XX. NITROGEN DISTRIBUTION IN THE TISSUES AND SOME OF THE FACTORS WHICH INFLUENCE IT.

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PREVIOUS WORK.

In the development as well as in the foundation of our knowledge the importance of technique can hardly be overestimated; a new method has been the sesame to many a hitherto closed door and has opened new fields for research. One need only refer to the discovery and elaboration of the methods for the determination and isolation of the individual amino acids by Fischer, Abderhalden and others, which threw a flood of light upon the structure of the protein molecule and revealed the astounding fact of its complete cleavage in the alimentary canal by the various processes of digestion. Linked to this was the necessary corollary that the animal organism was capable of resynthesising protein out of its cleavage products. There was everything in favour of the further assumption that it would be able to maintain itself and build up the required tissue protein if the protein of the food were entirely replaced by its cleavage products or by a mixture of its component amino acids. Abderhalden [1912, 1] in one of his papers, dealing with the synthetic capacities of the tissues, gives an account of the various steps by which he was able to feed a dog on completely cleaved protein and finally on an artificial mixture of amino acids for periods of eight and twelve days in two successive experiments; in both cases nitrogen equilibrium was established and there was a very slight increase in weight. An examination of these experiments shows that the trypsin digests from dog, ox and horse protein were more satisfactory substitutes for protein than the amino acid mixture, for the result of their administration was

frequently a marked retention of nitrogen accompanied by a gain in weight. A gliadin digest was markedly inferior to the animal digests doubtless owing to the relative amounts of the constituent amino acids differing widely from those of the tissues. One very interesting experiment [Abderhalden, 1913] demonstrated in a striking way that protein cleavage products were not only capable of replacing tissue waste but that they sufficed also for the new formation of tissue; a dog which had fasted for 26 days was shaved and then fed on a diet which contained all its nitrogen in the form of completely digested flesh. Not only did the dog put on 10 kilos. weight but it grew a new coat showing unmistakably that a synthesis of protein from the amino acid mixture had taken place. In some experiments which were carried out by Buglia [1912, 1] the meat in the dietary of young growing dogs was replaced by meat digests without affecting the retention of nitrogen or the gain in weight, provided that the carbohydrate in the food was given in a readily absorbable form. Frank and Schittenhelm [1910, 1911], in a series of experiments which will be referred to later, found that the digests of various forms of protein were almost as efficient as the proteins themselves, and in the case of one of the best utilised proteins, egg albumin, the digest was actually as efficient as the undigested albumin.

The attention of physiologists having been directed to the synthetic capacities of the organism, it was only a step further to inquire whether the amino acids themselves could be built up out of simpler forms of nitrogen. The work of Knoop and Kertess [1911] and of Embden and Schmitz [1912] acted as a fresh stimulus in this direction. Knoop and Kertess had found that the injection of an α -keto-acid or of an α -hydroxy-acid was followed by the appearance of the corresponding amino acid in the urine showing that nitrogen had been added on in the tissues. Embden and Schmitz perfused the isolated glycogen-free liver with the ammonium salt of an α -keto-acid and were able to recover from the perfusing fluid the corresponding amino acid, and they further obtained alanine after perfusing ammonium chloride through a glycogen-rich liver.

It is now generally accepted that glycocoll can be synthesised in the rabbit [Abderhalden and Hirsch, 1912, 1] but the results of replacing the protein of the food by an ammonium salt or urea have not been as satisfactory as might reasonably have been expected if the tissues can readily synthesise the amino acids necessary for the further construction of protein. The main workers in this field have been Grafe [1912, 1, 2, 1913, 1, 2, 3, 1914] and Abderhalden [1912, 1, 2, 1913]. Grafe seems

to have started out with the definite expectation of finding that some, at any rate, of the food protein could be replaced by nitrogen in the form of ammonia or urea, provided that an abundant carbohydrate fat diet was supplied, but the large number of experiments performed, coupled with the statement that some of them were not as clear as he wished, indicates that he had difficulty in convincing himself that an unequivocal synthesis had occurred. Grafe [Grafe and Schlpfer, 1912] planned his experiments with great care and the conditions under which they were carried out were practically always the same, viz., the animal was prepared by a previous fast of some days, following which there was a pre-period, in which the diet was nitrogen free and consisted of abundant carbohydrate and fat to the amount of 150 or more calories per kilo. body weight. The effect of this was of course to reduce the nitrogen metabolism to a minimum. The mean of the last three days of the pre-period and of the first three days of a similar after-period served as a measure of the endogenous metabolism, and was used to determine the nitrogen retention in the principal period during which nitrogen was added to the carbohydrate fat diet in the form of an ammonium salt or [Grafe and Turban, 1913] of urea. By this method Grafe was able to secure a well-marked reduction of a negative nitrogen balance and an occasional positive balance on particular days in the principal period. He claimed that very considerable amounts of nitrogen were retained, *e.g.* in one experiment [1912, 2] on a pig, 14 g. of ammonium citrate nitrogen were retained in 16 days. Young growing pigs proved more suitable for these experiments than dogs because they took the food readily and put on weight with astounding rapidity. It is noteworthy as showing how long life can be maintained in a state of complete nitrogen starvation, that two young pigs [1913, 3] of the same litter, one of which was fed on carbohydrate and fat alone, while urea and ammonium citrate were added to this diet in the case of the second, both died on the 67th day of the experiment although the animal which received no nitrogen began to fail much earlier than the other.

Grafe's [1913, 1] most favourable results were obtained in his later experiments by a modification of the procedure; after reducing the nitrogen metabolism to a minimum in the usual way, in a further pre-period he gave a small amount of protein equal to from 0.5 to 0.7 of the maintenance minimum, which was continued in the principal period during the administration of urea alone or of ammonium citrate and urea. The nitrogen balance remained negative in the pre-period in spite of the small quantity of protein

but it became definitely positive in the principal period, *e.g.* pig 8 had an average daily balance of -0.64 g. in the pre-period which became $+0.812$ g. in the principal period. Grafe points out the importance of making the difference between the nitrogen fed in the form of ammonium salt and urea on the one hand and the protein on the other as great in amount as possible, stating that it should be 3.5 times the value of the maintenance minimum and he criticises the small amount (1.3 times the maintenance minimum) used by Abderhalden and Lampe [1913, 1]. It is interesting to note that Grafe [1913, 2] found a negative daily balance of -0.18 g. in a pig fed for 40 days with 4.7 g. of urea nitrogen daily as the sole source of nitrogen. In a later paper [1913, 3] he ran parallel experiments with pigs of the same litter; pig 15 was given rather less than the maintenance minimum of protein during the principal period, pig 16, on the other hand, received in addition urea and ammonium citrate; both pigs gained in weight but the balance remained negative in pig 15 and became positive in pig 16. In a second experiment pig 17 got rather more than the maintenance minimum of protein in the principal period, while pig 18 received no protein but only ammonium citrate and urea; both pigs behaved in exactly the same way putting on weight and exhibiting a positive nitrogen balance.

Grafe [1914] found that a certain amount of nitrogen retention also occurred when an inorganic salt, ammonium chloride, was substituted for the organic salt, provided that it was given in small or medium doses; in larger doses it exerted an injurious influence. An endeavour to satisfy the nitrogen requirements of the animal by giving it in the form of nitrate [Grafe and Wintz, 1913] led to most contradictory results partly owing to the injurious effect of the nitrate itself. Abderhalden and Hirsch [1913] found that sodium nitrate was quantitatively excreted in the urine in the case of four dogs; in spite of this the nitrogen balance, exclusive of the nitrate, was affected favourably in two and unfavourably in the other two dogs.

Grafe [1913, 3] mentions three possibilities with regard to the nitrogen retained; ammonia may be retained as such, or the ammonia may be anchored, *i.e.* it enters into some form of combination not protein, and lastly ammonia may spare body protein in some way, *i.e.* ammonia is retained in some protein-like form. He decides in favour of the nitrogen being retained in a protein-like form.

