05	21,981	S:N 1:14.22	
31	8.316	.599	1:13.79	work stopped.		
1 Feb.	7.840	.588	1:12.72		8.194	.597
2	8.428	.604	1:13.95		S:N 1:13.72	
3	9.602	.654	1:14.68			
4	9.044	.549	1:16.47		9.100	.607
5	8.656	.620	1:13.97		S:N 1:14.99	

TABLE VIII (Continued).

Experiment 19.

Date.	T.N. grms.	T.S. grms.	S:N	Kgmts.work.
1929. 7 Oct.	10.598	.6866	1:15.43	
8	10.626	.5880	1:18.07	
9	10.220	.5550	1:18.41	
10	10.785	.5610	1:19.01	
11	10.808	.6265	1:17.20	23,338
12	11.088	.6375	1:17.39	22,206
13	11.328	.6592	1:18.18	22,448
14	11.032	.6155	1:17.92	23,050
15	10.878	.6045	1:17.99	22,448
16	11.116	.6045	1:18.36	work stopped
17	10.906	.6265	1:17.41	
18	11.407	.6100	1:18.70	
Experiment 20.				
18 Nov.	9.786	.7309	1:13.38	
19	10.052	.7692	1:13.67	
20	10.836	.7188	1:15.07	
21	10.108	.7419	1:13.64	
22	10.500	.8507	1:12.34	34,450
23	10.626	.7859	1:13.52	34,524
24	10.668	.7969	1:13.38	34,164
25	10.472	.7914	1:13.48	34,306
26	10.242	.7474	1:13.70	work stopped
27	10.778	.7878	1:13.68	

TABLE VIII.(Continued).

Experiment 21.

Date.	T.N. grms.	T.S. grms.	S:N	Kgmts.work.
1929. 22 Oct.	7.336	.6730	10.90	
23	8.120	.7089	11.45	
24	8.719	.7382	11.89	
25	8.721	.7360	11.84	
26	8.792	.8094	10.86	33,405
27	8.652	.7914	10.32	34,644
28	8.764	.7580	11.41	35,023
29	8.610	.7254	11.86	33,601
30	8.400	.6866	12.23	work stopped
31	8.288	.7748	10.69	
1 Nov.	8.386	.7639	10.97	

The work was carried out on a hand ergometer; the average amount of work done daily was 22,000 Kgmts. The original object of this experiment was to determine whether the rise in nitrogen output noted by Cathcart and Burnet when work was carried out for 6 days would fall if the work were carried out for a longer period. The experiment (Table VIII) thus consisted of 3 predays, 18 work days, and 5 post days. The basal nitrogen and sulphur outputs employed to calculate the excess of these elements excreted were taken as the average of the three predays and the last post day: the following are the figures employed:- 8.875 grms. N; .625 grms. S; S:N 1:14.2. The nitrogen and sulphur outputs tend to fluctuate irregularly above the basal values throughout the course of the experiment. In general, however, the nitrogen rises to a maximum of 11.368 grms. on the 11th day of work (23rd January) and then gradually falls below the basal value by the 30th January, the 17th work day. The three day average of nitrogen and sulphur outputs and S:N ratios for the 18 days of the experiment are shown in Table VIII. It will be noted that the average nitrogen and sulphur outputs rise to a maximum in the 3rd and 4th periods, then continue to fall to below the basal value on the first post period and to rise slightly in the last post period. The S:N ratio tends to fall from the preperiod (1:13.73) to 1:15.16 in the 3rd period and then with the exception of the 4th period rises to 1:13.72 in the first post period. More information may however/

however be obtained by calculating the excess nitrogen and sulphur above the basal value (8.875 grms. N and .625 grms. S). Below are tabulated the sum of the excesses and their S:N ratios in the three day periods.

Period I.	.926 grms. N. excess.		
	.052 " S. "	S:N	1:17.8
Period II.	1.8073 grms. N. excess.		
	.090 " S. "	S:N	1:20.6
Period III.	3.335 grms. N. excess.		
	.178 " S. "	S:N	1:18.8
Period IV.	4.567 grms. N. excess.		
	.376 " S. "	S:N	1:12.1
Period V.	1.347 grms. N. excess.		
	.058 " S. "	S:N	1:23.2
Period VI.	1.030 grms. N. excess.		
	.01 " S. "	S:N	1:103.0
Retention on two post days	1.015 grms. N.		
	.063 " S.	S:N	1:16.1

The maximum excess is, as was to be expected from the figures showing the total nitrogen and sulphur outputs, in Period IV. The S:N ratio of the excess rises to a maximum of/

of 1:12.1 in Period IV and then falls to a minimum of 1:103.0 in the 6th period. The retention in the two post days has a S:N ratio of 1:16.1, a figure approaching that of muscle tissue. The sum total of the excess nitrogen and sulphur in the 18 days' work period is 12.008 grms. N. and .764 grms. S., with a S:N ratio of 1:15.71, while the total loss to the body if the retention in the post period is subtracted from the excess, is 10.993 grms. N. and .701 grms. S., with a S:N ratio of 1:16.81. The influence of work has been in some way to cause a steadily increasing catabolism of protein increasing to a maximum on the 11th work day and then falling to a figure below the basal value. The S:N ratio of the material metabolised is not radically different from that of the basal S:N or the S:N ratio of muscle tissue although it tends to be lower than either of these two. From this experiment alone however it is not possible to say whether it comes from food or body protein. The figures however of the excess appear to indicate that possibly two processes are going on simultaneously, namely anabolism and catabolism. It seems that when work is commenced an increased rate of metabolism, i.e., catabolism and anabolism, is initiated, and that catabolism tends to exceed anabolism up to the fourth period. It is to be noted that the S:N ratio of the excess tends to rise to its maximum at the period of maximum catabolism. From Period

IV/

IV, although catabolism actually exceeds anabolism, the latter appears slowly to be overtaking it, and the S:N ratio of the excess output falls, indicating that the loss in sulphur is less than that of nitrogen relatively: this may be expressed otherwise by saying that sulphur retention is coming into play and would confirm the previous evidence that sulphur is the mobile unit. In the two post days the S:N ratio of the retained material is 1:16.1, almost identical with the ratio of the total material lost over the whole experiment (1:16.81). It is important however to visualise exactly what is taking place during the course of the experiment. The fact that at the end of the experiment the total nitrogen and sulphur output is down to or below the basal value does not mean that the rate of catabolism has decreased from what it was in Period IV. To the writer it appears that under the influence of work -- a catabolic phenomenon -- the catabolism of protein has gradually risen, and pari passu, but lagging behind it, the phase of resynthesis or anabolism has followed it up. In regard to the latter phase, two explanations as suggested by Cathcart are possible, depending on the nature of the processes supposed to take place in the active muscle. On the one hand it is possible that during activity the protein molecule is broken down and resynthesised again by means of the nitrogen free foodstuffs, particularly carbohydrate. On the other hand, it may be that there is an increase in the breakdown of protein during work, and this loss

is made up by a diversion of some of the exogenous food protein. If the latter assumption be true, a greater increase in the nitrogen excretion should be noted on a diet containing little or no protein. Thomas's experiments however showed a minimal increase in nitrogen output on a nitrogen free diet. It would then appear as if the first assumption is the more likely - namely resynthesis - but it is possible that both processes take place. The body hence as a result of work has increased the rate of its nitrogen turnover although the nitrogen output at the end of the experiment is the same as in the preperiod. This increased turnover has however been effected at the cost of a certain loss of material, whether of body or food protein will be seen in later experiments. The objection may be raised "Why is it that the catabolic phase was not at its maximum on the first day of work instead of 11 days later?" The energy turnover was the same on the first day of work as on the 11th, and it seems peculiar that the protein metabolism is not altered until much later. This delay would appear possibly to explain the rôle of protein in muscle work. In the first place the failure of the nitrogen output to increase on the first day of work seems to indicate that protein is not the source of energy for muscle work. The increased nitrogen metabolism must therefore have some indirect relationship to the increased energy exchange. Indeed it can not be altogether surprising that an increased energy turnover in the muscles should influence the other/

other functions of the body such as an increased rate of nitrogen metabolism. This increased catabolism and anabolism would favour the physiological hypertrophy not only of the muscles involved but also of the heart, both of which ultimately show an increased functional capacity after a period of prolonged work. Further, the increased nitrogen turnover would tend to show the solidarity and unity of the metabolic processes. The metabolism of the three foodstuffs tends to be considered each one separately, while in all probability the body metabolises all three as a unit although the proportion of each metabolised in any period of time may vary.

The experiment further shows the importance of investigating the problem on a normal human subject: experiments on rats or excised muscle can never give more than a partial view of the question. Experiment 18, however, gives no definite information as to whether there is an increased turnover in muscle tissue or possibly an increased rate of metabolism of food protein. It was therefore with this object in view that experiments were carried out with different proteins in the basal diet.

In Experiment 19 the following diet was employed in order to obtain a relatively sulphur poor mixture.

470 grms. bread.
50 grms. cheese.
100 grms. butter.
40 grms. gelatin.
250 grms. jam.
1 apple.

2,857 Calories.

The basal nitrogen and sulphur outputs were calculated on the average of the 4 predays (Table VIII), the figures obtained being 10.557 grms. N and .5974 grms. S; S:N = 1:17.66. The work was carried out on a hand ergometer under the same conditions as the previous experiment and was continued for 5 days. The following are the average figures for the preperiod, work period, and post period.

