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THE PHYSIOLOGY

OF

OTEIN METABOLISM

BY

E. P. CATHCART, M.D.

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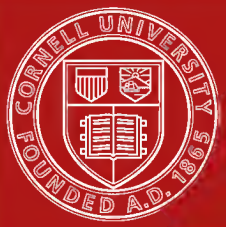

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EDITED BY

R. H. A. PLIMMER, D.Sc.

AND

F. G. HOPKINS, M.A., M.B., D.Sc., F.R.S.

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LONGMANS, GREEN AND CO.,
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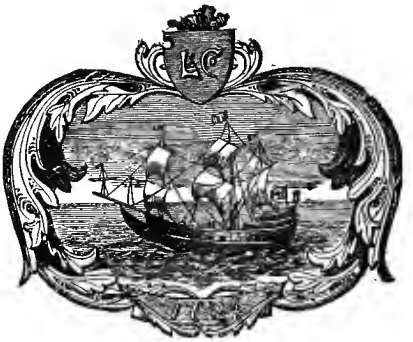
OF

PROTEIN METABOLISM

BY

E. P. CATHCART, M.D., D.Sc.

GRIEVE LECTURER ON CHEMICAL PHYSIOLOGY IN THE UNIVERSITY OF GLASGOW
RESEARCH ASSOCIATE OF THE CARNEGIE INSTITUTION, WASHINGTON



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1912

TO

D. N. P.

GENERAL PREFACE.

THE subject of Physiological Chemistry, or Biochemistry, is enlarging its borders to such an extent at the present time, that no single text-book upon the subject, without being cumbrous, can adequately deal with it as a whole, so as to give both a general and a detailed account of its present position. It is, moreover, difficult, in the case of the larger text-books, to keep abreast of so rapidly growing a science by means of new editions, and such volumes are therefore issued when much of their contents has become obsolete.

For this reason, an attempt is being made to place this branch of science in a more accessible position by issuing a series of monographs upon the various chapters of the subject, each independent of and yet dependent upon the others, so that from time to time, as new material and the demand therefor necessitate, a new edition of each monograph can be issued without re-issuing the whole series. In this way, both the expenses of publication and the expense to the purchaser will be diminished, and by a moderate outlay it will be possible to obtain a full account of any particular subject as nearly current as possible.

The editors of these monographs have kept two objects in view: firstly, that each author should be himself working at the subject with which he deals; and, secondly, that a *Bibliography*, as complete as possible, should be included, in order to avoid cross references, which are apt to be wrongly cited, and in order that each monograph may yield full and independent information of the work which has been done upon the subject.

It has been decided as a general scheme that the volumes

first issued shall deal with the pure chemistry of physiological products and with certain general aspects of the subject. Subsequent monographs will be devoted to such questions as the chemistry of special tissues and particular aspects of metabolism. So the series, if continued, will proceed from physiological chemistry to what may be now more properly termed chemical physiology. This will depend upon the success which the first series achieves, and upon the divisions of the subject which may be of interest at the time.

R. H. A. P.
F. G. H.

PREFACE.

More work has perhaps been done upon the digestion and assimilation of proteins than upon any of the other branches of metabolism. A monograph on Protein Metabolism would therefore be the first of a series, and it is to be hoped that volumes dealing with the other particular aspects of metabolism will soon be forthcoming which will furnish us with a complete survey of the present position of this portion of chemical physiology.

The present monograph does not pretend to cover the whole literature of protein metabolism; it consists rather of the discussion of the more important results published during the last decade and their bearing upon the work of the earlier investigators. The majority of recent writers have devoted their attention to the study of the metabolism of particular constituents of the protein molecule; an attempt has been made in this monograph to avoid laying undue stress on the fate of these since it is felt that a truer picture of the real course of protein metabolism can thus be drawn.

It is a pleasant duty to express my indebtedness to Miss G. D. Bostock, M.B., for her valuable assistance in the revision both of the manuscript and the proofs.

E. P. C.

CONTENTS.

CHAPTER VI.

CHAPTER VII.

CHAPTER VIII.

CHAPTER IX.

INTRODUCTION.

THE processes in the animal organism which are concerned with the destiny of the protein, whether of the food or of the tissues, are collectively known as protein metabolism. The metabolic changes are again arbitrarily divided into two phases, the anabolic and the catabolic, although at present there is but little evidence to show when one stage ceases and the other begins, or what products are the result of the action of the one and what the result of the other.

The problem of the metabolism of protein is one of the most complex and obscure in physiology, because the causes which bring about these changes are practically unknown. Carl Voit, even in 1902, after forty years of strenuous work in this field, could say no more than that "the unknown causes of metabolism are found in the cells of the organism. The mass of these cells and their power to decompose materials determine the metabolism."

We have at present a more or less detailed knowledge of the preliminary processes, which occur in the preparation of the food protein as a suitable pabulum for the tissues, during gastro-intestinal digestion. We know that the protein is practically completely disintegrated into very simple compounds before absorption and utilization take place. We have also a fairly complete knowledge of the products excreted in the urine—the waste products of protein metabolism—and we have been able to associate many of the variations in the metabolism, or, at least, alterations in the conditions affecting the organism, with variations in the output of these waste products. We know, for instance, that an animal fed on a food poor in protein excretes very much less nitrogen than one fed on a nitrogen-rich diet, and that there is a great diminution in the proportion of the nitrogen excreted as urea. We further know that creatine is never present, or only in minute amount, in normal urine, when the food taken contains no creatine, yet after a comparatively short period of fasting creatine is always to be found, and again we know that a high output of ammonia is closely associated with the production of acids in the tissues. Of

the various steps, however, of the intermediate metabolism which lead to the formation and the excretion of the different products we know but little.

Faced with the question as to the nature of the processes which lead to the building up of the material commonly known as "tissue protoplasm" we are at the very outset hampered and confined in our quest for accurate information by the imperfect knowledge which exists as to the very nature of the material formed. Can we with right assume that such a substance actually exists as a constant chemical entity, a substance immutable in form but variable in quantity? Does it wax and wane as does a crowd, the units constituting the whole inconstant in number but identical in nature, or is it a material unstable alike in form and amount? As Sir Michael Foster wrote in 1885: "He (the biologist) may speak of protoplasm as a complex substance but he must strive to realize that what he means by that is a complex whirl, an intricate dance, of which what he calls chemical composition, histological structure, and gross configuration are, so to speak, the figures; to him the renewal of protoplasm is but the continuance of the dance, its functions and actions the transferences of figures." Strive as we may, our insight into this intricate problem of the nature of living matter is but faulty. We see through a glass darkly.

Despite this scanty knowledge questions of such primary importance arise that their solution must be attempted. Such a question is that of the real demand for protein by the body. Does the organism require a large intake of protein or can it subsist on a relatively small one? We know by direct observation that under normal conditions the amount of nitrogen excreted is directly dependent on the amount of protein taken in the food, that is, if a definite amount of nitrogen in the form of protein be ingested a similar amount of nitrogen, in the form of waste material, from which practically all the energy has been extracted, will appear in the urine. What has happened to the nitrogenous material which left the lumen of the intestine in the form of "digest" products? Has it all been utilized for the necessary repair of tissue waste or has part of the material absorbed been stored and a corresponding amount of material present in the protoplasmic complex been excreted? Must this material taken up by the blood from the intestine be first converted into "living protoplasm" before it can be available for use in the tissues, or can disruption of the absorbed molecule occur without this synthesis? It is now more or less generally accepted, irrespective of the mode of such decomposition, that soon after absorption the protein products are split into a nitrogen-containing

and a nitrogen-free part. The use and value of the former portion is universally admitted, as nitrogen is an absolutely essential constituent of all living tissue, but does the non-nitrogenous rest play a special part in the metabolic processes which cannot be performed by non-nitrogenous material arising from the breakdown of either carbohydrates or fat? Does this non-nitrogenous part of the protein molecule simply serve as one of the sources of the energy supply, or does it, owing perhaps to a more highly specific nature, play an intimate part in the synthesis of fresh protein material?

CHAPTER I.

DIGESTION AND ABSORPTION OF PROTEIN.

General Methods of Investigation.

THE elucidation of the problems of protein metabolism has been attempted in various ways. The usual and most commonly employed method is that of studying the output of nitrogen in the urine, particularly the variations which occur in the partition of the different nitrogenous constituents. Since the careful and painstaking work of Folin (129) and the publication of his analyses of the composition of the normal urine upon different diets the conclusions have assumed an accuracy and weight which previously were lacking.

The variations in the output of nitrogen have been investigated under different conditions, thus (1) during complete starvation, (2) after definite alterations in the amount of one or more constituents present in the diet, as, for example, the production of nitrogen hunger on a diet which supplies the requisite number of calories, but which is lacking in protein or other substances containing nitrogen, (3) a diet which, although rich in nitrogen, lacks either carbohydrate or fat, (4) a diet in which the normal protein of the food is replaced by some nitrogen-containing substitute, i.e. by the addition of cleavage products of protein formed either by the action of a ferment, or an acid.

Observations have also been made when the protein has been introduced parenterally by intravenous, intraperitoneal or subcutaneous injection. The influence of various extraneous factors like work has also been investigated.

The problem has again been attacked in another fashion by means of perfusion of different organs and tissues with substances of known constitution or which give a well-defined reaction; thus, for example, repeated experiments have been carried out on the intestine and the liver in an attempt to discover the fate of the products of digestion after they leave the lumen of the intestine.

The subject has also been investigated by studying the fate of

particular substances when the organism is in a pathological condition. The products excreted in the urine in cases of alkaptonuria and cystinuria,[1] or in such a disease as acute yellow atrophy of the liver, have been examined, both after normal feeding and after the addition of different amino acids, etc., to the food. The course of metabolism has also been studied in pathological conditions artificially produced, as, for example, after phosphorus poisoning.

The discussion of the question of metabolism is opened with a short preliminary résumé of the present position as regards the course of gastro-intestinal digestion. This is inserted, as it is impossible to obtain a proper understanding of the subsequent fate of the protein without some knowledge of the degree of distintegration which it undergoes in the digestive tract.

Gastric Digestion.

Within the last decade a very considerable advance has been made in our knowledge of the course of digestion of protein. Actual digestion does not commence until the protein material has reached the stomach, where it is subjected to the action of gastric juice—pepsin and hydrochloric acid. A great deal of modern work has been done on the extent and degree of digestion which takes place here. It may be assumed that under normal conditions the digestion only proceeds as far as the peptone stage, but that all protein does not of necessity reach this stage of degradation. Lavroff (241), Langstein (240), and others, however, have clearly demonstrated that if the gastric digestion be allowed to go on long enough, *in vitro* at least, the breakdown of protein can be carried on to the formation of abiuret[2] products. This degree of digestion need not be considered here, for even under the most favourable conditions *in vitro* very many weeks are required.

It would seem, from many different observations, that proteoses form by far the largest part of the products of peptic digestion. The amount of proteose formed depends to some extent on the nature of the protein which has undergone digestion. The following table from an article by London shows this very clearly :—

[1] In the present monograph this side of the question will not be specifically considered. For further information Dr. Garrod's valuable book on "Inborn Errors of Metabolism" may be consulted.

[2] i.e. products of digestion which no longer give the biuret reaction.

Nature of Protein Fed.	Percentage of Proteose Formed.
Egg albumin .	72·5
Gliadin . .	67·7
Edestin . .	60·3
Caseinogen .	59·1
Gelatin . .	50·6
Serum albumin	46·1

London, "Handb. d. Biochemie," Oppenheimer III, p. 77.

According to Tobler (398), on the other hand, from 50 per cent. to 57 per cent. of the digested product reaches the intestine in the form of peptone, some 11 per cent. to 14 per cent. in the form of proteoses and from 30 per cent. to 34 per cent. in the form of soluble or insoluble protein. Zunz (430) concludes that three-fifths of the nitrogen of the products of protein digestion in the stomach enters the duodenum in a very simple form (mainly peptones) and about two-fifths in the form of proteoses. He is therefore inclined to agree with the work of Tobler. Zunz further shows that the condition in which the food is consumed plays a part in the degree of digestion reached. Thus when a dog is fed with cooked meat there is more proteose present in the stomach contents than when the same food (horse flesh or beef) is given raw. He also states that, in certain experiments which he carried out in dogs, where, previous to the digestion experiment, the ducts of the pancreas were ligatured, digestion in the stomach was more complete than when the pancreas was acting freely. He concludes that there may be at times an increased compensatory digestion in the stomach.

The degree of gastric digestion would not seem to be a matter of very great moment, however, as under normal conditions these changes in the stomach are only preparatory to the action of the pancreatic and intestinal juices. That this preparatory action is of importance, however, is shown by the *in vitro* experiments of Fischer and Abderhalden (126); they found that tryptic digestion took place much more rapidly and completely when the protein had been previously subjected to the action of pepsin and hydrochloric acid. They found that, if caseinogen were first digested with an artificial gastric juice before digestion with trypsin, they could isolate proline and phenylalanine, whereas if pancreatic digestion of caseinogen were carried out alone, these amino acids were not found in the free state but only in the polypeptide form. The polypeptide compound, however, was not completely decomposed by the double digestion. Acid hydrolysis, on the other hand, breaks up this polypeptide completely, both of the amino

acids being liberated. Fischer and Abderhalden (126) and Abderhalden, London and Voegtlin (43) showed later in a long series of experiments that a large number of the polypeptides were resistant to the action of the gastric juice, although they were rapidly broken down by the pancreatic juice (for further details see Dr. Plimmer's monograph in this series, Part II, pp. 43-46). Oppenheimer and Aron again (309) found that serum, which is normally resistant to the action of trypsin, could be digested by this enzyme if it had been previously subjected to the action of pepsin.

Gastric Absorption.

As regards the question as to whether absorption takes place in the stomach or not, there has been much discussion. London and his school deny that absorption ever takes place, whereas Tobler (398) states that after a protein meal he has observed the disappearance of from 22 to 30 per cent. of this material from the stomach. Salaskin (348) believes that Tobler is right in his contention that absorption of protein can and does take place in the stomach, but he does not bring forward any convincing evidence or experiment in support of his belief. If this absorption be as great as Tobler makes out, it is extraordinary that it has escaped the observation of so many of the competent workers in this field. Abderhalden, Prym and London (44) have for example shown that even if amino acids be given *per os* they leave the stomach practically completely through the pylorus, absorption first taking place in the duodenum.

The present general conclusion would seem to be that if any absorption of protein digestion products take place from the stomach, under normal conditions, it must be a small one.

Intestinal Digestion.

The main digestion of protein takes place in the small intestine, most actively at the upper end by means of the trypsin of the pancreatic juice and probably also by the erepsin of the intestinal wall. Trypsin acts on all forms of protein, which have been passed on from the stomach, and reduces them to simpler products. The question as to the extent of this splitting has been much discussed. Formerly it was believed that proteoses and peptone were the end products, but it is now generally held that the digestion proceeds to the formation of abiuret products in the form of polypeptides and the comparatively simple monoamino and diamino acids. (Abderhalden, Baumann, and London (42), Kutscher and Seemann (236), Cohnheim (94).) The difficulty has been to prove that the digestion proceeds to this extent, as (1) the disintegration of protein does not take place suddenly, in an explosive fashion, but proceeds gradually more like erosion, and (2) along with this slow decomposition there is a steady absorption of the simple products as they are formed. By the utilization of the polyfistular method, which has been elaborated by London (261, 262, 263), evidence of the thoroughness of the decomposition has been obtained. Abderhalden, London and Oppler (45), for example, traced the appearance of tyrosine and glutamic acid after feeding with gliadin. They found in the duodenum 0·75 grm. of tyrosine and 2·5 grm. of glutamic acid, in the jejunum 1·1 grm. of tyrosine and 20·9 grm. of glutamic acid, and in the ileum a mere trace of tyrosine and 33 grm. of glutamic acid. Very similar results were obtained, by the same method, after feeding with caseinogen and with meat. This work was repeated and confirmed later by Abderhalden, London and Reemlin (47). Not only then has it been proved that the digestion is gradual, but further, that the rate of digestion is greater than that which takes place *in vitro*. Abderhalden and Gigon (22) have demonstrated that the digestive ferments can combine with the amino acids formed in the course of digestion and in this way become inactivated. This inactivation is liable to occur during *in vitro* experiments, where the amino acids accumulate in the digestive fluid, but in the case of the intestine absorption is constantly taking place, and thus there is but little time for combination to occur with its resultant slowing of the rate of digestion. Abderhalden and Rona (13) have further shown that there is another difference between the actions of artificial juices, both gastric and pancreatic, and the real juices in *in vitro* experiments. They found that pepsin powder

can rapidly digest caseinogen giving rise to various amino acids and tryptophane, whereas the real gastric juice, when tested under like conditions, cannot do so. They also found that "pancreatin" will break down many polypeptides which are resistant to the action of natural pancreatic juice. This substantiates the contention of many writers that there is a considerable danger in drawing far-reaching conclusions as regards intravital action from mere test-tube experiments, important and useful though the latter be.

Intestinal Absorption.

It was not until the experiments of Salvioli, Hofmeister and others were carried out that a new explanation of the mode in which the proteins were absorbed from the intestine was sought. Salvioli (353) and Hofmeister (196) almost contemporaneously made the discovery that, if peptone were left in contact with the living intestinal wall, it disappeared or at any rate was so altered that it no longer gave the reactions by which it was characterized. Hofmeister concluded that the peptone on absorption was taken up by the leucocytes of the intestinal wall and by means of these was converted into protein and at once conveyed to the tissues (197). Heidenhain (179) repudiated this hypothesis, but both he and Shore (373) inclined to the view that the peptone was converted into protein, and that in this change the epithelial tissues of the intestine probably played an important part. In addition to this Hofmeister (195) and Neumeister (305) have shown that, if peptone were injected into the blood, it was wholly or in greater part excreted from the body as waste material, and further that no trace of peptone was ever found in the tissues, blood or lymph of animals even at the height of digestion.

It will be noticed that, although this expression of opinion as to the fate of peptone brought into contact with the living intestinal epithelium was practically unanimous, no definite direct proof of that fate was produced. Neumeister (305) again took up the question and again found that the peptone brought into contact with the intestinal mucous membrane lost its characteristic reaction, but whether this was due to the production of a higher product, a regeneration, or whether it was due to further decomposition he did not definitely conclude. He certainly found two decomposition products of protein, leucine and tyrosine—which suggested that further breakdown had taken place.

The presence of these amino acids, however, did not of necessity exclude the possibility of a subsequent synthesis—the condition might be analogous to the splitting of fats into fatty acids and glycerol which precedes the fat synthesis in the intestinal mucous membrane. Cohnheim (95) attempted to isolate the protein which he believed was synthetized in the intestinal wall. He, however, found that an increase of protein, i.e. a regeneration, never took place, but that the peptone was invariably broken down to simpler decomposition products; in other words that the characteristic peptone reaction disappeared not because protein had been synthetized but because crystalline decomposition products were formed. He further found and isolated the ferment which brought about this decomposition, and to it he gave the name erepsin. This erepsin, on further investigation, was found to be capable of breaking down the proteoses and peptones to their constituent amino acids—he was able to isolate leucine, tyrosine, lysine, histidine and arginine—but it could not attack native proteins, with the exception of caseinogen and fibrin. Kutscher and Seemann (236) on investigating the fate of protein in a dog with a fistula in the middle of its small intestine found that the protein—flesh—was reduced to amino acids of which leucine, tyrosine, lysine and arginine could be isolated, but neither proteose nor peptone was detected. This observation, that leucine and tyrosine could be isolated from the normal intestinal contents, was by no means new, as Kölliker and Müller as long ago as 1856 had discovered leucine and tyrosine in the intestinal contents, although of course they were unable to assign an explanation to their presence. They concluded that they were either absorbed or broken down further as they were unable to find them in the faeces.

Kühne (234) also detected leucine and tyrosine in the material collected from an isolated loop of intestine, and he rightly described them as being products arising from the breakdown of protein, but thought that they were rather by-products than normal digestion products on the way to absorption. Schmidt-Mulheim (357), who repeated Kühne's work, came, however, to the conclusion that although such a breakdown took place it was quite unimportant Sheridan Lea (371) came to similar conclusions as Kölliker and Müller. Macfadyen, Nencki and Sieber (272), who investigated the case of a woman with a fistula at the lower end of the small intestine, found that the intestinal contents contained soluble proteins and peptones, but no leucine or tyrosine.

Salkowski and Leube (352), on the other hand, put forward the suggestion that the leucine might be considered as a product which,

after absorption, could be used for rebuilding purposes, and which therefore might be regarded as a stage towards protein regeneration. They held that their view was strengthened by the fact that the increase of the excretion of nitrogen after the administration of leucine did not correspond with the amount of leucine fed, and further that an analogy existed in plant physiology where it was demonstrated that the decomposition products of protein, namely asparagine, leucine and tyrosine could be regenerated into protein when carbohydrate was also present. They suggested that a similar combination might occur in the liver of animals. Kutscher and Seemann (236) as the result of their experiments stated that they considered the above hypothesis very plausible, and concluded that the appearance of leucine and tyrosine, which they found, was the normal condition, and that these crystalline substances must be looked on as constituents, which, after absorption, would be utilized for the formation of tissue protein. They were unable to prove, however, that the hypothesis put forward by Salkowski and Leube was correct, as a series of experiments in which they performed a variety of Eck's fistula (cutting the liver out of the circulation), using a glass cannula for connexion between the portal vein and the vena cava, did not give decisive results. They further ligatured off the lower part of the vena cava, kidney vessels, carotids and subclavian, so that the blood circulated only through the intestine, heart and lungs. They were unable to detect amino acids in the blood even at the height of digestion. This question of the presence of digestion products in the blood will be dealt with more fully later, when the fate of the absorbed material is discussed (see p. 16). Bunge in his textbook argued on teleological grounds against the conversion of any considerable amount of protein into amino acids. He held that if any such conversion took place it must be small, as the dissipation of chemical energy firstly in the decomposition and secondly in the necessary building up processes would be considerable and quite contrary to nature. These teleological arguments of Bunge can now be shown to be false, as the loss of energy in the conversion of protein into digestion products is remarkably small, as Rubner's calorimetric estimation of Loewi's digestion products proved. Even in plants, in the process of utilization of the stored protein, the formation of crystalline decomposition products must take place before resynthesis is possible.

It would appear, then, that the material absorbed is taken up for the most part in the form of abiuret products, of which the greater part consists of simple amino acids.

Absorption of Undigested Protein from the Intestine.

The question as to whether the completely undigested protein can be absorbed must also be considered. Magnus-Levy has suggested that the body may absorb from the intestine a sufficient amount of unaltered protein for purposes of repair of tissue, but that the greater part of the protein is broken down to simple nuclei, which are simply burnt up without playing any part in the tissue metabolism. That the body can absorb protein in the natural form has long been known. Voit and Bauer (408) showed that the absorption of undigested proteins such as serum and uncoagulated egg albumin could take place, and their results have been extended and amplified by Heidenhain (180), Friedlander (149), Waymouth Reid (416), and others. It is not maintained, however, that this is the way in which most of the absorption takes place. Ascoli and Vigano (55), using the biological precipitin test, have stated that they were able to demonstrate that part of the protein was taken up unchanged. Abderhalden, Funk and London (41), under much better conditions than Ascoli, and also using the biological method, were quite unable to obtain any reaction. In all these experiments the protein was introduced into the intestine in excessive amount. This absorption, such as it is, would appear to be dependent to some extent on an increased permeability of the intestinal wall, such as is found in the young. It is also dependent to a certain extent on the presence of water or salt solution. Friedländer (149), for instance, has shown that, if all the water or salt solution be absorbed, the absorption of the proteins to all intents and purposes comes to a standstill. Naturally doubts have been thrown on this form of absorption. It has been suggested that the intestinal digestion had not really been suspended, although the intestine previous to the introduction of the protein solution had been thoroughly washed out, i.e. that a certain amount of pancreatic juice had been left which brought about a solution of the protein, and thus a natural absorption. This objection is not valid, however, as the protein was rapidly absorbed, and further the amount of enzyme which could have been present must have been small and its activity on native protein slight. For example, the figures quoted both by Heidenhain (180) and by Waymouth Reid (416) show that the intestine can deal with large amounts of protein in a very short period of time.

Omi (311) has made a curious observation in connexion with the absorption of native protein. He found that dog serum is readily absorbed from the dog's intestine, but that if horse or ox serum be em-

ployed absorption only takes place with difficulty and in small amount. If, however, the "ox" serum be put into the intestinal loop along with an equal amount of pancreatic extract from ox pancreas, absorption is quite marked. The greatest absorption of all, however, followed the placing of a mixture of dog serum and dog pancreatic extract in the loop.

Fate of Parenterally Introduced Protein.

Even supposing the protein can be taken up to any extent in an unaltered condition, the question naturally arises, can the body deal with native protein circulating in the body fluids—in other words is parenterally introduced protein of value to the organism? The work of many investigators, as Zuntz and Mering (428) and Neumeister (305), among the older workers, has shown that utilization of native protein introduced parenterally does take place. Sollmann and Brown (377) have demonstrated clearly that under favourable conditions egg albumin injected intravenously can be well utilized, in many instances only a mere trace of protein appearing in the urine. On the other hand, Gürber and Hallauer (161) hold that the non-appearance of the protein in the urine is no evidence of its utilization in the tissues. In their experiments they injected a solution of caseinogen intravenously and found that part of this material reappeared in the urine, but that part was also excreted into the intestine by way of the bile. This excretion into the intestine and subsequent digestion might of course account for the positive results which have been obtained. Then the recent work of Friedemann and Isaac (147) showed that, if subcutaneous injections of egg white or of serum were given to dogs and goats, the material was for the most part excreted in the urine in a non-coagulable form; in other words, although it was not utilized it had been attacked during its stay in the tissues. Not only this, but examination of the blood four hours after the injection of 400 c.c. egg albumin showed the presence of a non-coagulable biuret-giving body (? proteose) but neither polypeptides nor monoamino nor diamino acids were found. In goats, in a condition of starvation, they found that there was a retention of the injected nitrogenous material before immunity was induced (by the repeated injections of protein), whereas after the induction of immunity the result of further injections of the protein was to bring about a marked rise in the output of nitrogen in the form of urea, frequently even exceeding in amount that of the nitrogen injected. They

also carried out a few experiments in which they injected a dog fed on a carbohydrate diet (potatoes) and found that now a well-marked retention of nitrogen took place without any formation of precipitin. They continued their investigations (148) and found that the condition of the nutrition of the dogs and goat played a very important part in this utilization of protein. It was absolutely immaterial whether the injected protein was the animal's own serum, or foreign serum, or egg albumin, since the result was always the same, namely that if the material were injected parenterally there was an increase in the breakdown of protein with a rise in the nitrogen excretion above the amount injected. When, however, the animal was fed on a carbohydrate diet no increase was found, indeed a retention of nitrogen was observed. This retention was so great that they were able to keep an animal in nitrogenous equilibrium by protein administered parenterally. They could not prove, however, that any addition of actual body substance occurred. They came to the general conclusion that parenterally introduced protein would be broken down and utilized by the body, but that the precipitin reaction was of no value for these investigations, since although any excess of the injected protein might be eliminated in a day or two, the precipitin reaction persisted for a much longer period. Mendel and Rockwood (279) have shown that edestin and excelsin, when introduced into the circulation, can apparently be retained in the organism, for they are not eliminated unchanged in the urine; when introduced into the peritoneal cavity they were also found to disappear. Borchardt (67), like Gürber and Hallauer (161), found that the urine was not the only channel of excretion for parenterally introduced proteins; the non-appearance in the urine of the substance injected could not therefore be accepted as absolute evidence of utilization. This worker injected intravenously hemielastin; a part of it was found in the wall of the small intestine. He concluded, therefore, that this material was either on its way to the intestine for excretion or was present to undergo certain changes which would render it suitable for utilization by the body, or that it was in the process of absorption after excretion by way of the bile. This last hypothesis was not considered likely, as no trace of hemielastin was discoverable in the liver. Michaelis and Rona (282) made an attempt to replace part of the nitrogen in the diet (of meat, milk, etc.) by injecting an equivalent amount of caseinogen subcutaneously into an animal in nitrogenous equilibrium. They found, however, as Friedemann and Isaac had previously found, that there was an increase in the output of nitrogen in the urine. The caseinogen, however, was not excreted in the urine as such, and like

many previous workers, they concluded that it had been broken down. They suggested, as the result of some of their experiments, that the mammary gland might be looked on as a channel of excretion for caseinogen. They found later (283) that, if horse serum were injected into dogs, the nitrogen equilibrium could be maintained and they concluded therefore that the tissue cells could to a certain extent take on the specific function of the intestinal mucous membrane. They only got excretion of protein in the urine after a very large dose of serum. Heilner (183) has suggested that this utilization of injected protein is brought about by the generation of a special ferment He found that the injected serum was well utilized. In this connexion the recent work of Abderhalden and his pupils is of interest (see p. 18).