Abderhalden and his co-workers Hirsch and Lampe carried out their experiments on the same general principles as Grafe, but the experiments

differed in various details and were not planned with an equal uniformity. It is often difficult and tedious to follow the tables owing to the frequent absence of the average daily balance calculated for the various periods under consideration. The first results were published in 1912, immediately after the appearance of the paper by Grafe and Schlpfer; they dealt with the effects of superimposing ammonium carbonate or ammonium citrate on an abundant carbohydrate fat diet. Although the nitrogen balance remained negative some of the added nitrogen was undoubtedly retained. Loss of weight continued in spite of the feeding with ammonia, but in two instances when a blood or flesh digest was added to the nitrogen-free diet the weight rose and the balance became definitely positive. Shortly after this there appeared an account [Abderhalden and Hirsch, 1912, 2] of two experiments which lasted 103 and 111 days respectively in which ammonium salts were added to a nitrogen-free diet. The difficulty of the continued administration of ammonium salts due to vomiting and diarrhoea necessitated the frequent interruption of the ammonia periods and the intercalation of fast days. Sometimes the condition of the animal was such that feeding with meat powder or flesh digest for some days had to be resorted to, a procedure which was invariably followed by immediate recovery. A retention of some of the ingested ammonia undoubtedly occurred, although the authors urged caution in the interpretation of the results on account of the variations which were present in the daily nitrogen balance. They drew attention to the fact that a temporary gain in weight might be exhibited under certain conditions by an animal fed on a nitrogen-free diet. With regard to the utilisation of urea [Abderhalden and Hirsch, 1912, 4] any retention of nitrogen in the dog was followed by a washing out. In an isolated experiment [Abderhalden and Lampe, 1913, 2] on a pig there was not even a temporary holding back of nitrogen for the negative daily balance was practically the same as that of the succeeding fast days; it may be mentioned however that the nitrogen metabolism had not been reduced to a minimum by a pre-period of a nitrogen-free diet. A pig [Abderhalden and Lampe, 1913, 1] fed for 28 days on ammonium acetate, on the other hand, showed a definite protein sparing action of the ammonia although the balance remained negative.

Abderhalden and Lampe [1912, 2] criticised Grafe's findings and methods and complained that they were unable to obtain such favourable results. In their own experiments they stated that a positive nitrogen balance or nitrogen equilibrium was only transitory and was followed by an increased output. They admitted that ammonium salts exercised a protein sparing

action but considered that the facts were best explained by the retention of the nitrogen in a non-protein form. They doubted [1912, 1] the existence of any marked synthetic capacity on the part of the tissues on the ground that experiments on dogs, in which the nitrogen was supplied in the form of gelatin and ammonia, failed to show a positive nitrogen balance although there was undoubted retention of nitrogen. They maintained that if the cells could synthesise amino acids a much better result should be gained if the task imposed on them were merely that of synthesising the few amino acids which were lacking in the gelatin, than if they were required to synthesise the whole series present in the protein molecule.

Following on this paper came another [Abderhalden and Hirsch, 1912, 3] in which the effect of gelatin plus ammonia was compared with that of erepton (flesh digest) on a three months old dog. A positive nitrogen balance and a gain in weight always occurred when erepton was fed, whereas the weight fell and the balance became negative when it was replaced by gelatin with ammonia. Four young dogs were then fed for periods of seven days on (1) gelatin and ammonium acetate, (2) erepton, (3) an artificial mixture of amino acids, (4) erepton and (5) ammonium acetate successively. The weight of the animals and their general condition only was noted; the weight rose in the two erepton periods, remained stationary during the feeding with amino acids and fell when ammonium acetate was given either with or without gelatin. The animals were cheerful and well during the erepton periods but they slept all day and seemed definitely unwell during the administration of ammonia.

It is quite apparent from a consideration of the foregoing experiments that even if the organism can synthesise amino acids from ammonia and the products of carbohydrate and fat metabolism, it does not readily do so, for flesh digests are undoubtedly more satisfactory than ammonium salts or urea as sources of nitrogen supply.

In connection with the utilisation of ammonium salts Underhill and Goldschmidt [1913, 1] carried out some useful work and pointed out the advisability of estimating the ammonia as well as the total nitrogen. The type of experiment differed from those just described, for the dogs were fed on an adequate mixed diet and the particular ammonium salt was administered on one day only in three separate portions in gelatin capsules. A radical difference was found in the behaviour of the organic and the inorganic salts; none of the organic salt was excreted as ammonia and it was apparently quantitatively converted into urea. About 20 to 25 per cent. of the

inorganic salt, on the other hand, was excreted as urea while the remainder appeared as ammonia in the urine. There was, moreover, an increase in the nitrogen output and a lag in the elimination of the inorganic salt for the ammonia in the urine was increased for 48 hours after its administration.

A similar experiment [1913, 2] carried out on a fasting dog which had been freed from glycogen gave results in complete accord with the above. They [1913, 3] finally tested the utilisation of ammonium salts with a non-nitrogenous diet using Grafe's method. Their results were in keeping with those of previous workers in the case of the organic salts, which caused a retention of nitrogen and did not increase the urinary nitrogen, but contrary to the conclusion of Grafe and Schläpfer they found that ammonium chloride was incapable of acting as a source of nitrogen supply for the nutritional needs of the body, and not only was there an increased excretion of nitrogen, but for some days following the administration of the salt the ammonia in the urine was increased.

The authors criticise Grafe's technique particularly with regard to the absence of catheterisation, but in view of the duration and the number of Grafe's experiments and the care and consistency which characterise them his main results cannot be called in question.

Taylor and Ringer [1913] pointed out that the presence of carbohydrates was not an obligatory factor in the retention of nitrogen from ammonia. The administration of a single dose of ammonium carbonate to a dog, which had previously fasted three days, was accompanied by a retention of a considerable portion of it, the result being confirmed by two repetitions, while the retention in the case of two phloridzinised dogs was even greater. They claim that two administrations of urea were followed by a quantitative elimination of the amide, but in view of the variation in the daily nitrogen balance such a result requires confirmation. They suggest that ammonia nitrogen is retained because of a reversed reaction that leads to its combination with the α -ketonic and α -hydroxy-acids to form amino acids, which may be used in the synthesis or sparing of body protein. It cannot be said that these experiments have disproved Grafe's contention that the administration of abundant carbohydrate is the surest way of demonstrating a retention of ammonia nitrogen in the normal dog and pig.

Henriques and Anderson [1914, 1] attacked the problem of the utilisation of ammonium salts and urea in another way which excluded the action of the intestinal wall and of the intestinal bacteria. Using their method of

permanent intravenous injection in the goat they reproduced Grafe's experiments, *i.e.* there was a daily injection of two litres of fluid containing glucose and salts in a pre- and after-period, and intercalated between the two was a period in which urea or an ammonium salt was added to the injection. Although there was a diminution of the negative balance, a lasting retention of nitrogen was absent and the evidence indicated a retention followed by a washing out. When a meat digest was added to the fluid a positive balance was at once obtained emphasising the superiority of digests over ammonium salts to which attention has already been drawn.

It must not be forgotten that a great deal of work on the utilisation of ammonium salts and amides has been carried out on the herbivora and in their case no doubt exists as to the effect on the nitrogen balance. Among the recent workers Kellner, Eisenkolbe, Flebbe and Neumann [1910] conclude from their experiments that asparagine and ammonium acetate can replace the protein necessary for maintenance but that they cannot be utilised for protein formation even in starving animals.

Morgan, Beger and Westhauser [1910] state that ammonium acetate can be utilised in no inconsiderable way for the maintenance of life and even for milk formation if it be added to a diet containing sufficient starch but very deficient in protein. In a later paper [1911] they found that the nitrogen contained in ammonium acetate and asparagine in a diet poor in protein but with sufficient nitrogen-free food was utilised to the extent of 32 per cent. not only for the maintenance of life but also for the production of milk, flesh and wool. Probably the intestinal bacteria play an important part in the utilisation of these simple nitrogenous bodies in the herbivora for the value of these as food stuffs is admitted.

The complexity of the problems of protein metabolism is indicated by the apparent difference which exists between the processes of tissue repair and growth; various workers have shown that food which suffices for repair is not necessarily capable of producing growth. McCollum [1911] reduced the metabolism of a pig to a minimum by feeding it on a scanty starch and salt diet after a preliminary fast. The creatinine nitrogen under these conditions constituted $1/5.5$ of the total nitrogen and at this low level all the nitrogen was assumed to be of endogenous origin; the creatinine nitrogen $\times 5.5$ therefore was used as a measure of endogenous metabolism in the subsequent calculations and it was possible to determine how much of this endogenous or tissue nitrogen had been replaced by the nitrogen in the substance fed. It was found that zein was utilised to the extent of 80 per

cent. on an average for repair, but even when given in excess there was no evidence of the formation of body tissue. The average utilisation of gelatin for repair was 50–60 per cent., whereas when caseinogen was the only protein fed there were increases of body protein to the extent of 20–25 per cent.