Preperiod. 4 days.	10.557 grms. T.N. output.		
	.5974 grms. T.S. "	S:N	1:17.66.
Work Period. 5 days.	11.026 grms. T.N. output.		
	.6286 grms. T.S. "	S:N	1:17.54.
Post Period. 3 days.	11.143 grms. T.N. output.		
	.6103 grms. T.S. "	S:N	1:18.25.

It will be noted that the increase in the work period is not considerable, and the S:N ratio has hardly altered from that of the preperiod. In the post period, however, the sulphur output dropped while the nitrogen increased a little, causing a small fall in the S:N ratio from 1:17.54 to 1:18.25. The excess outputs of nitrogen and sulphur however bring out some definite features. Below, the excess for the first two days work, the last 3 days' work, and the 3 post days has been calculated.

1st two days' work.	.782 grm. N. excess.	
	.0692 grm. S. excess.	S:N 1:11.3
Last 3 days' work.	1.567 grms. N. excess.	
	.1070 grm. S. excess.	S:N 1:14.6
3 post days.	1.758 grms. N. excess.	
	.0487 grm. S. excess.	S:N 1:38.1
Total Excess	4.107 grms. N.	
	.2248 grm. S.	S:N 1:18.26.

The S:N ratio of the total excess is almost the same as that of the protein ingested as deduced from the S:N ratio of the urine in the preperiod 1:17.66. The S:N ratios of the various periods show however that the sulphur has been excreted in advance of the nitrogen. The ratio is 1:11.3 in the first two days and then tends to fall to 1:38.1 in the post period. The figures would appear to indicate that the catabolic phase has risen to a maximum about the last days of work and that from then on the anabolic phase is beginning to creep up on it in the post period in that the excess sulphur output has fallen considerably. It is to be noted however that the excess nitrogen output is actually slightly greater on the 3 post days than on the last 3 work days. It would appear that the catabolic phase has not reached its maximum until this latter period this however is not altogether surprising when it is remembered

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that the maximum nitrogen output in the previous experiment was on the 11th work day. The fact however that the sulphur output has begun to fall by the last 3 work days would indicate that the anabolic phase has commenced.

The source of the excess material may now be surmised from the results of these two experiments. In Experiment 18 with an average basal urinary S:N ratio of 1:14.2 the ratio of the excess material was 1:16.81. In Experiment 19 with an average basal S:N ratio of 1:17.5 the ratio of the excess was 1:18.26. The evidence would hence tend to indicate that the effect of work is to increase the metabolism of food protein or to put it more broadly to increase the turnover of the sum total of the food ingested. The S:N ratio of the excess appears in both these experiments to be slightly lower than that of the protein ingested: a possible explanation of this may be suggested when it is recalled that in the previous experiments on retention the tendency was to retain a material slightly poorer in sulphur than that ingested. In Experiments 1 and 3, Table I, with gelatin, it will be remembered that the nitrogen only was retained. In Experiment 7 where beef was superimposed the material retained was poorer in sulphur the higher the intake. If then work accelerates the metabolism of the protein in circulation it is to be expected that the S:N ratio of the material lost will be poorer in sulphur than that of the protein or proteins ingested. If this interpre-

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interpretation be correct, work may then be said to increase the rate of metabolism of circulating protein, or, according to Rubner, reserve protein, and consequently since there is a loss during a work experiment, to reduce the amount of material in transit. Further information on this point may however be obtained from the remaining experiments.

Experiment 20, Table VIII, was carried out on a beef diet not only on account of the value attributed to beef as a food pre-eminently suited for those doing physical work, but also as a necessary part of a complete diet. The diet consisted of the following:-

470 grms. bread.
250 grms. lean beef.
100 grms. butter.
200 grms. jam.
1 apple.

2,822 Calories.

The work in this experiment was done on a bicycle ergometer, 34,000 Kgmts. work being carried out in a 1-hour period. It was the intention in this experiment to see if increasing the amount of work would increase the nitrogen and sulphur outputs in the urine. The following are the average daily outputs in the three periods.

4 days' preperiod.	10.195 grms. T.N.	
	.7402 grm. T.S.	S:N 1:13.77
4 days' work.	10.566 grms. T.N.	
	.8062 grm. T.S.	S:N 1:13.10
2 days' post period.	10.510 grms. T.N.	
	.7676 grm. T.S.	S:N 1:13.82

It will be noted that the S:N ratio does not change much except for a slight rise in the work period. The maximum sulphur excretion is however in the work period, while the nitrogen output is practically the same in the work and post periods. The excess nitrogen and sulphur have been calculated in two day periods, the outputs in the preperiod being employed as basal figures. The following figures are obtained:-

1st two days' work.	.736 grm. excess N.	
	.1562 grm. " S.	S:N 1:4.7
2nd two days' work.	.750 grm. excess N.	
	.1079 grm. " S.	S:N 1:6.6
2 post days.	.630 grm. excess N.	
	.0544 grm. " S.	S:N 1:11.58
<u>Total excess</u>	2.116 grms. N.	
	.3184 grm. S.	S:N 1:6.6

The ratio of the total excess is remarkably high, but, as in the previous experiment, the major part of the sulphur has been excreted first. It would appear that in this experiment the anabolic phase has not commenced as in the previous experiments. This is possibly due to the increased amount of work carried out daily on the one hand or to there being only 4 work days. The strikingly high S:N ratios of the excess in this experiment in contrast to the previous ones merit some attention.

In Cathcart and Burnet's experiments the S:N ratio of the excess output was, in all but one, about 1:28.0. The exception in their case was with the beef diet where the ratio of the excess was 1:14.8, approximately twice as high as in their other experiments. Those workers also noted that the excess outputs of nitrogen and sulphur were greater in the post period than on the work days, while our figures show the excess nitrogen output on the two post days to be very little lower than on the last two work days. No explanation can be offered for this peculiarity in regard to beef except to point out that it is evidently no casual phenomenon as our figures for the ratio of the excess output on a beef diet as compared to the other diets show the same trend as Cathcart and Burnet's although the absolute ratios are higher.

Experiment 21, Table VIII, was carried out on diet containing eggs in order to have a relatively high sulphur intake.

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The following was the diet employed:-

470 grms. bread.
125 grms. butter.
4 eggs.
200 grms. jam.
1 apple.

2,803 Calories.

The same amount of work (34,000 Kgmts.) as in the previous experiment was done on the bicycle ergometer. The experiment was divided into the three periods, the basal outputs of nitrogen and sulphur being based on the average of the two predays. The following figures give the average daily outputs of each period:-

2 days' preperiod.	8.720 grms. N.	
	.7371 grm. S.	S:N 1:11.83
4 days' work period.	8.704 grms. N.	
	.7699 grm. S.	S:N 1:11.30.
3 days' post period.	8.358 grms. N.	
	.7417 grm. S.	S:N 1:11.26

It will be noted that as the diet was relatively sulphur rich the S:N ratio is throughout high: further, work has had no influence on the output of nitrogen except in the post period when a retention has taken place. The sulphur alone has risen in the work period only to fall almost to its basal value in the post period. The following figures show the balance:-

1st two days' work.	.004 grms. N. excess.
	.1262 grm. S. excess.
2nd two days' work.	.033 grm. N. retained.
	.0486 grm. S. excess.
3 post days.	1.086 grms. N. retained.
	.0138 grm. S. excess.
Total N. retention	1.115 grms.
Total S. excess	.1886 grm.

It will be noted that sulphur alone is lost while the nitrogen shows a positive balance. The excess sulphur it will be noted is mainly excreted on the first two work days while the nitrogen retention is almost entirely on the two post days. This would again tend to confirm the mobile rôle of sulphur in the catabolic phase. The problem however as to why there is no nitrogen loss is rather difficult to explain. It may be that the excess nitrogen is masked by a greater retention, indicating that the anabolic phase has actually overtaken the catabolic in the post period. If this is so, however, the question as to why there is no sulphur retention will immediately suggest itself. Several possibilities however can be offered in explanation of this peculiarity. In the first place it will be recalled that in Experiment 2, Table I, the capacity of the body to retain the nitrogen of

egg/

egg albumen was high - 70% - while the sulphur was rejected. If then in this work experiment there is an increased tendency to retention it is feasible that the nitrogen alone is retained. On the other hand it is possible that as the S:N ratio of the basal diet is high, the material in transit is relatively sulphur rich. In this connection it will be recalled that in Experiments 2, 4 and 6, although the sulphur of the egg albumen was rejected, it was metabolised very slowly, the maximum excretion being on the day following superimposition. Under those circumstances as the egg diet was being consumed daily, it is extremely likely that the material in transit was sulphur rich. If then there was a tendency for the body to consolidate some of this material into a substance resembling muscle tissue, sulphur would be eliminated and nitrogen retained. The previous experiments with the exception of Experiment 20 (beef diet) would tend to confirm this. In Experiment 18 with bread and cheese the S:N ratio of the basal urinary output was 1:14.2, and in all probability the S:N ratio of the material in transit was lower. If then there is a tendency to stimulate the catabolism of the material in transit (circulating protein) the S:N ratio of the excess would also be low, in this case 1:15.81; if also there were a tendency to a consolidation of this circulating material into muscle protein, one would expect the nitrogen to be rejected more than the sulphur. Experiment 19, where the/

the S:N ratio of the preperiod was 1:17.66, showed an excess output with a ratio of 1:18.26. In Experiment 21, on the other hand, the basal (preperiod) S:N ratio was 1:11.83, and correspondingly if there is a tendency to the consolidation of some of the material in transit a greater output of sulphur than nitrogen is to be expected. In short if muscle work tends to promote the catabolism and anabolism of protein, and if further in the anabolic phase the material built up was tissue protein with a S:N ratio of 1:14.0, it is to be expected that the excess excreted will have a ratio lower than 1:14.0 if the S:N ratio of the food is lower than this, and on the other hand if the S:N ratio of the food is higher than 1:14.0 the excess excreted will be correspondingly higher. The process may be likened to a discarding of certain moieties of the circulating material in order to approximate its composition to that of tissue protein. It is here possibly that Rubner's conception of an "Ubergangseiweiss" intermediate in stability between circulating and organised protein may find its justification. Muscle work may initiate a process of transformation of circulating protein into "Ubergangseiweiss" (transitional) protein with a discarding of the unnecessary elements. The question as to whether the endogenous turnover is increased appears to be unlikely in view of the following figures showing the S:N ratio of the excess nitrogen and sulphur output in relation to the S:N ratio of the urine in the corresponding experiment.