Freund and Popper (143) carried out a series of interesting experiments in which they examined the blood of animals with and without the intestine cut out of the circulation. They found five minutes after the intravenous injection of a solution of peptone or other product of protein digestion that about 50 per cent. of the injected material could not be recovered, due simply to the distribution throughout the body. Of the other 50 per cent. they found that, if the intestine were in the circulation, only 15 to 20 per cent. was recoverable from the blood after twenty minutes, whereas with the intestine out of circulation practically the whole 50 per cent. was recovered. They demonstrated further that in the first instance about 32 per cent. of the material recovered was so far broken down that it no longer gave a precipitate with tannic acid, whereas in the case with the intestine absent only some 12 to 18 per cent. was thus changed. They could obtain no direct evidence to show that any part of the injected material was changed into a coagulable form. This work, of course, is strong evidence in favour of the contention that the intestine plays some important part in the preparation even of parenterally introduced protein before it is utilized by the tissues.

CHAPTER II.

PROTEIN REGENERATION.

How and in what form is protein normally conveyed from the intestine to the tissues? It has been shown that it can be absorbed in a natural undigested condition (pp. 12, 13), and it has also been shown (p. 15) that protein which reaches the blood stream through other channels than passage through the intestinal mucous membrane can be dealt with probably through the agency of a special enzyme. But on the other hand entrance of protein by these channels cannot be considered the normal one.

Are Proteoses or Peptones Found in the Blood?

A certain number of modern workers still adhere to the old belief that the absorption takes place in the form of proteose or peptone, but, as has already been shown, the work of Cohnheim has rendered this extremely unlikely. In support of their contention, however, they make the statement that proteoses or peptones can be detected in the blood stream, particularly the portal stream, during digestion. Much contradictory evidence has been put forward with regard to this statement. After the work of Neumeister (305) it was generally believed, that no proteose or peptone could be detected in the blood, but in 1903 Embden and Knoop (117), as the result of their experiments on the fate of proteoses and peptones when brought into contact with the intestinal mucous membrane, stated that, if the tests were carefully enough carried out, these substances could be detected in the blood. They held that such absorption must take place, as they claim to have demonstrated that peptone is neither synthetized to a higher product —a coagulable protein—nor broken down to an abiuret product when brought into contact with the intestinal mucous membrane; they could not, however, always demonstrate the presence of this non-coagulable biuret-giving substance in the blood. Langstein (239) confirmed the

observation of Embden and Knoop, but at the same time admitted that the evidence adduced for the presence of proteoses and peptones in the blood was not absolutely convincing. Schumm (366) on the other hand was quite unable either in health or disease to detect proteose in the blood. Abderhalden and Oppenheimer (9) held that, if proteose were present, it was present in amount that could not be detected by the ordinary methods; they therefore maintained that it could not be regarded as a normal constituent of the blood even under the most favourable conditions. For instance, in their experiments three dogs, which had been starved for several days, were given a full meat meal and then killed at the height of digestion and absorption —six to eight hours after the meal. On thorough examination of the blood no trace of a biuret-giving substance could be detected. They put forward the view that the so-called presence of proteose—or at least of the biuret reaction—was due to the imperfect methods by which the blood was coagulated, i.e. that traces of the coagulable protein were left which sufficed to give a definite biuret reaction. Apart altogether, however, from imperfect coagulation it is possible that the biuret reaction is due to the presence in the blood of non-coagulable proteins or of proteins which can only be coagulated with the greatest difficulty. Such a protein was described by Zanetti (424) who found that, by the addition of a large volume of alcohol to the concentrated filtrate of ox blood, from which all ordinary protein had been removed by careful acidification and boiling, a substance was precipitated which gave all the usual protein reactions. Zanetti held that this body belonged to the class of the mucoids. K. A. H. Mörner (289) and Eicholz (112) have also discussed the presence of a mucoid substance in the blood, but do not believe that it is an entity (Mörner) but an artifact. Howell (205), however, decides in favour of the serum containing a protein which is not coagulable by heat—Chabrie's albumone —a substance which is neither a proteose nor a peptone. Still more recently Bywaters (84) has also reached the conclusion that the "proteose" described in blood is in reality seromucoid. He maintains that the above-mentioned behaviour of the proteoses present in blood agrees with the characteristics of this body. Bergmann and Langstein (63) maintained, however, that the biuret-giving substance was proteose, and that it must be regarded as a constant constituent. Kraus (229) also found that small amounts of proteose could be constantly detected in the blood. As the result of his experiments with hemielastin—an elastin proteose—Borchardt (67) came to the conclusion that proteoses of the food could be found not only in the blood but in certain

of the tissues. After feeding with hemielastin (artificially prepared) he found evidence of its presence in the blood, liver, spleen, muscle, stomach and intestinal wall. Later he fed elastin itself and thus allowed the elastin proteose to be formed in the normal course of digestion and confirmed his observation that the proteose could be detected in the blood and the tissues. It was not confined to the portal blood, but was also found in the systemic (carotid) blood. Abderhalden and Ruehl (38) were quite unable to confirm this work of Borchardt although they took every precaution and care. They found no trace of the hemielastin in either blood or tissue. They stated further that as a source of protein supply elastin could not be considered as it was very poorly absorbed. They admitted that it might, however, have a slight protein sparing action. Freund (141) maintained that the explanation suggested by Abderhalden and Oppenheimer was wrong, that the biuret reaction was due simply to imperfect coagulation. He held that Abderhalden by his method not only precipitated the proteins, but also every trace of proteose (Abderhalden 6). Finally Körösy (225) using the most modern and careful methods and Howell (204) using a method of dialysis where all protein was held back, were quite unable to detect the presence of proteose and peptone in the blood, even at the height of digestion. Abderhalden and Pincussohn (35) have further found that, after the injection of peptone, a ferment appeared in the plasma capable of decomposing the injected material. If peptone were a normal constituent of the blood it would be expected that the ferment would also be constantly present but such is not the case.

The conclusion must be reached that there is no very decisive evidence in favour of the presence of either proteose or peptone in the blood. If it be present, it must be merely in traces.

Fate of Protein after Absorption.

If the view be untenable that the protein enters the organism from the intestine in the form of proteoses and peptone in what form does it enter? At present there is a very marked difference of opinion on the question. One set of workers claim that immediately after absorption the products of digestion are synthetized to a coagulable protein, whereas the other set maintain that the absorption takes place in the form of very simple protein products—either as simple amino acids or groups of these—and that it is in this form that protein is conveyed in the blood stream, allowing each tissue to choose for itself

the food which it demands. As Leathes neatly puts it "the proteins circulating in the blood are a currency which is not legal tender".

Abderhalden, among the modern workers, has been the most active in upholding the view that a synthesis takes place in the intestinal wall immediately after absorption, and that therefore all the material is sent on into the animal organism in the form of coagulable protein. Abderhalden and his co-workers hold further that the protein, which is formed, is a neutral protein, probably serum protein.

They depend for their evidence very largely on the fact that the decomposition products of the protein have not in their opinion been clearly demonstrated to be present in the blood. Abderhalden, however, admits that the present methods for the estimation of small amounts of amino acids are very unsatisfactory.

If there be no immediate synthesis to protein, then the building material must be carried in a soluble form in the portal blood, and then distributed in the blood stream to the tissues of the body in order that these may take the material they require to satisfy their immediate wants. Neither those who contend that there is immediate resynthesis, nor those who adhere to the view that the material is absorbed from the intestine in an amino acid form, conveyed in the portal stream to the liver, possess evidence which is universally accepted.

If, on the one hand the presence of amino acids has never been satisfactorily demonstrated in the portal blood, there is no evidence on the other hand of the increase of coagulable protein in the same blood, such as would be demanded by the hypothesis of immediate resynthesis.

The great difficulty in reaching a final settlement of this question arises from the fact that, on account of the rate at which the blood flows through the intestinal vessels, the absorbed material is removed extremely rapidly and is present in so small an amount; a given amount of blood at a given moment contains only mere traces of the material to be tested for. Further it must be remembered, that the digestion and setting free of the soluble digestion products is not explosive in character but is a gradual process thus limiting the amount of material available for absorption. A number of estimations of the rate of intestinal blood flow have now been made. Cybulski (103) measured the rate of flow through the portal vein of a dog which weighed 9·5 kilos. and found the rate of blood flow to be about 9000 c.c. per hour or about 150 c.c. per minute. Burton Opitz (82) found in his experiments, on the rate of the blood flow in the portal vein of dogs, that the mean

amount which passed along this vein was 594 c.c. per kilo animal per hour, which works out about a third slower than the flow as estimated by Cybulski. Now Pflüger (330) showed in a series of experiments in which cats were fed with lean meat that the maximal rate of absorption of the protein for an animal of one kilo in weight was 1·14 grm. protein per hour. Pflüger (331) maintains that this is a good maximum for human beings as they cannot digest protein at the same rate as animals. In support of this statement he cites the case of a dog of 30 kilos which digested 2500 grms. of meat in twenty-four hours, whereas a man of twice the weight could hardly manage half this amount with comfort. If then the rate of absorption be taken at 1.14 grm. protein per kilo per hour, and using Burton Opitz's figures, the 1·14 grm. is contained in 594 c.c. of blood, thus the percentage concentration of protein digestion products in the blood is ·19. If on the other other hand we use the figures obtained by Cybulski the result is still lower, for here we have the 1·14 grm. dissolved in some 950 c.c. of blood, i.e. a concentration of ·12 per cent. Other workers, Bergmann and Langstein (63), for example, put the amount to be looked for as low as ·005 per cent. But the difficulty does not end here as in the first place we know only a fraction of the substances obtainable by the digestion of proteins, even leucine, the most abundant amino acid, common to most proteins, is present on the average only to the extent of 20 per cent., and in the second place this search for digestion products is being made in a fluid which already contains some 3 per cent. of nitrogen in the form of coagulable protein, and about ·03 per cent. nitrogen in the form of nitrogen non-precipitable by tannic acid. Unless the search for products of digestion be made in the portal blood the chances of detecting them must be small, as, in addition to the liver acting as an efficient filter and deaminizing organ, the tissues probably fix a large percentage of the circulating nitrogen at a very rapid rate.

In spite of all these difficulties, a certain amount of evidence does exist in support of the contention that the simple products of digestion are to be found in the blood more particularly in the portal stream at the height of digestion. Bergmann and Langstein (63) examined the portal and systemic blood of well-fed dogs for total nitrogen and non-coagulable nitrogen, and found that there was always a slight gain in the non-protein nitrogen—"residual" nitrogen—after a meat meal. The percentage amount of non-protein nitrogen of the total nitrogen of the blood varied between 7·7 and 14·7 with an average of 10·7. This coagulation method, however, cannot be regarded as satisfactory as the non-coagulable nitrogen cannot with certainty be pronounced

to be simple amino acids, polypeptides, etc., in the light of the statement that non-coagulable proteins are sometimes present in the plasma. Bergmann (62), utilizing the method introduced by Fischer and Bergell of shaking the material to be tested with β-naphthalene sulphochloride and separating out the amino acid compound which is formed states that he was able to obtain from the blood of a patient suffering from acute yellow atrophy a product which crystallized out, but which he could not identify. He also obtained evidence of the presence of bodies which could unite with the β-naphthalene sulphochloride, but which he was also unable to identify in the blood of dogs killed after an abundant flesh meal. Further in the blood and expressed juice from muscles and liver of an animal which had fasted he was unable to demonstrate the presence of any material which would combine with the β-naphthalene sulphochloride. Howell (204) used the same method, but, by an ingenious device he got rid of the serum proteins without the risk of losing part of his amino acids in the coagulum formed on heating. He enclosed the blood taken from fed and fasted dogs, in sacs of collodion and dialysed it against distilled water. Amino acids and substances as complex as the proteoses will pass through this membrane. Howell found evidence of amino acids being present in the portal blood of well-fed animals in greater amount than in the systemic blood. Even after fasting for fifty hours he states that a positive amino acid reaction may be obtained from the blood. He also found that the lymph collected from the thoracic duct after a meal gave a positive reaction. Although Howell obtained these positive reactions he did not obtain a pure crystalline body but a sticky substance which he could not identify. This may be and probably is due to the fact that the material present in the blood and which dialyses out is mostly in the form of mixed polypeptides and individual amino acids, none of them in sufficient quantity to enable purification by crystallization to be carried out.[1]

Hohlweg and Mayer (198) have also taken up the question of this "residual nitrogen". They found a constant increase of this residual nitrogen in the blood of fed dogs above that taken from the fasting animal. In fasting there was present in 100 c.c. serum 0·0525 grm. total residual nitrogen and 0·0384 grm. urea, whereas in the digesting animal there was present in the same amount of serum 0·0788 grm. residual nitrogen and 0·0567 grm. urea. In both cases urea formed

[1] Professor O. Folin has informed me that working in conjunction with Dr. Denis he "has traced in cats both urea and glycocoll from the small intestine into the blood and from the latter into the muscle". Jan. 1912.

about 73 per cent. of the total residual nitrogen. In using the tannic acid precipitation method they found an increase in the non-precipitable nitrogen in the blood of the digesting animal as compared with the fasting animal. The increase amounted to ·007 grm. nitrogen in 100 c.c. blood which equals, as they have calculated, ·0705 grm. leucine, or much less glycine. They carried out a couple of experiments in which they fed an animal with deutero-proteose, but they were unable to detect any increase of the proteose fraction in the serum above the amount present in a meat-fed dog.

Cathcart and Leathes (92) attempted to demonstrate the increase of the non-precipitable nitrogen (by tannic acid) in the blood following absorption, by performing a perfusion of the isolated intestine in which the same blood was repeatedly used, and in which therefore the products from absorption would accumulate. Owing, however, to the fact that the mucous membrane of the intestine ceased to functionate this method had to be abandoned. By utilizing the whole animal and allowing the absorption of peptone, proteoses or tryptic digestion products to take place from a limited portion of the intestine, a constant increase of the non-precipitable nitrogen in the blood was found. It was definitely shown that the whole increase was not due to urea and ammonia. In all the experiments control blood was taken from the animal before any digestion products were introduced into the intestine. The ammonia was found to account for about 4 per cent. and urea for nearly 50 per cent. of the non-precipitable nitrogen. By this method only some 15 per cent. of the nitrogen, which was absorbed, could be accounted for by the increase of the non-precipitable nitrogen in the blood, and when the liver was also examined about another 15 per cent. was found to be retained there. Further an attempt was made to detect the presence of an increase in the coagulable protein (which was to be expected if the immediate synthesis hypothesis were correct), but no such increase could be detected although full allowance was made for the dilution of the blood by fluid absorbed from the intestine. In carrying out these tests not only was the alteration of the dilution of the blood controlled by estimations of the total nitrogen of the blood, but the hæmoglobin content was also estimated. The conclusion reached was that the blood could not be looked on as a storage place for nitrogenous nutriment, but was concerned simply with the transport of such material. The question of deaminization is one of very great importance in this connexion, because if, as is probably the case, the amino group be rapidly split off from the greater proportion of the absorbed amino acids, a large increase in the non-precipitable

nitrogen cannot be expected in the blood, since the ammonia which results is rapidly converted into urea in the liver and excreted. If the amount of nitrogen excreted in the urine during the period of absorption were also considered in addition to the non-precipitable nitrogen found in the blood and liver, the total amount of nitrogen which had disappeared from the lumen of the intestine could be largely accounted for (over 70 per cent. in one experiment).

Abderhalden and his co-workers have produced a certain amount of indirect evidence against the view that the nitrogenous products after absorption pass on to the tissues in a non-protein form, but no direct evidence of value in favour of the view that immediate resynthesis takes places. Abderhalden, Funk and London (41) state that they were quite unable to demonstrate by the most modern chemical methods that either proteoses or amino acids were present in the blood of dogs with Eck's fistula when fed on different diets. Certainly a rise of ammonia in the blood was to be expected as the liver was cut out of the circulation, but no such rise was found. They believe these experiments to be direct evidence in favour of the hypothesis of immediate resynthesis. Gliadin was one of the proteins fed, but even under what must be considered very favourable experimental conditions, they could not account for the fate of the excess of glutamic acid—not even in this experiment could they detect a rise in the ammonia content of the blood. Abderhalden and London (28), as the result of a further series of experiments on a dog with an Eck fistula, reiterated the opinion that the experimental results—nitrogenous equilibrium maintained on fully digested meat products—afforded strong support for immediate resynthesis. These authors have further attempted to support the hypothesis by investigating the variation in the nitrogen content of the intestinal wall, but they failed to get conclusive evidence (31, 34). In their experiments they used control pieces of intestine from the same animal on which the experiment was carried out. London (264) also tried to prove that resynthesis of protein took place immediately after absorption, but the results which he obtained were negative. He argued that, if the intestinal mucosa were actively concerned in resynthesis, evidence of the resynthesis should be obtained by a comparative chemical analysis of the mucosa during hunger and at the height of digestion (of gliadin), His control animals gave a nitrogen content, for 50·1 grm. dried mucosa, of 3·15 grm. nitrogen, and 0·75 grm. glutamic acid hydrochloride and his digestion mucosa, for 50 grms. dried substance, 3·10 grm. nitrogen and 1·40 grm. glutamic acid hydrochloride. Thus, even at the height of

digestion, he was unable to prove that there was any accumulation of nitrogenous material. This is, of course, no proof that such a synthesis does not take place, as it is possible, although highly improbable, that the synthetic product is very rapidly formed (constant synthetic action) and just as rapidly removed. It does not, on the other hand, support the immediate resynthesis hypothesis. Körösy (225) carried out a large number of absorption experiments under different conditions, using a method very similar to that followed by Cathcart and Leathes. He pronounced, however, in favour of immediate resynthesis, on the grounds that in dogs with their circulation restricted to the intestine the non-precipitable nitrogen (by the tannic acid method) in relation to the total nitrogen of the blood is not increased after a protein meal in greater amount than that which is found in fasting. He was unable to detect any free amino acids or proteoses in the blood, and this he regarded as an additional argument for immediate resynthesis.

Cohnheim (96), on the other hand, produced some evidence which distinctly favoured the view that the absorbed protein material travelled in the blood in the form of amino acids. He carried out his experiments on the intestine of the Octopus and the Eledone moschata. He introduced solutions of peptone into the isolated gastro-intestinal tract which he floated in oxygenated blood. Not only was he able to prove that absorption took place, but that amino acids were absorbed, at any rate under the conditions of his experiment, as he was able to isolate from the blood at the conclusion of the experiment, which lasted twenty hours, leucine, tyrosine, lysine, arginine and ammonia. When, however, he carried out similar absorption experiments on the intact animal, he was unable to detect these amino acids in the blood.

The Nature of the Absorbed Material.

Thus there is no direct evidence which definitely determines the form in which the digestion products of protein reach and travel in the blood. Wherein, it might be asked, lies the benefit of converting the protein into simple products like the amino acids to have them immediately after absorption takes place converted into a neutral protein. The complete breakdown in the gastro-intestinal tract probably takes place either because the highly complex molecule is not readily dealt with as such by the tissues or because certain amino acids, as for example glutamic acid in gliadin, which are present in excess

in the molecule and which are not required for the building up of the tissue protein, must be eliminated. The view that the tissue proteins differ from one another, that they are specific bodies of definite constitution, and that therefore each requires a different amount and supply of building material is gradually being accepted. Abderhalden himself accepts this. What end then is served in having a single uniform pabulum formed when the demand is so varied? This is all the more questionable when it is remembered that there is no indubitable evidence which shows that one amino acid can be converted into another. Further, the belief is gradually gaining ground, as regards the protein requirements of the organism, that it is not so much the actual quantity as the quality of the protein supplied in the food, which is of importance. If the material supplied be uniform it necessitates a fresh breakdown by each tissue, perhaps by each individual cell. Although the tissues all probably possess this power of breaking down protein material by means of their intracellular proteolytic enzymes, still the extra work involved seems to negative the immediate resynthesis hypothesis, especially when the hypothesis of the circulating digestion product postulates the presence of the individual food material in the blood. As already remarked, the mere failure to detect these products in the blood does not give adequate reason for concluding that they are not present. The tissues certainly do not break down in regular sequence, nor are they left to fall to pieces for lack of repair material. Repair is among the most active functions of all tissues. Must, then, a tissue of highly complex structure keep destroying and digesting plasma, picking out from the debris the nuclei which it requires and letting the rest go? (Why, and this destruction is admitted by Abderhalden, are the superfluous amino acids not found in the blood?) What happens, for instance, in the case of the connective tissues with their demand for, say, glycine, where the food supply is not over-abundant as the circulation is poor, and the tissue not very suited for lymph perfusion? It will not do merely to say that there is no great breakdown of material here. If the demand for food exist how is it satisfied? Pflüger (331), in an interesting paper in which he combated this immediate resynthesis hypothesis, ascribed to the cells of the intestinal wall, with regard to the protein synthesis, the same capacity as the cells of all tissues, but denied that the synthesis of protein for the whole organism was carried out there. He held that such a hypothesis was contrary to all existent knowledge of physiological assimilation.

If immediate resynthesis take place, to what extent does it occur?

Does all the protein absorbed in the form of digestion products become converted into coagulable protein, or does the intestinal mucous membrane exert a certain selective action? The experiments of Abderhalden, London and Oppler (45) in which they showed that after feeding with gliadin, as the digested material descended the digestive tract tyrosine disappeared from the intestinal contents but glutamic acid increased in amount, certainly point to some such selective activity. In support of this work was that of London (264) who was unable to show that any marked accumulation of glutamic acid took place in the intestinal wall, even at the height of digestion, after gliadin feeding.

Further, how is the fact to be explained that following protein digestion and absorption there is a very great and rapid rise in the output of nitrogen in the urine, mostly in the form of urea, if immediate resynthesis take place? There is also the observation of Nencki, Pavloff and Zaleski (298) that, as the result of protein digestion, there is a marked rise in the amount of ammonia present in the portal blood. This rise in the ammonia content of the portal blood is supported by and supports the observed increase in the excretion of urea in the urine as the conversion of ammonia into urea certainly takes place for the most part in the liver. Of course one must admit that this deaminization presents also certain difficulties in the digest product absorption hypothesis. Does a selection of appropriate amino acids or amino acid groups which are allowed to pass on into the tissues unchanged take place or is this deaminization a mere protective mechanism—a certain concentration of amino compounds being permitted to pass but any excess immediately undergoing deaminization, just as in the case of carbohydrate a certain limited amount is fixed by the liver (and the tissues) any excess being excreted in the urine? In favour of the selective action are certain observations of Lang (238) who found that *in vitro* some tissues deaminized certain amino acids more readily than others (see page 52). If this be true for the living organism then one might regard the intestinal wall and the liver as successive layers of a highly protective filter which exerted a selective action on the material which they let through.

The balance of evidence seems to me to be in favour of the hypothesis that the synthesis of protein in the body is a function of each individual cell, and is not confined to one set of cells (those of the intestine). Further that the material which is utilized in this synthesis is not circulating in the fluids which bathe the tissues as a "whole" or "neutral" protein but in the form of amino acids or groups of these—the products of the hydrolytic decomposition of protein.

The Hypothesis of Freund.

Freund and his pupils also ascribe to the intestinal mucous membrane a somewhat similar relationship to protein resynthesis as that advocated by Abderhalden. They hold that not only does the digestion take place in the intestine, but that, after or during absorption, some kind of polymerization of the digestion products takes place which is absolutely essential before synthesis of protein can be effectively carried out by the tissues. Toepfer (399) found that, if the liver of an animal were perfused with its own blood, there was no increase in the blood of any decomposition products even after the addition of protein to the blood used in the perfusion. If an addition of Witte's peptone were made there was a slight increase in the amount of coagulable nitrogenous products at the expense of the proteose. If, on the other hand, the intestine as well as the liver were left in the circulating area an increase of decomposition products in the blood could always be detected. He came to the conclusion that both the liver and intestine were necessary to the proper breakdown of proteins. Freund and Toepfer (144) continued the investigation. They perfused the liver and intestines of two fasting dogs with (1) their own blood, (2) with the blood of a well-fed animal. They found that in both cases there was an increase in the nitrogenous decomposition products, but that the increase in the case of the perfusion with the blood of a well-fed animal was about twice that when the blood from the fasting animal was utilized. Freund (140) later, in a long and extremely indefinite paper, came to the conclusion that the liver played a very essential part in the breakdown of protein, but that before this action could be evoked the protein material must have passed through the intestinal wall. Even in starvation he held that the various autolytic products must first be excreted into the intestine and then be reabsorbed and altered in some mysterious fashion before they could be utilized. He believed, like Abderhalden, that the protein digestion products travelled in the portal stream in a coagulable form—chiefly as pseudoglobulin. Certainly in support of some such circulation as this are the observations of Horodynski, Salaskin and Zaleski (203) that during starvation the ammonia content of the portal blood is higher than that of the systemic, due it might be to deaminization of the reabsorbed autolytic products taking place. Freund's experiments are extremely difficult to understand, as not only is his explanation

very obscure, but the experimental data which he offers are difficult of interpretation even in the light of his own hypothesis.

Körösy (226) tested the hypothesis of Freund by using dogs in which practically the whole of the intestine was out of circulation. He then injected foreign serum into the blood stream in the expectation that under these conditions the protein would appear in the urine unchanged if the intestinal wall did perform certain essential preparatory functions. Protein was either absent or appeared only in traces, so that if we accept Körösy's interpretation of these experiments then the preparatory action of the intestine advocated by Freund cannot be very essential. In the course of his experiments Körösy made the curious observation, previously recorded by Slosse (376), however, that the mere cutting of the intestine out of the circulation leads to the appearance of a certain amount of protein in the urine. In a later paper (226 A) he fully confirmed his earlier observations.

Abderhalden and London (34) were unable to show that there was any excretion into the intestine after the subcutaneous injection of protein. In their investigation they used animals with intestinal fistulæ, where any excretion, even in traces, could be comparatively readily detected under fairly normal conditions. Still a certain amount of independent evidence exists, which might be regarded as lending support to this contention of Freund. Thus London and Polovzova (265) found that certain nitrogenous bodies were excreted into the intestine high up and were absorbed again lower down. Further Reach (335) found that, if he perfused a liver with a mixture of blood, Ringer's solution and a protein containing iodine, quite a large amount of the iodine-containing protein was retained in the liver. He believed that the fact, that but little proteolysis of the perfused iodine protein compound took place, supported to a limited extent the hypothesis of Freund. Abderhalden and Slavu (32) also found after the subcutaneous injection of certain compounds of iodine and polypeptides that iodine appeared in the intestine and was excreted in the faeces, in other words, that a definite excretion into the intestine had taken place.

A somewhat similar hypothesis to that of Freund was put forward for the carbohydrates by Croftan and may be mentioned here as it also bears on this excretion into the intestinal canal. Croftan (102) stated that dextrose by its passage through the intestinal mucous membrane underwent some alteration which rendered possible its polymerization into glycogen in the liver. He further stated that, if this passage were omitted, and the dextrose injected directly into the

mesenteric vein there was no increase in the glycogen of the liver, but that an actual excretion of sugar from the blood into the lumen of the intestine occurred. He held that the experiments of Grube, who showed that perfusion of the tortoise liver with dextrose could give rise to glycogen, were without value as they were carried out on a cold-blooded animal. Pflüger, it may be noted, held that this objection was not valid. Fischer and Moore (128) have also observed this excretion of carbohydrate into the intestine, but the conditions under which it occurs are more or less pathological.

Plastein Formation.

Intimately connected with the preceding work on protein regeneration are the curious observations on the so-called plastein formation. This work originated in the experiments of Danilevsky and Okuneff in 1895 (107), who showed that if rennin were brought into contact with a solution of proteoses a precipitate—plastein—was produced. They regarded this precipitate as a resynthetized protein. Kurajeff (235) observed the same formation of a precipitate when papayotin solutions were brought into contact with proteose solutions. In a later communication (235) he stated that he could obtain the formation of a coagulable protein from his plastein proteoses, if they were brought into contact with the gastric or intestinal mucous membrane. Nurnberg (306) found that the plastein formation was not limited directly to the action of the gastric rennin, for if autolytic organic extracts were brought into contact with protein solutions a precipitation resulted. This work was confirmed by that of Grossmann (159). Sawjaloff (354) also investigated this precipitation reaction. He came to the conclusion that the digestion in the gastro-intestinal canal formed a substance which, taken up into the circulation, was coagulated later when and where required. He stated that if the proteose solution were fractionated in the manner described by Pick, and if the individual fractions were then treated with the enzyme, no precipitation resulted, although the same proteose solution not fractionated gave the precipitation quite readily. He was firmly convinced that the reaction was a true synthetic one. He further held that the substance formed was a true substance of constant constitution. He gave to the substance the name plastein.