Osborne and Mendel [1912, 2] carried out experiments on the feeding of isolated proteins to rats. Gliadin, which contains relatively very little glycocoll, arginine, histidine or lysine and extremely large quantities of glutamic acid, proline and ammonia, was unable to support normal growth in young growing rats who were dependent on it for their sole nitrogen supply. The same diet however sufficed for the maintenance of the adult animals, which not only paired but also produced healthy young, which were suckled by the mother, so that in this case there must have been a synthesis in the maternal organism of the amino acids which were lacking in the gliadin. Of four young rats suckled by a gliadin-fed mother, the three which were taken away from her at the end of 30 days and were then fed on caseinogen, edestin and milk respectively, showed normal growth, while the fourth, which was left in the cage with the mother and had access only to the gliadin mixture, showed no growth. Hordein also was incapable of maintaining normal growth in contradistinction to caseinogen, edestin and glutenin. As Osborne and Mendel point out maintenance experiments alone are not sufficient to solve the problem of the biochemical value of dietaries. An account appeared elsewhere [1912, 1] of an elaborate investigation which led to an extension of the list of proteins which were capable of supporting normal growth. The soy bean was the only member of the Leguminosae containing a protein which fulfilled these conditions. Hordein and zein resembled gliadin in their inability to do so. With regard to the quantity of protein it was found that normal growth occurred if 18 per cent. of protein (or 14 to 15 per cent. calories) was present in the diet, but there was no increased growth if this quantity was increased. Frank and Schittenhelm [1910] investigated the question of the superiority of the “arteignes” protein of Michaud for tissue repair over allied forms of protein. They reduced the nitrogen metabolism of dogs to a very low level by the use of a nitrogen-free, carbohydrate-rich diet following a fast and then tested the relative values of dog, horse, pig, fish, goose and pigeon flesh. In two out of the five dogs, dog protein was the best utilised but in the other three dogs one of the other forms of protein held the first place. They concluded that “arteignes” protein had no definite superiority over other nearly allied proteins provided they could be readily broken down and that they yielded

much the same mixture of amino acids. Continuing this line of work [1911] they succeeded in maintaining without loss of weight two dogs for 6.5 and 4.5 months respectively on a diet in which the nitrogen minimum was given in the form of different types of animal protein or their digests. They found no marked difference in the utilisation of the different types of meat and the digests were almost as efficient as the undigested proteins. One dog with an intake of 1.2 g. protein was practically continuously in nitrogen equilibrium or else showed a positive balance, in the second dog a positive balance was not reached nearly so soon.

The method of introducing food stuffs intravenously has been practised by a number of workers and has had for its object the exclusion of the action of the intestinal wall. Buglia [1912, 2] injected meat digests intravenously in dogs and cited as evidence of their utilisation that only varying proportions were excreted in amino acid form in the urine. The experiments lack uniformity and are few in number but such a utilisation is in harmony with the results of the work of Folin and Denis [1912] and van Slyke and Meyer [1912, 1913, 1, 2, 3] who showed that the products of digestion were carried to the tissues as such without undergoing any preliminary change in the intestinal wall.

Henriques and Anderson [1913] elaborated the method of intravenous injection and were able to keep a goat alive for 20 days by the daily introduction of protein digests into the blood stream; the nitrogen balance under these conditions was frequently positive and a daily retention of 1 or 2 g. of nitrogen was quite common. Their use of this method in the investigation of the utilisation of urea and ammonia has already been referred to.

The same observers [1914, 2] used their method of permanent intravenous injection in goats after total extirpation of the bowel. They managed to keep such animals alive for $2\frac{1}{2}$ days. During this time the injection of trypsin + erepsin digests caused a considerable deposit of nitrogen, for example in goat 1 positive balances of 2.2 g. and 4.18 g. were obtained on the first and second day respectively.

Oehme [1914] injected meat digests intravenously on a single day in the case of two fed and two fasting dogs respectively, the diet of the former consisting of carbohydrate and fat. The amount of amino acid excreted unchanged in the urine was much smaller in the fed than in the fasting dog but even in the latter the protein cleavage products injected had been extensively utilised, for the maximum excretion of amino acid was only 29.5 per cent. of the amount injected. In view of the capacity of the tissues

to pick up amino acids, etc. from the blood stream the fact of a certain amount of the amino acid introduced not appearing within a given time in the urine certainly cannot be regarded as evidence of its utilisation. The real aim of the researches on the introduction of food stuffs intravenously was to obtain evidence that the intervention of the intestinal wall was not necessary and to disprove the hypothesis enunciated by Abderhalden of a synthesis of the products of digestion by the cells of the intestine.

When we come to the field of tissue analysis mention must first be made of the work of Grund [1910] and Diesselhorst [1911]. Grund endeavoured to find evidence of protein storage in the liver of the dog as it had been claimed by Seitz that such a storage of protein took place to a very marked extent in the liver of the hen. Grund estimated the total nitrogen, the total protein nitrogen and the total phosphorus in the liver, kidney and muscle of the fed and fasting animals, and compared the two values. When the total nitrogen was calculated as a percentage of the initial body weight, there was a great diminution in all these organs but particularly in the case of the liver as the following figures show:

		Liver	Kidney	Muscle
Mean of the fed dog	...	1.059 %	0.157 %	0.185 %
Mean of the fasting dog	...	0.570 %	0.098 %	0.129 %

If the percentage of total nitrogen in the liver of the fed and fasting animals be compared, however, the increase is in the fasting animal, due presumably to a diminution of glycogen in the latter which would lead to a relative increase in the total nitrogen content of the organ. Changes in the amount of moisture are not accountable for the increase. The kidney and muscle show practically no change in the percentage of total nitrogen. Grund mentions the fact that the ratio of "rest" nitrogen (= total soluble nitrogen) to total nitrogen expressed as a percentage of the latter showed great irregularities in the individual experiments but only moderate differences between the fasting and fed animals; the average figures are:

		Liver	Kidney	Muscle
Fed animal	...	11.6 %	15.7 %	15.1 %
Fasting animal	...	10.5 %	12.9 %	13.2 %

The ratio of the total phosphorus to the total nitrogen was practically constant in the organs of the fed and fasting animals. The difficulty of interpreting Grund's figures lies in the impossibility of getting a fixed basis for comparison, both the initial weight of the body and the weight of the individual organs

being subject to variation from various factors. As we shall see later it is much easier to detect changes in the "rest" nitrogen than in the total nitrogen.

Diesselhorst tried to determine whether the diet influenced the chemical composition of the tissues by a comparison of the muscles of a dog fed on a scanty carbohydrate fat diet and an abundant meat diet respectively. Two dogs were fed on the meagre nitrogen-free diet for $1\frac{1}{2}$ to 2 months, a hind leg was then amputated in the case of each animal and both were fed on an abundant meat diet for approximately three months. During this period they rapidly put on weight and at its termination they were killed. The main fact which emerges from the experiment is that the nitrogen : carbon ratio remains unaltered and is practically the same in the two dogs, 1 : 32 and 1 : 31.8; these figures agree closely with the ratio of 1 : 33.8 obtained by a previous worker, Stockhausen.

With the work of Folin and van Slyke we turn over a new page in the history of protein metabolism, for it has solved the problem of the form in which the products of digestion enter the blood stream and the theory of a resynthesis in the intestinal wall has been relegated to oblivion. Folin [Folin and Denis, 1912, 1] was the first to publish his results dealing with the application to the blood and tissues of his new micro-methods for the determination of total nitrogen, urea and ammonia nitrogen. In his investigation of the effects on the blood and tissues of the absorption of the products of protein digestion he selected the cat as being the most suitable animal for his experiments. After taking samples of blood and removing the gracilis muscle from one leg, he introduced into a ligatured loop of the intestine solutions of urea, glycocoll, egg albumin and a pancreatic digest respectively; samples of blood were withdrawn at varying intervals during the experiment and at its close the gracilis was removed from the other leg. The determination of the total non-protein nitrogen and the urea in the samples thus obtained showed that there had been a rapid absorption of the urea from the intestine, that it had entered into the blood stream unchanged and had actually accumulated in the muscle. In the case of the glycocoll, the increase of the non-protein nitrogen in the blood and muscle showed that it also had entered the blood stream and had travelled unchanged to the muscle, the urea content of the blood, however, was slightly increased towards the end of the experiment indicating a conversion of some of the glycocoll into this substance. In the same way there was evidence of the direct transference to the tissues of the egg albumin and protein digest. In a later paper [1912, 3] by allowing

a longer interval, 2.5 to 3 hours, to elapse before the final samples of blood and muscle were taken Folin gave ample demonstration of the conversion into urea of some of the absorbed alanine, glycocoll and peptone, for, in addition to the increase of the non-protein nitrogen, there was also an increase in the urea of the blood and tissues. The absorption of creatine and creatinine on the other hand was unaccompanied by any other change than the accumulation of the non-protein nitrogen in the blood and muscle. The author points out that the liver has not brought about any specialised deamination of the amino acids and emphasises the fact that the urea forming process is characteristic of all the tissues.

A few experiments [1912, 1] on the difference in the non-protein and urea nitrogen content of the blood in the fasting and the fed animals provided very scanty data for drawing satisfactory conclusions as to the influence of feeding. The amount of non-protein and urea nitrogen, however, in the blood of cats fed on a nitrogen-free, carbohydrate fat diet was undoubtedly less than in the case of meat-fed cats and this result was confirmed by data in a later paper [1912, 3] where very low values were obtained after feeding cats on a rice and cream diet.