Exp.18.		
S:N ratio of urine	1:14.2	
S:N of excess	1:16.81	Bread and cheese diet.
Exp.19.		
S:N ratio of urine	1:17.66	
S:N of excess	1:18.26	Bread, cheese & gelatin diet.
Exp.20.		
S:N ratio of urine	1:13.77	
S:N of excess	1:6.6	Bread and beef diet.
Exp.21.		
S:N ratio of urine	1:11.83	
S:N of excess, sulphur excess. N retained.		Bread and egg diet.

These results with the exception of Experiment 20 would then tend to show that the increased metabolism of protein is concerned primarily with the food protein and no indication is given that the endogenous metabolism of body tissue is increased. Two questions remain yet to be solved however -- (1) Is the excess nitrogen and sulphur output in any way related to the level of protein intake? (2) Is the excess output related to the amount of the work done?

The average daily excess nitrogen and sulphur outputs for the first four days of each experiment along with the corresponding basal nitrogen and sulphur outputs are as follows:-

Exp.18.

Basal N. output	8.875 grms.		Av. excess N	.424 grm.	
" S. "	.625 grm.		" S	.029	"

Exp.19.

Basal N. output	10.557 grms.		Av. excess N	.507 grm.	
" S. "	.5974 grm.		" S	.0273	"

Exp.20.

Basal N. output	10.195 grms.		Av. excess N	.3715 grm.	
" S. "	.7402 grm.		" S	.0410	"

Exp.21.

Basal N. output	8.720 grms.		Av. excess N	--	
" S. "	.7371 grm.		" S	.0437 grm.	

Cathcart and Burnet in general found that the higher the protein intake the greater the excess nitrogen in the urine. As the intake in their diets varied from 8 grms to 17 grms. nitrogen daily it would seem that one of the factors concerned in the excess output is the level of the intake. In the experiment recorded here the nitrogen intake did not vary much, but it would appear that not only the amount of protein ingested but its quality may also play a part.

In Experiment 19, for instance, on a bread and gelatin diet the average daily excess nitrogen output is .507 grms. N. and .027 grms. S., while in Experiment 21 with bread and eggs there is no excess nitrogen, while the average excess sulphur amounts/

amounts to .0437 grm.: further, the daily amount of work done in Experiment 19 was 22,000 Kgmts. as opposed to 34,000 Kgmts. in Experiment 21. In Experiments 1 and 2 (Table I) recorded at the beginning of this paper, it will be recalled that the retentions of the nitrogen of gelatin and egg albumen were 50% and 70% respectively, while the sulphur of both proteins was completely rejected. The egg albumen appeared also to be much more stable or resistant to metabolism than the gelatin in that five days or so elapsed before the nitrogen and sulphur outputs approached the basal figures. It would appear likely then that not only the level of protein intake but also the quality of the protein determine to a certain extent the amount of increase of the nitrogen and sulphur outputs under the influence of work.

In order to find whether there is any co-relation between the amount of work done and the excess nitrogen output, the total excess nitrogen output for each experiment including the post periods has been divided by the total work done and the result expressed as increased nitrogen output per 1,000 Kgmts. work. The following figures were obtained:-

Exp.18.	32.1 mgs.N per 1,000 Kgmts.work	Bread & cheese diet.
Exp.19.	36.4 mgs.N " " "	Gelatin diet.
Exp.20.	14.0 mgs.N. " " "	Beef diet.
Exp.21.	-	Egg diet.

These figures do not appear to indicate that the absolute increase in nitrogen output is a constant charge per unit of work

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and quite independent of the nature of dietary protein ingested.

The foregoing experiments would then permit the following tentative conclusions to be drawn. An increase in nitrogen output is evidently not associated directly or necessarily with the energy requirements of the working muscle. One worker, Mitchell, has maintained that the slight increases observed are due to an insufficient calorie intake or perhaps even a temporary local starvation of the cells. This idea is a priori unlikely as the influence - on this assumption - should be observed early in the experiment while as has been noted in Experiment 18 the maximum nitrogen output was on the 11th day of work.

It may thus be concluded that the influence of work is to increase the rate of catabolism and the processes of resynthesis of protein in the cells. The question arises then as to whether it is an increase in the endogenous metabolism of the tissues or of the exogenous metabolism of the foodstuffs. If the endogenous metabolism were increased one would expect the excess nitrogen and sulphur and S:N ratio of this excess to be the same in each experiment regardless of the quantity and quality of the protein ingested. This however is contrary to the observations noted here and those obtained by Cathcart and Burnet, and Thomas. The former workers found a tendency for the increase in nitrogen output to rise in general although not always with the protein intake. Thomas's experiment on a nitrogen free diet showed an insignificant rise in the nitrogen

output/

output after doing approximately 105,000 Kgmts. work daily for three days. Other workers - Dunlop, Paton, Stockman and Macadam - noted a rise in the uric acid output in untrained subjects after work, while Cathcart and Burnet found insignificant increases in the creatinine which they attributed to the protein of the diet rather than to the work itself. Mitchell, working with rats on a nitrogen free diet, found no increase in the endogenous wear and tear output after work.

The only other possibility is then that the rise in nitrogen and sulphur output is due to an increased rate of metabolism of the food protein ingested. The figures for the excess nitrogen and sulphur and S:N ratios of the basal urinary outputs would appear to indicate that the rate of turnover of the circulating protein or material in transit is accelerated. Evidence has also been adduced which tends to show that probably the catabolic phase is first stimulated and is later caught up by an equal or greater increase in anabolism. It also seems likely that the quality of the protein ingested determines in some degree the rate at which its catabolism is accelerated and possibly also its capacity for taking part in the anabolic phase. The evidence obtained from the experiments with gelatin and egg albumen respectively favour the view that there is a consolidating of the circulating protein into a material which approaches in its S:N ratio to that of body tissue. In the former experiment more nitrogen relatively

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to sulphur was eliminated, while in the latter the opposite was the case.

Taken all over, it appears that the metabolic processes in the body should be considered as a whole. The body does not appear to metabolise protein, fat, or carbohydrate as entirely independent processes: rather it would seem that if the energy turnover is increased the turnover of nitrogenous matter tends also to increase and similarly when the turnover of nitrogenous matter by means of additional dietary protein is increased a rise in the energy output follows as is illustrated by the specific dynamic action of protein. That there should be an increase in nitrogen turnover with an increase in energy output such as work is not altogether surprising in view of Rubner's contention that the protein needs of all warm blooded animals tend in general to be proportional not to the body weight but to the surface area and hence to its energy requirements. What the significance of this may be is not certain, but it is possible that the increased rate of turnover is advantageous to the organism, particularly in relation to the hypertrophy and improved physical well-being that ultimately follows after prolonged training.

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SECTION IV.

THE INFLUENCE OF WATER INGESTION ON PROTEIN METABOLISM.