On the other hand, Lavroff and Salaskin (245) held that there was no reason why the precipitate should be accepted as an entity—a re-

generated peptone. They believed that it was a mixture and suggested that "rennin proteose" should be the name given to it. They obtained it from the different proteose fractions. Sawjaloff, in a later paper (355), restated his position and again maintained that the formation of plastein was a true synthetic process, and that it could only be demonstrated in strong proteose solutions. He thought that in all probability it was evidence of a reversible action of pepsin. He regarded it as an assimilation product of first-rate importance, the intermediate product between digested protein and the formation of blood proteins. This change took place, he believed, immediately after absorption. He was convinced, that when proteins have reached what may be called the plastein level in enzymic degradation, they are of uniform constitution. Lavroff (242, 243) certainly obtained the precipitation as other authors have done, and he called the substance shortly "coagulose". He found that it could be produced from digestive products from which the hexone bases had been removed, and that the product which was formed, contained no such bases. It was also formed from material in which these bases were present, and in this case the coagulose contained them. He was not therefore inclined to regard them as specific substances. In a later paper (244) he demonstrated that, by the peptic digestion of caseinogen, two series of "coagulose" substances were formed, one conforming to a proteose type, the other to a polypeptide type.

Sacharoff (345) held, that this plastein formation was not synthetic in origin at all, that it was only an intermediate substance in the process of digestion, precipitation taking place simply because the physical conditions for solution were not suitable. Bayer (56) also stated that, in his opinion, the substance formed was no true synthetic product, and thought that its protein-like character was probably due to impurities. He believed that it was a member of the so-called peptoid group of Zunz, and that it was a body therefore of comparatively simple constitution. Herzog (191) and Volhard (412) maintained that this plastein formation was not the result of the action of rennin at all, but might be regarded as another example of the reversibility of reaction of proteolytic ferments (Herzog) or of pepsin (Volhard).

The question as to (1) whether this substance is a new synthetic product differing from the protein from which the proteoses are derived, or (2) whether it is merely a resynthesis of the original protein from which the proteoses are obtained, or (3) whether it is no synthetic product at all but merely a substance—a digestion product—on the road to complete solution is not definitely settled. The work, how-

ever, of Levene and Van Slyke (252, 253) would incline one to the view that the substance belonged more to the proteose than the true protein group of bodies. Levene and Van Slyke carried out a complete hydrolysis of the material, with subsequent isolation of the amino acids, and found that it contained at least thirteen amino acids. It must, therefore, be regarded as a fairly complex body. The evidence (253) obtained by the investigation of the viscosity also pointed rather to its proteose than its protein nature, precipitation taking place owing to mere difficulties of solution, as Sacharoff had already suggested.

Synthesis in the Gastric and Intestinal Mucous Membranes.

Closely allied to this work on plastein formation is that of Hofmeister and his pupil Glaessner some of whose observations were made previous to the publication of the work of Danilevsky and Okuneff. Hofmeister (197) stated that if proteoses were left in contact with the gastric mucous membrane they were converted into protein. In his experiments he divided the stomach of a dog, which had been killed at the height of digestion, into two approximately equal parts. One of these parts he immersed at once in boiling water, and the other he placed for two hours in an incubator. He then estimated the amount of proteose and peptone obtainable from each part employing the biuret reaction colorimetrically for the purpose. A diminution, even a complete disappearance, of proteose and peptone, was observed in the incubated half. He concluded therefore that the proteose and peptone had been converted into protein through the agency of the gastric mucous membrane. He found that, if he had previously warmed the part of the stomach to be incubated to 60° for a short period, it lost its synthetic power. Glaessner (154) confirmed these experiments of Hofmeister using, however, more exact methods. He killed dogs three to fourteen hours after a heavy meat meal, and immediately removed the stomach, which he carefully freed from its contents, then divided into two approximately equal parts. In one part the proteose content was at once determined whilst the other part was placed in a moist chamber at 40°. He found, like Hofmeister, that there was a very marked diminution in the amount of proteose to be obtained from the incubated part and at the same time no increase in lower digest products. He also concluded that a true resynthesis of the proteose to protein had taken place—a synthesis which began

soon after the commencement of digestion and which reached its maximum between the fifth and sixth hour and then gradually decreased. He thought that the change was brought about by a proteo-synthetic enzyme but he did not think that rennin played a part, in other words, the product which he obtained was not "plastein". Other observers, however, have criticized these observations of Glaessner and have offered other explanations of his findings. Embden and Knoop (117) repeated Glaessner's work using intestinal mucous membrane (a tissue which had also been previously used with success by Hofmeister). They, however, could find no trace of protein resynthesis, either in the natural intestine or in an intestine freed from trypsin by previous ligature of the pancreatic duct to prevent digestion of any newly formed material. At the same time they found no evidence of the further breakdown of the proteose. Cohnheim (96) believed that the difference found by Glaessner between the amounts of proteose present in the two parts of the stomach, depended, to a certain extent at least, on the fact that fresh tissue coagulated only with difficulty. It is questionable if this argument is valid. Salaskin (347) suggested that the changes which were observed might depend on the alterations which take place in the cells during the resting period—that the apparent synthesis was nothing more or less than the normal cell restitution. Neither of these series of experiments is very convincing, but on the whole the evidence from digestion and absorption experiments generally does not indicate the occurrence of any marked resynthesis in the stomach wall, at least during normal digestive processes.

Synthetic Experiments *in vitro*.

Not only have the above workers maintained that a synthesis took place in the gastro-intestinal mucous membrane when proteose and peptone are brought into contact with it, but a purely synthetic action has been assigned to pepsin and trypsin as another phase of their activity. A. E. Taylor (392), for example, stated, that if the concentrated products of the tryptic digestion of protamine were subjected to the further action of fresh trypsin for five months reformed protamine could be obtained. It is true that the amount of resynthetized product was not large, as from the digestion products of 400 grms. protamine he obtained only 2 grms. of synthetized material. The trypsin employed was obtained from the liver of clams. In a later

paper (393) he confirmed his original finding and adduced additional experimental evidence. Brailsford Robertson (337, 338) found that by the action at 40° C. of a concentrated solution of pepsin on an acid concentrated solution of the products of peptic digestion of caseinogen a substance was precipitated within a few hours which was identical in properties and phosphorus content with a substance related to paranuclein. This material was only formed by the action of the pepsin on the caseinogen digest, since if both these substances were kept separate under similar experimental conditions no precipitate was formed.

The Rôle of the Leucocyte.

Another view of the process by which protein digestion products are dealt with after absorption has been put forward by Hofmeister (197). He believed that the peptone after absorption was taken up by the leucocytes and then either through their own agency or through that of the adenoid tissue it was converted into protein. This contention was largely based on the marked leucocytosis which was found to occur after a meal and not on the direct estimation of the contents of the leucocytes. In support of this hypothesis of Hofmeister, Pohl (332) found that during digestion, in addition to the postprandial leucocytosis, there was an excess of leucocytes in the mesenteric veins as compared with the mesenteric arteries. Paton, Goodall and Gulland (321) showed that there was no detectable difference between the number of white cells in the veins and the arteries. They, however, confirmed the postprandial leucocytosis and showed that the most marked percentage increase occurred in the lymphocytes. There was also some increase in the polymorphonuclears but practically no change in the number of the eosinophiles. The maximum increase in the number of the leucocytes took place about four hours after food. Paton and Goodall (322) later demonstrated that the leucocytes did not arise in the intestinal lymphatic tissue as Hofmeister believed, but that they orginated in the bone-marrow. Erdély (120) also worked at this problem and found that the intestinal wall was richer in leucocytes after a meal than after a period of starvation. He believed that alterations in the nature of the diet brought about variations in the nature of the leucocytosis. Cramer and Pringle (100) have also supported the hypothesis that the leucocytes played a very important part in the assimilation of the protein food products from the intestine, and Cramer (99) believes that even in the case of protein introduced parenterally assimilation is the result

of the action of the leucoctyes. Pavy (324) held that the whole conversion of the food protein into tissue protein was brought about by the lymphocytes. He maintained that the products of protein digestion were resynthetized at the seat of absorption by the lymphocyte growth and that the lymphocytes in turn were resolved by autolysis into the proteins of the blood and in this way the fresh food material was brought within reach of the tissue cells, in other words he believed, like Abderhalden, that the proteins of the blood formed the pabulum for the tissue cells.

None of these workers, however, support their hypothesis by any observations on the variation in constitution of the leucocytes before and after a meal. There is no doubt about the postprandial leucocytosis, but it is not yet proven that these leucocytes are engaged in the manufacture of the new food for the tissues. Halliburton (164) has offered an excellent criticism of the leucocyte synthesis theory. He pointed out that the number of the lymphocytes available was not commensurate with the work to be done. He calculated that a man of eighty kilos had about four kilos of blood of which some forty per cent. was in the form of corpuscles, that is about 1600 grams. Now as the ratio of white corpuscles to red is about 1 : 500 it means that about 3·2 grms. of leucocytes are present. Of this amount lymphocytes form at most thirty per cent., and therefore in the blood there would be about one gram of lymphocytes. If this amount were doubled during digestion, "it is difficult to see how two grams of lymphocytes can tackle the enormous burden which every meal must impose upon them". Even using the figures of Gulland, who stated that the rise in the number of leucocytes might be as much as four times, the difficulty in ascribing so large a synthetic action to this comparatively small number of white cells is great, more particularly if, as Pavy supposed, the newly formed protein was liberated by a complete autolysis of the cell in which the synthesis took place.

CHAPTER III.

FEEDING EXPERIMENTS WITH ABIURET PRODUCTS OF DIGESTION.

The Value of Abiuret Products of Digestion.

INTIMATELY connected with the question of the extent of protein digestion and the form in which the digestion products are absorbed are the feeding experiments with pre-digested protein. These important experiments, first carried out by Loewi (259), have yielded valuable results. They have clearly demonstrated that a food, in which the nitrogen consisted wholly of protein digestion products which no longer gave the biuret reaction, was capable not only of maintaining life but of keeping the animal in a state of nitrogenous equilibrium and even of leading to a certain retention of nitrogen and a rise in weight. An interesting fact which set at rest some of the objections to the complete breakdown of protein in the intestinal canal was discovered in the course of Loewi's investigations. A calorimetric estimation of 1 grm. of the digestion products used by Loewi in his experiments was carried out by Rubner. This amount was found to yield 4·599 calories, a figure very close to that for albumin. In Loewi's experiments the rest of the animal's diet was made up of fat and carbohydrate. He found that, if he fed the digestion product with fat alone, no nitrogenous equilibrium resulted, but that this took place as soon as carbohydrate was added. Lesser (251), who was one of the earliest workers to repeat Loewi's work, was unable to confirm it. Lesser used in his experiments both peptic and tryptic digestion products, but was unable to get a positive nitrogen balance, although the products acted as sparers of protein. In spite of Loewi's bad results with fat alone Lesser omitted carbohydrate from his diets. Henderson and Dean (184) were the first to use acid hydrolytic products in their experiments. They found that they got nitrogen retention but were not at all certain that it indicated protein synthetic action. As the result of the line of research opened up by the original experiments of Loewi a great number of experiments have been carried out mainly by Abderhalden and his co-workers in Germany and by

Henriques and Hansen in Denmark. Abderhalden and Rona (8) carried out a number of experiments on mice using different preparations of caseinogen :—

(1) Caseinogen digested for two months with pancreatin. The preparation gave a faint biuret reaction and contained about 15 per cent. of polypeptides.

(2) Caseinogen digested for one month with pepsin-hydrochloric acid mixture, then for two months with pancreatin. The preparation gave no biuret reaction and contained only about 8 per cent. of polypeptides.

(3) Caseinogen hydrolysed ten hours with 25 per cent. sulphuric acid. The preparation contained no polypeptides.

(4) Unaltered normal caseinogen.

Mice were fed with the various preparations mixed with sugar. Oil was omitted, as when it was present the mice refused the food. They found that the mice fed with preparations (1) and (4) lived about the same length of time. Mice fed on diet (2) died earlier than these but lived longer than mice fed on sugar alone, and mice fed on diet (3) died at about the same time as those fed on sugar alone. Thus it will be noted that the least broken down (15 per cent. polypeptides) of the digestion products behaved most like the normal caseinogen, that next came the preparation with 8 per cent. of polypeptides, and finally the preparation which to all intents and purposes could not be regarded as a food, the acid product with no polypeptides. This evidence is certainly in favour of the hypothesis that certain nuclei are left more or less intact during digestion *in vivo*. But experiments carried out on mice can never be regarded as very reliable unless very large numbers of them are used as controls, as the individual differences in the powers of resistance to diet, starvation, etc., are so great that the results of separate experiments are hardly comparable. Abderhalden and Rona (10) repeated these experiments on a dog, and found unmistakably that part at least of their earlier work on mice was correct. They found that the biuret free digest could completely take the place of protein in the diet, but that the acid hydrolytic product could not do so. In the digest product used in these experiments about 10 per cent. of the nitrogen was in form of polypeptide. The diet contained both fat and carbohydrates. Abderhalden and Rona offered as an explanation of their negative results with the acid hydrolytic product, that there had been first a complete disintegration of important and necessary compounds, and secondly that racemization of the amino acids had also in all probability taken place.

The same workers later (13) tried to replace the protein in the diet by a mixture of single amino acids, but the attempt was quite unsuccessful. But little value need be attached to this experiment as many of the amino acids ordinarily found in proteins were absent.

Henriques and Hansen (187) carried out a series of experiments contemporaneously with much of the German work. They also found that acid decomposition products could not replace protein in the diet although digestion products, which resulted from the long-continued action of trypsin and erepsin, could not only prevent the loss of nitrogen but could even lead, as Loewi had found, to retention. They further found that the loss of nitrogen could be prevented by feeding with the fraction of the digest products which was *not* precipitated by phosphotungstic acid, i.e. the monamino acid fraction. They also obtained the same result when the products of a tryptic digest soluble in warm 96 per cent. alcohol were used, whereas the alcohol-insoluble products could not prevent loss of nitrogen. The animals which they used for their experiments were rats, and the food in addition to the digestion products contained fat and carbohydrate. The same objection applies here as to Abderhalden and Rona's experiments with mice; far-reaching conclusions from the metabolism of rats should not be drawn as their nitrogen exchange is too small. But for this objection this piece of work would render the hypothesis put forward by Abderhalden and Rona, that the digestion products are active on account of the higher groups they contain, untenable. The majority of the polypeptides are precipitated by means of phosphotungstic acid and yet the filtrate from this precipitation sufficed to keep the animals alive.

Sörensen (378), however, has suggested that these positive results of Henriques and Hansen with the monoamino fraction alone were due to this fraction still containing the essential polypeptides which were not precipitated with phosphotungstic acid (Pflaundler (327) has shown that such exist). Sörensen showed that about 20 per cent. of the total nitrogen of the "monoamino fraction" was still in polypeptide form. In a later paper Henriques and Hansen (188) showed clearly that although the products of acid hydrolysis of protein could not replace protein in the diet as efficiently as the products produced by the action of enzymes, they were nevertheless excellent protein sparers, a fact already demonstrated by Henderson and Dean. They concluded, however, from another series of experiments that these same digestion products, if fed along with a protamine (clupeine sulphate), could bring an animal into a state of nitrogenous equilibrium.

Protamine given alone would even seem to exert a certain protein sparing power.

The polypeptide group does not appear to be of paramount importance in spite of Abderhalden's earlier work; in an experiment carried out by Abderhalden and Oppler (20) a dog was kept alive for thirty-eight days, during which time the nitrogen supply was in the form of an abiuret digest mixture consisting almost solely of amino acids. Abderhalden and Rona (21) fed a young dog for three weeks on completely digested meat—biuret free—and found not only a retention of nitrogen, which was to be expected under normal conditions with the growing animal, but a distinct increase of weight. Abderhalden (2) was also able to keep a bitch in nitrogenous equilibrium with fully digested meat during lactation. Even more astonishing was the result of an experiment carried out by Abderhalden and London (28) on a dog with an Eck fistula, as not only did they get their animal into a state of nitrogenous equilibrium, but it retained a certain amount of nitrogen, although the protein part of the diet consisted wholly of fully digested meat. The body weight of the animal slowly sank, however, during the course of the experiment. Abderhalden and London used this experiment as a weighty piece of evidence in favour of the synthesis of the protein digestion products taking place immediately after absorption. They also stated in conclusion that the liver must not be considered as an organ of absolute importance in protein synthesis.

Henriques (185) returned to the question of the difference in nutritive value which exists between ferment and acid hydrolytic products. He found, although unfortunately the experiments were again carried out on rats, that ferment digestion products (trypsin and erepsin) heated with 20 per cent. sulphuric acid for six hours in a boiling water bath could keep the animals in nitrogenous equilibrium, and even bring about a retention of nitrogen, yet the same products heated in the same way with the acid for seventeen hours utterly failed to produce this sparing effect. The only difference which he could detect between the two preparations was that after the six hours' acid hydrolysis the tryptophane reaction could be obtained, but that in the seventeen hours specimen it had disappeared. In this connexion the work of Miss Willcock and Hopkins (199) is of interest (see p. 73).

Recently Abderhalden returned to the question why acid hydrolytic products failed and ferment digest products succeeded in bringing about nitrogenous equilibrium. As just mentioned, Henriques found that if the acid hydrolysis were not too prolonged, if the tryptophane

reaction had not disappeared, such a product could bring about nitrogenous equilibrium. Abderhalden and Frank (36) put this observation to a further test. They completely hydrolysed horse flesh by boiling with sulphuric acid and then added to the prepared products, before feeding them to dogs, 0·5 per cent. tryptophane. They found it was then possible to keep one dog in nitrogenous equilibrium for twelve days and another for fourteen days. During these periods the body weight remained fairly steady. E. Voit and Zisterer (411) have worked out the actual relationship between undigested caseinogen and caseinogen hydrolysed (*a*) by pancreatin, (*b*) by acid, as sources of protein supply. Like other observers they found that "whole" caseinogen acted better than either of the hydrolysed products, and that again the ferment digest was superior to the acid one. The ratio between the three as sparers of protein worked out as follows:—

Caseinogen	Caseinogen (Pancreatin)	Caseinogen (Acid)
100	107	127

On the assumption that the work of Abderhalden and others was correct they concluded that the acid hydrolytic products must be accepted as more than protein sparers—that such products, provided they be not too fully hydrolysed, can take part in actual synthetic processes. Like the majority of workers, they held that in gastro-intestinal digestion there was a complete degradation of the food protein to the simple amino acids or groups of these, and that certain nuclei (probably polypeptide in nature) were absorbed unchanged. It was with the help of these, and probably certain other nitrogen-containing and nitrogen-free groups that the new protein was built up.

Abderhalden (5) also showed that it was impossible to prevent tissue waste with caseinogen minus tryptophane. Three diets were used (1) fully digested caseinogen, (2) fully digested caseinogen from which the tryptophane had been removed, (3) fully digested caseinogen from which the tryptophane had been removed and then the proper amount again added. Nitrogenous equilibrium was obtained with (1) and (3) but not with (2). In support of these experiments are the further experiments of Abderhalden (3) in which he showed that nitrogenous equilibrium could be obtained by the products of incomplete acid hydrolysis. He used edestin partially hydrolysed by treatment for five days at 20° C. with 70 per cent. sulphuric acid; this product consisted of curious polypeptide substances containing glutamic acid, tryptophane and leucine. Similar results were obtained when elastin, haemoglobin and keratin were treated in like manner. From

the extremely interesting experiments of Abderhalden and Olinger (29) the nature and constitution of the *whole* digest evidently plays its part. They starved a dog and within seventeen days the weight of the animal fell from 8820 grm. to 7120 grm. They then gave the animal 3·03 grm. of nitrogen in the form of fully digested caseinogen, but after six days of this diet there was no increase of the animal's weight. The nitrogen intake was increased to 3·99 grm. of the same digest, but still without effect. Then the caseinogen digestion products were replaced by an equal amount (3·99 grm.) of nitrogen from fully digested horse flesh, and after twenty-one days on this diet the weight of the animal rose from 7000 grm. to 8400 grm. That this was not due to mere storage of unutilized nitrogenous material was clearly demonstrated by the fact that when the animal was starved again the daily loss of nitrogen was quite similar in amount to that which took place in the first instance without any previous protein storage. If this had been a mere accumulation of nitrogenous products in the tissues a great loss of nitrogen during the first days of starvation might have been expected. In all these experiments the diet was made up of a mixture of fat and carbohydrate in addition to the protein digest. Abderhalden, Messner and Windrath (48), however, in contradistinction to all previous experience, state that they managed to keep an animal in nitrogenous equilibrium with a diet of digestion products and fat *minus* carbohydrates. Of course the explanation here may lie in the fact that the protein was given in such amount that it (or possibly the fat) was partially converted into carbohydrate. And later Abderhalden and Suwa (37) maintained that they were able to keep a dog in nitrogen equilibrium and to obtain an increase of weight on a protein digestion product alone. Their experiments are, however, very unsatisfactory, as great trouble was experienced in the feeding of the dog with the result that the nitrogen intake was not constant.

As the fact had now been abundantly proved that digestion products could not only maintain nitrogenous equilibrium but even lead to storage of protein in the lower animals, it was of interest to see whether the same result could be obtained in man. Abderhalden, Frank and Schittenhelm (49) were fortunate enough to obtain a human subject, a boy of twelve, with a stricture of the oesophagus and on whom gastrotomy had been performed. The experimental material used was flesh completely digested by trypsin and erepsin. The result of feeding per rectum for fifteen days with these digestion products as the main source of protein was that nitrogenous equilibrium was at-

tained and there was even a certain retention of nitrogen. The body weight increased and the general condition was good. Unfortunately the experiment could not be prolonged as towards the end the enemata were not well retained and this was of course followed by a diminution in absorption.

The Value of Asparagine.

The nutritive value of amides like asparagine is another question very closely related to that of the maintenance of nitrogenous equilibrium and the growth of animals fed on mixtures of amino acids. It has been very actively discussed by agriculturists, as amides play a large part in the nutrition of the herbivora. It is, however, one of great interest from a general point of view, particularly as regards the question of resynthesis in the body; if it can be proved that an animal can thrive when fed on a single amide or an amide mixture it is indirect evidence in favour of a transformation taking place of amino acids one into another.

Mercadente (280) was one of the earliest workers to suggest that the formation of protein could take place from asparagine, particularly in plants. He believed that a decomposition first took place. Schulze (363) also recognized the possibility of such a synthesis occurring, although he found it difficult to believe that the simple direct union of the amide with the nitrogen-free substance could yield protein. Sachse (346), on the other hand, thought that asparagine formed protein simply by the addition of fatty aldehydes. O. Loewi (258) believed that asparagine was converted into protein in the presence of carbohydrate by a series of condensations.

As regards animal metabolism, Zuntz (426) suggested many years ago that in the herbivora before utilization the amide was built up into a protein by the aid of "Pansen" bacteria and that the animal lived on the protein thus formed. Müller (291) states that he has definitely proved that this hypothesis of Zuntz is correct. He found that these bacteria could form, not only from asparagine, but also from ammonium tartrate, higher molecular nitrogenous substances which in part resemble native protein, and in part peptone. The peptone formation represented about 39 per cent. of the asparagine used, and the active protein about 10 per cent. In the ammonium tartrate experiment after twenty-four hours' incubation about 28 per cent. of the total

nitrogen present was in the form of native protein and 67 per cent. in that of peptone. In support of his work he quoted Gerlach and Vogel (152) who stated that protein-forming bacteria were widely distributed in nature (in the soil) and that if the conditions were suitable these bacteria acted rapidly and well. They found that nitrogen in the form of nitrates was converted quantitatively into insoluble protein in the presence of glucose. Ammonium salts could also undergo the same change, but it proceeded at a slower rate. Müller further carried out a series of feeding experiments with this bacterial protein which he prepared in sufficient amount and found that if it were added to the diet of a dog it could entirely replace the ordinary protein supply. Schulze (364) in a general discussion of this question holds that this conversion of the amide into protein is probably correct. He thinks the fact that ammonium acetate acts as well as the asparagine strongly supports this contention. He does not come to any final decision as to how the bacteria really bring about this synthesis.

Much other work exists in favour of asparagine acting as a partial substitute for protein, and it would seem that the form in which it is given, plays quite an important part. Thus Lehmann (249) found that he could obtain a greater protein sparing effect and even a retention of nitrogen, if the asparagine were given embedded in celloidin, in order to cause slow utilization, than if the asparagine were simply given free in the food to the same animal. Müller (293) confirmed this work. He found that when the asparagine embedded in the celloidin was given to dogs there was a retention of nitrogen in the body practically equal to twice that found when asparagine was given free. He also stated that if equal amounts of asparagine and serum albumin were given by this celloidin method (the difference in caloric value between the two being made up by means of carbohydrate), they acted equally well in bringing about retention of nitrogen. Kellner (218), on the other hand, in his experiments was quite unable to find any difference between the action of free asparagine and asparagine embedded in celloidin.

Voltz (413) and Voltz and Yakuwa (415) tested the effect on dogs of the addition of different nitrogenous substances, ammonium acetate, acetamide, glycine and asparagine and a mixture of all four, to a ground ration consisting of meat, rice, lard and bones. They found that there was no marked retention of nitrogen after the addition of asparagine and that glycine was more or less indifferent in action. Acetamide, on the other hand, caused a retention of nitrogen of about 0·2 grm. per diem, and ammonium acetate a retention of 0·4 grm. nitrogen per diem. Munk (294) denied that asparagine could be even

considered a protein sparer in dogs. Mauthner (277) also tested asparagine as a protein sparer on the dog, but without decisive result. On the whole, he inclined to the belief that there was evidence of a limited sparing action.

As regards the herbivora asparagine is claimed by many workers to be a very efficient protein sparer (Weiske, 418). Voltz (414) maintained that amide bodies could replace about two-thirds of the protein in the food of adult ruminants. He concluded that, in all probability, the herbivorous organism could build up its highly complex protein out of a comparatively limited selection of amide bodies. The figures given, if not absolutely convincing, are of great interest. Von Strusiewicz (389) also showed that sheep could have a very large proportion of the protein in their diet replaced by amide nitrogen.

On the other hand, there is much contrary experimental evidence to the retention of nitrogen in animals fed on asparagine as their source of nitrogen. Politis (333) could not obtain any evidence of the protein sparing power of asparagine, when this substance was fed to rats as the sole source of nitrogen, with an otherwise abundant diet, and the same result was obtained by Henriques and Hansen (189). These workers found, however, that although amide substances obtained from young growing plants conld not replace the nitrogen of the food, they could exert to a limited extent a protein sparing action.

In a very interesting paper on this question Kellner (219) pointed out that the protein sparing action of asparagine could only be demonstrated on a protein free or protein poor diet. If it were given with a diet rich in nitrogen there might even be a stimulation of the nitrogen metabolism. He also observed the same sparing action of ammonium acetate when in a protein poor but carbohydrate rich diet. Lüthje (269) demonstrated that it was impossible to keep rabbits alive on an abundant carbohydrate diet when the sole source of nitrogen was the protein free amide material obtained from fresh potatoes.

Viewing the evidence as a whole it would appear that amides and even certain ammonium salts are capable of replacing a certain amount of protein in the diet, i.e. acting as protein sparers, but are certainly not capable of replacing all the protein in the diet. The results of these experiments do not then contribute much towards the elucidation of the question of the transformation of the amino acids. It is always possible that even in those experiments in which the amide was shown to have a marked sparing action that the positive result was due to the presence of the essential substances in the other protein material given. In the case of the ammonium salts

where protein sparing action has been exhibited there must apparently have been a certain amount of conversion (see Embden and Schmitz' work, p. 119) or transformation of ammonium salts into amino acids as, unlike the amides, these salts could not bring about sparing action by contributing to the energy needs of the body.

The Fate of Amino Acids.