Folin had always been interested in the question of the deamination of the products of protein digestion in the intestinal wall and he succeeded in demonstrating in a striking way that the ammonia in the portal blood did not originate in any such process. He [Folin and Denis; 1912, 2] compared the ammonia content of the blood in the mesenteric branches of the portal vein and discovered that it was two or three times greater in the blood coming from the large intestine than in that from the small intestine, owing to the extraordinarily high ammonia content of the faeces in the large intestine, particularly after a meat diet. This coupled with the fact that the injection of a pancreatic digest, glycocoll or asparagine caused no increase in the ammonia of the portal blood was conclusive proof of the source of the portal ammonia. In connection with the question of absorption Folin [Folin and Denis, 1912, 4, 5] also showed that a certain amount of absorption both from the stomach and large intestine occurred after the introduction of solutions of amino acids, urea or peptone.

Van Slyke's work dealing with the fate of protein digestion products in the body was made possible by the elaboration of a new method [1913, 1] for the determination of amino acid nitrogen and is of the highest importance as it gives a direct method of demonstrating the presence of amino acid nitrogen—in Folin's work the presence of the amino acid was only indirectly

inferred from the increase in the non-protein nitrogen. Van Slyke established the following facts in his first paper [van Slyke and Meyer, 1912], which was essentially preliminary. The blood of normal dogs, which have fasted for 20 to 24 hours, contains from 3 to 5 mg. amino acid nitrogen per 100 cc. When 12 g. of alanine were injected into the circulation of a 14 kilo. dog in 13 minutes, the added amount of amino acid nitrogen in the blood diminished very rapidly, only a relatively small portion of the alanine (1.5 g.) being excreted in the urine. It was calculated, on the assumption that the total volume of the blood was $1/20$ of the body weight, that five minutes after the injection only 1.47 g. of alanine remained in the circulation. When alanine was injected into the small intestine its direct absorption was proved by the increase of the amino acid nitrogen in the blood of the mesenteric vein. The amino acid content of the blood five hours after a meal of 1 kilo. meat was practically doubled in amount. In view of these facts van Slyke is amply justified in his conclusion that the amino acids are not synthesised in the bowel wall but are offered to the tissues directly. The elaboration of these results in a later paper [van Slyke and Meyer, 1913, 1] showed that the amino acid nitrogen of the tissues was increased by an intravenous injection of amino acid. Apparently however there is a saturation point which sets the limit to absorption, as the authors say they were never able to force the figure for muscle up beyond 80 mg. per 100 g., the saturation point for liver is much higher and amounts to 125 to 150 mg. By increasing the length of the experiment [van Slyke and Meyer, 1913, 2] and comparing the amino acid content of samples of the blood and tissues taken 30 minutes after the cessation of the injection with that of samples taken several hours later, it was found that the amino acid content of the liver rapidly decreased. Although the latter might have been practically doubled in amount by the injection, the liver was able to desaturate itself completely in two or three hours, whereas the amino acid content of the muscle remained practically unaltered during this period. The disappearance of the amino acid from the blood was accompanied by an increase in the amount of urea.

A short study [van Slyke and Meyer, 1913, 3] of the effects of fasting and feeding on the amino acid content of the tissues lacks sufficient data to establish any very definite conclusions, in view of the variations present in both fasting and fed animals. The figures show that the amino acid nitrogen in the tissues of dogs fed on a protein-rich diet was not greater than the amount found in some of the fasting dogs. A nitrogen-free, abundant carbohydrate fat diet was not included in the list but it is extremely likely

in view of Folin's work that under such conditions the amino acid nitrogen of the blood and tissues would have been lower than in either the fasting or the fed animal.

The following researches owe their origin to the work of Folin or of van Slyke. In their attempts to prove that the liver is not the main site of urea formation and to belittle its activity in this direction, Fiske and Sumner [1914] performed some experiments on cats, in which they completely excluded the liver from the circulation and subsequently introduced an amino acid into the blood stream. Under these conditions they produced a certain amount of evidence that urea formation still goes on. They claim that the accumulation of urea per unit mass of blood and tissues is as great in the absence of shock when the liver and other abdominal viscera are excluded from the circulation as when they are in their normal relations. It may be pointed out that the initial variation in the urea content of the blood and tissues is astounding, and one is tempted to ask whether the small increase in the values in the case of the muscle is not within the limits of experimental error.

In conjunction with Karsner, Fiske had previously [1913] carried out a few perfusion experiments on the cat's liver, ammonium carbonate or glycocoll being added to the perfusing fluid, but the experiments are of little value. The failure to find evidence of urea formation from the glycocoll can be attributed to the short duration of the experiments and to the relatively small amounts of glycocoll used. The whole amount of ammonia perfused was not converted into urea, and simply from a consideration of the figures for the total soluble nitrogen the authors regard it as doubtful if the liver has bound any ammonia as such. It may be pointed out that indirect calculations of this kind are valueless in the absence of a direct tissue analysis; the fixation of ammonia and of amino acids by the liver is an established fact.

Matthews and Nelson [1914] devised a method for determining the exact character of the metabolic changes taking place in muscle tissue. They removed practically all the viscera with the exception of the kidneys and about 20 to 25 g. of liver in decerebrate dogs, and then injected continuously into the veins a diuretic salt solution to wash all the nitrogen out of the tissues and to keep up an even flow of urine; the "zero" urine thus obtained had a very low nitrogen content and was ammonia-free. 10 g. of erepton containing only traces of ammonia and no urea were then injected in 30 minutes into the muscles. From a comparison of the urine secreted after

the injection with the zero urine the authors claim to have obtained positive evidence that the amino acid mixture injected is broken down and appears in the urine largely as ammonia and urea. The data are very scanty however and the results are not concordant, *e.g.* in dog 3, Table II, the urea is actually diminished after the injection of erepton, and in dog 4 the increase is trivial.

Marshall and Davies [1914] showed that the concentration of urea in the blood and tissues was approximately the same both normally and after the injection of large quantities of urea. The following figures may be quoted:

Normal dog				After repeated injections of ascending doses	After injection of 5 g. urea in 7 minutes
Blood	...	...	28 mg. }	1065 mg.	—
			27 " }		
Blood serum	...	...	29 " }	1034 "	493 mg.
			29 " }		
Liver	...	...	32 " }	930 "	354 "
			23 " }		
Muscle	...	...	25 " }	914 "	377 "
			25 " }		

According to these workers the tissues take up with great regularity about 90 per cent. of the amount of urea injected within three minutes after the injection, whether the concentration in the body be high or low. Diffusion of the urea was practically instantaneous and was complete in all parts of the body in 15 minutes. The rate of elimination by the kidneys was very rapid and rose to 16 g. per kilo. body weight or higher per day; when the excretion of urea was prevented the entire amount was stored in the body. No evidence was found of the conversion of urea into any other substance.

McLean and Selling [1914] give some interesting data regarding the urea and non-protein nitrogen in human blood; they found that the concentration of these two forms of nitrogen was not constant in normal individuals but fluctuated from hour to hour and varied within wide limits owing to such factors as diet and the amount of fluid ingested. The figures for the urea nitrogen in their tables range from 10 to 23 mg. per 100 cc. giving a variation of ± 35 per cent. (*i.e.* the total variation is expressed as a percentage of the mean of the two extreme values), the figures for the non-protein nitrogen vary from 23 to 36 mg. per 100 cc.

Paul György and Edgard Zunz [1915] have recently studied the amino acid content of the blood of normal dogs using the van Slyke method. This value, *viz.* 4 to 5 mg. per 100 cc., proved remarkably constant, it was not affected by a meal of potato puree but after a meat meal (25 g. meat per

kilo. body weight) the average rose to 11.9 mg. A comparison of the whole blood with the blood plasma showed that this increase was more marked in the case of the blood corpuscles than of the plasma.

Jansen [1915] as a result of perfusing the livers of 26 cats and dogs with blood of varying dilution to which, with two exceptions, additions of leucine, glycocoll, alanine, ammonium carbonate or erepton were made, came to the conclusion that, contrary to the opinion of Fiske and Karsner, the liver played an important part in the formation of urea from amino acids.

In considering the significance of the nitrogen retention which undoubtedly occurs when ammonium salts are introduced into the dietary, the rôle of ammonia in maintaining the normal neutrality of the tissues must not be forgotten. The effect of alkali on autolysis *in vitro* is to retard the process, and acid on the other hand has an accelerating action. Schryver [1906] obtained practically the same results in the living animal and he believed that the rôle of ammonia in retarding the activity of the proteolytic enzymes of the tissues was a very important one. That large amounts of acid can be introduced into the body by the use of certain foods with a preponderance of acid ash is evident from the work of Shermann and Gettler [1912]; these observers were able to introduce the equivalent of 21.7 cc. and 32.7 cc. N. acid per day on two occasions respectively by substituting rice for potato in a man's diet. The extent to which the acid excreted in the urine is neutralised by the ammonia present in the body is still the subject of conflicting views; in the experiment just mentioned Shermann and Gettler found that the ammonia excreted only accounted for one-quarter to one-half of the acid excreted. Janney [1911] claims that by giving large doses of alkali he was practically able to banish ammonia from the urine.