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The problem of the rôle of water in metabolism has for long been a source of recurring interest. This is in large measure due to its practical importance in the treatment of disease at the various Spas. When however it is realised that some 70% of the body weight is water and that all the chemical reactions of the living organism take place in this medium, its supreme significance can scarcely be overemphasised. Henderson has already pointed out the ideal properties of water for several of the functions which it subserves in the animal economy such as those concerned in heat regulation, to mention one instance. Further, according to Weiland, water in virtue of its dissociation is absolutely essential for one of the fundamental phenomena of life, namely oxidation. The necessity of water is again illustrated in the chemical changes associated with synthesis and hydrolysis, to which all the foodstuffs are subjected in varying degree in the living organism. It is then to be anticipated that the metabolic process will not be uninfluenced by factors tending to alter the water content or balance of the organism. With justification water has been classed as a food in that without water life is impossible for more than a few days. Further, the fact that it is ingested and excreted in large measure by the same channels as those concerned in the digestion of food and the elimination of their waste products renders possible the following of the

water/

water balance. It is however surprising that in spite of its forming a large percentage of the body weight, the capacity of the organism to withstand water deprivation is limited to only a 3 or 4 days. On the other hand however it is to be remembered that the daily turnover or mobilisation of water in the body is considerably greater than that eliminated or ingested. Bidder and Schmidt were the first to appreciate this large internal circulation of water in the body, particularly in relation to the secretion of the various digestive juices. It is probably no exaggeration to say that during the 24 hours a volume of fluid equivalent to at least the blood volume is secreted into the alimentary canal. Further, it has been shown by Haldane and Priestley, and Priestley that the ingestion of large quantities of water causes only a very minute change in the composition of the blood although the kidney may be simultaneously secreting urine at a considerably increased rate. If the water content of the tissues can be kept as constant as that of the blood it might be questioned if water ingestion in large quantities could have any influence on the chemical or physical processes taking place in the cells. It is known however that muscle tissue, for instance, can increase their water content after work. In view of the constancy of the composition of the blood it is to be expected that some tissue or tissues must have a capacity/

capacity to act as temporary water reservoirs. Engels in a carefully planned experiment found that the percentage water content of muscle and skin could be increased by 3.8% and that of the liver by 2.3% without endangering the life of the animal. As the muscular system forms some 40% of the body weight, it was found that of 100 volumes of retained water 67 vols. were held in the muscles, 17 vols. by the skin, and the remainder distributed over the rest of the tissues.

These observations of Bidder and Schmidt, Haldane and Priestley, and Engels would then tend to show that the water of the body is undergoing a continual mobilisation, and further that its distribution to the tissues is very definitely controlled. Water, in spite of its chemical stability, evidently plays no passive rôle in the organism. The ingestion of water is however not the only way in which the water content of the body may be increased. The continual oxidation of the foodstuffs in the cells supplies a steady source of metabolic water tending to dilute the cell fluids. In this respect the foodstuffs differ in the amount of metabolic water produced on oxidation; fat, for instance, yields 107 grms. while carbohydrates yield only 55 grms. water per 100 grms. dry weight. It should be noted however that on an isodynamic basis the amount of water formed from each is approximately the same. The retention of water is

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however not entirely conditioned by the amount of water ingested or formed by oxidation. The salt intake and the nature of the food ingested are both important factors controlling a water retention by the tissues. Baird and Haldane have shown that a water retention in man is favoured by the ingestion of salt: in this type of retention it was noted that the skin was playing an important part as oedema tended to develop in the lower limbs and under the eyes. It is probable that although the tissues may vary in their water content the osmotic pressure may be maintained more or less constant by a corresponding retention of sodium chloride or some other osmotically active molecules. As NaCl is the most important food salt as far as quantity is concerned, the problems of water and salt metabolism are intimately associated. The type of food ingested appears also to influence the water balance. Benedict and Carpenter found that a change on to a carbohydrate rich diet was associated with a retention, while a change back to a fat diet was associated with a considerable water loss. Fischer and Wishart also noted a marked but temporary reduction in the urine volume of a dog after giving glucose dissolved in water. The addition of glucose appeared to delay the diuresis until it was all absorbed from the bowel.

The influence of water on the metabolism of the body has in general been investigated by two different methods. The majority of workers - Bidder and Schmidt, Bischoff, Voit, Oppenheim/

Oppenheim, Heilner, Dubelir, Fowler and Hawk, Grosser, Abderhalden and Bloch, Orr, Howe and Hawk - observed the influence of increased or excessive water ingestion on the output of nitrogen in the urine. Spiegler and Straub on the other hand studied the effect of water deprivation induced directly by limiting the intake or causing a diuresis by salt ingestion. The main issues with all investigators were whether increased water ingestion caused a mere flushing out of the tissues or whether a stimulation of protein metabolism occurred. Hoesslin, Straub, Bidder and Schmidt observed an increased urea output after excess water ingestion and concluded that it was the result of an increased transudation in the tissues. Bischoff noted that the greater the diuresis after water ingestion the more urea was excreted, but no comment was made on the cause of the increase. Forster obtained a 90% increase in the urea output in a dog after giving 3 litres of water by stomach tube: the large increase was associated with an increase in urine volume from 171 c.cs. to 2,010 c.cs. per diem. Voit determined the influence of water ingestion on a fasting dog and noted an increase in urea output if the urine volume were increased. He stated that an increase in urea output took place only if a diuresis were provoked, but did not consider that it was a washing out effect. Voit pointed out that a retention of urea does not readily occur and further

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that as the chlorides are present in higher concentration in the blood than urea, the increase in chlorides should be more marked than that of urea if it were a mere flushing out of the blood. The increase in chlorides in Forster's experiment was less proportionately than that of urea and Voit on this basis maintained that water ingestion caused an increase in the breakdown of protein provided there was a rise in urine volume. Oppenheim, experimenting on the human subject, noted a considerable increase in the urea output and concluded that it was due partially to a washing out and partially to a stimulation of urea formation from its precursors associated with an acceleration of the processes of digestion and absorption. Heilner observed an increased nitrogen and chloride output after ingestion in fasting dogs; the increase in chloride was continued however into the next day when the urine volume was back to normal. The conclusions drawn were that the increase in nitrogen and chlorides was due to an increase in the breakdown of protein, but that the salt was more firmly retained than the nitrogen and hence its excretion was delayed into the next day. Heilner also noted that the respiratory exchange was increased, indicating an increased oxidation of protein, fat and carbohydrates. He hence compared the influence of water to the specific dynamic action of protein. Howe and Hawk gave water to a fasting dog and noted an increased nitrogen output/

output and the appearance of creatine in the urine. The output of creatine - on the basis of the creatine content of muscle - was found to be more than sufficient to account for all the extra nitrogen in the urine. It appeared then as if water had stimulated the catabolism of muscle tissue, the creatine being excreted and a portion of the other nitrogenous bodies retained or possibly transported to other parts of the body. Howe and Hawk carried out a similar experiment on a dog which had fasted 60 days. The daily water intake was increased from 700 c.cs. to 2,100 c.cs. for 4 days and then reduced again. The total nitrogen in the urine was increased 77% on the first water day but by the 4th day it was at the basal level again. An increase in the allantoin nitrogen was also noted but this was more than compensated by a decrease in the purine nitrogen. The authors concluded that increased water ingestion causes a stimulation of protein catabolism and an increase in the oxidation processes as evidenced by the allantoin increase and purine decrease in the urine: they also stated that there may be a washing out of the tissues as well. Hawk and Fowler carried out a most comprehensive study on the influence of water ingestion on man not only on the nitrogen output in the urine but also on the total nitrogen and bacterial nitrogen of the faeces. A constant nitrogen intake was given until equilibrium was attained, and then followed a water period of 5 days, on each of which 3 litres of extra water were ingested, 1 litre at each meal. The nitrogen

output/

output was increased on the first day and then gradually fell to below the value on the preperiod: the fall was observed to extend into the post period when the water intake was reduced. The decrease in nitrogen output on the later days more than compensated for the increase in the early days of the experiment and hence the nitrogen balance over the whole experiment was positive. Creatine appeared in the urine on the water days but the increase in total nitrogen output was insufficient to account for the creatine if muscle tissue were supposed to be broken down completely with elimination of the creatine and its associated nitrogen.

The total nitrogen and bacterial nitrogen of the faeces was found to be decreased throughout the water period. They concluded that water ingestion improved the digestion, absorption and utilisation of protein and further that its catabolism was stimulated. Abderhalden and Bloch investigated the influence of 5 litres of extra water for one day on the urinary output on an alkaptonuric subject. They found that while the total nitrogen output rose, the homogentisic acid output remained constant and concluded that water caused only a washing out of the tissues. Grosser investigated the influence of water on the nitrogen output of an infant and found it to be decreased: he maintained that water ingestion promoted the retention of nitrogen. This investigator, working

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on an alkaptonuric, noted that water ingestion increased the output of both water and homogentisic acid; the quotient $\frac{\text{nitrogen output}}{\text{homogentisic acid}}$ remained constant however: on the day following the water ingestion the outputs of both those substances were reduced. The final conclusion in the face of those results was that water stimulated both the catabolism and anabolism of protein. Burns and Orr reinvestigated the influence of water on the output of creatine and found that if the technique of creatine estimation was carefully controlled no evidence of a creatine excretion was observed even after the ingestion of 9 litres of water. Orr reinvestigated the influence of water on the nitrogen output on the human subject, with diets whose nitrogen content varied from 6 grms. to 40 grms. per diem. Three litres of extra water were ingested for one day in all the experiments except the one with the high nitrogen intake where 9 litres of extra water were ingested. It was noted that the greatest absolute and relative increase in nitrogen output after water ingestion was on the low nitrogen intake. On the highest protein intake even after 9 litres of extra water no increase in urinary nitrogen was observed. On the day following the extra water ingestion the nitrogen output tended to be above the basal level except in the high protein intake where it fell below the average output for a few days. There was an increased percentage of the

nitrogen excreted as urea and ammonia which the author attributes to a more complete catabolism of the protein molecule, but as there was an absolute increase in the urea output on the high protein intake while the total nitrogen output was not increased, the author doubts if water necessarily caused an increased catabolism of protein. The ammonia output was increased in all experiments in proportion to the total nitrogen output in the urine. This is held to be due to the primary products of deamination being carried off and excreted before their synthesis into urea could take place. The author further points out that the increase in urea excretion often lasts for a few days after the diuresis has passed off and tends to negative the conception that the water causes a flushing out of the tissues. His final conclusions are that water consumption influences both the catabolic and the anabolic phases of protein metabolism. Von Noorden on the other hand in his review states that water ingestion causes a mere flushing out of the tissues. This inference was drawn from the observation that the increase in urea output ran <u>pari passu</u> with the diuresis, a fact which Orr showed to be incorrect for urea and Hoesslin for NaCl excretion after water ingestion. Dubelir on the other hand could find no increased output of nitrogen whether a diuresis was produced by water drinking or salt ingestion. Straub found that the administration of NaCl to dogs on a constant diet caused a decrease in the nitrogen output followed by an increase

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when the salt was discontinued: he stated that salt caused a protein sparing action and probably an increased nitrogen catabolism as well. He explains the increase in nitrogen excretion after the salt has been discontinued to be due to a want of free water to excrete the waste products during the period of salt administration. His experiments on water fasted but fed dogs showed a rise in the nitrogen output which was more marked in the after period when water was again given: he concluded from this that water deprivation caused an increased catabolism of protein.