So far consideration has been given to feeding experiments with digestion products of proteins—mixtures of amino acids, simple and compound, known and unknown. The point now to be discussed is the fate of the amino acids introduced into the body either singly or in groups, and either by the mouth or parenterally. It would appear, that amino acids are utilized when the active form natural to the body is fed or injected, whilst the other form is excreted unchanged. Likewise when a racemic amino acid is given, the natural form is burned and the abnormal one appears unchanged in the urine. Levene and Meyer (255), for instance, found after feeding an animal with natural alanine that all the nitrogen appeared in the urine within twenty-four hours as urea, 90 per cent. of it being excreted within nine hours. On the other hand the optical antipode to the natural alanine (l. alanine) was only partially converted into urea (68 per cent.); the rest appeared in the urine as such. Natural l. leucine was more slowly broken down, about 54 per cent. appearing as urea in the first twenty-four hours, the remainder in the following twenty-four hours. Natural l. phenylalanine was all converted into urea, but the operation as in the case of leucine was slow; d. phenylalanine was only converted into urea to the extent of 31 per cent. Again the nitrogen of aspartic acid was removed to the extent of 86·6 per cent. in twelve hours, while the optical antipode to the natural l. aspartic acid was only excreted in the form of urea to the extent of 31·6 per cent. Arginine nitrogen was found to be excreted as urea to the extent of 97 per cent. Thompson (395) also found in the case of arginine that the nitrogen was largely excreted as urea. Differences in the fate of injected polypeptides have been noted mainly by Abderhalden and his coworkers. Abderhalden and Bergell (7), for instance, found that although glycine when injected subcutaneously into the rabbit was burned completely, yet when the dipeptide glycylglycine was injected glycine appeared in the urine, but on the other hand if glycyl-l.

tyrosine (Abderhalden and Rona (11)) were injected combustion was apparently complete, as neither substance was discovered in the urine. Later as the result of both feeding and injecting a dog with the simple amino acids, glycine and alanine, with polypeptides like glycylglycine and diglycylglycine and alanyl-alanine and diketopiperazines, like glycineanhydride and alanineanhydride, they showed that the breakdown of these substances in the body of this animal was complete, the nitrogen of the different products injected being excreted for the most part as urea. They concluded further that the breakdown of protein in the tissue resembled that which went on in the intestine. Apparently however the proteolytic activity of the tissue enzymes was greater than that of the intestinal ferments as polypeptides which were resistant to the action of trypsin, glycylglycine and leucylglycine, for example, were broken down to urea (Abderhalden and Babkin (14)). Another point noted was that the proteolytic tissue enzymes of the dog are apparently much more powerful than those of the rabbit. In support of this observation they quoted Schittenhelm and Katzenstein (356) who found that if d. l. alanine was injected into a dog only a small part of even the l. alanine was excreted in the urine, whereas Wohlgemuth (421) found when working with the rabbit that only the natural forms of the amino acids were combusted, the abnormal forms being excreted (Abderhalden and Teruuchi (15)).

These same authors (15) tested extracts obtained from the tissues, and found that liver extract could break down glycylglycine and leucylglycine, both of which are resistant to trypsin. Abderhalden and Hunter (16) also tested the juices obtained by great pressure from different tissues (liver, muscle, kidney) and found that they could decompose various dipeptides (d. l. leucylglycine, glycyl-d. l. alanine and glycylglycine). The decomposition took place asymmetrically, the amino acid *not* found in nature being the one which was not decomposed. Tissue juices were further tested by Abderhalden and Teruuchi (17) with like result. Dog serum was also shown to be capable of splitting one dipeptide at least, glycyl-l. tyrosine. Abderhalden and Rona (18) attempted to discover whether these ferments might not be reversible in action, but they found no synthesis. Abderhalden and Schittenhelm (19) tested again the question of the fate of racemic simple amino acids in the body. They found that in the dog the abnormal amino acid was excreted in part. Abderhalden, Gigon and London (46) carried out an excellent experiment on the injection of d. alanine into the jugular vein of a normal dog and of one with an Eck fistula. In each case part of the amino acid was

recovered from the blood which flowed from the upper cut end of the jugular vein during the period that the amino acid was being injected into the lower end. More alanine was recovered from the blood of the normal animal than of the one with the Eck fistula (i.e. with the liver cut out of the circulation) and more alanine from the urine of the operated animal than from that of the normal. Even when the alanine was fed to one of the operated dogs the alanine could be isolated from both the blood and the urine. As regards the fate of the diketopiperazines (glycineanhydride and alanineanhydride), neither in dog nor man was there definite evidence of either being attacked, whereas in rabbits the breakdown took place with the subsequent excretion of part of the amino acid in the urine. Abderhalden (1) thought that the anhydride compound was probably first converted into the dipeptide, then to the simple amino acids. Abderhalden and Walker (30) showed later that part of the anhydride appears as such in the urine. Among other workers in this field are Stolte (387), Plant and Reese (326), Friedmann (146). Their results confirm the data previously given.

The Administration of Amino Acids as a Test of Functional Activity.

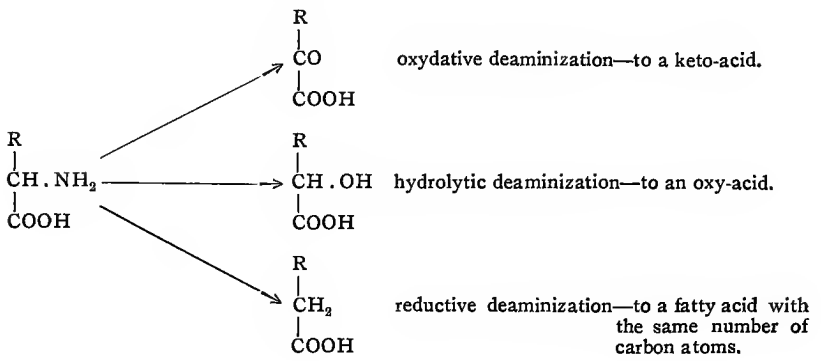
The fact that the administration of certain amino acids leads to a rise in the output of urea in the urine has been known for many years (Nencki and Schultzen (299), Salkowski (350)), but not until the β-naphthalene sulphochloride method was introduced was there any practical means of ascertaining whether part of the administered amino acid was excreted unchanged. With the introduction of this method which is roughly quantitative, many new observations have been made. Glaessner (155), for example, has made practical use of this utilization of amino acids in the body for testing the functional activity (particularly of the liver in his opinion) of the tissues and organs. He determined first the amounts of amino acids which could be dealt with by the normal tissues (liver?), and found that 25 grm. alanine, 25 grm. aspartic acid, 25 grm. glycine and about 20 grm. leucine could be dealt with, the nitrogen of the amino acids given appearing as urea. He then tested the tissues under different pathological conditions, and obtained the results given in the following table:—

Pathological Condition.	Amino Acid Given.	Dose.	Result.
Fatty liver.	Aspartic acid.	20 grm.	Marked rise in output of amino acid in the urine.
Syphilitic ,,	Alanine.	20 grm.	Marked rise in output of amino acid in the urine.
Cirrhotic ,,	Glycine.	25 grm.	Rise in output of amino acid equal to about 70 per cent. of the glycine taken.
,, ,,	,,	25 grm.	Rise equal to about 51 per cent. of the glycine taken.
,, ,,	Aspartic acid.	25 grm.	Rise in output equal to about 40 per cent. of the aspartic acid taken.
,, ,,	,,	25 grm.	About 87 per cent. of the aspartic acid taken excreted.
,, ,,	,,	25 grm.	About 90 per cent. of the aspartic acid taken excreted.
,, ,,	Alanine.	20 grm.	About 51 per cent. of the alanine taken excreted.
Phosphorus Poisoning.	Glycine.	20 grm.	Very marked rise in the output of amino acid in the urine.

Bergell and Blumenthal (61) have also demonstrated that, under pathological conditions, an amino acid, tyrosine, which was not excreted as such after subcutaneous injection into the normal animal, appeared unchanged in the urine if the injections were made after removal of the pancreas.

Mode of Catabolism of the Amino Acids.

In the present monograph it is not intended to discuss the course of the catabolism of the amino acids from the purely chemical standpoint, as this will be dealt with in the monograph by Dr. Dakin. It may be said that after the removal of the amino group by the deaminizing ferments (see p. 52) the fate of the nitrogen-free residue resolves itself into the fate of an ordinary fatty acid. Within recent years there has been a most active discussion concerning the chemical processes which are involved in deaminization. Among the most active workers in this field are O. Neubauer and his pupils. Neubauer (300) has published an excellent paper dealing with the problem and he has drawn up the following scheme of the different forms of catabolism which the amino acids undergo :—



Sugar Formation from Amino Acids.

One problem connected with the disposal of the non-nitrogenous rest which is of considerable interest may be briefly referred to here, namely the production of carbohydrate from this rest. Although it is generally supposed that the carbohydrate of the food is mainly concerned with the energy needs of the organism it is gradually becoming apparent that carbohydrate is closely concerned with other tissue requirements. Lusk (268) has carried out much important work on this subject. He has demonstrated that glycine and alanine are converted into glucose and that three of the carbon atoms contained in aspartic and glutamic acids are also so converted. As regards the sugar production from meat he has found that fifty-eight parts of glucose may be formed from one hundred parts of meat, and he has further calculated that forty-five per cent. of the total sugar production from protein in diabetes may arise from the four amino acids glycine, alanine, aspartic and glutamic acids. Among other workers who have investigated this problem are Embden and his pupils (113, 114, 115, 116), Glaessner and Pick (156), and Halsey (166).

CHAPTER IV.

DEAMINIZATION.

General.

THE question of deaminization of the protein molecule must now be dealt with. Evidence has been gradually accumulating which points to the deaminizing capacity of the tissues, as being one of the fundamentally important activities. It has long been known that shortly after the ingestion of protein there follows a marked rise in the output of nitrogen in the urine for the most part in the form of urea (see p. 77). The cause of this rise has long been a matter of speculation: did this nitrogen come from the protein ingested, or did it come from the tissues? The work of Nencki and his school helped to show with some degree of certainty the important part played by the ingested protein digestion products, but it was not until modern experimental methods were introduced, such as those of Lang, that more or less direct evidence was obtained in favour of the view that the urea could come direct from the ammonia liberated by the deaminizing activities of certain of the tissues. To meet the objection that the energy loss would be considerable if an extensive deaminization took place, direct estimations of the energy value of certain amino acids and of the products resulting from deaminization have been carried out. The following table drawn up by Leathes[1] shows that no material loss occurs:—

	Calories per 1 Gram.	Calories per 1 Gram Molecule.	Difference Per Cent.
Leucine	6·52	854·9	} 3
Caproic acid	7·16	830·2	
Leucic acid	—	—	—
Alanine	4·36	389	} 5·7
Propionic acid	4·95	366·9	
Lactic acid	(?) 3·7	(?) 338	15
Glycine	3·13	235	} 10·4
Acetic acid	3·49	209	
Glycollic acid	2·10	160	22

[1] Leathes, "Problems in Animal Metabolism," p. 154.

These results show that the loss is inconsiderable, even as low as 3 per cent. What most workers lose sight of in considering this question is the fact, that the actual demand for nitrogen by the body is not high, and that the fundamental use of nitrogenous food stuffs is to repair protein tissue waste and not to supply energy in the form of a compound of a fatty acid and ammonia. Protein must be regarded simply as a suitable and convenient compound for the introduction of a certain amount of organized nitrogen into the tissues. Too much stress is laid on the quantity of protein introduced. Protein is of importance to the tissues not because of any inherent virtue in itself, but merely because it contains within its molecule certain compounds of nitrogen more or less ready for building purposes.

Lower forms of life can build up the amino acids for their protein formation from ammonium salts or nitrates present in their nutritive mixtures, although they utilize the amino acids and flourish amazingly on them if given the opportunity. Abderhalden and Rona (12) found that *Aspergillus Niger* on a potassium nitrate medium could form its protein which contained glycine, alanine, leucine, glutamic and aspartic acids. Plants work on the same principle, but are even more adaptable, as certain members can utilize the atmospheric nitrogen when required, a power which animals never possess, as Oppenheimer (308) and Krogh (231) have so clearly shown.

This deaminizing activity is fundamental in its nature, probably it is essential for synthetic activity. The idea that such a splitting of the protein molecule into a nitrogenous part and a non-nitrogenous part takes place is by no means a new one. Voit many years ago accounted for the comparatively rapid output of nitrogen and the comparative slow output of carbon after a protein meal on the grounds that soon after absorption there was a splitting of the protein molecule into a nitrogen rich part which was rapidly dealt with, and a nitrogen poor part which was more slowly utilized.

The Presence of Ammonia in the Portal Blood.

The first real experimental advance made as regards the rapid deaminization which takes place during or soon after the process of absorption was the work of Nencki (298) and his pupils. These workers, when experimenting with dogs on which the Eck fistula operation had been carried out, found that the portal blood contained three to four times more ammonia than the systemic blood. Further that the ammonia content of the systemic blood approximated to that of the portal after an Eck's fistula was made, i.e. the liver, cut out of the circulation, no longer acted as a check to the entry of ammonia into the systemic blood. They also noted that the gastric and intestinal mucous membrane contained more ammonia at the height of digestion than when at rest. Horodynski, Salaskin and Zaleski (203) confirmed this work, and found that, although there was a definite increase of ammonia in the portal blood compared with the systemic blood during digestion, even during starvation the portal blood contained more ammonia than the systemic. They also noted that the ammonia content of the tissues and organs (the intestinal mucous membrane, liver, etc.) intimately connected with the absorption and utilization of food was diminished during starvation. Biedl and Winterberg (64) denied that the portal blood contained, as a rule, more ammonia than the systemic, although they admitted that the portal blood contained at times more than the average amount of ammonia. They maintained that the previous results of Nencki were largely due to faulty technique. As their work was carried out in 1902, and a reliable method is still lacking for the quantitative estimation of ammonia in protein tissues and fluids, their objection cannot be too seriously considered. The comparatively recent work of Cohnheim with living tissue (97) gave almost direct evidence of deaminization taking place during absorption. He found that if isolated fish intestine, into which he had placed proteose solution, were floated in Ringer's solution, partial deaminization took place, as shown by the fact that ammonia and an unknown base appeared in the Ringer solution. In conjunction with Makita (98) he repeated this experiment introducing into the intestine glycine and tyrosine as test substances. Glycine yielded ammonia, or perhaps more correctly a volatile base, and from tyrosine ammonia was obtained. Similar experiments were tried with the intestines of dogs and cats, but without much success.

Deaminizing Capacity of Tissues.

As regards the presence of enzymes in the tissues which led to the formation of ammonia, the work of Loewi (260) and of Jacoby (211) was the earliest, although their evidence was not very complete. Jacoby found that in the fluid which he obtained by pressure from pulped liver tissue the amount of ammonia increased after incubation at 40° C. This ammonia, he believed, was derived from substances like amino acids which were not deaminized by boiling with acids. Loewi (260) showed that the amino group in glycine was converted by the action of liver pulp into a substance which, although it was not urea, resembled this substance, particularly as regards the ease with which it could give up its ammonia. Lang's paper (238) was the first serious attempt to attack the question of deaminization by testing the action of the different tissues on the amino acids themselves. Lang obtained results which showed that the deaminization in the tissues was extremely active, although there was a certain degree of specificity, i.e. some amino acids were quite untouched by one tissue, whilst actively broken down by another. Thus glycine was much more readily broken down by the intestine than the liver, and it was not attacked by the spleen tissue at all. He found, too, that the amides, asparagine and glutamine, gave up their ammonia very readily in the presence of any tissue. The short experiments which he carried out with fresh tissue under aseptic conditions gave better results than the long experiments, in which he used material preserved by means of an antiseptic.

Although a great deal of stress has been laid on these experiments of Lang, which were carried out in 1905, so far as I am aware, no full and direct confirmation has been published. The facts observed fitted in with the current beliefs—strengthened them indeed—with the result that they received but little criticism. Miss Bostock (71) has recently reinvestigated this question, and has repeated some of Lang's experiments. She found that Lang's main contention was true that deaminization took place, but she also found that the degree of deaminization was much less marked than Lang described. She further found that Lang's results with tissue pulp with and without antiseptic could not be fully substantiated. Like Lang, she showed that the amide bodies yielded their nitrogen with greater readiness than the amino acids. Of course, on account of the well-known capacity of many bacteria to deaminize, one of the objections which has been raised against the so-called "aseptic" autolytic experiments is that they are not really

aseptic and that the breakdown found is due to bacteria. Miss Bostock found that it was almost impossible to get a real aseptic test. She demonstrated, however, that although part of the ammonia given off might be due to the bacterial action, additional deaminization due to ferments present could be proved to take place. She also carried out some observations on foetal tissues, and found that the deaminizing activity started at a very early age.

Both Lang's and Miss Bostock's experiments are at one in showing that after digestion with the different organ pulps an amide like asparagine gives up its nitrogen with greater ease than an amino acid like glycine. These *in vitro* observations are quite contrary to the results which have been obtained when these two particular substances are given by the mouth. Levene and Kober (254), for example, found that if glycine were given *per os*, practically the whole of its nitrogen appeared rapidly in the urine as urea, whereas in the case of asparagine there was quite a distinct retention of part of the nitrogen. (It is admitted that this is no direct evidence against complete deaminization of asparagine and the subsequent retention of part of the nitrogen liberated, but such a condition is not likely to occur. If retention take place it almost certainly occurs in a compound form, i.e. part of the nitrogen is still in connexion with the carbon chains.) Further, they noted that if asparagine were given to a protein starving animal nitrogen was retained, whereas when glycine was given no retention of nitrogen took place. Miss Bostock also carried out a few experiments in connexion with this retention, and she confirmed the observation of Levene and Kober. She found that within eight hours practically all the nitrogen of glycine when given *per os*, appeared in the urine as urea, but in the case of asparagine in the same time only about 63 per cent. of the nitrogen reappeared as urea in the urine. Apparently then in the digest conditions *in vitro*, the amino group attached to the carboxyl group of an amide, is much more readily split off than the amino group in the α position of an amino acid, whereas *in vivo* a certain protection is given to the amide which is not extended to the amino acid.

At any rate this evidence, slight as it is, suffices to show that the method of research instituted by Lang for the investigation of the deaminizing capacity of tissues is by no means representative of the changes which go on within the normal living tissues.

Evidence from the Fate of Amino Acids.

Other evidence in support of deaminization being a normal intra-vital change has come from the many experiments which have already been given in detail (p. 44) carried out on the feeding of animals with different amino acids. Thompson (395) found in the case of arginine that the nitrogen was largely excreted as urea; the amount excreted, however, differed to some extent in the different animals, and apparently with different diets. Part of the urea was excreted at once, part more slowly. He believed the latter—the slow excretion—came from the deaminization of the ornithine moiety of the arginine molecule. If the arginine were injected subcutaneously there was a much greater proportion of its nitrogen excreted as urea. Neuberg and Langstein (304) also showed that after the administration of large doses of alanine to rabbits, lactic acid, the denitrified product, could be found in the urine. The appearance of the oxyacid, lactic acid, is contrary to what one would have expected from the work of Neubauer, who maintains that pyruvic acid, a keto acid, is formed on deaminization of alanine. In experiments which exclude any possible bacterial action, Mayer (281) has shown that after the subcutaneous injection of diamino-propionic acid there was a small output of glyceric acid (a di-oxy acid). The formation of homogentisic acid from tyrosine and phenylalanine in cases of alkaptonuria is direct evidence of deaminization taking place readily. Neubauer and Falta (301) believed that the change consisted in the formation of a keto acid and that the original observation of Blendermann, that after the administration of tyrosine, p. oxy-phenyl-lactic acid was found in the urine, was wrong. This contention of Neubauer about the keto acid formation, however, is not universally accepted. Kotake (227), for instance, maintained that p. oxy-phenyl-lactic acid could be obtained from the urine only after giving a large amount of tyrosine. Neubauer and Fischer (302) have shown that phenylglyoxylic acid is formed, if the isolated liver be perfused with fluid containing phenylaminoacetic acid. In purine metabolism also deaminization is one of the stages in disintegration as for instance during the conversion of adenine and guanine to hypoxanthine and xanthine.

Deaminization in the Lower Forms of Life.

If we turn now to the lower forms of life we find that deamination is a normal function of both moulds and the micro-organisms of putrefaction. Shibata (372) made a dry acetone preparation of *Aspergillus Niger* and tested its deaminizing activity on various substances. It split off ammonia from urea, biuret, acetamide, asparagine and alanine but did not decompose guanidine, allantoin and uric acid. In the case of asparagine the yield of ammonia was small, and he concluded on quite insufficient evidence that in this case the ammonia came from the amino acid NH_2 group and not from the amide NH_2 group. The deaminizing capabilities of bacteria have been investigated by Brasch and Neuberg (74), Neuberg and others (303), Brasch (73), Borchardt (69), whilst the action of yeast has been studied by Ehrlich (109). Weinland (417) in an extremely interesting paper on the excretion of ammonia by fly larvæ gave excellent evidence in support of deamination. The Calliphora larvæ are flesh eaters, and Weinland found that in the course of larval development, when much flesh was eaten, ammonia was given off in large amount.

From all the evidence cited we may conclude that the process of deamination is a fundamental one in the metabolism of proteins, and particularly of that portion of the protein which serves for dynamic purposes.

CHAPTER V.

INFLUENCE OF THE FOOD ON THE COMPOSITION OF THE TISSUES.

THE disposal of the protein, both in its natural and in the abiuret condition has now been dealt with. The question now arises, if the food be of a highly specific nature will it influence in any way the composition of the protein of the tissues.

Evidence from Feeding Experiments.

Abderhalden and Samuely (26) were the first to offer direct evidence on this question. They attempted to influence the composition of the serum protein by feeding a horse with gliadin which contained 36·5 per cent. of glutamic acid, whereas the serum globulin of the horse contained only 8·5 per cent. and the serum albumin 7·7 per cent. The tyrosine content of gliadin is 2·37 per cent. In their experiments they tested the tyrosine and glutamic acid content of (1) normal serum protein, (2) serum protein after starvation, and (3) serum protein after feeding with gliadin. The horse was firstly thoroughly bled, it then fasted for eight days, and was subsequently fed with gliadin, which was given in large quantities, for two days (Experiment I). After a period of rest it fasted again for six days (Experiment II). Blood was removed from the horse in Experiment I both during and after the gliadin feeding, but only after feeding in Experiment II. The tyrosine and glutamic acid content of the serum protein removed at the different periods is given in the following table :—

	Experiment I.				Experiment II.		
	Percentage of Substance Present in the Blood Removed During—						
	Before Period.	Hunger Period.	Gliadin Feeding Period.	After Period.	Before Period.	Hunger Period.	After Period.
Tyrosine.	2·43	2·60	2·24	2·52	2·50	2·55	2·48
Glutamic Acid.	8·85	8·20	7·88	8·25	9·52	8·52	8·00

It will be noted that, under the above conditions, the food has had no influence on the composition of the proteins of the serum. The authors held that the necessary change took place in the intestinal wall, the extra glutamic acid having been split off and probably absorbed separately. Abderhalden, Funk and London (41) again investigated this question. In this series of experiments they used dogs with Eck's fistula and fed the animals with food of known composition (of known glutamic acid content). The blood was examined before and after the special feeding. The foods used were flesh, egg albumin and gliadin. As the result of their experiments they concluded that no food protein was to be found in the blood and that by the chemical method as before they were unable to discover any influence of the constitution of the food on the constitution of the serum protein, more especially as regards the content in glutamic acid. They also examined the nature of the protein in the blood cells but found that it too was unchanged. Then Abderhalden, Gigon and Strauss (40) showed that although the animals were fed on widely differing foods, the tissues of cats, rabbits and hens were practically identical in constitution, i.e. with about 3·3 per cent. of glycine and over 13 per cent. of glutamic acid. Orgelmeister (310) again has shown that the arginine content of tissues could not be altered by feeding the animal on foods rich in arginine. Nor was he able to alter the arginine content of the tissues by the administration of substances like benzoic acid which might have been expected to combine with the arginine.

Gitkins (153) studied the composition of the blood during hunger, and found, like Burckhardt (79), Lewinski (256) and others, a distinct increase of the globulin fraction of the serum during hunger. He also noted that if bread were used in the subsequent feeding of the animal the blood more rapidly returned to its normal composition than when a meat diet was given. He suggested that the albumin of the serum came from the food, and the globulin from the tissues. It must not be forgotten, however, that Moll (287) has stated that he could convert *in vitro* albumin into globulin with the greatest of ease by heating the serum to 58° for a short period.

Composition of the Tissues.

Pflüger and the majority of workers in this field held that the composition of the tissues did not appreciably alter by altering the nature of the food. Rubner, on the other hand, maintained that the composition of the tissues could be altered if they be deprived of certain food materials. Stockhausen (386) carried out elaborate analyses of the tissues of two sets of dogs. An adult and a young growing dog were fed on a protein (flesh) diet and an adult and young growing dog were fed on a diet of rice. He found that the muscles of both the flesh-fed dogs contained more nitrogenous material than the rice-fed; those of the adult dog fed on rice contained 88 per cent. of nitrogen, on flesh 94 per cent.; those of the young dog fed on rice contained 93 per cent. nitrogen, on flesh 94 per cent. A like condition was also noted in the case of the liver. The liver of the adult dog fed on rice contained 65 per cent. of nitrogenous material and that on flesh 88 per cent.; in the case of the young growing dog when rice-fed the liver had 66 per cent. and when flesh-fed 91 per cent. of nitrogenous material. Stockhausen further made some interesting observations on the relation of nitrogen to carbon in the tissues of his rice- and flesh-fed dogs. He found that in the case of the rice-fed dogs, taking both together, the ratio of nitrogen to carbon was as 1 : 3·30 and that in the flesh-fed dogs it was as 1 : 3·28. Therefore in young growing dogs, when the tissue growth and exchange was extremely active, very decided change in diet caused but little or no alteration in the composition of the tissues.

Müller (292) carried out a very interesting experiment in this connexion. He fed a dog for six weeks on a meat-free diet of rice, lard, salts and water, and at the end of this period of feeding he amputated a hind limb the muscles of which were used for an examination of the composition of the tissues. After recovery from the operation he fed the animal for another six weeks on large quantities of horse flesh, and at the end of the period the animal was killed and again the appropriate tissue was taken for examination. As a result of his analysis Müller came to the conclusion that a special nitrogenous store material was formed which differed in its elementary composition from muscle protein, as it was particularly rich in nitrogen. He believed that the formation of this nitrogen-rich carbon-poor store substance accounted for the large retention of nitrogen which had been observed from time to time after a diet rich in protein.

Zisterer (425) has also investigated this question. In the first place

he compared the composition of various proteins, gliadin, glutenin, and caseinogen, with muscle protein (syntonin) and has compiled the following table of the amino acid (partial) content in 100 grms. of protein :—

	Syntonin.	Caseinogen.	Gliadin.	Glutenin.
Alanine	4·0	0·9	2·7	0·3
Leucine	7·8	10·5	6·0	4·1
Glutamic acid . . .	13·6	11·0	31·5	24·0
Tyrosine	2·2	4·5	2·4	1·9

He argues that if these different proteins, after decomposition in the intestine and absorption were utilized for the formation of the muscle, then a variation in the constitution of the muscle protein ought to be found. In order to test this point small quantities of these different proteins close to the minimal protein requirement were given but no very marked differences were found in the minimum amount required to get the dog into nitrogenous equilibrium. This result did not correspond with what was expected from an interesting calculation which he gave at the end of his paper as regards the relationship in amino acid content of the different proteins used. Putting down 100 as the constant for each amino acid in syntonin, the following figures are the values for his other proteins :—

	Syntonin.	Caseinogen.	Gliadin.	Glutenin.
Alanine	100	22	67	8
Leucine . . .	100	134	78	52
Glutamic acid . .	100	81	232	177
Tyrosine . . .	100	205	109	49

From these figures he calculated the following :—

Syntonin.	Caseinogen.	Aleuronat.
100	444	267
100	74	154
100	124	49
100	47	102

i.e. four to five times as much caseinogen and two and a half times as much aleuronat must be given in order to yield the amount of alanine

necessary to form syntonin, and so on for the other amino acids. This calculated result is extremely interesting in the light of the actual result. It suggests that a certain transmutation of amino acids can and does take place (see later p. 61).

Growth of Moulds.

Some interesting work has also been carried out on the influence of the nutritive medium on the growth and composition of moulds. Abderhalden and Rona (12), for example, found that there was no apparent alteration in the composition of the protein obtainable from *Aspergillus niger*, when it was grown upon culture solutions in which the nitrogen present was in three different forms: (1) potassium nitrate, (2) glycine, and (3) glutamic acid.

Variation in the Composition of Proteins.

Another question of fundamental importance in discussing the minimum requirement of protein, more especially in the light of Michaud's (284) work, is whether any marked difference can be detected in the constitution of proteins, obtained from different sources in the animal and vegetable kingdom. Osborne and Jones (315) have carried out some extremely valuable work along this line. They examined the muscle obtained from chicks (312), from fish (313), and from the scallop (314). When the various muscles were compared very marked differences in their amino acid content were found. They also showed that the constitution of syntonin was very different from that of muscle itself. They obtained a smaller yield of leucine, proline, phenylalanine, aspartic and glutamic acids and lysine from the former. They came to the general conclusion that glycine, alanine, valine, leucine and proline increase in proportion as we go from the lower to the higher forms of life. The table on page 61 copied from their paper demonstrates their conclusions very clearly.

These workers (315) have also found that the different vegetable proteins differ markedly in composition. The proteins of the leguminous seeds most nearly approximate in composition to muscle protein. They suggest that this is probably the explanation why these seeds have proved to be such valuable food stuffs. [1]

[1] For fuller details, see Plimmer's monograph, Part I., 2nd edition in this series.