Lawrence Henderson [1911] finds that the alkalinity of the urine is always lower than that of the blood, and the amount of alkali which must be added to the urine to equalise the two represents the amount of alkali saved to the body. Under normal circumstances he states that the two factors, the physico-chemical separation of acid and ammonia secretion, are probably of the same order of magnitude and are strictly additive. Gammeltoft [1911] turned his attention to the relation of ammonia to the acid secretion of the stomach and continued the investigations of previous workers. Corresponding with the secretion of an acid juice after a meal he found a diminution in the excretion of ammonia in the urine, while following on the subsequent pouring out of an alkaline juice the ammonia excretion rose again.

In view of these facts it is evident that ammonia is constantly needed

to replace the amount excreted in the urine. Now as the only source of ammonia under normal conditions is the protein of the food, it follows that whenever protein is wholly withdrawn from the dietary a shortage of ammonia in the body must occur which will become more pronounced the longer the protein-free diet is continued. Under these conditions the introduction of organic ammonium salts would inevitably lead to a retention in order to restore the normal equilibrium in the tissues. The same argument applies when a shortage of other forms of nitrogen, *e.g.* amino acid nitrogen, has been produced by withholding all nitrogen from the food; given such a condition of nitrogen hunger, the introduction of protein itself or of its cleavage products will almost certainly lead to a retention of nitrogen. In all these experiments in which the nitrogen metabolism of the animal is reduced to a very low level by the administration of nitrogen-free diet, whether it be preceded by a fast or not, this consideration must be borne in mind otherwise the results will receive too favourable an interpretation. Attention has been called to the influence of the previous state of nutrition on the course of metabolic experiments by Pfeiffer and Kriske [1911] in their investigation of the effect on sheep of a protein-rich and a protein-poor diet respectively. It was found that the retention of nitrogen continued longer and was greater in amount in the case of those animals which were in the poorest state of nutrition at the outset of the experiment. In the course of this research, which combined tissue analysis with metabolic work, the authors made an important observation which has a direct bearing on the interpretation of metabolic experiments in general; they stated that the nitrogen retention recorded in the metabolism experiments was usually greater in amount than could be accounted for by the subsequent analysis of the tissue on the slaughter of the animal.

From the experiments of Folin and van Slyke it would seem that the tissues have no selective action with regard to the various forms of nitrogen carried to them by the blood stream, but that they take up amounts of amino acid, urea and ammonia which depend chiefly on the concentration of these bodies in the blood, the higher the concentration the greater the probability will be of finding a fixation on the part of the tissues. In the experiments already mentioned in which ammonium salts were fed, the concentration of ammonia in the blood would certainly be increased above the normal immediately after its absorption and such a condition in itself would favour a retention of nitrogen. A consideration of the facts which have been enumerated should be sufficient to show what caution is needed in

the interpretation of the result of metabolic experiments particularly with regard to attributing a retention of nitrogen to protein synthesis.

Turning to the tissues themselves, as we have seen under normal circumstances, a definite percentage of ammonia, amide and amino acid nitrogen is present which can readily be extracted, and although there is a considerable degree of variation in the actual and relative amount of these various substances the tissues never get entirely depleted. It is to be supposed that the amino acids present have been mainly derived from the proteins of the food and have been absorbed as such, while a small proportion has probably come from the catabolic processes of the cells themselves. Some of these amino acids must be destined for constructive and others for degradation purposes, only a very small proportion being normally excreted unchanged in the urine. Possibly the percentage of amino acid normally present in the tissues represents the point of equilibrium between the anabolic and catabolic processes in the cell. The amide has presumably originated in the tissues themselves mainly from the amino acid of the food but in a lesser degree as a degradation product of cell metabolism. The percentage is much lower than that of the amino acid, possibly indicating that it takes part in fewer transactions particularly of a synthetic nature; there is no evidence that it can be utilised for synthetic purposes beyond the feeding experiments previously described, in which urea caused a marked retention of nitrogen, and the outstanding feature of these investigations was the inability of the amide to act as an adequate substitute for protein.

The percentage of ammonia, the simplest form of tissue nitrogen, is again very much smaller than that of the amide. Does this small quantity of ammonia indicate participation in a very small number of metabolic transactions or does it represent the minimum necessary for purposes of neutralisation, *i.e.* to regulate the reaction of the tissues? There is no doubt that ammonia is mainly derived from protein degradation and the evidence of its resynthesis into protein in the body, as we have seen, is certainly not of a nature to suggest that such a process occurs to any extent under normal circumstances.

It will be shown later that there is a higher percentage of amino acid and of amide nitrogen in the liver than in the muscle, the reason for which may be that a larger proportion of the amino acid of the food is metabolised in the former than in the latter.

These three forms of nitrogen are so readily washed out of the tissues that one cannot imagine them to be in chemical union with any of the cell constituents. The same consideration applies to the fixation by the tissues

of these same nitrogenous bodies when introduced directly into the blood stream. They are so easily extracted that the nature of their union with the cell seems much more likely to be physical than chemical. The process of adsorption offers at once the simplest and most feasible explanation of the bond, and the release of the adsorbed substance would immediately follow on lowering the concentration of the solution in the process of extraction. Bayliss [1915] points out that adsorption is a necessary preliminary to many chemical reactions and certainly in the body with its colloidal solutions there must be an infinite number of chemical processes which are initiated in this way.

COMPOSITION OF NORMAL TISSUES.

When the present investigation was started four years ago the interest was focused on the capacity of the liver to fix and absorb ammonia after its introduction into the blood stream, and on the question of whether the ammonia content of the tissues was affected by different diets. It was hoped by adopting the following method that the same set of experiments would throw light on both points. Previous to the actual experiment the dogs were fed on two diets which differed as widely as possible in their nitrogen content, the one consisting of lard and tapioca and the other of meat with or without an addition of dog biscuit. It was expected that a comparison of the initial tissue analyses in the case of animals fed on the two different diets would furnish data as to the part played by diet in the ammonia content of the tissues and that a comparison of the tissue analyses after the injection would determine the changes in the ammonia content caused by the injection. The actual experiment was carried out under complete ether narcosis; after one or more lobes of the liver had been ligatured off for the initial tissue analysis, the ammonium salt was injected intravenously. The animal was killed by bleeding shortly after the cessation of the injection and the remaining lobes of the liver were then utilised for the determination of the final ammonia content.

After the first few experiments the research began to widen out, muscle tissue was analysed as well as liver, and in addition to the ammonia, total soluble or non-protein nitrogen, amino acid and amide nitrogen were determined with a view to finding out the initial nitrogen distribution in the tissues and the effect produced on it by intravenous injection. An investigation of the disappearance of ammonia from the blood was quickly given up

because of the impossibility of carrying out so many estimations single-handed, and for the same reason an analysis of the intestinal mucosa was only carried out in four of the earlier experiments. A further question arose after the first seven experiments, viz. was there any evidence of protein synthesis after the intravenous injection of ammonia, and this entailed a determination of the total nitrogen of the tissues before and after injection together with an estimation of their water content.

As the initial variations in the nitrogen content of the tissues were found to be very wide, the attempt to determine the influence of diet upon it was abandoned after the fifteenth experiment (dog G); the variations up to this time had no definite relation to diet and were therefore judged to be physiological, and the correctness of this deduction was confirmed by the subsequent work in which the variations were about equally wide although a uniform diet of porridge and milk was employed¹.

For purposes of comparison and control a short series of experiments was carried out to ascertain whether the intravenous injection of physiological salt solution caused any change in the nitrogen content and its distribution or in the water content of the tissues. In the later experiments the effect of injecting glycocoll and urea was also investigated. Unfortunately the unavoidable interruption of the research led to its extension over more than two years, and as the later experiments were planned in the light of the earlier work, they naturally furnished on the whole more concordant results and required fewer repetitions. In dealing with the methods employed it is only necessary to describe the procedure in the later experiments as any modifications introduced have only been slight. The results of the experiments are all tabulated in Tables I–XV.

Technique and methods.