The present investigations were carried out in order to gain more information not only as to the rôle of water in protein metabolism, but also to gain more insight into the nature of protein metabolism as a whole. In this investigation not only was the distribution of nitrogen in the urine but also the sulphur output estimated. It was expected that the sulphur balance in view of the part it probably plays in metabolism would be of considerable significance.

In Experiment 22, Table IX, a basal diet consisting of the following was ingested:-

470 grms. bread.
50 grms. cheese.
125 grms. butter.
200 grms. jam.
1 apple.

The average daily water ingestion was about 1,000 c.cs.

and/

TABLE IX.

Date.	Diet.	Urine Vol. c.cs.	T.N. grms.	Uric acid mgs.	T.S. grms.	P_2O_5 grms.	S:N.
1924. 3 Nov.	Basal Diet	660	8.592	488	.615	2.399	1:13.9
4	"	640	8.540	512	.604	2.428	1:14.1
5	"	640	9.072	540	.654	2.442	1:13.8
6	"	630	8.932	546	.665	2.338	1:13.4
7 Exp.22	2 litres H_2O	1600	9.982	537	.670	2.712	1:14.7
8	Basal Diet.	750	8.708	540	.604	2.634	1:14.3
9	"	640	8.848	536	.601	2.385	1:14.5
10	"	550	8.736	545	.621	2.442	1:14.0
11	"	560	8.764	505	.626	2.343	1:13.9
12. Exp.23 Day.	2 litres H_2O	1196	5.339	231	.317	1.378	1:16.8
Night.	Basal Diet.	630	4.284	-	.383	1.278	1:11.4
Total.	-	1826	9.623	-	.700	2.656	1:13.7
13	"	900	8.036	461	.588	2.201	1:13.7
14	"	700	8.492	526	.560	2.560	1:14.4
15	"	700	8.456	532	.615	2.428	1:13.7

TABLE IX (Continued).

Date.	Diet.	Urine Vol. c.cs.	T.N. grms	Urea N. grms	NH_3N grms	Uric acid mgs.	T.S grms	P_2O_5 grms.	S:N	Urea N% T.N.
23 Oct.	Basal Diet	900	9.800	8.287	.494	547	.593	2.428	1:16.0	84
24	"	1000	9.772	7.985	.414	500	.637	2.130	1:15.3	81
25	"	800	9.800	7.940	.571	612	.665	2.272	1:14.7	80
26 Exp.24	2800 c.cs H_2O	2600	10.080	8.636	.604	615	.676	2.424	1:14.9	85
27	Basal Diet	800	9.204	7.834	.398	370	.632	2.130	1:14.5	85
28 Exp.25	2800 c.cs H_2O	3200	10.136	8.758	.538	526	.615	2.840	1:16.9	86
29	Basal Diet.	900	8.988	7.431	.437	472	.626	2.456	1:14.3	82
30	"	800	9.268	7.558	.364	487	.593	2.399	1:15.7	81
2 Nov.	Basal.Diet	700	7.868	6.204	.375	475	.588	2.499	1:13.3	78
3	"	700	7.672	6.333	.302	370	.604	2.485	1:12.6	82
4	"	700	7.948	6.661	.325	421	.670	2.683	1:11.8	80
5 Exp.26	2800 c.cs H_2O	2940	8.148	7.022	.538	486	.643	2.683	1:12.6	86
6	Basal Diet	1100	7.098	5.630	.370	487	.610	2.619	1:11.6	79
7	"	980	7.364	6.338	.330	482	.626	2.627	1:11.6	86
8	"	620	7.600	6.120	.347	555	.648	2.556	1:11.7	80
9 Exp.27	2800 c.cs H_2O	2300	8.064	7.168	.588	516	.626	2.641	1:12.8	88
10	Basal Diet	700	6.748	5.432	.319	487	.626	2.201	1:10.7	80
11	"	660	7.128	5.678	.342	482	.621	2.485	1:11.4	79

and on the 7th and 12th November 2 litres of extra water were ingested. The average nitrogen and sulphur and S:N ratios of the 4 predays were calculated as a basis on which to compare the two water drinking days: the basal figures obtained were 8.884 grms. N, .623 grm. S, S:N = 1:14.2. On the 7th November as a result of the water ingestion the urine volume rose from 630 c.cs. to 1,600 c.cs.; the total nitrogen increased 1.198 grms. and the sulphur .037 grm. above the basal values, while the S:N ratio fell to 1:14.7. On the following three days the nitrogen and sulphur outputs fell below the basal values,which would appear to indicate a retention of those materials. The following figures show the balance:-

7th Nov.	1.198 grms.	N. above basal.	
	.037 "	S. above basal.	S:N excess = 1:32.1
8th Nov.	.176 grms.	N. below basal.	
	.019 "	S. below basal.	S:N retention 1:9.2
9th Nov.	.036 grms.	N. below basal.	
	.013 "	S. below basal.	S:N retention 1:2.8
10th Nov.	.148 grms.	N. below basal.	
	.002 "	S. below basal.	S:N retention 1:74.0

Excess	1.198 grms N.	.037 grm. S.
Total retained	.360 grm. N.	.034 " S.
N. loss.	.838 grm. N.	.003 grm. S.

It will be observed that the over-all loss is nitrogen only: the daily figures are however of interest. On the day of water ingestion the S:N ratio of the material lost is 1:32.1, and on the following day there is a retention of a sulphur rich material of S:N 1:9.2; on the three days following the 7th November the sulphur loss on that day is almost entirely made up: the tissues are hence relatively richer in sulphur. It should also be noted that although the sulphur output on the 7th November is in excess of the average of the preperiod it is actually only .005 grm. above that of the preday: the P_2O_5 output on the other hand is increased on the 7th and 8th November and then falls to its pre-level. On the 2nd day of water ingestion, 12th November, (Experiment 23) the urine output was followed in two 12-hour periods: the total 24 hours' urine volume rose to 1,826 c.cs., of which the major two-thirds was excreted during the day. It will be observed that the nitrogen output is higher in the day period than in the night. This, quite apart from the water ingestion and diuresis, might be expected as the diet is ingested during the day: the peculiarity is however that the sulphur output was actually less during the day than at night. The following figures show the positive and negative balances for the water day and three post days; the same average basal figures were employed as in the previous experiment.

12th Nov.	1.739 grms.	N.	above	basal.	
	.077 "	S.	"	"	S:N = 1:22.5
13th Nov.	.848 grm.	N.	below	basal.	
	.035 "	S.	"	"	S:N = 1:24.2
14th Nov.	.392 "	N.	below	basal.	
	.063 "	S.	"	"	S:N = 1:6.2
15th Nov.	.428 "	N.	below	basal.	
	.008 "	S.	"	"	S:N = 1:53.5

Excess N	= 1.741 grms.	Excess S	= .077 grm.
Retention	= 1.668 "	Retention	= .106 "
N. loss	.073 "	S.retained	.029 "

In this experiment the nitrogen loss has been compensated by the retention on the next three days, while the sulphur balance is actually positive: the outcome is in principle the same as in the previous experiment, namely the tissues are richer in sulphur. The figures for the urinary nitrogen and sulphur on the day of water ingestion in each of these experiments might at first suggest that it is simply a washing out of nitrogenous waste products. It is quite probable that this may be one of the factors involved, but the figures for the post days show that some other process has come into play; it will be noted that in both experiments there is a tendency to a retention of both nitrogen and sulphur which in the latter case has exceeded the loss on the day of water/

water ingestion. In Experiment 22 the tissues have become relatively sulphur rich by a loss of nitrogen, while in Experiment 23, although there is practically no nitrogen loss, there is an actual retention of sulphur. Provisionally it would appear that the first effect of water ingestion is to cause an increased catabolism of protein followed by a stimulation of the processes of retention, sulphur again playing the mobile part in being preferentially retained.

In order to gain further information two sets of experiments were carried out with diets of different sulphur contents, the average S:N ratio in the urine in one case being 1:15.5 and in the other 1:12.6. An estimation of the urea and ammonia excretion was also made. In Experiments 24 and 25, Table IX, a diet of bread, butter and gelatin was ingested until equilibrium was attained and then 2,800 c.cs. of extra water were ingested in each experiment. The same basal figures were employed in both experiments, namely the average of the 23rd, 24th and 25th October - 9.791 grms. N; .631 grms. S; S:N 1:15.5. The following figures give the daily balance (Experiment 24).