Substance.	Scallop.	Fish.	Chicken.	Ox.	Syntonin Ox.[1]
Glycine . . .	0·00	0·00	0·68	2·06	0·50
Alanine . . .	+ ?	+ ?	2·28	3·72	4·00
Valine . . .	+ ?	0·79	+ ?	0·81	0·90
Leucine . . .	8·78	10·33	11·19	11·65	7·80
Proline . . .	2·28	3·17	4·74	5·82	3·30
Phenylalanine . .	4·90	3·04	3·53	3·15	2·50
Aspartic acid . .	3·47	2·73	3·21	4·51	0·50
Glutamic acid . .	14·88	10·13	16·48	15·49	13·60
Serine . . .	?	?	?	?	?
Tyrosine . . .	1·95	2·39	2·16	2·20	2·20
Arginine . . .	7·38	6·34	6·50	7·47	5·06
Histidine . . .	2·02	2·55	2·47	1·76	2·66
Lysine . . .	5·77	7·45	7·24	7·59	3·26
Ammonia . . .	1·08	1·33	1·67	1·07	0·83
Tryptophane . .	Present	Present	Present	Present	?
Total . . .	52·51	50·25	62·15	67·30	47·11

Variations during Development.

Abderhalden and Kempe (23) carried out some experiments on the composition of the egg at different periods of incubation. They found that tyrosine was the only amino acid which altered in amount and that this alteration was very small: 100 grms. dried fresh egg contained 1·82 grm. tyrosine; after ten days incubation at 40° there was 2·11 per cent. of tyrosine and after twenty days' 2·25 per cent (in these analyses the embryo was also included). The amount of glycine and glutamic acid, the other two substances estimated, hardly varied during the incubation. They concluded that there was no new formation of amino acids during development.

Transmutation of Amino Acids.

Another important question, in this connexion, is whether the organism always breaks down a specific protein in the same fashion—does any variation ever occur in the amount of individual amino acids or the compound polypeptides?

Supposing the ingested protein contains 10 grms. of leucine, and the tissue protein contains double this amount, can only 50 grms. of tissue protein be formed from the 100 grms. of the food protein, or can

[1] Analysis by Abderhalden and Sasaki. "Zeit. f. physiol. Chem." 1907, 51, p. 404.

more than 50 grms. of tissue protein be formed by the conversion into leucine of other amino acids which are present in excess in the protein fed? In the case of certain of the amino acids which resemble one another very closely it is more than probable that such an interchange can take place.

This question has been investigated by Magnus-Levy (274) and others by the study of the ratio of the amount of glycine nitrogen to total nitrogen, hippuric acid being used as the product for the estimation of the glycine. With the exception of gelatin the body proteins contain on an average 4 per cent. of glycine.[1] Now as protein contains some 16 per cent. of nitrogen, and glycine 18·7 per cent. there is present in 100 grms. protein-nitrogen rather less than 5 per cent. glycine-nitrogen. As Magnus-Levy points out if in the benzoic acid experiments a higher value for the quotient (glycine-nitrogen : total nitrogen) be found than this figure (5 per cent.), then there is evidence of the production of glycine from a source other than preformed glycine. Magnus-Levy found that the quotient was larger, therefore a synthesis of glycine had taken place somewhere in the tissues. He has also pointed out as further evidence in favour of such a synthesis the fact that the protein food of a suckling animal was remarkably poor in preformed glycine—he estimated that milk proteins, at the outside, contain only from ·1 to ·3 per cent. of glycine, and yet it is found that from 100 grms. of this protein a suckling calf can in a short period build up 78 grms. of tissue protein containing about 2·5 grms. of glycine. It is suggested that in the formation of this glycine two courses are open, either (1) the *in vivo* breakdown of protein is identical with that *in vitro*, and that the other amino acids formed are converted by oxidative processes into glycine, or (2) that the *in vivo* breakdown of protein is not the same as that observed *in vitro*, in that a greater amount of glycine is formed. Magnus-Levy rather inclines to the first hypothesis. As an example of his experiments the following may be cited. A rabbit weighing 1500 grms., containing thus some 200 grms. protein with about 6·6 grms. of glycine, excreted after treatment with benzoic acid 8 grms. of glycine. During the course of the experiment the weight of the animal had fallen to about 1250 grms.; a loss of 250 grms. which (even supposing the total loss were due to protein utilized) would have only yielded a little over 1 grm. of glycine. Magnus-Levy later suggested that the glycine which was excreted in the previous ex-

[1] This value is probably less than the true value, for according to Osborne the esterification method by which the amino acid content of tissues has been estimated gives too low results.

periments might have been formed from other amino acids, such as leucine and alanine, which had combined with the benzoic acid, the resulting product having been then converted by a process of oxidation into hippuric acid. He carried out a series of experiments to test this hypothesis, but did not obtain positive results. He tested some ten preparations, but in none did he get conversion into hippuric acid.

Abderhalden and Funk (33) also investigated this question, and came to the conclusion that the only amino acid of which definite proof of formation or synthesis *in vitro* can be obtained is glycine. They also utilized the evidence obtained by feeding with caseinogen in which there is no glycine; the animal was rapidly got into a state of nitrogenous equilibrium. It must therefore be concluded that synthesis of glycine can be readily brought about with little or no strain on the protein anabolic processes. They were unable to confirm an observation of Henriques (186) on the synthesis of lysine in the animal body (rats). Henriques stated that although zein, which lacked glycine, lysine and tryptophane, could not maintain life when given in the food as the sole source of nitrogen, yet under the same conditions this could be successfully accomplished by gliadin, which lacked lysine. The deduction was that either lysine was not required or that it could be synthetized from some of the other amino acids present. Abderhalden and Funk repeated this experiment on a dog and found that nitrogenous equilibrium could be obtained, but their analyses showed the presence of a small amount of lysine in gliadin.

Wiechowski (420) also found that there could be a greater output of glycine after dosing a rabbit with benzoic acid than could be accounted for by the amount of glycine present in the tissue proteins. He came to the extraordinary conclusion that in the rabbit glycine was the principal source of the urea, and that all the other amino acids were converted into glycine before their final destruction in the body. Such a conception does not seem necessary to explain the facts already known as regards the fate of the amino acids in the body.

On the other hand Brugsch (76) stated that the amount of glycine which was excreted after giving benzoic acid was equal in amount to that which existed as such in the tissues and which could be obtained by hydrolysis *in vitro*. Tsuchiga (400) obtained results which varied considerably and no definite conclusion could be reached. He maintained that according to the dose and the method of administration of the benzoic acid, the amount of glycine excreted as hippuric acid fluctuated greatly.

The evidence then is in favour of a synthesis of glycine taking

place in the tissues, and this change in all probability is brought about by the decomposition of some other amino acid with a longer carbon chain. Cohn (93A) has suggested that the glycine arises in part by the union of acetic acid and ammonia. Magnus-Levy, however, does not consider such a synthesis very probable.

Can a synthesis, however, of a more complex amino acid take place in the same fashion? As has already been shown (p. 63) the only evidence in favour of such a synthesis rests on a false analysis. Against such a synthesis are the feeding experiments with gelatin, which lacks most of the aromatic group of amino acids, and with zein, which lacks glycine, lysine and tryptophane. It is impossible to use either of these substances as the sole source of nitrogen for the body, and therefore we must conclude that the tissues cannot have an efficient mechanism for the conversion of lower amino acids into higher. This conclusion is not quite fair, since the aromatic groups play apparently a special rôle in the tissues, or the mechanism which controls their breakdown (perhaps also their formation) is a specialized one, as shown by the anomaly in metabolism of alkaptonuria. It is possible that those proteins, which lack certain groups, are not able to form ordinary tissue protein, but are able to form a protein or a protein-like body of a simpler type like protamine or histone. Such a substance might be retained in the tissues either as a protein reserve or as store material for building up and repairing new tissue when the necessary amino acids from some other source, e.g. a fresh intake of food, are available.

Formerly it was believed that Miescher's (285) experiments on the Rhine salmon during their stay in fresh water at spawning time afforded absolute proof of the change of simpler monamino acids into the more complex diamino acid, arginine, which was required in large amount for the formation of the protamine found in the genitalia of the salmon. Miescher found that during the growth of the testes and ovary there was a great wasting of the muscle tissue, but did not believe that it contained sufficient preformed arginine to supply the needs of the new forming protamine. Weiss (419), however, in Kossel's laboratory, demonstrated quite clearly that the salmon muscle contained an amount of arginine which amply sufficed for the formation of the protamine. In other words, a mere transference of arginine can occur in the body without the necessity of any synthetic action. Dunlop (108) has also shown that all the protein nitrogen lost by the salmon during the ascent of the rivers for spawning is not required for the formation of the testes and ovary. It may therefore be assumed that the whole

change which takes place in the salmon during the development of the genitalia is a mere change of position rather than the result of synthetic action. Noël Paton (318) has shown that the amount of fat which the salmon accumulated in its muscles during its sojourn in the salt water is not only amply sufficient to yield all the fat required by the growing sexual glands, but is also sufficient to yield the energy for an enormous amount of muscular work. In this way the protein of the muscle tissue apart from the ordinary wear and tear, is not called on to supply the energy required for movement. It must also be remembered that the salmon starts from the estuary for its ascent of the river with a very large store of some soluble protein in its tissues, and that this steadily diminishes in amount (Boyd (72)). Is this soluble protein a highly specialized one eminently suited for the formation of protamine or is it ordinary protein filling out the cells and which is used either for energy or building purposes?

On the other hand, the evidence adduced by Henriques and Hansen (187, 188) in connexion with an entirely different question must not be forgotten. These workers stated that they were able to get rats into a state of nitrogenous equilibrium when they fed them solely on the monamino acid fraction of a digest. Now if these experiments be accepted—many workers, as already mentioned, doubt the accuracy of metabolic experiments carried out on rats—then we must at the same time accept the synthesis in the organism of the diamino acids, not to speak of other complex nitrogenous bodies precipitated by the phosphotungstic acid, from simple amino acids and simple polypeptides.

Again, the experiments which have been carried out where asparagine has formed the sole source of the nitrogen supply (see Chap. III., section 2) synthesis not only of the diamino, but also of the other monamino acids, aliphatic, aromatic and heterocyclic. It may be that in these experiments the bacteria alter the asparagine before absorption takes place.

And finally the growth of moulds like *Aspergillus Niger* on a medium containing potassium nitrate or glycine (12) as the sole source of nitrogen is evidence of the synthesis of amino acids, for a protein of complex structure is formed from which glycine, alanine, leucine, glutamic and aspartic acids can be isolated.

CHAPTER VI.

PROTEIN REQUIREMENTS.

A QUESTION which must now be considered is that of the minimum amount of protein required daily by the body. The subject is one not merely of scientific, but also of economic importance. Two questions require answers. (1) Can the organism not only subsist but also thrive on an intake of protein nitrogen equal to that which is excreted during starvation? (2) Is there a single protein minimum intake?

The Protein Minimum.

The physiological protein minimum is the quantity of protein which must be ingested in order to prevent loss of protein from the body.

Voit and Korkunoff (410) were amongst the earliest workers who investigated this question. Taking the output of nitrogen of a dog on the third day of fasting as the standard for the amount of protein catabolized daily, they found that the amount of nitrogen required to prevent loss of nitrogen from the body varied very markedly with the nature of the diet, i.e. whether it consisted of pure protein (washed flesh), or protein plus carbohydrate, or protein plus fat. The physiological protein minimum was always found to be greater than the amount of protein catabolized in the tissues during hunger. Thus for every 100 grms. protein catabolized in starvation 368 grms. of pure protein, 157-193 grms. of protein when mixed with fat, and 108-134 grms. of protein when mixed with carbohydrate, had to be consumed in order to prevent loss of nitrogen. Thus the addition of fat lowered the amount of protein required from 368 grms. to 157 grms., a decrease of 57 per cent., and the addition of carbohydrate reduced it from 368 grms. to 108 grms., a decrease of 70·6 per cent. Cremer and Henderson (101) repeated some of this work, but they were unable to obtain

the extreme values of Voit and Korkunoff. Michaud (284), who has since dealt with this problem, attributed the previous failure of workers to get nitrogenous equilibrium with the fasting nitrogen output to the fact that the protein fed differed too markedly in composition from that of the tissue protein. He nearly reached the hunger minimum if he fed a protein which was of exactly the same constitution as the tissue protein, i.e. if he fed dogs on dogs' flesh. As a result of his experiments, he drew up a scale of the amounts of protein required. This scale varied according to the similarity in composition of the protein to that of the tissue. Flesh of another animal—horse flesh—fed to a dog was less valuable than dog flesh, caseinogen was still less, and vegetable protein the least. He did not believe that the apparent unsuitability of vegetable protein depended on the lack of extractives present in these proteins. Zisterer (425) has published experiments which substantiate this hypothesis of Michaud. He agreed with the general results reached, although he thought that Michaud was not careful enough in establishing his hunger value. Zisterer's figures for the ratios of the different protein material as regards the minimal requirements were as follows:—

Dog's Flesh.	Horse Flesh.	Caseinogen.	Nutrose.	Edestin.	Gliadin.
100	108	128	121	153	163

These figures bear out the conclusion reached by Michaud, that an an animal can subsist on an intake of protein which is lowest when the protein given resembles most closely the tissue protein. Recently Frank and Schittenhelm (49) have carried out some work along the same line as Michaud. They found, however, that all their dogs did not give the same results. Thus, one dog, which was tested with a protein mixture prepared from the "whole" animals of the most varied species, did not reach the lowest level of nitrogen intake with the dog preparation. On the other hand, two other dogs most unmistakably showed a diminution in nitrogen output when placed on dog flesh. They also tested a boy with goose, fish and ox flesh, and found that goose flesh took first place, and fish flesh next. Busquet (83) in a somewhat similar set of experiments on frogs found that if frogs were fed with frog muscle the gain in weight by the animal was much greater than when they were fed with the same amount of ox flesh; Billard (65) found that tadpoles fed on frogs' liver developed even better than those fed on calf-liver or algae.

Siven (374, 375) carried out a long investigation on the question of the possibility of reducing the protein intake employing an entirely different method. He experimented upon himself, using diets contain-

ing varying amounts of protein. He came to the conclusion that the fully grown human organism, for a short period at least, and without any increase of the caloric intake over the normal, could remain in nitrogen equilibrium with an intake of nitrogen of 4·52 grms. (i.e. 28·3 grms. protein, of which only about 12·5 grms. was in the form of pure protein). If the amount be reckoned per kilo of body weight, then the nitrogen requirement is 0·08 grm., of which only 0·03 grm. requires to be in the form of pure protein nitrogen. This amount of nitrogen is considerably smaller than that which was excreted on the third day of complete starvation by Succi: it was not until the third week of fasting that values in any way comparable with the figure of Siven were obtained. It is certainly true that if at the close of a fast the subject be put on a protein-free diet the output of nitrogen—an output which may reasonably be taken to represent the real protein catabolism—may reach a still lower figure. Thus it was found (89) in the case of Beauté that the output of nitrogen in the urine on the third day after a fast of fourteen days, when he was fed on a diet of starch and cream, amounted to only 2·84 grms. Landergren (237), Folin (129) and Cathcart (90) have also obtained in the normal individual remarkably low outputs of nitrogen when the subject was confined to a diet rich in carbohydrate but practically free from protein. Far-reaching conclusions concerning the actual amount of protein required daily cannot be drawn from such experiments unless it be shown that these positive results are not due to the very short duration of the experiments. The importance which is to be attached to results obtained from feeding experiments of short duration, more particularly those in which the diet lacks one or more of the normal constituents or contains them in abnormal proportion or form, is still a subject of dispute.

As regards the uniformity of the protein minimum it may be definitely stated that there is no single minimum—common to all men and to all conditions. Rubner (342), Caspari (85) and others also hold firmly to this opinion. Caspari quotes the work of Larguier des Bancels in 1903 in confirmation of this belief in the existence of multiple protein minima. The facts which can be cited against a common minimum are many in number. Thus the caloric value of the diet given influences very definitely the protein minimum intake required by the organism. Again, the nature of the food which is fed with the protein influences very materially the amount of nitrogenous material required, as is shown, for example, in the experiments of Voit and Korkunoff. Then, as Rubner has pointed out, the temperature influences quite markedly the course of protein metabolism. Finally,

another factor of considerable importance may be mentioned, the activity of the organism.

The Work of Chittenden.

As regards the practical protein minimum for an everyday dietary there is a great difference of opinion, more particularly since the interesting work of Chittenden was published. Voit had laid it down as the result of repeated experiment and observation that the daily intake of protein should be about 120 grms. Chittenden (93) believes that this amount is much too abundant, and that any person who lives up to this standard and who encourages others to do so is encouraging individual and race suicide.

He was able to maintain nitrogen equilibrium on diets which contained about 6 grms. of nitrogen, equivalent to some 40 grms. of protein, and which were in addition of very low caloric value, 27 to 28 calories per kilogram. Chittenden's investigations were not merely confined to experiments on himself, but were arranged on a large scale and carried out on three different classes of men: (1) professional men engaged for the most part in laboratory work, (2) on student athletes who were in training for various university contests, and (3) on soldiers who performed a series of regulated exercises daily. The period during which these men were under investigation was also a prolonged one, thus giving the experiment a good chance of failure. Chittenden maintained that the results were excellent both physically and mentally in all classes of individuals on an intake of protein on the average about half that of the so-called Voit standard.

As a matter of fact the intake of protein in the diet of the average individual, as evidenced at least by the output of nitrogen in the urine is not so large as is commonly believed. It has become a habit to assert that the average intake of protein in the food of the average man is large, but this assertion is probably not true, particularly in the case of those who follow sedentary occupations. Thus Hamill and Schryver (168), for example, examined the urine of seven individuals in the Physiological Laboratory of University College, London, who, during the period of examination, maintained their everyday existence, no care being exercised in the choice of food, and they found that the output of nitrogen corresponded to an intake of some 90 grms. of protein per diem. From my own experience this amount is probably a good average value.

In one recommendation at least Chittenden is absolutely at fault. He recommends a dietary containing 50 grms. of protein and of about 2500 calories as sufficient for a soldier doing hard work. This is quite an inadequate diet when judged by the standard laid down by Lieut.-Colonel Melville (278) as the result of observations on soldiers doing hard work similar to that done under service conditions. Melville found that his soldiers did well on a diet containing 190 grms. of protein and of about 3400 calories. "I have no doubt in my own mind," he writes, "that this allowance is ample, and if it errs does so on the side of generosity." But on the other hand he thought that 145 grms. of protein in a diet containing 3500 calories was as low as it was advisable to go, "and might well be increased especially when hard work is demanded of men under conditions of exposure". But even apart from these practical observations in the field it can be shown by direct estimation and calculation of the amount of work done by Chittenden's soldiers that the 2500 calories were quite inadequate for the work done. The simple fact that some of the soldiers lost as much as 8·5 kilograms in weight during the period of the experiment, which lasted about six months, showed that as the material for the supply of energy was not obtainable from the food it must have been drawn from the subjects' tissues.

Further, the work of Major McCay (270) on a people—the Bengalis—who naturally conform to the so-called Chittenden standard—shows that as compared with races which have a higher intake of nitrogen they are of inferior physique, of low endurance and activity, and are not long-lived.

Chittenden also showed that animals, dogs, fed for long periods on a diet containing a low proportion of protein did not die off suddenly, but on the contrary thrived. Benedict (59) offered a trenchant criticism of Chittenden's work in which many facts against the propriety of a low protein intake were pointed out, e.g. those of Haecker, who showed that the resistance diminishes if an animal were kept for a prolonged period on a low protein intake. Two herds of cows, ten in each, were under observation for three years, one herd being fed on a diet containing the normal proportion of protein, and the other on a diet poor in protein. No marked difference was observed between the two herds at the end of the second year, except that those on the low protein diet were somewhat less in weight; during the third year, however, the animals fed on the low protein diet began to fail, and as the animals became so ill the experiment was discontinued.

Jägeroos (213) has also investigated the problem whether the organism suffered either directly or indirectly by living on a minimal protein intake. Two pregnant bitches, in both of which the lowest limit of nitrogen exchange was about 0·2 gram per kilo, were studied; one lived for about ten months and then died suddenly, probably from some infection following abortion; the other also died suddenly after about six and a half months. (Both animals were on the diet before pregnancy commenced.) In each the food was well utilized throughout, and the general condition continued excellent almost up to the end. He discussed the question whether death was due to lack of resistance towards infection induced by the nature of the diet, or whether the resistance was lowered by the unnatural life (in the cage) and the lack of exercise. (Confinement in cages is frequently accredited with producing failure in nitrogen retention.) He concluded that the fatal results were not due to low protein intake, and that the dangers commonly associated with such a scheme of feeding were greatly over-rated, and that if a diet fulfilled all "hygienic" conditions as to amount, digestibility, etc., little attention need be paid to the amount of protein present—as long as there is enough to satisfy the tissue needs.

Quantity or Quality of Protein ?

It will be shown later (p. 77) that a rapid elimination of nitrogen follows immediately on the ingestion of protein. This being so why should so high an intake of protein be regarded by Voit and others as essential? What is the real daily protein requirement of the body? It is evident that the tissues require a fairly abundant supply of protein for purposes of repair, but it is not yet clear what exactly the daily needs are. As has already been pointed out, the tissues evidently exert a certain selective action as in the gliadin feeding experiment. Again, the different tissues differ in composition and presumably require a varying supply of protein in order to satisfy the different repair requirements. As it has not yet been definitely proved that one amino acid can be changed into another if we give a low intake of protein, we may be either starving the animal in whole or in part. To put it in another way, if we remember that the protein molecule is built up of a series of amino acids which for the present we may call A,B,C,D, and so on, and if on a certain day the body requires for purposes of

repair of a certain protein tissue x amount of amino acid K, it does not matter apparently whether the body in getting this amount of K has to discard ten times x amount of say amino acid B, and thirty times x amount of amino acid M. As Zisterer (425) has pointed out (p. 59) supposing syntonin is the substance to be formed and caseinogen be fed, then in order to obtain the necessary amount of alanine required in this synthesis between four and five times more caseinogen is required than if syntonin itself be given or, in other words to yield the necessary amount of preformed alanine for 100 grms. syntonin, 444 grms. caseinogen must be consumed. Zisterer, of course, in his calculation assumes that the amino acids required are present in the proteins and are not synthetized. Obviously, it is not so much the quantity but the quality of the protein given which is of prime importance, and it follows that on the whole it is safer to give a relatively large intake of protein than a small one. There is the further consideration that in order to obtain the requisite daily caloric intake protein must be taken in fair amount. There is a definite limit to the intake of both carbohydrate and fat on account of the difficulties of digestion attendant on too full meals of these substances. At most carbohydrate and fat supply only about four-fifths of the caloric requirements, and the balance must be made up by the addition of protein.

Feeding Experiments with "Abnormal" Proteins.

Attempts have been made to maintain life by replacing the ordinary protein of the diet by other proteins, which may be termed abnormal forms of protein, e.g. gelatin. It is, of course, a well-known fact that the members of the aromatic group of amino acids and cystine are practically completely absent from the gelatin molecule. Feeding experiments have been carried out with gelatin, and with gelatin plus the missing amino acids, but complete replacement of protein has not yet been achieved. Whether this be due simply to the amino acids not being added in proper amount, or in proper combination, or whether it be due to the absence of some other essential substance is not clear. Kaufmann (215) carried out very complete experiments on himself and dogs. He found, like many others, that only a comparatively small amount of the protein can be replaced by pure gelatin (in dogs between one-fifth and one-fourth of the protein of the diet). He stated, however, that if he replaced the caseinogen

nitrogen in his own diet by 93 per cent. of gelatin nitrogen plus 4 per cent. tyrosine nitrogen, 2 per cent. cystine nitrogen, and 1 per cent. tryptophane nitrogen, the mixture almost sufficed to prevent protein waste (no trace of the amino acids was found in the urine). In the case of two dogs which he also tested, he found that from one-half to one-third gelatin nitrogen plus 4 per cent. tyrosine and 2·5 per cent. tryptophane partially prevented loss of tissue protein. Murlin (296 A) held that, quite apart from the addition of these missing amino acids, the retention of nitrogen during gelatin feeding depended to a large extent on the nature of the remainder of the diet. He believed that the presence of carbohydrate in large amount was of paramount importance. If carbohydrate were given with the pure gelatin it was possible to replace, for a short period at least, 63 per cent. of the total nitrogen, and even to obtain a small retention of nitrogen. Rona and Müller (340), on the other hand, came to the conclusion that the capacity of gelatin to replace protein was not enhanced by the addition of 4 per cent. tyrosine and 2·5 per cent. tryptophane. In only one of their experiments was there the slightest evidence of increased sparing of protein due to this addition. About two-fifths of the protein nitrogen could be replaced by gelatin with the addition of the amino acids. Abderhalden and Bloch (24) found that, in the case of alkaptonuria, if the amino acids (tryptophane, cystine, tyrosine, phenylalanine, leucine, alanine, glutamic and aspartic acids) were added to the gelatin to make up the deficiencies, about one-half of the protein could be replaced. The experiments of Hopkins and Miss Willcock (199) on the protein-replacing power of zein, a protein which contains no tryptophane in its molecule, yielded evidence similar to that obtained from the gelatin-feeding experiments. These observers fed mice on a diet of zein, carbohydrate and fat. They found that 77 per cent. of the mice died within twenty days. After the addition of tyrosine to the food 70 per cent. died within the same period, but after the addition of the missing tryptophane only 20 per cent. died in the twenty days, and nearly 70 per cent. of the remaining animals lived over thirty days. Not only did the mice fed with the zein and tryptophane live longer, but their physical condition was much better than that of the other two series of animals.

The Influence of a "Pure" Diet.

Apart from this difference in composition, other factors influence the food, particularly the protein requirements of the body to a great extent. Experiments in which one form of protein has been given as the sole source of nitrogen for a long period demonstrate that, in spite of the abundance of nitrogen in the diet, the animal ceases to thrive.[1] This may be due to the lack of certain "minimum" nitrogenous substances in the food, but other factors apparently equally potent play a part. Much of the earlier work in this connexion was faulty owing either to the manner in which the experiments were carried out, or to the fact that the diets could not be regarded as "pure," i.e. the protein used was not absolutely free from impurities. Notwithstanding this it has been found that if animals be kept for a prolonged period on one diet they invariably die in spite of an abundant caloric intake. The early work of Lunin (267) in which the diet consisted of caseinogen, fat, cane sugar, and the ash of milk, showed that death took place much earlier if no ash were present. Further, mice fed on this artificial diet died in from twenty to thirty-one days, whereas mice fed on simple dried milk were still living at the end of two and a half months. Hall (162) who also fed mice on a mixture consisting of caseinogen, fat, starch, cellulose and ash of milk, found that death resulted within forty days. All his animals fed greedily at first. Steinitz (384) fed dogs on the same form of diet, but after a fortnight or three weeks vomiting supervened, and the experiment had to be broken off. Röhmann (339) also gave a so-called pure diet, but with greater variation in the non-protein part. He found that under these conditions his animals (mice) remained well for weeks. The diets he used cannot unfortunately be considered as "pure". The animals were practically fed on an ordinary mixed diet. He made the suggestion that the various proteins might vary greatly as regards their nutritive value and that in this variation lay the explanation of many of the poor results previously obtained. Jacob (210) found that both pigeons and rats could be kept for long periods (one of the rats lived 124 days) on a diet of caseinogen, fat, starch and salts, but that eventually death resulted. Falta and Noeggerath (125) fed rats on mixtures of absolutely pure proteins (egg albumin, casein-

[1] Recently Osborne and Mendel have published a long series of experiments which, if they be confirmed by future work, are of fundamental importance. They found that pure proteins like edestin or excelsin when fed to rats with protein-free milk suffice to keep the animals in good condition over a long period. (Carnegie Report, 1911.)

ogen, blood albumin, fibrin, hæmoglobin and blood globulin) pure fat, and pure carbohydrate, with a definite amount of salts. They also found that in spite of the variety it was impossible to keep the animal alive for any length of time. Knapp (221) fed rats on a food containing seven varieties of pure protein, together with cholesterol, lecithin, carbohydrate, fat, and salts, but despite this fact the animals died in a few weeks (nine and a half to sixteen weeks). At first they greedily consumed their food, but in the end they gradually pined and died. Finally he confirmed the statement that rats at least could not subsist on milk powder, and that even on a fat-free horse flesh or dog biscuit diet there was a tendency to early death. Death in Knapp's opinion was due partly to the diminished intake of food, the result of a diminished appetite from the monotony of the food, and partly to the concomitant defective digestion and absorption of protein.

Stepp (385), on the other hand, suggested that much of the former work on pure proteins was defective, and that the failure to maintain life was due to the lack of "lipoid" and not to the nature of the protein. He carried out a long series of experiments which clearly demonstrated that mice died when fed on milk bread, which had been extracted with alcohol, ether or sometimes with chloroform as well, whereas mice fed on the unextracted bread lived and thrived. The animals fed on the extracted bread ate with avidity for the first fortnight, but thereafter their appetites failed and they died. Another series of mice were fed on extracted bread plus the extracted material, but although the animals lived longer than mice fed on extracted bread alone they did not survive like the control animals. He held then that a certain amount of fat "lipoid"—which is not lecithin—was necessary in the diet. In this connexion the suggestion of Glikin is of interest (157) that lecithin is a material of first-class importance in the tissue metabolism, especially in connexion with growing tissues.