After a preliminary hypodermic injection of morphia the animal was anaesthetised with chloroform followed by ether. The jugular vein was first exposed and ligatures were placed in position, the left common iliac artery and vein were then ligatured to facilitate amputation of the thigh, which was performed as high up as possible in order to furnish the maximum amount of muscle tissue for analysis; the bleeding was completely controlled by including all the muscles above the seat of amputation in three interlaced tape ligatures. If the bladder contained any urine it was emptied by direct

¹ This does not imply that diet has no influence on the composition of the tissues but that no appreciable change could be attributed to the diet in these particular experiments.

pressure or failing this by an incision, which was then closed by a purse-string suture. Two or even three lobes of the liver were then ligatured with tape and removed, and immediately after this the cannula was inserted into the jugular vein and the intravenous injection was started. The solution was warmed before being placed in the burette and was run in continuously in the case of the urea and glycocoll but the injection of the ammonia had to be frequently interrupted because of its toxic effect. The animal was killed by bleeding about ten minutes after the cessation of the injection; a few exceptions in the case of some of the ammonia experiments were due to the death of the animal from ammonia poisoning during the injection¹. After the death of the animal the rest of the liver was at once removed and the other thigh was amputated. If the bladder contained any urine it was ligatured off and removed to prevent any loss of the contents. The blood was carefully and thoroughly squeezed out of each lobe of the liver, immediately after its removal, by firm pressure with the fingers; three small portions from about 0.2 to 0.3 g. of tissue were quickly excised and placed in weighing bottles of known weight, the latter were immediately replaced in the desiccator and were weighed as soon as possible. When practicable 100 g. of liver were used for the determination of the non-protein nitrogen but sometimes a much smaller quantity only was available, viz. 50 to 60 g. After weighing, the tissue was plunged into 100 cc. of acidified boiling water and after being cut up in the fluid into small pieces with scissors in order to ensure complete coagulation of the protein it was boiled for five minutes. (This particular modification of the earlier technique, in which the tissue was boiled in acidified water after being ground up with glass, was adopted from the work of van Slyke.) The muscle tissue was treated in the same way.

The further treatment of the tissue set aside for the determination of the non-protein nitrogen and its constituents was as follows: the liquid was poured off into a porcelain basin and the tissue was pounded with ground glass in a mortar and was then transferred to the liquid in the basin with an additional 100 cc. of water, the whole was then boiled with constant stirring to prevent bumping. After cooling the precipitate and filtrate were transferred to a 500 cc. cylinder and the volume was made up to 400 cc.

¹ One of the five dogs destined for the injection of normal saline solution died before the injection started apparently from an overdose of the anaesthetic, the tissues were worked up in the usual way however, duplicate samples of liver and muscle being taken for the determination of the soluble nitrogen and its constituents. Although both values have been given in the tables only the figures in the column of initial values have been included in the calculation of the various averages relating to the whole series or to the particular group of experiments.

Aliquot portions of the filtrate were taken for the various analyses, 25 cc. for the non-protein nitrogen and 50 cc. for the ammonia, amide and amino nitrogen. Duplicate analyses of the ammonia and amide nitrogen could not be carried out owing to the number of determinations necessary, but a sufficient check was established by the repetition of the experiments. The analytical methods employed were the same as those used in the previous work [Bostock, 1912, 1] on the nitrogen distribution in autolysis and were as follows: non-protein nitrogen by the Kjeldahl method, ammonia by the Kruger-Reich-Schittenhelm method, amide by a modification of the Benedict-Gephart autoclave method and amino acid by the Henriques-Sørensen method, the initial neutralisation being carried out with litmus as indicator.

The total nitrogen was at first estimated on the moist tissue in duplicate, in all the later work, however, it was estimated in triplicate on the dried tissue; the same samples having been previously weighed in the moist condition served also for the determination of the percentage of dried matter in the fresh tissue, drying being carried out at a temperature of from 100° to 103°.

There is no record of any observations dealing primarily with variations in the composition of the normal tissues and little attention has been drawn to the fact that very considerable differences do occur, although the initial tissue analysis in certain types of experiment furnishes some data on the subject. Emmet and Carroll [1911] however have pointed out some most interesting facts with regard to the physical constants of fat; there was no change produced in the fat of an animal by feeding it with different amounts of protein but great differences were found in the fat of the same animal in different regions of the body, *e.g.* the melting point was 34.1° for the back fat and 45.7° for the kidney fat. Van Slyke [1913, 2] drew attention to the fact that the amino acid content of the gracilis muscle was normally about 10 to 20 mg. less per 100 g. than that of the triceps, and elsewhere [1913, 1] he stated that when different samples of an organ such as the liver were taken as duplicate samples or when symmetrically placed organs such as the right and left kidney gracilis were used, a variation up to 10 per cent. of the amount of amino nitrogen determined must be expected. The following instances may be quoted: lobe (1) 34 mg., lobe (2) 29 mg. [van Slyke and Meyer, 1913, 1], and again, lobe (1) 59 mg., lobe (2) 55 mg., lobe (3) 64 mg. [van Slyke and Meyer, 1913, 3].

That variations exist in the non-protein nitrogen of the blood in different individuals of the same species may be seen from an examination of Folin's

figures; in the first series of experiments [Folin and Denis, 1912, 1] these range from 30 to 40 mg. per 100 cc. of portal blood, while in a later series [Folin and Denis, 1912, 3] the extent of the variation is even greater, the limits per 100 cc. of blood being 29 and 67 mg.; the minimum values it may be mentioned in the last named experiments are unmistakably due to the diet.

The nitrogen of the tissues is of course mainly in the form of coagulable or protein nitrogen but a small percentage can be washed out of the tissue, which is neither coagulable by heat nor affected by the ordinary precipitants of protein. The amount of this soluble nitrogen varies slightly according to the method used, in the case of that adopted in the present research it amounts to about 11 per cent. of the total nitrogen. The main constituents of the soluble or non-protein nitrogen are the amino acid, amide and ammonia nitrogen and together they constitute about 60 per cent. of the soluble nitrogen.

The term *nitrogen distribution* in this paper is used to designate the actual and relative amounts of these different forms of nitrogen, and the present series of experiments will furnish data regarding the particular arrangement existing in different tissues and the changes produced by the intravenous introduction of various forms of nitrogen. The variations which occur in different individuals in the amount of the various nitrogenous bodies and in the relations existing between some of these may be conveniently described under the heading of *physiological variation*, and it will be necessary to consider its limits before proceeding to the study of the effects produced on the nitrogen distribution in the tissues by the introduction of different types of nitrogen into the blood stream.

The difference between the maximum and minimum values in a given series of figures furnishes the total variation for the series and if this figure be halved the total $\pm$ variation is obtained; the significance of the variation can only be accurately gauged, however, by expressing it as a percentage of the average value for the whole series of figures under consideration and such a procedure also renders it available for purposes of comparison. This method of treating the total $\pm$ variation has been adopted in the present paper. For example, the maximum and minimum values for the series of figures 4, 3 and 2 are 4 and 2 respectively, the total variation is 2 and the total $\pm$ variation is ± 1 ; the mean of the series is 3 and therefore the percentage variation is ± 33.3 per cent. $\left(\frac{1 \times 100}{3}\right)$.

In the case of figures taken from the work of other investigators, the mean of the maximum and the minimum values has been substituted for

the mean of the whole series as it was more readily arrived at and did not seriously affect the resulting value.

The physiological variation in the normal tissues will be considered under the following headings:

1. Percentage of dried material in 100 g. fresh tissue.
2. Total nitrogen in 100 g. fresh and dried tissue.
3. Total insoluble or protein nitrogen in 100 g. fresh and dried tissue.
4. Total soluble or non-protein nitrogen in 100 g. fresh tissue.
5. Relationship of the total protein and non-protein nitrogen to the total nitrogen.
6. Amino acid nitrogen in 100 g. fresh tissue.
7. Amide nitrogen in 100 g. fresh tissue.
8. Ammonia nitrogen in 100 g. fresh tissue.

1. *Physiological variation in the percentage of dried tissue in 100 g. fresh tissue.*

It is evident that the percentage of moisture in the tissues will affect the figures for the nitrogen content of the fresh tissue and it might conceivably be responsible for the variations in the latter. It will be seen from Table I that there are considerable differences in the percentage of dried matter in the fresh tissue of both liver and muscle in different individuals. The limits for the liver are 28.4 per cent. and 21.9 per cent. giving a total variation of ± 3.2 or of ± 12.1 per cent., which is less than the percentage variation in the values for every variety of nitrogen in the fresh liver, and therefore the amount of moisture cannot be the determining factor in variations of the nitrogen content. The average value for the whole series is 26.64 per cent. and may be compared with the average for Grund's whole series which is 25.5 per cent. In the case of the muscle the limits are 28.4 per cent. and 22.3 per cent. giving the same percentage variation of ± 12.1 per cent. as in the liver; here again the variation in the moisture is smaller than the variation in any of the forms of nitrogen present in the fresh muscle. The average value for the muscle is 24.8 per cent. and is slightly higher than Grund's average, which works out at 23.9 per cent. It may be mentioned that it by no means always follows that a high percentage of moisture in the liver is associated with the same condition in the muscle; in about 47 per cent. of the cases the value is above the average in the one and below it in the other tissue and neither the maximum nor minimum value for the liver is

associated with the corresponding maximum or minimum value in the muscle.

TABLE I.

Giving percentage of dried matter in 100 g. fresh tissue before and after injection.