26th Oct.	.289 grms. N above basal.	.045 grms. S above basal.
27th Oct.	.587 " " below basal.	.001 " " above basal.

Excess N	= .289 grms.	Excess S	= .045 + .001
Retained N	= .587 "		= .046 grms.
Total Retention	= .298 "	Sulphur loss	= .046 "

It is to be noted that the rise in urine volume is much greater than in the previous experiments, namely from 800 c.cs. to 2,600 c.cs. due to the greater water intake. The average daily nitrogen output in the urine was also greater in Experiment 24, yet the increase in nitrogen output under the influence of water on the 26th October was very much less.

On the following day however, the 27th October, the retention of nitrogen more than compensated for the loss on the day of water ingestion. The selective retention of sulphur however has not been noted. Possibly this may be due to a deficiency of gelatin in this respect. It will be remembered in Experiment 1, Table I, where gelatin was superimposed for a day, a retention of nitrogen only was observed.

On the 28th October, 2,800 c.cs. of extra water were again superimposed (Experiment 25) and the following balance obtained.

28th Oct.	.345 grm. N. above basal.	.017 grm. S. below basal.
29th Oct.	.803 " " below basal.	.006 " " "
30th Oct.	.423 " " " "	.038 " " "
Excess N	= .345 grm.	Excess S = nil.
Retained N	= 1.226 grms.	Retention = .061 g
Total retention	= .881 "	Total retention .061 "

S:N retained material = 1:14.4

In this experiment there has been an over all gain of a material whose composition approaches that of muscle. The effect of the extra water ingestion on the 28th October has been not only to stimulate the catabolism of protein but also to initiate the anabolic phase as is to be observed by the sulphur retention followed by a nitrogen retention on the next day, 29th October. It should be noted that it is quite possible that on the day of water ingestion the sulphur output is initially increased but followed within the 24 hours by a more than equivalent retention.

This would be in keeping with the more mobile rôle which sulphur appears to have in metabolism: it further illustrates how the 24 hour experiment may obscure certain details of what is taking place.

In the 2nd set of experiments (Experiments 26 and 27, Table IX) the diet was sulphur rich, consisting of bread, butter and eggs. The basal figures for both experiments were the average of the 2nd, 3rd and 4th November - 7.829 grms. N; .620 grms. S; S:N ratio 1:12.6.

On the 5th November, Experiment 26, 2,800 c.cs. of extra water were ingested and it will be noted that the urine volume rose fourfold and was above the average volume even on the following day. The following figures show the balance obtained.

5th Nov.	.319 grms.	N above basal.	.023 grm.	S above basal.
6th Nov.	.731 "	" below basal.	.010 "	" below basal.
7th Nov.	.465 "	" " "	.006 "	" " "
8th Nov.	.229 "	" " "	.028 "	" " "

Excess N	= .319 grm.	Excess S	= .023 grm.
Retention	= 1.425 "	Retention	= .042 "
Total retained	1.106 "	Total retained	= .019 "

S:N retained material = 1:58.2

Again the influence of water has been to cause an over all retention although the material retained is sulphur poor. The mobile rôle of sulphur in regard to its preferential retention is not observed here, but it is to be noted that as the diet and probably also the material in circulating was sulphur rich it is possible that the retention process might tend to even up the circulating protein to a composition approaching that of muscle. If this assumption be correct, it is to be expected that the retention of nitrogen would be relatively greater than that of sulphur. (Experiment 27).
On the 9th November, 2,800 c.cs. of extra water were again superimposed and the balance was as follows:-

9th Nov.	.235 grm.	N. above basal.	.006 grm.	S. above basal.
10th Nov.	1.081 "	" below basal.	.006 "	" " "
11th Nov.	.701 "	" " "	.001 "	" " "

Excess N = .235 grm.

Retained N = 1.782 grms.

Total Retention 1.547 " .013 grm. S. lost.

Again it will be noted that in spite of the urine volume being considerably increased the excess nitrogen output on the 9th November was extremely little, while over 1 grm. of nitrogen was retained on the next day. These experiments would appear to indicate that the influence of water is more than a mere washing out process. It will be observed that in Experiments 24 and 25 with gelatin the loss in nitrogen tends to be about the same as in Experiments 26 and 27 with the egg diets, while the retention on the post days in the gelatin experiments is less than that with the egg diet. It seems as if the quality of the protein in the diet influences the anabolic effect of water ingestion. In Experiments 1 and 2, Table I, it will be recalled that the retention of egg albumen N. was 70% and that of gelatin 50%. It is hence not surprising that the retention in the post period should be greater with the egg diet. A somewhat similar phenomenon was noted in the work experiments where there was no loss of nitrogen on the egg diet in contrast to a loss with gelatin.

The results of all those experiments do not appear very uniform, but several features stand out. In the first place/

place there is always a compensatory retention of nitrogen on the post days which in some experiments falls short of the loss, while in others it exceeds it. In general where the nitrogen retention falls short of the loss there is either no loss in sulphur or a slight gain. On the other hand where the nitrogen retention in the post days exceeds the loss on the day of water superimposition, there is a slight tendency to a retention in the gelatin,(Experiments 24 and 25), and a tendency to a sulphur loss in the experiments on an egg diet.

It would appear that the first effect of water is to cause an increased catabolism of protein and possibly a slight washing out of nitrogenous end products. The compensatory retention on the following days is, it appears, a response of the organism to the increased catabolism, in the form of a stimulation of the anabolic phase.

It is further to be noted that the higher the average nitrogen output the less the excess on the day of water superimposition: the following figures show the excess outputs. (Table IX).

Exp.22 Basal N output = 8.884 grms.
Excess N 7th Nov. = 1.198 grms. 2,000 c.cs extra water
Total retention in post days = .360 grm.

Exp.24. Basal N. output = 9.791 grms.
Excess N 26th Oct. = .289 grm. 2,800 c.cs.extra water
Total retention in post days = .587 grm.

It will be seen that not only is the excess output greater the lower the average basal nitrogen output, but also that the retention is less on the lower output. It would thus appear that the higher the protein intake the more is the catabolic phase obscured by the anabolic: further the fact that the excess output in Experiment 22 is 1.198 grms. in contrast to only .289 grm. in Experiment 24 where more water was actually ingested indicates that the anabolic phase must certainly have been initiated on the day of water ingestion. The greater retention on the higher protein intake is probably due to there being a greater pabulum offered to the tissues for storage; although as has already been mentioned the retention after water ingestion depends to a certain extent on the quality of the protein in the basal diet.

The nitrogen distribution in the urine after water ingestion shows however that qualitatively the metabolic processes are altered under the influence of water ingestion. The following figures give the increase in total N, urea N, and ammonia N on the day of water ingestion. The increase in urea and ammonia is based on the average of the same predays as those employed for the basal nitrogen output.

	Increase in T.N.	Increase in urea N.	Increase in NH_3N.	Increase in urea + NH_3N.
26th Oct.	.289 grm.	.566 grm.	.108 grm.	.674 grm.
28th "	.345 "	.688 "	.042 "	.730 "
5th Nov.	.319 "	.624 "	.204 "	.828 "
9th "	.235 "	.769 "	.254 "	1.023 "

These figures clearly indicate that waste products which would have otherwise been excreted in some unknown form are under the influence of water excreted as urea and ammonia. The figures for the 9th November, Experiment 27, are the most striking: the increase in total nitrogen output was only .235 grm., while the increase in urea N. was .769 grm. and of urea + ammonia N. 1.023 grms. Apparently not only has water stimulated catabolism but catabolism is also more complete. This influence outlasts the day of water ingestion as can be seen in Table IX. It will be noted that the increased percentage of urea nitrogen lasts for a day or two following the water ingestion although it is partially obscured by the fact that the total nitrogen output on the first few post days is reduced; this, quite apart from water ingestion, tends to decrease the percentage of urea N. Hawk and Fowler noted this increase in ammonia and held that it was produced to neutralise the excess HCl produced in the stomach in response to the water ingestion. Orr however pointed out that the increase in NH_3 is related to the protein content of the diet and not to the amount/

amount of water ingested. Orr explained it as due to some of the products of deamination being carried away from the tissues by the increased rate of water diffusion and excreted before they were synthesised to urea. This explanation is however problematical in view of Benedict's discovery that the kidney is probably the only organ to form ammonia by the hydrolysis of urea. The increase in urea must certainly be due to an increased efficiency in the catabolism of nitrogenous metabolites and probably associated with an increase in the oxidation processes in the cells. Howe and Hawk noted in the dog an increase in allantoin at the expense of the other purines. The uric acid output in the experiments recorded here although irregular do not appear to decrease (if any) sufficiently to account for the increase in urea and ammonia nitrogen.

The increase in phosphates is observed usually on the day of water ingestion and may possibly account for the increase in ammonia if the increase is in the form of acid phosphate. Under these circumstances the kidney would form ammonia from urea according to Benedict in order to neutralise the acid excretion. This is rendered likely by the fact that in almost all the experiments the increase in phosphorus and ammonia is on the day of water ingestion only. Another possibility is however the washing out of intermediary acid products from the tissues. If this be true it is not likely that the acid products are lactic or oxybutyric acids as the ammonia increase

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is greater the higher the nitrogen intake.