Folin (132) has suggested the existence of a special tissue metabolism which in all probability would necessitate a further supply of special food products for the maintenance of nitrogenous equilibrium in the living tissues. He suggests, for example, that creatine may be such a substance and that in consequence muscle tissue is found to be rich in this material.

Hopkins (200), from his experiments on feeding with zein, has suggested that certain materials taken in the food are essential to the organism and are capable of utilization without in the slightest degree contributing to the tissue formation or structural maintenance; the formation of adrenaline from some of the aromatic nuclei of the pro-

tein molecule is an instance. That such a formation may take place has been suggested by Halle (163), who stated that after digesting suprarenal pulp with tyrosine more adrenaline could be obtained than from control experiments carried out without such addition. This work, however, has been questioned by Ewins and Laidlaw (121) who found no evidence of the conversion of tyrosine, or even of the more closely related bases, parahydroxyphenylethylamine and dihydroxyphenylethylmethylamine, into adrenaline by ferment activity.

From the above evidence, it is clear that apart from the caloric intake and the protein, carbohydrate and fat content of the food, there is some factor or factors which influence the utilization perhaps also the amount of food required. This evidence practically points to the presence of some "minimal" substance or substances in normal food which are absent in "pure" food.

The Psychic Influence.

Still another factor, of which at present we know but little, is probably of fundamental importance, namely the psychic factor. Owing to the nature of the experimental diet it is frequently monotonous in flavour, or may be even nauseous, and it causes a certain amount of reluctance on the part of the animal to eat it. This distaste leads directly to a failure in appetite which eventually results in faulty digestion and incomplete absorption and utilization. In other words, in these pure diets there is a lack of those stimuli which Pavloff has proved to be so essential for gastric digestion at least, and which in all probability will be found to be necessary for the other organs. McCollum (271) investigated this question with a certain amount of success, and reached the general conclusion that the palatability of a diet was the most important factor. He found that, even with many of the simple mixtures of pure proteins, if sufficient care were taken to change the character and the palatability of the food, by altering the flavour for example, it was possible to induce an appreciable retention of nitrogen. Very young animals, probably due to the fact that their metabolism is so active and hunger so predominant a feature of their daily lives, are found to adapt themselves more readily than adult animals to a ration of a comparatively low degree of palatability.

The Rise in the Output of Nitrogen after a Meal.

It has already been mentioned that there is a rapid rise in the output of nitrogen in the urine soon after a protein meal. This rapidity of output naturally leads one to doubt the necessity of a large intake of nitrogen. It has been repeatedly shown that after a protein meal there is an output of about 70 per cent. of the ingested protein within eight hours. This output does not occur in a single steady rise following the protein meal, but in a series of maxima, as a rule, three. Tschlenoff (401), Veraguth (405), and Hass (171) all found that the first rise took place about the second hour, the second between the fifth and sixth hours, and the third, if present, about the seventh hour. In all probability the first rise is due to the increased blood flow causing a washing out from the tissues of accumulations of waste material; the second and the third, when present, represent the results of actual protein catabolism. The cause of the fall in output between the first and second rise may be due either to absorption and subsequent deaminization proceeding slowly, or it may be that during this period the tissues are taking up the necessary nitrogenous material for purposes of repair, i.e. that a physiological retention occurs, and that the subsequent rise is due simply to the excretion of surplus and effete nitrogen. Graffenberger (158) has shown that the rate of output varies very definitely with the nature of the nitrogenous material which is fed. Curiously enough a partially digested material like peptone does not cause so rapid a rise in the output of nitrogen as proteins like fibrin and gelatin, and although asparagine is a freely soluble substance it is retained for a considerable period in the body. This slow excretion may have been partly due to the fact, although it is extremely improbable, that part of the asparagine was taken in solid form. Koraen (224) found in his experiments on feeding with protein (8·3 grms. nitrogen) that about 50 per cent. of the ingested nitrogen was excreted in five hours, and that up to 100 per cent. appeared within nine hours. When he lowered the nitrogen intake to 3·2 grms. all the nitrogen ingested had not appeared at the end of five hours. It is, however, extremely questionable if these percentage figures of Koraen are right. If, as seems probable from his figures, he has taken the endogenous nitrogen output along with his exogenous, on deducting the endogenous nitrogen figure (the fasting nitrogen output which he gives for the subject used) his nitrogen output during five hours amounts only to about 26 per cent. and for nine hours to a little over 50 per cent.

As regards the individual amino acids it has already been pointed out that Levene and Meyer (255) and Miss Bostock (71) among others have demonstrated that within a few hours (eight to nine) about 90 per cent. of the ingested nitrogen of alanine and glycine is excreted, for the most part, in the form of urea. Here again, as in the case of the protein, there is some variation in the rapidity of excretion depending on the amino acid used. For instance, in the case of leucine only about 54 per cent. of the nitrogen appears in the first twenty-four hours. In a series of experiments, not yet published, which I have carried out recently, where various forms of nitrogenous material were introduced directly into the small intestine, and the urine was collected directly from the ureters for a period of several hours, it was found in some cases, for example with a simple amino acid like alanine, that about 70 per cent. of the nitrogen introduced could be recovered from the urine of the animal within five hours. The rapidity with which the nitrogen was eliminated again, depended largely on the nature of the substance tested, and on the rate of its absorption. In all my experiments the endogenous output of nitrogen was estimated and subtracted from the total nitrogen excreted, so that the figures obtained represented the actual degree of catabolism of the absorbed protein or amino acid.

The Effect of the Partition of the Diet on the Output of the Nitrogen.

As Voit pointed out long ago the nitrogen output depends for the most part on the amount of nitrogen contained in the food consumed. The rate of this output seems to be influenced, however, by the partition of the food—the giving of the food in several amounts instead of at a single meal. Adrian (51, 52) found that this partition sometimes increased and sometimes decreased the amount of nitrogen excreted. The variation he observed was probably due to faulty methods. Munk (295) also with poor methods found a slight increase in the nitrogen output, but Krummacher (233), on the other hand, using a fairly reliable method, showed that the division into meals brought about a slight decrease due to the fact that there was a constant succession of maxima of absorption—one supply of food coming in before the previous supply was completely dealt with, with the result that there was some slight decrease in the absolute amount of material metabolized. Gebhardt (151) also found that the partition of the food into separate meals

brought about some decrease in the amount of total nitrogen excreted. My own results rather suggest that division produces a slight increase in the output of nitrogen. In this connexion the work of Leathes (247) is interesting. He showed that there was a well-marked tide in the output of total nitrogen; in the twenty-four hours the highest output was between ten p.m. and four a.m. and the lowest output between four a.m. and ten a.m. Osterberg and Wolf (316) also observed the same diurnal and nocturnal variations.

Storage of Protein.

Another question of great importance is whether protein is ever stored in the body in the same way, or approximately the same way, as carbohydrate and fat. No one doubts now that excess of carbohydrate from the food is stored in the liver and other tissues, principally the muscles, as glycogen, and that the fat is stored as fat. Both these stores can give up their supplies with great readiness when required. But the question of protein storage has not yet been definitely settled. There is no doubt that the body requires daily a certain supply of protein material to repair tissue waste, but if there be no supply from without can the body for a short period, as is the case with carbohydrates, or for a longer period, as in the case of the fats, draw this necessary protein from stored material, or must it break down its protein tissues? If the demand for endogenous protein be prolonged, as in the case of a fast or in protein starvation, then the protein for the most part comes from the breakdown of protein tissue. Voit and Chossat have both shown that there is a general wasting of certain protein tissues like muscle, during a fast. But if the deprivation of the supply of protein from without be only for a short period—a day or two at most—the answer is not so clear. Unfortunately the amount of experimental evidence in this field is not large. Pflüger (329) as the result of observations, which he made during his well-known experiments on glycogen formation and storage, came to the conclusion that the liver must be regarded as a storehouse for protein. He held that the hepatic cells not only manufactured this reserve protein from the food proteins, but also that this protein, just as is the case with glycogen, was held in reserve in the liver to meet urgent demands from the active tissues. Schreuer (361) by the study of the respiratory quotient, also came to the conclusion that there was a certain limited

storage of nitrogenous material in the tissues. He suggested that after liberal feeding with protein food there was an increase in the functionating cell material in dogs, but that this increase of active cell material did not last long. The only condition, which altered this natural tendency to get rid of the stored material rapidly, was when an increase in the amount of muscular work had to be carried out at the same time as there was the increased supply of protein. Bornstein (70) had previously reached much the same conclusion. He could always get a marked retention of nitrogen in the tissues, if simultaneously with the increased protein supply much work was carried out. Thus, when he added to a ground ration of 13·21 grms. nitrogen per diem 6·75 grms. nitrogen in the form of caseinogen, and at the same time did 17,000 mkg. of work daily, there was a definite retention of nitrogen, which he believed was in the form of flesh. The nitrogen retained was equal to about 800 grms. flesh; the calculated increase of weight during the period of experiment (eighteen days) amounted to 773 grms.; the actual increase, ascertained by weighing, was 800 grms. He concluded that if this "activity hypertrophy" were to take place readily then there must be an abundant supply of protein food. Seitz (368) has demonstrated by actual analyses of the liver that storage of protein material took place in the hepatic cells. This worker carried out two series of experiments on hens and ducks. Four birds in each experiment were starved for eight days, then two were killed and the livers immediately analysed, whilst the other two were fed on cod flesh free from both fat and glycogen:—

Exp.			Liver.			Liver N. in Per Cent. of the Total N. Content of the Bird.
			Weight in Grm.	N. in Grm.	N. in Per Cent.	
I.	Starved hens	1	34·241	1·1700	3·4170	1·592
		2				
	Fed hen .	3	54·060	1·4240	2·633	—
	,, ,, .	4	50·822	1 5900	3·1290	3·952
II.	Starved hen	1	26·024	0·8697	3·342	2·446
	,, ,,	2	24·262	0·8305	3·423	2·536
	Fed hen .	4	57·298	1·7620	3·075	4·710
III.	Starved ducks	1	24·382	1·6690	3·353	1·853
		2	25·397			
	Fed duck .	3	102·359	3·368	3·290	5·967
	,, ,, .	4	89·092	2·838	3·186	5·151

These figures, although perhaps far from conclusive, show at least that in the case of the livers of the fed birds there is a greater content of nitrogen than in those of the starved ones. Of course this does not settle in any way the form in which the protein is stored. Schryver

(362), on the other hand, who examined the livers of fasting cats and those killed about five hours after a full meal, obtained results exactly contrary to those of Seitz. Schryver found that in practically every liver examined (fourteen on record, eight fasting and six fed) there was more total nitrogen present in the liver of the starving animal than in that of the fed one. Not only was the total nitrogen increased under this condition, but there was also a slight increase of the non-coagulable, or as he called it, the residual nitrogen. It must be remembered that in Seitz's experiments the birds were carefully fed on a high protein diet for many days before they were killed, and further that retention could only be shown after more or less prolonged and careful dieting. Schryver's experiments were not carried out to demonstrate this point, and therefore no care about the previous feeding was taken. Cathcart and Leathes (92) have also shown that during active absorption from the intestine there is a definite amount of storing of nitrogenous material in the liver. Reach (335) carried out a series of experiments to demonstrate this retention of protein by the hepatic cells, but the method he employed was quite different. He perfused the liver with a mixture of blood, Ringer's solution and a protein-iodine preparation. He found that usually there was a very marked retention of the iodine protein compound in the liver in the form of the iodine protein itself; very little breakdown of the protein took place, as no free iodine could be detected.

A certain amount of histological evidence has been adduced in support of the view that the hepatic cells form one, at least, of the protein depots. Amongst the modern workers Boehm (70 A), in an interesting investigation of the histological appearance of the hepatic cells after different kinds of food and digestion products, found that the size of these cells varied. The smallest cells were found after hunger, and the largest after protein feeding. He further found that the structure of the protoplasm differed with different foods. Feeding with alanine and asparagine had no definite effect on the hepatic cells. Reichenau (336 A), maintained, however, that the histological evidence was valueless, that, for example, the proteoses could not be shown to have any marked influence on the activity of the liver. He further raised the question as to the form in which the protein was retained. Was it retained as protein or as protein digestion products? (see later p. 83).

Johansson and Hellgren (213 A) have also stated from experiments on men that all the food material including protein was not used directly it was absorbed, but that after absorption it was laid down in certain depots and only drawn on as required. The work of Gruber

(160) and Falta (122) on the prolonged excretion of nitrogen which sometimes follows the administration of protein is also in support of this storing of nitrogen. Abderhalden (4) has also produced a certain amount of evidence in its support. He found that a high intake of protein before a fast was not necessarily followed by a big output of nitrogen on the first day of the fast. He held, however, that the retention of nitrogen did not take place in a protein form, and further, that even in starvation there was an attempt by the animal to hold on to this "free" nitrogen. Morawitz (288 A) has also given some contributory evidence in his work on the formation of the blood proteins. He found after thorough bleeding and replacement of the removed blood by a suspension of blood corpuscles in Locke's solution rendered viscid by gum, albumin and globulin did not re-form at the same rate. The albumin gradually appeared in the first few hours after the bleeding, but the globulin much later. He concluded that the initial supply of albumin must have come from some store in the tissues. This reformation of blood proteins took place also during starvation, but in this condition he stated that the re-formation of the globulin was if anything more rapid than the albumin.

Probably the only other condition in which this retention of protein can be readily demonstrated (in addition to that caused by training or work already referred to) is during growth and convalescence from a wasting disease. Rubner (344) has dealt exhaustively with the problems of retention during growth, and there are a large number of clinical papers bearing on the latter point, as for instance that of W. Hale White and Spriggs (420 A) who showed that there was a daily retention of over twelve grms. of nitrogen in a woman who increased from 39·2 kilos (after inanition from worry) to 52·49 kilos in 55 days.

Siven (374) in his experiments has given a very practical example of the difficulty of getting protein stored in the body even when the conditions for such retention might have been held to be particularly favourable. After his feeding experiments with a low nitrogen diet when he had lost almost 1 kilo of muscle—32·41 grms. N., he suddenly increased the daily nitrogen to 13 grms., but found after fourteen days' feeding a storage of only 14·49 grms. N., and of this 11·93 grms. were retained during the first four days. He then raised the nitrogen intake to 22·5 grms. daily and found only about 6·15 grms. nitrogen retained in six days, most of it again being retained on the first day. In fact, on three days out of the six there was actually a negative balance. For the whole experiment there was thus only a retention of 20·64 grms. nitrogen, or about two-thirds of what the organism had previously

lost, i.e. 32·41 grms. Apparently, then, tissue restitution must be a very slow process. Probably if a certain amount of training had been carried out retention would have been more marked.

In what Form is Protein Retained?

An observation first made by Voit, and which has repeatedly been verified, seems to be in direct contradiction to this difficulty of protein storage. When the subject, or animal, under investigation is suddenly changed from a protein poor to a protein rich diet the nitrogen output does not immediately jump up to the new protein level; during the first few days there is less nitrogen excreted than is taken in the food, but eventually nitrogenous equilibrium is established. The same is true when the change is from a protein rich to a protein poor diet, during the first few days succeeding the change more nitrogen is excreted than is contained in the food. Two questions naturally rise in connexion with these observations. In what form is the protein or rather the nitrogen retained? and What is the cause of the retention?

The first of these questions has been frequently discussed by Gruber (160), Falta (122), Ehrström (111) and others. The general opinion is that the retention takes place in the form of some protein-like material and not in the form of extractives or of end products. The amount of extractive or non-protein nitrogen in the body, however, is by no means an insignificant amount, as Schöndorff and others have shown. Thus Schöndorff (359) found that the muscle tissue of a 22 kilo dog killed after abundant meat feeding contained 40 grms. of nitrogen in the form of extractive bodies soluble in water and that there were from 15 to 20 grms. of urea present in the total organism.

Rubner and Burgi (341 A) have further stated that, in their opinion, part of the nitrogen from meat extract can be retained in the body when the conditions are suitable as, for example, after the administration of the extract at the end of a period of starvation. They found that this retention was always associated with a great retention of water. As a rule, however, the nitrogen of the meat extract was rapidly excreted. Thompson (396) has also shown that a retention of nitrogen given in the form of meat extract can occur.

The retention certainly does not take place mainly in the form of end or waste products such as urea, as it has been shown that if urea

be given by the mouth it is quantitatively and rapidly excreted. This was demonstrated by Voit (VII) many years ago in the dog, and again recently by Achard and Paisseau (50 A), who gave large doses of urea to a man and found that the output of nitrogen rose immediately and fell just as suddenly when the urea was stopped. Rosemann (339 A), however, was inclined to believe, from his experiments, that a retention of nitrogen in the form of end products could take place. His investigation was carried out on a subject who could scarcely be regarded as normal and his results remain unsubstantiated.

As already stated in an earlier section, Abderhalden thinks it highly improbable that the retention occurs in the form of protein (p. 82). Von Noorden also holds that so long as the nitrogen retained is small in amount it is not necessarily in the form of tissue protein; a small amount of retained nitrogen may be distributed in the blood and lymph, and part somewhere as reserve material. Pflüger held (330) that if there were a retention of carbon there must also be a retention of nitrogen. He believed that a kind of intermediate substance was formed from the protein absorbed and stored as a reserve protein, a substance which was richer in carbon and poorer in nitrogen than the ordinary protein found in the tissues and tissue juices. This storing of material was an actual storage, i.e. it did not lead to a new formation of cells, but a mere filling out of the cells already existent—a physiological hypertrophy—an eutrophy as it has been called. Even the activity hypertrophy has been stated not to be due to an actual increase in the number of tissue cells, but to a mere thickening or distension of existent elements.

As the result of their study of the nitrogen : phosphorus ratio in experiments where a great retention of nitrogen had taken place Lüthje and Berger (268 A) came to the conclusion that part of the nitrogen was certainly retained in the form of "flesh" and that the rest of it was stored in the form of dead intracellular protein just like so much glycogen or fat.

Pharmacology offers examples of a similar capacity for retention. Thus the retention of bromine, iodine and fluorine has been demonstrated by Tappeiner and Brandl (cit. 57) and that of chlorine by Belli (57). In each of these cases the retention took place in the same step-like fashion characteristic of nitrogen.

What is the Cause of the Retention?

The cause of the retention is the need of the organism for nitrogen, but the laws which govern this demand and the nature of this retention and which regulate the rate and the manner of the output are not fully understood.

As an explanation of the nature of the retention Ehrström (111) has suggested that there are certain "affinities" in the tissues to which the absorbed material attaches itself in a more or less labile union, this union lasting for very variable periods. The duration of the retention, he believes, is absolutely independent of the chemical form assumed by the retained material. On the other hand, Gruber (160), without entering into any vague theorizing, sums up his opinion in the sentence "that this temporary retention of protein is simply the result of the superposition of the hourly curves". Assuming that the hourly curve of the output of nitrogen of one day is identical with the next (always if the protein be provided in the same amount and fashion), and that as a result the nitrogen from the daily protein catabolism is excreted in the same proportion in each 24, 48, 72 and 96 hours, Gruber has put forward an extremely interesting hypothesis which offers a very probable explanation, of the step-like increase or decrease in the output of nitrogen produced by variations in the protein intake (see Table). He suggested that 80 per cent. of the food nitrogen was catabolized on the day of ingestion, 13 per cent. on the day following, 5 per cent. on the third day, and 2 per cent. on the fourth. Thus, after an increase in the intake of protein there would be a steady step-like rise in the output of nitrogen until equilibrium of intake and output was reached. The same explanation holds good with a decreased nitrogen intake.

	Feeding Period.					Starvation Period.		
Days.	1	2	3	4	5	1	2	3
1	80	13	5	2	—	—	—	—
2	—	80	13	5	2	—	—	—
3	—	—	80	13	5	2	—	—
4	—	—	—	80	13	5	2	—
5	—	—	—	—	80	13	5	2
	80	93	98	100	100	20	7	2

Magnus-Levy (III.) has improved on this table of Gruber. He

pointed out that even when the organism was in a state of nitrogenous equilibrium a certain amount of the protein ingested was required to replace broken down tissue protein. A certain amount was also retained in the organism to replace store or labile protein which had been utilized. In other words, the nitrogen excreted on a particular day did not wholly represent the nitrogen ingested on that day, part came from the food protein and part from the protein already present in the organism. Magnus-Levy, therefore, subtracted from the 80 parts of the nitrogen said to be disintegrated by Gruber on the day of ingestion the amount which would be required to replace the effete tissue protein. He estimated this amount as 30 parts. He devised two tables, one of which presumed a moderate protein intake with a corresponding small retention of store protein, and the other a large intake of protein and a corresponding increase in the amount of labile protein retained. The table dealing with the moderate intake is given here :—

Of 100 Dcgm. Food Nitrogen of the 1st, 2nd, 3rd, etc., Days—	On the Last Hunger Day.	There were Excreted in the Urine										These were Stored as Stable Prntein for the Replacement of the Organized Tissue Prntein and so not Excreted for a Long Period.
		On Food Day.					On Hunger Day.					
		1st.	2nd.	3rd.	4th.	5th.	1st.	2nd.	3rd.	4th.	5th.	
1st food day	—	50	13	5	2	—	—	—	—	—	—	30
2nd ,, ,,	—	—	50	13	5	2	—	—	—	—	—	30
3rd ,, ,,	—	—	—	50	13	5	2	—	—	—	—	30
4th ,, ,,	—	—	—	—	50	13	5	2	—	—	—	30
5th ,, ,,	—	—	—	—	—	50	13	5	2	—	—	30
Total from food and labile protein	—	50	63	68	70	70	20	7	2	—	—	—
In addition from stable tissue protein	30	30	30	30	30	30	30	30	30	30	30	—
N. excretion in urine	30	80	93	98	100	100	50	37	32	30	30	—

This regularity of storage, or excretion, only occurs when there are no disturbing factors present. If, for instance, the subject had undergone a period of nitrogen starvation previous to his nitrogenous intake experiment then there would be very marked retention of nitrogen, presumably for purposes of repair.

All proteins are not broken down with equal readiness, and probably as a result there is considerable variation in the period in which nitrogen

equilibrium is reached. Thus Falta (122) superimposed different proteins on to a standard diet, whose effect on the metabolism was known, and found in his experiments a variation from about three to six days in the rate at which the nitrogen was excreted. This observation was made on man, but in the carnivora the differences in the rate of excretion did not hold good, or at any rate were much less marked. This step-like excretion is not due to slowness of absorption, as the excretion is spread over days, but must be due to a step-like breakdown of protein in the body. Falta has suggested that in the absorption of protein, part is taken up in some high molecular form, and part in a small molecular form. According to the immediate needs of the organism part is retained and part burnt, the low molecular form being much more labile than the high. A certain amount of evidence in support of this step-like breakdown is available, and will be considered presently. In the light of Falta's results, then, it is not only the amount of protein present which influences the amount of nitrogen excreted, but also the nature of the protein given plays quite as an important part. As Ehrström has remarked, the capacity which the body possesses of catabolizing protein is constant, not variable. The variable factor lies in the differing chemical constitution of the products of digestion and their varying resistance to such catabolic action.

Examination of the Nitrogen : Sulphur Ratio.

Evidence of the rate of protein catabolism has also been put forward from quite another point of view, namely from the investigation of the sulphur output. Sulphur, just like nitrogen, is found in practically all proteins, but in more varying amount. It had been suggested that from the study of the ratio of the sulphur : nitrogen output evidence might be obtained of differences in the rate and form of the breakdown of proteins. This ratio has accordingly been fairly fully investigated by several workers. Von Wendt (419 A) carried out some extremely interesting and careful work which demonstrated clearly that the amount of protein catabolized can be calculated quite as accurately by estimation of the sulphur as of the nitrogen output. Using as a factor 9·3—the ratio of nitrogen to sulphur excretion in a nitrogen starvation experiment—and multiplying the daily output of sulphur by it, figures were obtained which were practically identical with those obtained by the Kjeldahl nitrogen method. The drawback of course to the utiliza-

tion of the sulphur output for estimating the protein breakdown is a technical one—the method of estimation is comparatively slow. Von Wendt held that it was only by the combined examination of the nitrogen and sulphur output that a true picture of the total protein exchange in the body could be obtained; individually they only indicated the excretion of certain decomposition products. He found that the tissues first burned and excreted the sulphur-rich digestion products of the protein, and that the nitrogenous compounds, which were retained in the body, were poor in sulphur. The maximal output of sulphur preceded the maximal output of nitrogen. It was suggested that this retained material might be some decomposition product which, although it was not a true protein, could not be washed out of the tissues. Rubner also found that the sulphur output preceded the nitrogen output.

Gruber, on the other hand, found that the output of nitrogen and sulphur ran parallel, and concluded that the protein material stored for use in the tissues resembled normal protein. Sherman and Hawk (371 A) and Siven (374) also found that the output of nitrogen and sulphur ran very nearly parallel. Ehrström (111) found that the nitrogen and sulphur output ran nearly parallel, but that on the whole the sulphur adapted itself more rapidly to changes in the intake. He suggested that the protein products most deeply split were richest in sulphur, and possibly that they underwent oxidation in the tissues first, thus accounting for the more rapid excretion of sulphur sometimes observed. His work, therefore, lends support to that of Von Wendt.

Hämäläinen and Helme (167) have also investigated the rate of the nitrogen and sulphur output, using the superposition method introduced by Falta, in which the material to be tested is added to a standard diet, or ground ration. Their standard diet was one poor in nitrogen, and on this they superimposed at different times egg white, proton, and roast veal. Of the three diets apparently the veal alone was able to supply the deficiency in nitrogen and thus replace the wasted tissue protein. This was only to be expected, in accordance with the later work of Michaud (284), as veal protein more closely resembles tissue protein than either of the other two substances. They held that their results supported the hypothesis of Von Wendt that the sulphur-rich products of protein digestion were more rapidly burnt and excreted than those which contained less sulphur.

The following table details their most important results:—

Egg Albumin.			Proton.			Veal.		
Percentage Rate of Excretion.			Percentage Rate of Excretion.			Percentage Rate of Excretion.		
Day.	N.	S.	Day.	N.	S.	Day.	N.	S.
1	21·0	41·4	1	64·0	90·0	1	56·0	74·2
2	21·3	32·2	2	10·5	10·0	2	26·1	17·8
3	22·0	13·4	3	12·8		3	17·8	8·0
4	11·2	4·3	4	12·8				
5	13·7	5·5						
6	10·8	2·4						
7	—	0·8						

In each case within the number of days indicated the nitrogen output had returned to the original level.

Apparently, then, in addition to the splitting of the protein molecule into a nitrogen-rich and a nitrogen-free portion, there is a like separation of the sulphur-containing fraction. The early breakdown of the sulphur combination may also account for the rapid increase in the output of carbon dioxide which has also been observed after the ingestion of food (Frank and Trommsdorff 134).

Examination of the Nitrogen: Phosphorus Ratio.

The rate of the output of phosphorus has also been frequently compared with that of the nitrogen output. It has been found that the phosphorus output does not bear the same intimate relation to the rate of protein catabolism that sulphur does. This is not a matter for surprise, as in all probability the phosphorus, or the greater part of it, is associated with the special nucleo-proteins of the food and the tissues, and may undergo a special form of catabolism. As will be shown later in discussing the course of catabolism during fasting, nucleo-proteins apparently are more resistant to the action of proteolytic enzymes than the ordinary tissue protein. The curve of the phosphorus excretion has been found to run more or less parallel to the nitrogen curve, but behind it. The output of phosphorus has been investigated by Sherman and Hawk (371 A), Siven (374), Ehrström (110), Tigerstedt (397), Hämäläinen and Helme (167), and others.

CHAPTER VII.

THEORIES OF PROTEIN METABOLISM.

MANY hypotheses have been advanced to account for the various changes observed during the course of protein metabolism, but until about five years ago only two were really considered. These two were put forward by Voit in 1867 and by Pflüger in 1893 respectively. Previously the theory advanced by Liebig was almost universally accepted. Liebig considered that the protein of the food was the one essential material, that it entered the organism without having undergone any very serious change during digestion, and that it immediately and directly replaced the effete material of the tissues.

Voit.

Voit (VII) put forward the view that the protein of the food after absorption circulated in the tissue fluids—became "circulating protein"—and was utilized (catabolized) by the living tissues without first becoming an integral part of them. This "circulating protein" was readily broken down, whereas the "tissue protein" was resistant. A certain amount of the tissue protein constantly died and was excreted, and was replaced by material drawn from the "circulating" or food protein. No chemical difference existed between the "circulating" and the "tissue" protein. One of the facts which led Voit to the conclusion that protein existed in two forms was that during starvation a bare 1 per cent. of the tissue protein of the body was broken down per diem, whereas, if protein were fed in amount equal to 12 per cent. of the body protein, the breakdown was fifteen times greater than in hunger.