Experiments		Liver		Muscle	
		Before	After	Before	After
Early ammonia	*	23.41	25.54	24.59	24.57
"	A	26.1	26.2	28.4	28.4
"	B	26.3	25.9	27.7	25.9
"	C	27.72	25.95	26.25	25.55
"	D	26.92	24.33	23.8	23.78
"	E	27.25	26.98	24.32	23.11
"	F	27.71	23.87	24.46	23.68
"	G	27.68	25.92	26.23	25.17
Last ammonia	I	21.9	21.7	23.1	23.5
"	II	26.7	24.5	22.3	22.6
Sodium chloride	I	27.71	28.43	26.66	26.67
"	II	27.97	26.23	26.04	26.88
"	III	28.07	24.68	24.49	25.55
"	IV	26.38	21.77	24.89	25.14
"	V	26.82	24.61	25.06	24.73
Glycocoll	I	24.73	22.14	23.69	23.49
"	II	24.38	24.57	24.61	23.33
"	III	26.94	22.15	24.39	24.11
"	IV	26.8	24.9	24.17	24.7
"	V	27.61	25.40	25.11	25.66
"	VI	26.64	24.34	24.46	24.36
Urea	I	26.5	27.1	22.8	23.5
"	II	25.5	25.7	25.05	24.62
Average for whole series		26.64	25.05	24.89	24.72

2. Total nitrogen calculated on the fresh tissue.

These figures will be found in Table II. The average value for 100 g. liver is 3.064 g. and there is a total variation of ± 0.443 , or of ± 14.4 per cent. The average value for the muscle is slightly higher and is 3.397 g., with a total variation of ± 0.516 g. or of ± 15.1 per cent. Neither the maximum nor the minimum values for the liver are found in conjunction with the maximum or minimum values for the muscle, and the lack of any close correspondence between the relative amounts of the total nitrogen of the two tissues is borne out by the fact that in 38 per cent. of the experiments a high value in the one tissue is accompanied by a low value in the other. Buglia and Constantino [1913] give 3.240 g. and 3.390 g. for the total nitrogen of the muscle of two fed dogs; these figures coincide closely with

the average value given above. Diesselhorst's figures [1911] in the case of two dogs are as follows:

Rice diet: Dog 1, 3.08 g. ... Dog 2, 3.21 g.
Meat diet: Dog 1, 3.27 g. ... Dog 2, 3.46 g.

TABLE II.

Giving the total nitrogen in g. in 100 g. fresh and dried tissue before and after injection.

Experiments		Fresh liver		Dried liver		Fresh muscle		Dried muscle	
		Before	After	Before	After	Before	After	Before	After
Early ammonia	*	3.192	3.287	11.23	12.88	3.599	3.631	14.63	14.77
"	A	2.990	2.890	11.45	11.03	4.081	3.318	14.36	11.68
"	B	2.891	2.289	10.99	8.84	3.346	3.069	12.08	11.85
"	C	2.590	2.947	9.34	11.35	3.365	3.328	12.81	13.02
"	D	3.165	2.029	11.75	8.33	3.302	3.252	13.87	13.67
"	E	3.058	3.370	11.22	12.49	3.269	3.150	13.44	13.63
"	F	2.793	2.933	10.08	12.28	3.328	3.188	13.60	13.46
"	G	2.591	2.950	9.36	11.38	3.366	3.328	12.83	13.22
Last ammonia	I	3.128	3.011	12.42	13.25	3.247	3.351	13.48	14.04
"	II	2.918	3.037	10.92	12.39	3.048	3.112	13.62	13.69
Sodium chloride	I	3.251	3.489	11.73	12.27	3.482	3.471	13.05	13.01
"	II	2.697	2.859	9.64	10.90	—	—	—	—
"	III	3.009	2.952	10.71	11.95	3.277	3.312	13.37	12.96
"	IV	3.185	2.730	12.02	12.52	3.492	3.485	14.02	13.90
"	V	3.043	3.022	11.59	12.42	3.409	3.398	13.60	13.79
Glycocoll	I	3.070	2.970	12.41	13.41	3.290	3.352	13.88	14.27
"	II	3.202	3.333	13.13	13.56	3.494	3.606	14.19	15.45
"	III	3.107	2.746	11.53	12.39	3.448	3.579	14.13	14.84
"	IV	3.467	3.209	12.93	12.87	3.381	4.131	14.19	16.67
"	V	2.960	3.046	10.71	11.96	3.504	3.597	13.91	14.01
"	VI	3.476	3.299	13.04	13.55	—	—	—	—
Urea	I	3.419	3.578	12.88	13.17	3.213	3.326	14.04	14.13
"	II	3.271	3.322	12.82	12.88	3.409	3.426	13.60	13.91
Average for whole series		3.064	3.012	11.47	12.07	3.397	3.396	13.65	13.80

Calculated on 100 g. dried tissue the average value for the liver is 11.47 g. with a total variation of ± 1.89 g. or of ± 16.4 per cent. The minimum value coincides with the minimum value for the fresh tissue and Table III shows that there is a general correspondence between the values for the moist and dried tissue in the different groups of experiments, a high or low value in the one being accompanied by a correspondingly high or low value in the other with the exception of the last ammonia group. The average value for muscle is 13.65 g. with a total variation of ± 1.275 g. or of ± 9.3 per cent.

Buglia and Constantino's values for the dried muscle are 14.08 g. and 13.87 g. and those of Diesselhorst are:

Rice diet: Dog 1, 12.82 g. ... Dog 2, 11.44 g.

Meat diet: Dog 1, 11.54 g. ... Dog 2, 12.88 g.

The maximum and minimum values for the dried muscle do not coincide with the corresponding values for the moist muscle and this lack of concordance is evident in two out of the five groups of experiments in Table III, where a high value in the dried tissue is associated with a low value in the fresh and *vice versa*.

TABLE III.

Average value in g. for the different groups of experiments of the total nitrogen in 100 g. moist and dried tissue before and after injection.

Experiments	Fresh liver		Dried liver		Fresh muscle		Dried muscle	
	Before	After	Before	After	Before	After	Before	After
Early ammonia ...	2.908	2.836	10.67	11.07	3.407	3.283	13.40	13.16
Last ammonia ...	3.023	3.024	11.67	12.82	3.147	3.231	13.55	13.86
Sodium chloride ...	3.000	3.080	11.41	11.88	3.389	3.393	13.34	13.25
Glycocoll ...	3.214	3.100	12.29	12.95	3.423	3.653	14.06	15.04
Urea ...	3.345	3.450	12.85	13.0	3.311	3.376	13.82	14.02
Mean value for the whole series	3.064	3.012	11.47	12.07	3.397	3.396	13.65	13.80

There is a rather closer agreement between the relative amounts of the total nitrogen of the liver and muscle than was found in the case of the total nitrogen calculated on the moist tissue, for in 33 per cent. only of the experiments is a relatively high value in the one tissue associated with a relatively low value in the other. In comparing the percentage variation, it will be seen that it is greater in the case of the dried liver than in that of the fresh organ but the reverse is true of the muscle, showing that in the latter case the water content of the tissue is responsible for some of the variation found in the analysis of the fresh material.

3. *The total insoluble or protein nitrogen.*

The figures for the insoluble nitrogen are obtained by subtracting the total soluble nitrogen from the total nitrogen, the average value for 100 g. liver is 2.772 g. and the total variation is ± 0.398 g. or ± 14.3 per cent. The average value for the muscle is 3.018 g. with a total variation of ± 0.528 g. or ± 17.4 per cent. The maximum value for the one tissue does not coincide with the maximum for the other and the same holds good

of the minimum values; in 55 per cent. of the experiments the value is above or below the average respectively in both liver and muscle while in the remaining 45 per cent. a high value in the one is associated with a low value in the other.

TABLE IV.

Total insoluble or protein nitrogen in g. in 100 g. moist and dried tissue respectively before and after injection.