From the foregoing it will be realised that the influence of water on metabolism is by no means as simple as might at first appear. It is possible however to distinguish between the direct effect on metabolism of water ingestion on the one hand and the total results on metabolism on the other. It is probable that water directly stimulates the catabolism of protein only, and that the anabolic phase which follows is a biological response not to the water ingestion itself but to the increase in catabolism. This would tend to show the self-regulating trend of the metabolic processes always striving to maintain an equilibrium between intake and output.

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SECTION V.

THE LABILITY OF THE SULPHUR FRACTION IN METABOLISM.

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The experimental work discussed up to the present has tended to show that sulphur plays a definite rôle in the metabolism of protein. The evidence would certainly appear to justify the conclusion that in catabolism the sulphur moiety is the first to be dealt with; it seems also that in the anabolic phase the sulphur takes the lead although the evidence on this point is not so striking. It must be remembered however that the nature of the metabolic processes renders the separate analysis of those two phases extremely difficult. In the living cell under normal conditions those two phases of metabolism tend to be in equilibrium and the mobile rôle of sulphur can not be observed. It is only when either anabolism or catabolism is predominant that the lability of the sulphur moiety can be noted. It should further be kept in mind that an increase in anabolism, i.e., a retention, tends to be followed sooner or later by an increase in catabolism which tends to obscure the phenomena taking place.

If however sulphur is the mobile unit it may be asked in what phase of its metabolism is its lability exhibited. As one is completely in the dark as to the various stages of anabolism, the investigation of this aspect can not be prosecuted. It is possible however to attempt to find out at what stage in its catabolism the sulphur fraction is more easily attacked. It should be noted that the sole evidence in favour of/

of the preferential catabolism of sulphur lies in its early excretion in the urine for which several factors might be responsible. If it is assumed that ingested protein is built up into some complex before being metabolised, there are three different phases in the catabolism and excretion of sulphur one of which or all three might be responsible for its early excretion in the urine. In the first place if some complex is built up from the ingested food protein, the process of catabolism may split off some amino acids before others. This question has not been investigated in vivo so far, but Abderhalden has shown that in a tryptic digest of protein tyrosine is split off more quickly than glutamic acid, while cystin is said to appear early in the digest. If this holds good in vivo, then the early excretion of sulphur is possibly due simply to the cystin being available for catabolism before most of the other amino acids. In the second place however it is possible that cystin is much more readily catabolised than the other amino acids and hence the sulphur excretion in the urine would tend to precede the nitrogen. There is yet a third possibility, namely that all the amino acids are equally labile, but that sulphates are more quickly eliminated by the kidney than urea or ammonia. It is to be noted that the two latter possibilities might explain the early excretion of sulphur in catabolism, but they would give no indication of the mobile rôle of sulphur in anabolism. The first possibility

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on the other hand might be invoked to explain not only the preferential catabolism of sulphur but also its leading rôle in the anabolic phase, if it could be proved that in protein synthesis the sulphur is the first to be built up into the protein molecule.

Experiments were therefore planned to find out if either of the two latter hypotheses was true in regard to the catabolism of the sulphur fraction. The question of the preferential excretion by the kidney of sulphate over urea was first investigated. The experiments on this aspect of the problem were carried out on a female dog weighing 14 Kgs. The principle of the method was to feed ammonium sulphate and note the relative rate of excretion of sulphur and nitrogen. It is known that ammonium sulphate is changed in the body and excreted as the alkali salt of the sulphate, while the ammonia is combined with CO_2 to form urea although some of it may be excreted as such.

A perineotomy had been done on the animal in order to render catheterisation easy and it was kept in a metabolic cage during the course of the experiment. Two experiments in all were performed. In Experiment 28, Table X, the animal was fasted for three days and on the morning of the 4th day a solution of ammonium sulphate was given by mouth. Owing to the fact that a stomach tube could not be passed a record of the/

TABLE X.

Experiment 28.

	T.N. grms	T.S. grms	S:N	Excess N. grms	Excess S. grms.	S:N of excess	
Preperiod 6 hours.	.7530	.0349	1:21.50				Fasting
Period I, 6 hours.	1.484	.4135	1:3.58	.731	.378	1:1.93	$(NH_4)_2SO_4$ superimp.
Period II, 6 hours.	1.550	.351	1:4.41	.797	.316	1:2.52	Fasting
Period III, 12 hrs.	1.121)	.208)	1:5.38	-	.069		"
Average per 6 hours.	.560)	.104)					
Period IV, 6 hours.	.460	.0357	1:12.88				"

Experiment 29.

Preperiod 24 hrs.	2.748	.1264	1:21.7				Basal Diet
Experimental Period, 24 hours.	4.048	1.2644	1:3.2	1.300	1.138	1:1.19	7 g $(NH_4)_2SO_4$ superimp.
Post Period 24 hrs	2.987	.2164	1:10.3	.239	.0898	1:2.66	Basal Diet

TABLE X (Continued).

Date.	Diet.	T.N. grms	T.S. grms	Excess N above basal grms.	% of excess N excreted daily.	Excess S above basal grms.	% of excess S excreted daily.
14 Jan.	Basal Diet	7.882	.491				
15	"	7.627	.480				
16 Exp.30	250 g.beef superimp.	10.301	.739	2.303	75	.239	87
17	Basal Diet	8.960	.533	.962	25	.033	13
18	"	7.784	.464				
19	"	7.952	.454				
20	"	8.072	.524				
21 Exp.31	70 g.gelatin superimp.	11.961	.659	3.963	55.9	.159	65.9
22	Basal Diet	9.996	.533	1.998	28.2	.033	13.6
23	"	8.562	.516	.564	7.9	.016	6.4
24	"	8.554	.533	.556	7.8	.033	13.6
25	"	7.851	.511				
26	"	8.285	.533				
27 Exp.32	250 g.beef superimp.	11.544	.791	3.546	46.4	.291	63.9
28	Basal Diet	10.301	.607	2.303	30.1	.107	23.4
29	"	9.293	.527	1.295	16.9	.027	5.9
30	"	8.201	.527	.203	2.6	.027	5.9
31	"	8.282	.505	.284	3.7	.005	1.0

TABLE X (Continued).

Experiment 33.

Date.	Diet.	T.N. grms	T.S. grms	S.N.	Excess S grms.	% S excreted in excess daily
1928.						
1 Oct.	Basal.N.Free.	8.232	.4396	1:18.72		
2	"	5.241	.2912	1:17.99		
3	"	4.558	.2748	1:16.58		
4	"	4.172	.2582	1:16.15		
5	"	3.864	.2473	1:15.62		
6	Basal + 4 g.cystin	4.816	.8352	1:5.76	.5776	48.4
7	Basal.N.Free.	4.480	.555	1:8.07	.2968	24.9
8	"	4.064	.500	1:8.12	.2418	20.2
9	"	4.648	.3354	1:13.85	.0772	6.4

Experiment 34.

Date.	Diet.	T.N. grms	T.S. grms	S:N	Excess N. grms.	%excess N. excr. daily.	Excess S. grms.	% excess S excreted daily.
1928. 31 Oct.	Basal.N.Free	4.065	.348	1:11.65				
1 Nov.	"	3.654	.268	1:13.24				
2	Basal + 4 gr. cystin + 250 grms. beef.	6.258	1.044	1:5.99	2.604	51.5	.776	62.3
3	Basal.N.Free	5.348	.621	1:8.66	1.694	33.5	.353	28.3
4	"	4.410	.368	1:11.97	.756	14.9	.100	8.0
5	"	3.654	.283	1:12.11	-	-	.015	1.2

the exact amount of the salt was not obtained. This however does not invalidate the results as the object was to determine the S:N ratio of the excess material excreted over the preperiod. In order not to miss the early excretion of sulphur the urine was collected in two 6-hour periods and one 12-hour period over the 24 hours following the administration of the salt. In Experiment 29, Table X, in order to get the animal to take a larger quantity of the salt a standard diet was given daily and on the experimental day 7 grms. of the salt were added. The urine in this experiment was collected over the 24 hours. The excess nitrogen and sulphur and S:N ratio were calculated and compared with that of the ingested salt.

The standard diet fed was as follows:-

60 grms. fat.
100 grms. beef.
100 grms. tapioca.
10 grms. bone ash.

In Experiment 28, Table X, it will be noted that the ratio of the excess is 1:1.93 in the first 6 hours and 1:2.53 in the second 6 hour period. In the subsequent 12 hours the nitrogen output was below the basal while the sulphur was still being eliminated. There is no indication of the preferential excretion of sulphur as judged by the excess S:N ratio which does not even reach the theoretical for the salt ingested (1:0.87). In Experiment 29, Table X, in/

in a 24 hour period the same phenomenon was noted and it may be concluded that some factor other than the selective renal excretion of sulphur is responsible for the early elimination of sulphur in protein catabolism.

A series of experiments were therefore planned to test the second possibility, namely that cystin is more readily metabolised than the other amino-acids. If the food protein is absorbed and catabolised directly as a mixture of amino-acids, a comparison of the rate of elimination of sulphur after cystin ingestion with the rate of elimination of nitrogen after a protein has been superimposed should show whether cystin is catabolised more quickly than a mixture of sulphur free amino-acids.