Pflüger.

Pflüger in 1893 subjected the view advanced by Voit to a very severe criticism. He thought that the food protein must first become an integral part of the living protoplasm before it could be utilized; In other words, the absorbed food protein, unlike the living tissue protein, was not readily catabolized. He believed that a fundamental difference in chemical constitution existed between the two forms of protein and that the greater lability of the living tissue protein was in all probability due to the presence of cyanogen radicles in it. Verworn was inclined to agree with these views, but Vernon (406 B) could obtain no experimental evidence which supported them.

Pflüger based his views largely on some experimental work carried out in his laboratory by Schöndorff. Blood was taken from a starving dog and was circulated through the hind limbs and liver of a *well-fed* dog; the urea content of this blood was increased at the end of the experiment. Blood was taken either from starved or well-fed dogs and circulated in the same fashion through the hind limbs and liver of a *starving* dog; no increase in the urea content was detected. Folin (131) severely and justly criticized these experiments, pointing out that the evidence furnished by them was by no means unassailable. He worked out the details of one of Schöndorff's experiments, of which full protocols were given, and showed that the actual amount of catabolism during the four and a half hours of the experiment corresponded to 25 mg. of urea nitrogen only, instead of to the 125 per cent. increase calculated by Schöndorff. During the four days preceding the experiment the dog had catabolized about 35 grms. of nitrogen per day; this 25 mg. gain therefore amounts to less than one-tenth of one per cent. As Folin says "Considering the numerous sources of error and uncertainty necessarily attached to an experiment of this kind it seems very strange that the extraction of 25 mg. of urea nitrogen from the hind legs of a dog killed while engaged in digesting 700 grms. of meat should be accepted as proving not only that protein catabolism did occur during the experiment, but also that it occurred in the bioplasm and not in the circulating protein".

Pflüger's theory does not satisfactorily explain the fact that very soon after the ingestion of protein there is a rapid rise in the output of nitrogen in the urine. One would have to assume an extraordinarily rapid synthetic process followed at once by an equally rapid catabolism.

Pflüger looked on protein as the food preferred by the organism to all other foods and the one which it would assimilate rapidly.

It seems improbable that all the nitrogen taken in is required or even utilized for protein synthesis. It is extremely difficult to obtain any direct evidence decisively in favour of one or the other of these very divergent theories. It is indeed highly probable that the differences which exist between the tissue and food protein are in degree and not in kind.

Rubner.

Rubner (343) advanced a theory of protein metabolism, in 1908, in which he maintained that the study of metabolism could not be divorced from the study of heat production, therefore that metabolism must be considered in association with the energy exchange. He referred all the metabolic changes of protein to the production of energy. He believed in a "store" protein resembling Voit's "circulating" protein, and in a "wear and tear quota" necessary for the repair of tissue waste. The greater part of the protein after absorption was rapidly disintegrated into a nitrogen-free and a nitrogen-containing part. The fate of the nitrogen-containing moiety, as it played but little part in the energy exchange, was disregarded. The nitrogen-free part formed the dynamic quota of the protein ingested. In this splitting of the protein a certain liberation of energy—of heat—occurred which was of no value as a source of energy for the cells and was therefore lost. This liberation of energy was termed the specific dynamic action of the protein.

A highly speculative hypothesis explained how the various changes took place. All protoplasm was not regarded as being of the same type, one kind might be thermolabile, another thermostable, but all varieties had in common a certain molecular grouping which acted as a kind of nucleus to which other protein groups (for example those which were thermostable or thermolabile) could attach themselves. The mechanism of the energy exchange, which is characteristic of activity, was effected by a distinct vibratory movement of the whole or a definite part of the protoplasm. Owing to this specific oscillation, the protoplasm had the power of bringing about the breakdown of contiguous food stuffs. The "affinities" (specific oscillations) must be of a specific nature for each tissue and were probably somewhat akin to ferment action. Thus, in diabetes, the "affinities," which brought about

the breakdown of carbohydrates, were for some reason or another in a state of suspension, inoperative or actually destroyed, whereas those which dealt with the catabolism of fat were active. The direct effect of the approximation of the foodstuffs to the "affinities" resulted in an atomic rearrangement and the entry of oxygen. The potential energy of the foodstuff now became available and caused a complete alteration in the "affinities"; an absorption of energy into the living substance took place at the moment of the catabolism of the foodstuff. The internal oscillations and changes in the cells, however, gradually used up all the energy, which was converted into heat and lost, and there was a return to the original condition, the "affinities" being again ready to begin work. The rate of the change depended on the nature of the living substance, the temperature, nervous influences and the conditions of the organism itself.

Speck.

Speck (380), in 1903, also expressed his belief that two forms of protein existed, but his idea of their subsequent fate was rather curious. The part of the protein of the food, which was not utilized for the formation of new tissue (living protein), was broken down into a nitrogen-containing part, which was rapidly converted into urea, and a nitrogen-free rest, which was readily used as a source of energy. The tissue protein, after the death of the cell, was also converted into a nitrogen-containing and a nitrogen-free part, but the subsequent fate of these two parts was different. The nitrogen-free part under normal conditions was converted into a fat or carbohydrate-like substance and utilized for energy purposes. The nitrogen-containing part was not immediately converted into urea, but it formed a great variety of substances, which played an important part in metabolism and which were eventually excreted as urea. He believed that oxygen deficiency played an all-important rôle in the breakdown of the tissue protein.

Folin.

Folin (131), in 1905, advanced an extremely interesting and valuable theory based on the laws which governed the composition of the urine. He carried out a very elaborate series of analyses of normal urine obtained from subjects on standard diets, (1) rich in nitrogen, and (2) poor in nitrogen, both diets being practically free from purine, creatine and creatinine. He said that there were two forms of catabolism which were essentially independent and quite different. "One kind is extremely variable in quantity, the other tends to remain constant. The one kind yields chiefly urea and inorganic sulphates, no creatinine and probably no neutral sulphur. The other, the constant catabolism, is largely represented by creatinine and neutral sulphur, and to a less extent by uric acid and ethereal sulphates. The more the total catabolism is reduced the more prominent become these representatives of the constant catabolism, the less prominent become the two chief representatives of the variable catabolism." To the constant type he has given the name of *tissue* or *endogenous* metabolism, and to the variable *intermediate* or *exogenous* metabolism. The following table of Folin's demonstrates his views very clearly:—

	Nitrogen rich diet.	Nitrogen poor diet.
Volume of urine	1170 c.c.	385 c.c.
Total nitrogen	16·8 grm.	3·60 grm.
Urea nitrogen	14·70 ,, =87·5 per cent	2·20 ,, =61·7 per cent
Ammonia nitrogen	0·49 ,, = 3·0 ,,	0·42 ,, =11·3 ,,
Uric acid nitrogen	0·18 ,, = 1·1 ,,	0·09 ,, = 2·5 ,,
Creatinine nitrogen	0·58 ,, = 3·6 ,,	0·60 ,, =17·2 ,,
Undetermined nitrogen	0·85 ,, = 4·9 ,,	0·27 ,, = 7·3 ,,
Total SO_3	3·64 ,,	0·76 ,,
Inorganic SO_3	3·27 ,, =90·0 ,,	0·46 ,, =60·5 ,,
Ethereal SO_3	0·19 ,, = 5·2 ,,	0·10 ,, =13·2 ,,
Neutral SO_2	0·18 ,, = 4·8 ,,	0·20 ,, =26·3 ,,

Folin held that the arguments advanced for the immediate resynthesis hypothesis of absorbed protein were not valid, as they were based on purely teleological grounds. He considered that his observations necessarily led to the view that only a small amount of protein was required by the organism, namely that necessary for the endogenous metabolism. He was further inclined to accept the evidence put forward by Chittenden as incontrovertible. He overlooks, however, the essential fact regarding protein requirements that in all probability it is not so much the quantity as the quality of the food provided which is of importance, so long as we have not a perfect food supply, i.e. food

which will provide in exact amount the different materials requisite for protein tissue repair.

Folin's view as regards the two forms of metabolism has been generally accepted, although exception has been taken to some minor points. He is perhaps inclined to separate his endogenous and exogenous metabolism too completely; other workers have shown that although his products of endogenous metabolism are characteristic of this form, they are also found in the exogenous form. Noël Paton (319) thought that some at least of Folin's results might be explained by variation in the activity of hepatic metabolism. Thus, on a protein-poor diet the hepatic metabolism would be sluggish and must therefore fail to convert a large amount of the waste nitrogen into urea, while on a protein-rich diet with hepatic stimulation the conversion must be much more complete. He also thought that urea must be considered a definite end product of both endogenous and exogenous metabolism., Folin himself, however, is prepared to allow this, as he says that "the fact that the urea and inorganic sulphates represent chiefly the variable catabolism does of course not preclude the possibility that they also represent to some extent the constant catabolism".

The statement that the output of creatinine is quite unaltered by alterations in the amount of the intake of nitrogen may be called in question. Both Noël Paton and I have found, particularly in the dog, that the output of creatinine is subject to variations, which, however, are usually small in amount, by altering the amount of protein ingested even when this protein is creatine and creatinine free.

CHAPTER VIII.

STARVATION.

MOST of the evidence offered up to this point in connexion with protein anabolism and catabolism has been drawn from experiments on feeding with individual amino acids, mixtures of these acids, single foodstuffs, or combinations of foodstuffs. But evidence of great value, particularly as regards the catabolism of protein, has been obtained by the investigation of the products excreted in the urine during complete or partial fasting. The value of this evidence has steadily increased, especially since the appearance of the work of Folin.

Output of Total Nitrogen.

Starvation experiments have been carried out both on men and on animals. In the case of the men the majority of the fasters have been professional "Hungerkünstler". The metabolism of the best known of these "Hungerkünstler," Succi, has been investigated at least five times, by Luciani (266), Lo Monaco (288), Daiber (105), E. and O. Freund (142) and finally by Brugsch (76). Lehmann, Müller, Munk. Senator and Zuntz (249) studied Cetti and Breithaupt. Hooven and Sollmann (202) have investigated the metabolism of inanition in a man during hypnotic sleep. Complete studies of fasting metabolism with special reference to the nitrogenous waste products, in which the fasting period was proceeded by a period of feeding on a definite diet, have been carried out on the professional faster, Beauté, by myself (89), and on two non-professional fasters (university demonstrators) by Hawk and his pupils (173). Studies on fasting women have been carried out by Van Hoogenhuyze and Verploegh (201), Bönniger and Mohr (66), and by Benedict and Diefendorf (60). Benedict has published from the Carnegie Nutrition Laboratory (58), a very large volume on metabolism during inanition but it is mainly concerned with the energy output and carbon metabolism.

Starvation is characterized by a slow but steady fall in the output of nitrogen. The amount of the nitrogenous output in the early days, as Voit showed years ago, is directly proportional to the amount of the previous nitrogenous intake. A uniform output of nitrogen is practically always reached by the seventh day of the fast, irrespective of the nature or amount of the food taken before the fast. In practically every experiment the output of nitrogen is lower on the first day, or two, than during the two or three subsequent days. This is almost certainly due to the body utilizing its stores of free carbohydrate at this period, as Prauznitz (334) in a long series of short experiments on students has clearly demonstrated. Benedict's work supports such an interpretation.

The main interest, however, in these starvation studies, when the organism is living on a purely endogenous supply of protein, is not in the total nitrogen output but in the partition of the nitrogenous constituents.

The Output of Urea and Ammonia.

Urea gradually falls both in relative and absolute amount. The same result has been observed by Folin and others when a subject is put upon a diet practically nitrogen-free. In the case of Beauté, during the fasting period the urea output fell to 71 per cent. of the total nitrogen. Hawk (173) in his subjects found the lowest percentage fall in the output to be 75·8. E. and O. Freund observed on the twentieth day a much lower percentage output, viz. 56 per cent. Now it has been shown by Von Schroeder and many others that the main source of the urea is the ammonia, which is formed in the body either from the deaminization of part of the ingested amino acids, or from some other metabolic process taking place both in hunger and feeding. It is also a well-known fact that one of the symptoms of starvation is acidosis, as Brugsch (77), in his investigations of Succi, and also Bönninger and Mohr (66) have very clearly demonstrated. This acidosis is due presumably to the imperfect combustion of fat; under these circumstances, as one of the protective mechanisms of the body, the ammonia formed is used for the neutralization of the acids. The fall in the urea output is accordingly accompanied by a well-marked rise in the output of ammonia. In my investigation, the percentage output of ammonia in relation to the total nitrogen rose from 3·16 per cent. on the last day of feeding to a maximum of 14·88 per

cent. on the eighth day of the fast. The Freunds, although they obtained so conspicuous a fall in the output of urea could not detect any greater increase in the ammonia output than 2 per cent. If this figure be correct, it is extremely difficult to explain the enormous fall in the percentage output of urea which they observed. Brugsch found in the urine of Succi on the twenty-ninth day of fasting a maximum ammonia output of 35·3 per cent. of the total nitrogen.

The Output of Creatine and Creatinine.

The output of creatinine does not maintain the regularity which it exhibits under normal conditions. The investigations of Benedict and Diefendorf (60), Van Hoogenhuyze and Verploegh (201), Hawk (173) and myself (89) show a decrease, which is more marked in some experiments than in others. Thus, in Beauté, this fall was quite definite, whereas in the subjects of Hawk it was only slight. Accompanying the creatinine output there is in starvation an output of creatine (Cathcart, Benedict, Hawk and others). In my subject, the amount of creatine excreted first rose, then fell slightly, and subsequently remained more or less constant to the end of the fast. Hawk observed the same course in one of his subjects, whereas in the other the creatine excretion reached its maximum in the first day, and then steadily fell. Benedict noted in his experiment that the output of creatine gradually increased during the fast. If the combined output, creatinine and creatine, be considered, there is no marked fall, although the general tendency is towards a decrease. Hence the decreased creatinine output is compensated for by the output of creatine.

From a study of the relationship of the output of creatine nitrogen to that of total nitrogen in the urine of the fasting bird, Noël Paton (320) holds that light is thrown on the nature of the course of protein metabolism in the muscles and other organs. He thinks that if the muscle "flesh" catabolized as calculated from the creatine excreted be greater than the total "flesh" disintegrated as calculated from the total nitrogen eliminated, the conclusion may be drawn that there has been a retention of some of the muscle protein nitrogen, due either to resynthesis in the muscle or to transference to some more essential organ. On the other hand, when the "flesh" catabolized, calculated from the output of total nitrogen, exceeds that calculated from the output of creatine, the stored nitrogen, i.e. the circulating, or surplus protein of

Voit, has been broken down. He supports his hypothesis by evidence obtained from geese fed in varying fashion before the fasting period. Thus, a fat young growing gander, abundantly fed previous to the fast of three days, catabolized during this time mainly non-muscle protein —i.e. the reserve protein had been adequate to tide the bird over the period of deprivation; a fully grown bird, which was fed on a comparatively nitrogen-poor diet previous to the three-day fast, had only a sufficent store of non-muscle protein for one day, and on the two subsequent days subsisted on muscle protein. Even when the reserve protein was all utilized and the bird was existing on its muscle protein, there was a retention of part of this muscle nitrogen.

These facts are extremely interesting, particularly the latter with reference to the utilization of a stored protein, as it is further evidence in support of the view that, as is the case with carbohydrates and fats, there can also be a reserve store of protein for emergencies (see p. 79).

Noël Paton maintains that the same calculations can be applied in the case of human subjects, if the combined creatine and creatinine output be considered. Using the data of Van Hoogenhuyze and Verploegh, of Benedict and of Cathcart, on starving subjects, he finds that muscle flesh is the protein material utilized during the fast, and that as in the case of birds, there is a very well-defined retention of the muscle nitrogen as the fast progresses.

This alteration in the ratio of the output of total nitrogen to creatine which Noël Paton regards as indicative of the retention of muscle nitrogen is of considerable interest. One must infer that the creatine or creatine precursor is present in the muscle in a more or less labile form and that it is not directly required for the resynthetic processes. Urano (403), it may be pointed out, has shown that under certain conditions (*in vitro*), particularly if the tissue has undergone slight autolysis, creatine can be readily dialysed out of muscle tissue. As creatine is only present in considerable amount in muscle tissue, and as muscle tissue, according to the observations of Voit and others, is much reduced during the course of starvation, it must be concluded that it is the liberated creatine which is excreted and that the creatine-free nitrogenous rest is utilized for the building up of tissues more immediately essential to the animal.

Certain experiments which Hawk and Fowler (172) carried out on the effect of water drinking must be considered here, as the results were interpreted by them as indicating the possibility of removing creatine from muscular tissue without any accompanying total catabolism of the muscle. Hawk (173) in his fasting experiments found that less

than 25 per cent. of the total nitrogen output—i.e. protein catabolized —could be accounted for on the basis of the creatine eliminated. Thus in subject E. the amount of muscle protein catabolized, calculated from the creatine output, was equivalent to an output of only 20·06 grms. of nitrogen, whereas the actual output of total nitrogen was 86·44 grms. He and his co-workers have found that the creatine content of muscle may be decreased as much as 66 per cent. by fasting, with but slight reduction in the total nitrogen content. They concluded, therefore, that it may be logically inferred that part of the creatine had been removed from muscular tissue which was still functioning in the body. Unless future experimental results are more conclusively in favour of such an inference, to my mind the more probable explanation of the results will still be the partial catabolism of muscular tissue and the reutilization of the creatine-free nitrogenous rest in synthetic processes elsewhere.

In this connexion two observations of Abderhalden are of interest. Abderhalden, Bergell and Dörpinghaus (39), with the present methods of analysis, could demonstrate no difference in chemical constitution between the tissues obtained from a normal and from a fasting animal. Abderhalden (4) also found distinct evidence of the retention of nitrogen in the tissues of an animal during fasting, the retention not being necessarily in the form of protein. He reached this conclusion from the observation that if a fasting animal were given a large amount of fluid there was a marked rise in the output of nitrogen. This rise in the output of nitrogen might, of course, be due to increased catabolism of protein due directly to the giving of the water, but Abderhalden considered such an explanation as highly improbable. In support of his conclusion he cited some previous work carried out by himself in conjunction with Block (24) on an alkaptonuric patient. They gave a large quantity of water (5 litres) to the subject of the experiment who was on a fixed diet. The output of total nitrogen in the urine rose markedly but without any accompanying rise in the output of homogentisic acid. From this constancy in the homogentisic acid output Abderhalden concluded that the protein metabolism as such was quite uninfluenced by the giving of water and that the increased output of total nitrogen was due simply to the washing out of "free" nitrogen—probably end products from the tissues. Hawk (174), on the other hand, found that the giving of four and a half litres of water on two successive days to a man in nitrogenous equilibrium brought about a rise in the output of nitrogen in the urine on both days. As the output was greater on the first day than

on the second day, Hawk thought there was a washing out of waste products from the tissues, but at the same time also an increase in the protein catabolism, as even on the second day there was an increased output of nitrogen.

In starvation particularly, it is almost certain that both sources of nitrogen must be considered. In all probability, in this condition, much of the so-called "free" nitrogen is the result of autolytic action in the tissues and is material in transit for utilization. If this repair and fuel material be washed out, as life must be maintained, then there will be a further breakdown of protein to supply the deficiency. Probably the same hypothesis holds true in the fed animal provided the intake of nitrogen be not too large. Here by the excessive ingestion of water and resultant diuresis the absorbed material is washed away before it can be utilized. In order to supply the deficiency in the organism, which is now in a state of partial nitrogen starvation, there will be an increase in the catabolism of tissue protein. That some such balance as the one just presented does occur is borne out by the following observations. Straub (388) was unable to get an increase in the output of nitrogen after two litres of water in a man in nitrogenous equilibrium on a large intake of protein, whereas Hawk (174) on a diet containing half the amount of nitrogen did get an increase. Heilner (181, 182) was unable to get an increase in the output of nitrogen after giving water to well-fed dogs, although, on the other hand, he found 2000 c.c. of water given to a fasting dog increased the output of nitrogen and at the same time increased the output of chlorine by about 30 per cent. This increase, he maintained, was due not to mere washing out of the tissues but to increased catabolism of the protein. He based his conclusion on the fact that the increase in the output of chlorine was extended over several days, whereas the nitrogen output rose at once with the increased diuresis.

The loss of fluid from the body by hæmorrhage from injury also seems to be associated with an increase in the output of nitrogen (Hawk and Gies (175) and Haskins (170)), but on the other hand the natural hæmorrhage of menstruation is said to be accompanied by a well-marked retention of nitrogen in the body (Schöndorff (359) and Schrader (360)).

The Output of Purines.

The ratio between the output of the purines and the total nitrogen has also been used to investigate the nature and the rate of protein catabolism. In the case of Beauté it was found that the total purine (bases and uric acid) output fell at first during the fast for the first three days, then gradually increased in amount until a figure slightly greater than the endogenous output during the previous feeding on a purine-free diet was reached. Now Nemser (297) has stated that it is the nuclein part of the tissue which resists disintegration in starvation longest, and this is not at all improbable, as the nucleins are a component part of the nuclei and are therefore intimately connected with cell regeneration.

As the fast progresses there will therefore be a rise in the output of endogenous purine. Apparently in starvation the body first utilizes tissues which, at the time, are of least value, preserving as long as possible those which are of greater importance. Thus it happens that in the early days of a fast the output of urinary purine (in my case there was no fæces to examine) is much diminished—the general protoplasm, as well as the non-nitrogenous bodies, the carbohydrates and fats forming the principal food supply, i.e. material containing but little or no purine nitrogen. As the fast progresses, the body can no longer obtain this material so freely, as is shown by the gradual diminution in the amount of total nitrogen excreted, and consequently coincident with this fall in total nitrogen, there is a rise in the output of purines. This increase is probably due to the fact that the body has now to draw upon its more valuable reserves—the nucleins, proved sources of purine supply—to supply their quota of energy along with the other tissues. Burian and Schur (81) look upon the hypoxanthine of the muscle as the important source of urinary purine. It may be that this hypoxanthine is the sole or chief source during the early days of the fast, but as the period of starvation lengthens other sources must be drawn upon. An attempt (89, page 127) was made to calculate from the total nitrogen the amount of endogenous purine which might be expected to be excreted.

The table (p. 103) shows a steady decrease in the calculated amount of purine nitrogen, whereas the columns giving the actual output of purine nitrogen show a steady and constant rise. From these figures it may be inferred, either that tissues, or parts of tissues, rich in the precursors of the endogenous urinary purine are being increasingly

Day of Starvation.	Protein Tissue = Total Nitrogen × 6·25 × 5.	Calculated Yield of—		Actual Excretion of—	
		Purine Nitrogen = 0·03 Per Cent (Muscle).	Purine Nitrogen = 0·06 Per Cent (Muscle and Gland Tissue).	Uric Acid.	Total Purine.
1	328·5	0·098	0·197	0·12	0·15
2	449·4	0·134	0·269	0·06	0·11
3	428·8	0·128	0·256	0·06	0·09
4	428·8	0·128	0·256	0·08	0·14
5	353·1	0·106	0·212	—	—
6	336·5	0·100	0·200	0·10	0·14
7	302·2	0 090	0·180	0·12	0·15
8	297·5	0·089	0·178	0·12	0·15
9	293·4	0·088	0·176	—	—
10	261·8	0·078	0·156	0·16	0·20
11	265·3	0·079	0·158	0·16	0·20
12	274·0	0·082	0·164	0·17	0·19
13	280·3	0·084	0·168	—	—
14	243·1	0·073	0·146	0·17	(0·19 ?)

drawn upon as the fast progresses, or, assuming the purine content of the tissues to be constant and that there be a steady total catabolism of these tissues, that there is retention of part of the protein nitrogenous constituents, as in the case of creatine. In their experiments on La Tosca, Van Hoogenhuyze and Verploegh (201) also observed the gradual rise in the output of purine after a short preliminary fall.

The Output of Sulphur.

As stated already, the investigation of the sulphur output yields a certain amount of evidence as to the course of protein metabolism. It will be recalled that Von Wendt (419 A) went so far as to maintain that it was only by considering the output of sulphur in relation to the output of nitrogen that a true idea of the course of protein metabolism could be obtained (p. 87). The course of sulphur output in starvation has been the subject of investigation on two or three occasions. As the ratio of nitrogen to sulphur in muscle is as 14 : 1 the figures obtained in starvation bear out the statement that a large part of the protein supply in this condition comes from muscle tissue. In my investigation the ratio of nitrogen to sulphur gradually fell during the course of the fast from about 17 to 1 to 14·5 to 1. Halpern (165) found at the end of a fast of twenty days a nitrogen : sulphur ratio of 14·61 to 1. Presumably, then, towards the end of the fourteen-day fast of Beauté the main source of protein supply was muscle. As the out-

put of both creatinine (and creatine) and purine indicated the possibility of a certain retention of muscle tissue having taken place, it may be inferred that the partial splitting of the protein molecule, which led to the liberation of these substances, could not have proceeded so far as to set free the sulphur. On the other hand, it must be remembered that, according to Ehrström, Falta and others, the sulphur-containing part of the molecule must be looked on as the labile part. It is possible that all the sulphur was liberated from the muscle protein, and that the balance of nitrogen excreted was made up from other sources. Until, however, reliable analyses of tissues are available it is impossible to come to any definite conclusion. Voit and Korkunoff hold that the nitrogen excreted in hunger does not come from actual tissue protein alone, but may also originate from the extractives of the organs. They found that the tissue mass of a goose after five days' starvation contained 15·39 per cent. nitrogen. Of this 12·55 per cent. (81.55 per cent. of the total nitrogen) was in the form of protein, and 2·84 per cent. (18·45 per cent. of the total nitrogen) was of extractive nature.

The Influence of Non-nitrogenous Substances on the Rate of the Protein Breakdown.

The fact must not be lost sight of that the amount of nitrogen liberated—protein utilized—directly depends on the supply of nitrogen-free food, like fat. So long as there is a sufficiency of fat present in the organism the nitrogen output slowly falls, but as soon as the fat store is all utilized, there is a rise in the output of nitrogen. This fact has been clearly demonstrated by a large number of workers, by C. Voit (VII), Schöndorff (359), Rubner (341), E. Voit (409) and others. It has also been clearly demonstrated that an animal with large stores of fat will resist starvation better than one which is thin. Schulz and his pupils (365), on the other hand, hold that the premortal rise in the output of nitrogen is not due to the lack of fat, or other nitrogen-free food material, but to a general breakdown of the cells themselves as the result of injury due to the hunger. E. Voit denies this. He holds that death in starvation arises not from the death of the total cell mass of the body, but from nutritional disturbances of essential organs. Reicher (336) also is inclined to accept this view. This worker was never able to detect any evidence of necrosis. He

quotes some work of Loeb in which it was shown that cell disintegration first took place after the removal of certain important lipoids (compare the work of Glikin and Stepp, p. 72). Schulz holds that the premortal rise takes place even if the animal be given fat or carbohydrate in sufficient amount to prevent loss of fat from the tissues. Kaufmann (215), who repeated some of this work, found that rabbits in a fasting condition die in three to four days with a persistently high output of nitrogen, even if they be given oil. On the other hand, when cane sugar was introduced the results obtained were much more satisfactory. He was able to keep the animals alive for as long as nineteen days with a constant decrease in the amount of nitrogen excreted.

The Capacity of the Starving Organism to deal with Injected Amino Acids.

Some modern work has also been carried out on the power possessed by the fasting animal of retaining and breaking down amino acids. Thus Hirsch (192) finds that the organism deals as usual with amino acids after injection into the tissues. Later Brugsch and Hirsch (78), working with a professional faster, found, so far as they could judge, no increase in the amino acid excretion during hunger even after the administration of comparatively large amounts of amino acids; on the contrary a retention of nitrogen might even occur when amino acids were injected.

Autolysis.