The principle of the method was similar to the previous ones, and the experiments were carried out on myself as subject. A basal diet of bread and cheese was ingested until equilibrium was attained and the protein was then superimposed for one day. The proteins employed were beef 250 grms. (Experiment 30), gelatin 70 grms. (Experiment 31) and beef 250 grms. (Experiment 32), Table X. The excess nitrogen and sulphur outputs were calculated in the usual way, the basal values being obtained from the average of the two predays, 14th and 15th January, and the last two days of the period, 30th and 31st January. The percentage of the total excess excreted/

excreted daily has been calculated for each experiment separately. (The total excess excreted over the basal for each experiment has been taken as 100 and its distribution over the 2 to 5 days calculated).

It should be remembered that the assumption in these experiments is that ingested protein is absorbed and metabolised as a mixture of amino-acids. The main object of the experiments was simply to note the rate of excretion of the nitrogen alone: the sulphur outputs however have been given in addition as they may also be compared to the sulphur output after cystin ingestion. It will be noted in these three experiments (Experiments 30, 31 and 32, Table X) that the percentage of sulphur eliminated on the day of ingestion is higher than that of the nitrogen. This again confirms what has repeatedly been noted, namely the preferential catabolism of sulphur. It will also be observed that the excess nitrogen and sulphur metabolised in Experiment 30 with beef is relatively small, and consequently the percentage excreted on the day of superimposition is high. In Experiment 32 with beef the excess metabolised is greater and is spread over 5 days. In Experiment 31 with gelatin the excess excreted is spread out over 4 days: it will thus be seen that the percentage of both nitrogen and sulphur excreted in each day varied for the different experiments according to the number of days over which the catabolism of the ingested material/

material was spread. It remains now to compare those figures with the rate of elimination of sulphur after cystin ingestion. A nitrogen free diet was employed in Experiment 33, Table X, and on the 6th October 4 grms. cystin containing 1.09 grms. S and .49 grm. N. were superimposed. The excess sulphur was calculated by employing the sulphur output on the preday, 5th October, as a basal value. Over the 4 days 1.1934 grms. of excess sulphur were excreted and hence there was a slight loss. The percentage of the total excreted on the day of superimposition was 48% and dropped to 6.4% on the 4th day. If these figures are compared with the percentage nitrogen excreted on each day after protein ingestion it will be seen they are less than those in Experiments 30 and 31, and approximately the same as that in Experiment 32. In Experiment 34 4 grms. cystin and 250 grms. of beef were superimposed for one day on a similar nitrogen free diet. It will be noted that of the total excess sulphur excreted, namely 1.244 grms., 62% has been eliminated in the first day and 1.2% on the 4th day. It should be observed that the total excess sulphur is scarcely greater than in the previous experiment in spite of the fact that an extra .55 grm. of sulphur were ingested as beef along with the cystin. Apparently the rate of elimination of the excess sulphur has been accelerated by beef ingestion as the per cent of total excess sulphur in Experiment 34 is 62.3% as opposed to only 48.2% in Experiment 33 with cystin alone. It

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is more than probable however that this increased percentage of sulphur excreted on the day of superimposition in Experiment 34 is due to the sulphur of the beef being metabolised in advance. In Experiment 32, Table X, with beef alone the per cent sulphur excreted on the day of ingestion was 62.3%. The evidence from these experiments does not support the hypothesis that cystin is more readily catabolised than the other sulphur free amino-acids. This is perhaps not altogether surprising in the light of our knowledge of the catabolism of cystin. Lewis and Root and Hill and Lewis have shown that cystin is not metabolised unless its amine group is free: in other words deamination precedes oxidation of the sulphur. If the process of deamination was the same for all the amino-acids one would expect that all the amino-acids including cystin would be of equal lability or stability as the case may be. In view of the evidence which shows that the sulphur moiety is possibly the mobile unit both in anabolism and catabolism, it would appear probable that the ultimate solution of its lability will explain both aspects on a uniform basis. To the author it appears better to assume that the lability of the sulphur is a biological one. In the process of building up of protein or protoplasm and the consequent phase of breakdown, the sulphur fraction may play the leading rôle. The early excretion of sulphur in the urine may hence be due to the cystin or sulphur fraction being available/

available for final demolition before the other nitrogenous constituents of protoplasm. The preferential retention of sulphur would correspondingly be due to the sulphur forming perhaps not the nucleus but the keystone of the protein or protoplasmic molecule. The sulphur moiety on this analogy would hence both confer and condition the stability of the living biological unit. The lability of sulphur would therefore be due not to any inherent property of the cystin or sulphur containing moiety itself, but to the position assigned to it in relation to the other metabolites in the living cell.

SECTION VI.

CONCLUSION.

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The experiments recorded in this paper have all been directed towards investigating the problem from as many different aspects as possible. Further, the experimental conditions observed are to a large extent comparable to those under which the community live. Variations in the quantity and quality of the protein ingested, increase in the energy turnover such as work, and increase in the water consumption are all factors which play an important rôle in the life of man. The essential experiments moreover have all been carried out on the one human subject. This in itself is both an advantage and a disadvantage. It is a disadvantage in that one can not apply the results universally without due discrimination. The commonplace saying "One man's meat is another man's poison" is still very true. The habits, environment, and hereditary make-up of individuals differ considerably and it may be said with truth that an ideal diet does not exist. The term 'ideal' indeed has only relative significance as applied to food requirements. On the other hand the fact that the investigations have been carried out on one subject renders them comparable inter se in that they represent the unit biological response of an individual. It may then be asked what conclusions can be drawn as to the nature of the metabolic processes taking place in the organism, and what factors have to be borne in mind in prescribing the protein requirements of man.

The issues as they were expounded by Voit and Pflüger have already/

already been discussed in the introduction and it remains to sum up in the light of the evidence brought forward. The results of these investigations have shown definitely, as proved before by Voit, that the nitrogen output is intimately dependent on the intake. An increase in the intake is followed by a corresponding increase in the output and associated with a retention of what Voit called circulating protein which is characterised by its relative lability as compared to body or organ protein. Further, it has been shown in the experiments where beef was superimposed that when equilibrium was attained the material excreted was similar to that ingested: in short what goes in comes out. The question to be asked is then, "Is this food material in transit a pabulum for the cells or is it actually living substance for the time being?" To the author the latter appears the more realistic conception. If we assume the food to be a pabulum for the cells a distinction is made between living substance and its nutriment: the latter assumption does not distinguish between food on the one hand and the living cell on the other: both are for the time being one. Food may be looked upon as a pabulum before ingestion only, while after its assimilation into the body it loses its identity and merges with that of the tissues completely. This conception does not however imply that all the tissues are in an equal state of flux.

Voit was convinced that under normal conditions there

was no disintegration of cell structure. It is more probable that of the total nitrogen or protein content of the organism only a relatively small moiety is mobile in that it is the seat of the active flux of matter. This mobile protoplasm or Voit's circulating protein may be considered to be material manifesting its activity by a continual breakdown with elimination of heat and nitrogenous and other waste products. Its composition is probably variable, and is built up of protein, fat, carbohydrate and other metabolites -- living protoplasm in short. It appears a more satisfactory conception to view this material as being in equilibrium with the more stable body tissue, being built up from the food under normal conditions and in fasting kept up by a transformation of body protein.

The experiments discussed appear also to show how the body tends to preserve its integrity. It has been shown in the observations on work and water ingestion how relatively small is the variation in the nitrogen output. A small increase is noted followed by a retention, a catabolic is followed by a compensatory anabolic or resynthetic phase. It would appear as if the self-regulating mechanism is very finely adjusted to any influence tending to disturb it. The increase in nitrogen output after protein ingestion is no exception. An increase in the intake is followed by an increased output in order to keep the/

the integrity and "milieu interne" of the cells constant.

Evidence has further been adduced which appears to indicate that the course of anabolism and catabolism is probably an ordered one. The sulphur moiety of protein as far as the present investigations go would seem to be the mobile unit in the process.

The practical issue that arises from these experiments is by no means new. The time old problem as to how much protein should be ingested daily in order to maintain optimum nutrition has not yet been solved. The want of agreement on this point is largely due to a lack of more complete knowledge as to what protein effects in nutrition, particularly in the case of the adult, and also to the protean nature of protein itself. The advocates of the low protein intake such as Chittenden and Hindhede gain support for their contention in the fact that equilibrium can be maintained at a low level as well as at a high. The question to be faced however is "Is the level at which equilibrium is maintained of no significance to the organism?" It would appear to the author that it is of distinct advantage to have at least a moderate flux of nitrogenous material passing through the tissues. If the material built up from the food is protoplasm, in all probability this latter substance in comparison to the more stable structural elements is plastic and nascent: it is hence more readily diverted or rendered available for the fluctuating needs of

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the organism as they arise. The circulating protein and the more stable body tissue might be compared to capital in current account and capital as plant. The former is mobile and quickly available for current needs as they arise, while the latter is stable and indispensable, but not readily realised as liquid capital for adventitious demands.

And yet a final word: Is the issue between the views of Voit and Pflüger of real and practical importance? Does it arise from the nature of things in themselves, or is it the outcome of a difference in the temperament and intellectual outlook of man? Nature after all is observed, questioned and interpreted by and through the mind of Man. Kant's words to the philosopher may perhaps with justice be applied to the scientist -

"To know what questions we may reasonably propose is in itself a strong evidence of sagacity and intelligence."

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