The body can make use of its stores of protein during starvation, owing to the fact that each tissue contains proteolytic enzymes, capable of bringing about a disintegration of the protein tissue, apparently similar in every way to that produced by the action of pepsin, trypsin and erepsin. It is more than probable that these enzymes play an active part during the normal life of the organism, that they are proteosynthetic as well as proteoclastic. For such a conception there are many analogies; take, for example, the proved reversible action of maltase and of lipase, the work of Robertson (l.c.) and Taylor (l.c.) on pepsin and trypsin, and the probable reversible action which goes on in the liver, the conversion of sugar into glycogen, and of glycogen back into sugar. Knoop (222) has also shown that the first phases of the oxydative breakdown of amino acids in the tissues are reversible. What are the peculiar conditions, then, under which this catabolic function gains the upper hand in starvation? Is it because the constitution and amount of the blood proteins are interfered with? Burckhardt (79), Lewinski (256), Gitken (153), Inagaki (206) have all found a reduction in the amount of protein present in the blood in fasting, the decrease being mainly in the albumin fraction. Hedin (177) and Cathcart (87) have both shown that the serum possesses active anti-proteolytic properties and that this anti-proteolytic action is associated with the albumin fraction, thus it may be that the increased autolysis in fasting is due in part to the disappearance of the serum albumin. Schryver (362), on the other hand, holds that the autolytic enzymes are held in check by purely chemical means. In a series of experiments he demonstrated that a diminution of the alkalinity of the tissues and fluids of the body from any cause was followed by an increase in proteolytic activity. He found that acids, especially lactic acid, accelerated the process of cellular disintegration. The formation of acid, and particularly of lactic acid, is one of the constant phenomena in autolysis. Mochizuki and Arima (286), Inouye and Kondo (207), Frew (145), Turkel (402), Arinkin (54) have all come to the conclusion that both organic and inorganic acids increase the degree of autolysis in the liver. Schryver believes that the ammonia arising from the breakdown of protein is largely responsible for maintaining the protective alkalinity. The normal blood serum is not only resistant to the action of trypsin, but is capable of inhibiting the normal action of this enzyme on other proteins which are present, and which

would be fully digested in the ordinary course of events. Oppenheimer and Aron (309) think that something more than the mere presence of an "anti-trypsin" is involved in this resistance of normal serum. In support of this contention they quote the observations of Schwartz (367). This worker held that protein was resistant because of a peculiar combination of aldehyde groups in the protein molecule which offered but few points of attack for the enzyme. Fischer has also shown in another connexion that a certain degree of specificity of formation in the substrate can exist, and of course it is possible that such an explanation may be the correct one here. It may be that the proteins under certain unnatural conditions become so altered that they are open to the attack of enzymes to which at other times they are immune. Until, however, our knowledge of the nature and constitution of the protein molecule is more intimate it is useless to speculate with any degree of certainty along such lines as these.

As regards the presence of the proteolytic enzymes in tissues, it was first pointed out by Salkowski (351) that fresh tissues kept (antiseptically) at body temperature slowly dissolved, and the protein was replaced by various amino acids. To this change he gave the name of auto-digestion. Jacoby (211) carried these investigations further. He showed that a variety of the known decomposition products of protein could be detected in the liquefied tissue and that the change took place much more rapidly under aseptic conditions. He gave the name of autolysis to the process of disintegration. Hedin and Rowland (178) and Hedin (176) were able to demonstrate the presence of proteolytic enzymes in juices expressed under high pressure from various tissues and organs. Further, Hedin found more than one form of autolytic enzyme in the spleen. Vernon (406) has also proved the presence of an erepsin-like ferment in the tissues. Finally Leathes (246), Dakin (106) and Cathcart (88), using the most modern methods, have shown that the various amino acids formed in the process of autolysis are apparently identical with those produced by the action of the true digestive ferments.

Abderhalden and Prym (25) have demonstrated that the liberation of the different amino acids during the progress of autolysis is only gradual; even after fifty days' autolysis a fair amount of the products are present in a more complex form. Abderhalden and Lussana (27) have further shown that the expressed juices from different tissues can decompose various polypeptides. Recently Abderhalden and his pupils (50) have introduced some new polariscopic methods for the

investigation of the intracellular or other ferments found in the plasma. They demonstrated that when the occasion arises the organism can secrete into the plasma a ferment or ferments to deal with products which are not normally present in this fluid. A long series of observations were made after the subcutaneous or intravenous injection of various proteins. It was invariably found that a ferment was formed, which was not specific, however, for any definite type of protein. Moreover, if the protein, for example, raw egg albumin, were taken in large amount by the mouth, the part, which, as already mentioned (see p. 12), was absorbed unchanged, generated a specific ferment in the plasma. As the normal plasma does not contain any proteolytic ferment the amount of protein absorbed in an unchanged form must be, under normal conditions, remarkably small.

CHAPTER IX.

WORK.

The Influence of Work on the Output of Nitrogen.

As the endogenous output of nitrogen during rest is so small it might naturally be expected that it would be increased when the conditions were altered, i.e. when more active metabolism took place, as during work. Practically, however, every investigator of this problem of the extent of metabolism during exercise has found that work leads to little change in the output of nitrogen in the urine, provided always that the supply of food, particularly of carbohydrate and of oxygen, be sufficient. The source of energy during work has been the subject of investigation for a great many years, and naturally the most divergent views are found. Thus, Voit and his school looked on fat as the most important source of energy; Chauveau and Seegen considered that carbohydrate was the important material; Pflüger at first held that the sole source of energy lay in the protein, but later he modified the idea to some extent. Others held that all three foods played a part in the supply of energy.

No one has yet been able to show clearly that during work there is any great utilization of protein, as evidenced by a marked increase in the output of nitrogenous waste products in the urine. Thus, Voit (VII) found that, if a fasting animal were exercised, there was only a small increase in the output of nitrogen. In a thin young animal the increase varied from 8 to 16 per cent. of the total amount, and in an old fat animal, after eight hours' hard continuous work, the increase was from 1 to 8 per cent. of the total amount of nitrogen excreted. The differences noticed between the effect of exercise taken on a full, or an empty, stomach were only slight. Voit and Pettenkofer (VII) confirmed this negative effect on the nitrogen output in work both during hunger, and feeding, periods in a man. Voit was inclined to ascribe these slight rises after work to the complete utilization of the nitrogen-free foodstuffs which was followed by the burning up of some of the protein tissue, owing to the lack of fuel. Oppenheim (307), who

only found an increase in the nitrogen output after work when dyspnœa was induced, regarded the rise as secondary, and not as the direct result of an increased catabolism of protein due to the actual work. Fränkel (137, 138) obtained the same result, and ascribed it simply to the lack of oxygen. Voit (407) also observed this rise of nitrogen excretion after dyspnœa, but thought that the muscular work, arising from the struggle for breath, utilized all the available nitrogen-free material, and then drew upon the tissue protein. Argutinsky (53) observed a rise in the output of nitrogen, but not until some three days after the work had been carried out. Liebig previously had suggested that the rise in the output of nitrogen would not be observed on the day of work, but later. This is apparently due to the fact that a certain amount of damage is done to the cells by work, and that cell restitution with the coincident excretion of effete material is not a sudden act, but a comparatively slow one. Zuntz (427) held that, so long as the nitrogen-free substances were present in the food in abundance, the rise in the output of nitrogen would be but slight. Munk (296) was also of opinion that the work was done solely at the expense of the nitrogen-free food substances, and that it was only when these were exhausted, or when dyspnœa was induced, that work was followed by a rise in the output of nitrogen. He explained all positive experiments in which work was followed immediately, or later, by a rise in the nitrogen output on this hypothesis. Kaup (216) found that, provided the supply of nitrogen-free food was adequate, there was no increase in the breakdown of protein during work. Hirschfeld (193, 194) found an increase in the nitrogen output following work, only if the diet were deficient in amount. Unfortunately Hirschfeld did not devote much care to the nitrogen analysis of his foodstuffs and therefore his results cannot be regarded as conclusive.

Pflüger (328) fed a dog of about 30 kilos weight, doing severe work for some seven and a half months, on flesh which contained only a mere trace of fat and sugar. He concluded that protein alone was sufficient to supply all the necessary energy, and that indeed protein was the food par excellence—fat and carbohydrate would only be utilized, when all protein supplies failed. In this animal a slight rise in the output of nitrogen was always observed after work, but it was certainly not commensurate with the amount of work done. Further, the rise, such as it was, did not take place on the day of work, but on the second and third days following the exercise. In his conclusion Pflüger stated definitely that there was no work with-

out some increase in the catabolism of protein as an accompaniment. Pflüger (329) admitted later that non-nitrogenous food might play some part in contributing energy for muscle work, although the nitrogen-containing material played the really important part.

Noël Paton and others (323) found that, after moderate work, the rise in the output of nitrogen was small, but after excessive work, there was a marked rise in the nitrogen excretion. This marked rise might have been due to the complete utilization of the non-nitrogenous food material bringing about the breakdown of protein. Krummacher (232) found that there was an increase in the output of nitrogen in a man doing measured work, and that the increased output took place even when a very large amount of protein was ingested. Further, he showed that the possible energy, calculated from the nitrogen excreted, did not equal the energy expended in the work. Frentzel (139) likewise demonstrated that, even if the total nitrogen excreted on the day of work, and not only the excess of nitrogen excreted, were regarded as coming from protein utilized during work, the material utilized would not be sufficient to furnish the energy expended. In one experiment the amount of nitrogen excreted accounted for only about two-thirds of the energy expended. Zuntz and Schumburg (429) found in marching that the increase in nitrogen excretion took place two or three days after the work. They also noted that other factors besides the actual work influenced this output of nitrogen—that the amount of work, and the degree of protein catabolism, did not run exactly parallel. Thus a much greater excretion of nitrogen followed a march with a light load on a warm day than with a full load and a normal temperature. Caspari (86) alternately rested and worked dogs in nitrogenous equilibrium and found a slight increase in the nitrogen output after work. Even this small rise he was inclined to ascribe to faulty dietetic conditions—not to the supply of the food being insufficient to cover the energy expended.

Shaffer (369) carried out a series of experiments in which the effect of increased and decreased muscular activity was tested, and found that, with a sufficient supply of food, work had no effect on catabolism, as indicated by the nitrogen and sulphur excretion in the urine. The creatinine output was also quite unaffected, although it is generally held that there is some close relationship between the creatinine output and the amount of active muscular tissue in the body. I found that there was a distinct increase in the output of nitrogen, when the subject was made to do hard work on an abundant, but nitrogen-poor diet. Garratt (150) found that as the result of exercise there was a slight

increase in the output of nitrogen. He observed that the rise in the nitrogen output was preceded by a rise in the output of sulphur. Engelmann (119) also found an early rise in the output of sulphur as the result of exercise.

Frank and Gebhard (135) attacked the problem in another way. They argued that if the muscular metabolism were reduced below normal, by the injection of curare, there would be some effect on the endogenous nitrogen exchange. Accordingly they curarized a dog and found that the nitrogen output was reduced about 25 per cent. They held that their experiments demonstrated that a certain preparation, or storing, of material took place, so that the organism was always ready for activity, but their argument is extremely difficult to follow. Frank and Voit (136) had previously shown that the carbon exchange was hardly altered by the giving of curare.

Differences between Voluntary and Involuntary Muscle Contraction.

There would seem to be, however, the possibility of two different forms of work which affect the metabolism differently—that associated with the voluntary contraction and that with the involuntary. Leathes and Cathcart (248) demonstrated that the whole course of purine excretion can be modified by alterations in the intensity of the involuntary work. In these experiments shivering was utilized as the form of involuntary work. It was found that if a subject doing the minimum amount of voluntary work were exposed to cold, so that severe shivering was induced, a very marked rise in the output of uric acid followed, whereas when involuntary work was reduced to a minimum and voluntary work carried out even to excess, there was just as marked a fall in the output of uric acid. In both sets of experiments the food was identical, sufficient in amount, and purine-free.

Another interesting point of difference in muscular metabolism which has been pointed out by Graham Brown and Cathcart (75) and by Pekelharing and Van Hoogenhuyze (325), is that white muscle always contains more creatine than red muscle. The latter observers calculate that there is at least one-fourth more creatine present in white muscle than in red.

The Influence of Work on General Metabolism.

Bornstein (70) has found that work plays some part in the general metabolism of protein, even if it have no marked action on the catabolism of this substance. He found that there was quite a marked retention of nitrogen if protein were fed during the period in which work was being carried out. He held that the nitrogenous metabolism on a constant protein intake was alike both for rest and work, but that there was a difference in the nature of the protein consumed in the two conditions—a difference in the amount of tissue and circulating protein utilized in the nomenclature of Voit (or in the amount of old and fresh organized protein in the terminology of Pflüger). There might be an increase in the amount of old organized protein broken down, but so long as there was a supply of material available for the formation of the new protein, there would be no variation in the output of nitrogen. Hypertrophy of muscle due to activity takes place more freely when the amount of material present for building purposes is large. Von Noorden believes that part of the retention even under these conditions may be in the form of an inert reserve protein.

Why does Work have so Little Apparent Influence on the Catabolism of Protein?

What explanation is to be offered for the apparent anomaly that work, which presumably increases the metabolism of the tissues, which are mainly composed of protein, is accompanied by but little increase of nitrogenous waste products in the urine? Examination of the muscle itself has shown certain differences after work. Thus, Burian (80) and McLeod (273) have shown that the purine content is altered, Brown and Cathcart (75) have shown that there is an alteration in the creatine content, and Pekelharing and Van Hoogenhuyze (325) have found that a chemical change takes place, after stimulation, and in rigor mortis, which leads to the formation of creatine in muscle tissue. It is a fundamental law in mechanics that every piece of machinery wears with work and that the greatest amount of wear takes place in those parts which are most frequently in use, and where friction is greatest. It is distinctly interesting if the tissues—muscular tissue in particular—which are most deeply involved both at rest and at work do not obey this law. Are they to

be considered as unwearable, like the jewelled bearing of a watch? Such a conception hardly can be true, for, if the work be carried out under unfavourable conditions, there is soon evidence of use in the increased output of nitrogen in the urine. Why then, under absolutely normal conditions, is there little or no evidence of use? Either (1) the actual wastage of protein is small in amount; the protein tissue as it is broken down separates into two distinct portions, one of which, the non-nitrogenous part, is used solely for dynamic purposes, whereas the nitrogen-containing moiety is reutilized—resynthetized—within the body; or (2) the protein tissue is actually broken down, but an equivalent amount of nitrogen is taken from the food supply to replace that wasted, with the result that there is little increase in the amount of nitrogen excreted.

Which of these two hypothesis approximates more closely to the true condition is well-nigh impossible to state. The first presupposes only a small requirement of protein for the body. It fits in extremely well with the facts observed in connexion with the output of endogenous nitrogenous waste products, and in those feeding experiments where the daily intake of protein is small. The small increase in the output of nitrogen during work and the apparently small endogenous exchange do apparently balance one another.

The second hypothesis has little or no experimental evidence to support it. It seems to me it would practically entail the acceptance of Pflüger's statement that the body needs are satisfied mainly by protein. As already pointed out, there is direct evidence that the energy needs of the body are not solely supplied from a protein source during work. It would also involve the acceptance of the statement that the amount of nitrogen excreted represents exactly the amount of protein catabolism which has taken place in the tissues. Such a belief cannot now be adhered to. Evidence is steadily growing which shows that *all* the nitrogen ingested is not necessarily converted into urea with subsequent rapid excretion, but that part of it may be retained in the body, either by resynthesis into fresh tissue protein, or in some simple form not yet definitely understood.

Although resynthesis certainly takes place, still every case of nitrogen retention cannot be attributed to it without further evidence. Thus Kovalevsky and Markevićz (228) have shown that when ammonium carbonate is introduced into the blood stream of dogs it rapidly disappears—the ammonia content of the blood soon returns to normal. The question is what is the fate of this ammonia? Salaskin (349) put forward the hypothesis that ammonia can be stored, or fixed,

as such in the tissues, although this had been denied by Biedl and Winterberg (64). Kovalevsky and Markevićz support the hypothesis of Salaskin, and state that the ammonia, which disappears, is taken up by the tissues and fixed there in some loose combination. In proof of this contention they show that, after the disappearance of the ammonia from the blood, the ammonia content of the tissues is increased. They found an increase in the ammonia content of the liver, muscles, and intestine, but not of the kidney. The work of Uschinski (404) in this connexion is of interest, as he has shown that the tissues can take up from the blood, after injection, various salts and sugars; urea was also taken up. Even more interesting is the recent work of Knoop (222) and Embden (118) showing that ammonia can apparently be directly used in the tissues (liver?) in the synthesis of amino acids.

Nevertheless, the resynthesis of the nitrogen-containing part of the protein, which is presumably catabolized during work, is extremely probable. Such a reutilization of the tissue nitrogen must and does take place during starvation, as certain tissues and organs, even when doing steady work, as the heart, retain practically their original weight up to the last (Voit, Chossat). This idea of a resynthesis of the locally catabolized nitrogenous material taking place within the tissues, particularly the muscles, is by no means a new one. Hermann (190), in 1867, put forward the hypothesis that the protein in all probability was decomposed into a nitrogen-containing part, which was reutilized in some way, and a nitrogen-free part, which was burnt. He believed further that an increased output of nitrogen took place only when the work done was very prolonged and severe—when an actual destruction of the muscle fibres was brought about. Pflüger (328) has also suggested that the protein molecule might break down into two distinct parts, one containing the nitrogen, which might be reutilized for the formation of new protein, and the other nitrogen-free, which could be used to satisfy the dynamic needs. Verworn ("Textbook of General Physiology") supports such a view, believing that, under certain circumstances, regeneration of the nitrogenous residues can take place at the expense of other foodstuffs and oxygen. He maintains that this economical use of the costly nitrogen is wholly in accord with the other economies of nature. Cathcart (90) also carried ou some experiments to gain further light on this problem of resynthesis It was found that creatine, a substance which is not present under normal conditions in the urine, always appeared (see p. 98) during starvation. It was assumed that the appearance of this substance was

due to the absence from the tissues of some material which directly, or indirectly, caused its retention. Investigation showed that the creatine, present in the urine as the result of a fast, always disappeared, if carbohydrate food were given, but not if either protein, or fatty food without carbohydrate, were given. Fats given for several days, however, may bring about the disappearance of the creatine from the urine of a dog, or at least a reduction in the amount present, but so far this has not been observed in man. It is probable that the canine metabolism as regards fats is more adaptable than the human. Pari (317) has also obtained certain experimental data from feeding dogs with fat after starvation which might also be interpreted as evidence of the possibility of adaptation taking place.

It is believed that these experiments showing the appearance of creatine in the absence of carbohydrate and its disappearance when carbohydrate is added to the diet are good evidence in support of the hypothesis that a re-utilization of nitrogenous substances set free by the decomposition of tissue protein does normally take place. It is not meant to be inferred that the form in which the nitrogen is re-utilized is actually creatine, in all probability, it is a more complex molecule containing perhaps creatine, or a creatine precursor, as a constituent which takes part in the resynthesis, and when resynthesis does not occur, owing to the absence of carbohydrate, this complex material is decomposed with a concomitant appearance of creatine in the urine. The appearance of creatine under these conditions is merely to be regarded as an index of faulty metabolism in general, and perhaps more particularly of the non-occurrence of resynthesis.

The Part Played by Carbohydrates.

That carbohydrates play an exceptionally important part in the utilization of protein generally has been repeatedly demonstrated. The best example of this is found in the feeding experiments with abiuret digestion products where, unless there be an abundant supply of carbohydrate, there is no retention of nitrogen. The experiments of Lesser (see p. 35), who attempted to repeat the work of Loewi, give a most excellent demonstration of this fact. It will be remembered that this worker was unable to confirm the findings of Loewi, but an examination of his protocols showed that he used fats only to make up the caloric deficiences of his dietary, omitting carbohydrate com-

pletely. So far as I am aware Abderhalden, Messner and Windrath (48) are the only workers who have ever offered experimental evidence of a retention of nitrogen without carbohydrate being also present in the diet. As has been already suggested (p. 40) this result is in all probability due to the fact that the necessary carbohydrate was obtained from the protein, or the fat, in the diet. Lüthje (269) has also clearly demonstrated, in his feeding experiments on rabbits, that the carbohydrate moiety of the diet is of absolute importance in the utilization of protein. He suggested that some form of amino sugar was first formed. Ross Taylor and Cathcart (394) have shown that, if glycosuria be induced by injections of phloridzin, there is an immediate appearance of creatine in the urine, which only lasts as long as the glycosuria exists, thus agreeing with the previous observations on the close relationship between the output of creatine and carbohydrate metabolism. These observations have been fully confirmed and extended by Krause and Cramer (230), and by Wolf (422). Ross Taylor (393 A), and Krause and Cramer (230) have also shown that in diabetes mellitus there is an appearance of creatine in the urine.

Falta, Grote and Staehelin (124) found that when the metabolism of carbohydrate was interfered with after the removal of the pancreas, there was a very great rise in the breakdown of body protein as evidenced by the increased output of nitrogen. They hold that carbohydrate is essential for the general metabolic processes of the body, and if the animal cannot get it directly, then it obtains it indirectly from the protein; the sudden increase in the output of nitrogen is thus accounted for.

Why should carbohydrate be continually produced in these cases of experimental diabetes even to the extent of breaking down protein tissue after all the free sugar has been excreted? It is hardly probable that it is formed merely to be turned out again—a mere disturbance in the normal mode of catabolism. It must be produced as the result of a definite call of the cells for carbohydrate—a substance essential to their very existence. Carbohydrate is surely a substance of vital importance from the fact that the last traces of glycogen in hunger disappear but slowly, more especially from the muscles, and that sugar, although it is so readily utilized, is never absent from the blood, even at the end of starvation. It is probably a provision for the proper re-utilization of the products of protein digestion which arise from the autolysis of the tissues during this condition and which are required as foodstuffs by the heart and other essential organs. Falta and Gigon (123) carried out a series of experiments on the utilization of proteins

in the presence of carbohydrates and fats, and they also reached the conclusion that carbohydrate was absolutely essential to the animal organism. They state definitely that retention of nitrogen only takes place in the presence of carbohydrates. Shaffer and Coleman (370) have demonstrated clearly that the "toxic" destruction of body protein in fever may be largely prevented by the intake of carbohydrate.

The observations of the botanical physiologists also support such a contention. Hansteen (169), Ivanoff and others have repeatedly demonstrated that the presence of carbohydrate is absolutely essential before protein synthesis can take place in plants. Ivanoff (209) found that the synthesis of the organic phosphorus compounds in yeast did not take place in the absence of the decomposition products of sugar which are formed during alcoholic fermentation. The phosphoric acid is supposed by him to combine with an aldehyde-ketone group. Czapek (104) found that moulds did not grow well in a medium containing an abundant supply of amino acids, or other suitable nitrogen compound, if carbohydrate were absent. He found that the carbohydrate, which was most readily utilized, was glucose. Kinoshita (220) and Suzuki (390) carried out a series of experiments in plants and concluded that ammonia could be taken up from without and be converted into asparagine, if a sufficiency of carbohydrates were present.

Intracellular Synthesis. (*a*) *In vitro* Experiments.

Until recently no experimental work on the possible nature of this intracellular synthesis existed. Pflüger had suggested that the part of the decomposed protein molecule which was nitrogen-rich combined with "alcohol radicles" to form new protein. There is much *in vitro* work which offers valuable suggestions as to the probable nature of the processes which occur. It has long been well known that aldehydes form compounds with many different substances containing nitrogen, e.g. aldehyde ammonia. Sugars have also been shown to react like the simple acetaldehyde; Lobry de Bruyn (257) made compounds of sugar with ammonia. Other nitrogenous substances, more complex than ammonia, have also been shown to unite with different aldehydes: Morrell and Bellars (290) have obtained a definite compound of glucose and guanidine, and Wolfe (423) has prepared the compound with aminoguanidine. Schoorl (358) has made a compound of glucose and urea, and Jaffe (212) a compound of creatine or creatinine and formalde-

hyde. Sörensen (379) has shown that the simple amino acids react with formaldehyde with the formation of methylene compounds. Irvine (208) carried out some very interesting work on the nature of the condensation which took place between sugars and amino bases. He has found that, in the case of glucose anilide, the simple aldehyde condensation did not occur, as was formerly believed. Spiegel (381) suggested that certain polypeptide groups present in the protein molecule were linked together by means of carbon atoms, and he attempted to bring about a synthesis of protein from protein decomposition products and formaldehyde. He stated that he obtained products which suggested that a synthetic change had taken place.

It is more than probable that it is the union of the nitrogen-containing radicles with the reactive aldehyde, or ketone, groups which brings about the protein synthesis in the body. Very reactive aldehydes and ketones such as methylglyoxal and glyceric aldehyde can certainly be formed in the decomposition of the carbohydrates, and indeed they probably arise during the normal course of carbohydrate catabolism in the organism; at any rate various products known to arise during the decomposition of carbohydrates *in vitro* have also been recovered from animal tissues.

(*b*) *In vivo* Experiments.

A certain amount of experimental work has been carried out *in vivo*. Thus, Spiro (382) found that, if glycine and fructose were injected into an animal simultaneously, a dicarboxylic acid could be isolated from the urine which was not present when either substance was injected alone. Quite recently Knoop (222) has made an extremely valuable contribution to this question. He found that, after the injection of an *α*-keto acid, nitrogen could be added on and an *α*-amino acid formed within the tissues. The synthetized substance was asymmetric. It would thus appear that the first phase of the oxydative breakdown of the amino acid is a reversible process. He also found that *α*-oxyacids could be converted into *α*-amino acids. He held that this proved the possibility of the union of the decomposition products of sugar and ammonia to form a protein nucleus. Knoop and Kertess (223) have fully confirmed this previous finding. Embden and Schmitz (118) attacked the problem in another fashion and have contributed valuable confirmatory evidence. These workers

perfused the glycogen-free liver with blood to which had been added different keto- and oxy-acids and found that the corresponding amino-acid was formed. In this way they demonstrated the formation of tyrosine, alanine, phenylalanine and probably leucine. The first two amino acids were present in their natural optically active form. They also showed with a glycogen-rich liver that, if a small amount of ammonium chloride be added to the perfusing fluid, there was a comparatively free synthesis of alanine. Alanine was also formed in small amount even in the glycogen-poor liver, if lactic acid were added to the perfusing fluid.

It may be taken as practically proved that carbohydrate in some form, or other, is absolutely essential for the synthesis of protein within the tissues.

Carbohydrates and Fats as Sparers of Protein.

Throughout the previous pages the question of the relation of the non-nitrogenous portion of the ordinary diet to the requirement and the fate of protein has been dealt with indirectly (see pp. 35, 97, 113, 119). It is apparent that, when fat and carbohydrate are fed at the same time as protein, there is a smaller demand for that substance; in other words the size of the protein minimum is more, or less, dependent on the amount of the non-protein food consumed. The same sparing power, particularly of carbohydrate, is also found in early days of starvation, as the many experiments of Prausnitz (334) and of Benedict (58) have shown. The effect on the catabolism of protein of the intake of carbohydrate in contradistinction to the fat in the food is very marked as is evidenced by numerous experiments, such as those of Siven (374, 375), Landergren (237), Kayser (217), Tallquist (391), Folin (129), Cathcart (90). This influence of the intake of non-nitrogenous food is very definitely shown when it is given at the conclusion of a fast. Thus, in the case of the professional faster Beauté, at the conclusion of the fasting period of fourteen days the subject was given a diet consisting of cream and starch with the result that within three days the output of nitrogen had fallen to a third of the output on the last day of the starvation period. Rubner (V) also observed the same effect in the case of dogs.

As Landergen (237), whose results were confirmed by Cathcart (90), has pointed out, there is a very great difference in the power of

sparing protein between carbohydrate and fat. Both Landergren and Cathcart have shown that, if a subject be put on a purely carbohydrate diet, the output of nitrogen steadily falls during the experiment. Unfortunately, owing to the nature of the diet, it is impossible to continue it for more than five or six days at a time. If the carbohydrate be now replaced by another diet, also nitrogen-free but consisting solely of fat, there is an immediate rise in the output of nitrogen, although the caloric intake is ample to suffice for all the body requirements. The following table very clearly shows this effect of carbohydrate and of fat on the output of total nitrogen :—

Carbohydrate.						Fat.					
Landergren.		Cathcart.				Landergren.		Cathcart.			
		I.		II.				I.		II.	
1	8·91	1	6·40		Only two days on this diet.	5	4·28	5	4·83	3	5·25
2	5·15	2	4·77			6	8·86	6	8·13	4	9·01
3	4·30	3	4·79	1	8·12	7	9·64			5	13·30
4	3·76	4	4·39	2	6·65						

Voit (VII) had previously demonstrated that the ingestion of fat by a starving animal if anything rather increased the protein catabolism.

Even when protein is fed with the fat as in the experiments of Voit and Korkunoff (410) the fall in protein catabolism, although it reaches a level well below that for the protein alone, is not so marked as in the case of protein and carbohydrate. It is impossible at present to state what is the true cause of this increase in protein catabolism as the result of feeding on a purely fat diet. It may be that the degree of acidosis produced by the faulty combustion of fat plays a part, or it may be, as Falta, Grote and Staehelin (124) have suggested, that the demand for sugar is so urgent, that protein tissue is broken down to obtain it. Landergren (237) has offered a very similar explanation. He holds that for some unknown reason (as has been pointed out in a previous section the probable reason is that carbohydrate is essential for protein synthesis) carbohydrate is in constant demand by the tissues. He further holds that, under physiological conditions at least, fat cannot supply this want and that proteins are broken down to yield the sugar which is lacking with a resultant rise in the output of nitrogen.

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INDEX.

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