Experiments		Fresh tissue				Dried tissue			
		Liver		Muscle		Liver		Muscle	
		Before	After	Before	After	Before	After	Before	After
Early ammonia	*	2.809	2.934	3.239	3.264	9.88	11.5	13.7	13.2
"	A	2.778	2.653	3.727	2.971	10.63	10.25	13.08	10.4
"	B	2.656	2.037	2.963	2.734	10.48	7.8	10.6	10.6
"	C	2.374	2.681	2.973	2.943	8.70	10.2	11.3	11.5
"	E	2.833	3.145	2.905	2.789	10.48	11.5	11.8	12.0
"	F	2.577	2.695	2.986	2.845	9.35	11.2	12.2	12.0
"	G	2.425	2.759	3.052	3.003	9.52	10.6	11.6	11.9
Last ammonia	I	2.885	2.765	2.852	2.948	13.80	12.7	12.4	12.5
"	II	2.554	2.667	2.670	2.726	9.56	10.8	11.9	12.0
Sodium chloride	I	2.965	3.183	3.100	3.088	10.7	11.1	11.8	11.2
"	II	2.426	2.581	—	—	8.7	9.8	—	—
"	III	2.654	2.571	2.951	2.967	9.44	10.4	11.8	11.6
"	IV	2.830	2.409	3.085	3.102	10.76	11.0	12.4	12.0
"	V	2.774	2.726	3.061	3.055	10.4	11.0	12.2	12.1
Glycocoll	I	2.677	2.615	2.944	2.988	10.8	11.7	12.3	12.7
"	II	2.939	3.020	3.110	3.229	12.0	12.2	12.6	12.9
"	III	2.742	2.422	3.036	3.159	10.6	9.7	12.3	12.8
"	IV	3.147	2.830	3.011	3.738	11.75	11.3	12.4	15.1
"	V	2.637	2.665	3.132	3.202	10.36	10.4	12.3	12.5
"	VI	3.171	2.937	—	—	12.33	12.0	—	—
Urea	I	3.085	3.191	2.803	2.855	11.62	11.7	12.3	12.1
"	II	2.980	3.005	3.016	2.987	11.7	11.2	11.9	12.1
Average for whole series		2.772	2.701	3.018	3.013	10.4	10.8	12.0	12.1

The average value for the liver is smaller than for the muscle and in only 15 per cent. of the experiments is the reverse condition found. Grund's [1910] figures for the protein nitrogen are slightly lower than mine; they vary between 2.2 g. and 2.5 g. for the liver and between 2.5 g. and 2.8 g. for the muscle in the case of his six fed dogs.

It is of interest to note that the percentage variation of the insoluble nitrogen is much smaller than that of the soluble or non-protein nitrogen. Calculated on the dried tissue the average for the liver is 10.4 g. with a variation of ± 2.55 g. or ± 24.5 per cent. For the muscle the average is 12.0 g. with

a variation of ± 1.55 g. or ± 12.9 per cent. In the case of the liver the variation on the dried weight is considerably greater than on the fresh tissue, which proves conclusively that the main source of the variation does not lie in the percentage of moisture in the tissue. There is only a single exception, namely Experiment ammonia I, to the rule that the protein nitrogen of the dried liver is smaller than that of the muscle.

4. *Total soluble or non-protein nitrogen.*

The figures for the total soluble nitrogen of the fresh tissue will be found in Table V, and glancing at them one is at once struck by the very varying amounts present in the livers of different individuals; while the average for the whole series is 292 mg. in 100 g. fresh tissue there is a total variation of ± 144.5 mg. or of ± 49.4 per cent. The average for the muscle is 379 mg. with a total variation of ± 86.5 mg. or of ± 21.6 per cent. Buglia and Constantino [1913] found almost identical values for the non-protein nitrogen in the case of two dogs, the actual figures being 370 mg. and 390 mg. in 100 g. muscle. Folin's figures [Folin and Denis, 1912, 3] for muscle show a variation of ± 31 per cent. but in this case the diet is certainly responsible for the minimum values. The figures of Fiske and Sumner [1914], who used the methods of Folin, show a total variation of ± 55.5 mg. or of ± 21 per cent. The actual values for the non-protein nitrogen are lower than in my experiments, due probably to the different species of animal and the different method employed. Although in the present research the highest values for the liver and muscle occurred in the same dog and the lowest values were similarly found in one and the same animal, a relatively high nitrogen content in one tissue was by no means always accompanied by high content in the other, for in 50 per cent. of the cases a value above the average in one tissue is associated with a value below the average in the other.

5. *Relationship of the protein and non-protein nitrogen to the total nitrogen.*

The relation of the protein and non-protein nitrogen to the total nitrogen will be found in Tables VI and VII expressed as a percentage of the latter. The average ratio of the non-protein nitrogen to the total nitrogen in the liver for the whole series is 9.5 per cent. with a total variation of ± 3.19 or ± 33.5 per cent., the average for the muscle is 11.1 per cent. with a total variation of ± 2.01 or of ± 18.1 per cent.; in this relation therefore the limits of variation are also narrower in the case of the muscle than of the liver. Although the average value is rather smaller in the case of the liver than of

TABLE V.

Giving the non-protein, amino acid, amide and ammonia nitrogen in mg. per 100 g. fresh tissue before and after injection.

Experiments	Liver										Muscle																	
	Total soluble N				Amino acid N				Amide N				Ammonia N				Amino acid N				Amide N				Ammonia N			
	Before		After			Before		After			Before		After			Before		After			Before		After					
Early ammonia	I	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
"	II	—	240	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
"	III	313	358	—	—	—	48.7	52.9	—	—	—	—	—	—	—	—	46.4	44.7	—	—	—	—	—	—	—			
"	IV	261	239	—	—	—	24.6	21.8	7.0	20.1	—	—	—	—	—	—	23.5	27.3	10.0	11.2	—	—	—	—	—			
"	V	257	263	—	—	—	27.4	38.0	10.6	8.9	—	—	—	—	—	—	32.7	31.9	12.0	12.6	—	—	—	—	—			
"	VII	445	414	72.9	71.3	48.6	58.2	58.4	11.6	11.6	—	—	—	—	—	—	55.4	52.0	34.9	37.0	9.8	12.8	—	—	—			
"	VIII	350	327	52.0	57.7	57.2	58.4	58.4	3.2	5.6	—	—	—	—	—	—	39.7	33.4	43.3	41.9	8.4	13.1	—	—	—			
"	*	383	359	—	—	—	—	—	—	—	—	—	—	—	—	—	360	367	—	—	—	—	—	—	—			
"	A	212	237	78.3	72.4	29.4	37.2	37.2	8.3	11.4	—	—	—	—	—	—	75.3	68.6	33.1	29.6	10.8	8.4	—	—	—			
"	B	235	252	70.7	73.0	27.7	39.1	39.1	5.6	9.5	—	—	—	—	—	—	74.0	64.8	31.6	31.3	9.2	14.2	—	—	—			
"	C	216	266	54.1	62.2	33.4	37.6	37.6	5.6	21.7	—	—	—	—	—	—	63.5	69.5	36.6	37.8	9.2	10.9	—	—	—			
"	D	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
"	E	225	225	58.0	47.9	33.5	40.9	40.9	7.0	17.0	—	—	—	—	—	—	66.0	56.6	35.0	37.0	9.3	17.6	—	—	—			
"	F	216	238	76.0	72.4 = amino acid + ammonia N	—	—	—	—	—	—	—	—	—	—	—	91.0	90.2 = amino acid + ammonia N	—	—	—	—	—	—	—			
"	G	166	191	—	—	—	—	—	—	—	—	—	—	—	—	—	314	325	—	—	—	—	—	—	—			
Last ammonia	I	243	246	76.1	65.2	37.0	39.8	39.8	5.6	18.9	—	—	—	—	—	—	89.8	87.5	36.9	43.1	9.5	14.0	—	—	—			
"	II	364	370	73.2	67.1	50.7	62.8	62.8	5.2	26.4	—	—	—	—	—	—	85.8	84.2	53.1	57.8	12.1	14.9	—	—	—			
"	III	236	240	63.3	42.0	43.2	40.1	40.1	5.0	37.5	—	—	—	—	—	—	72.1	76.8	32.2	42.7	18.9	20.3	—	—	—			
Sodium chloride	I	286	296	72.6	71.8	—	—	—	10.7	12.9	—	—	—	—	—	—	—	—	—	—	14.9	14.9	—	—	—			
"	II	271	278	—	—	—	—	—	4.4	7.8	—	—	—	—	—	—	71.2	70.9	—	—	7.8	8.9	—	—	—			
"	III	355	381	100.7	107	42.6	43.1	43.1	13.3	10.1	—	—	—	—	—	—	81.8	77.4	36.1	37.3	9.9	9.9	—	—	—			
"	IV	355	321	98.8	80.5	—	—	—	7.0	7.0	—	—	—	—	—	—	73.3	80.3	38.0	34.7	9.5	10.0	—	—	—			
"	V	269	296	78.6	84.2	41.4	41.4	41.4	6.7	6.7	—	—	—	—	—	—	83.8	82.0	30.1	29.4	9.8	9.8	—	—	—			
Glycocoll	I	393	355	74.2	110.6 = amino acid + ammonia N	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
"	II	263	313	67.0	80.8	—	—	—	13.3	22.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
"	III	355	324	96.5	116.6	—	—	—	5.6	12.8	—	—	—	—	—	—	82.4	82.3	—	—	10.6	10.0	—	—	—			
"	IV	320	351	83.7	156.2	58.2	64.3	64.3	10.0	17.3	—	—	—	—	—	—	89.6	94.7	62.7	63.9	8.4	8.9	—	—	—			
"	V	323	354	77.2	138.9	44.1	51.5	51.5	10.2	10.2	—	—	—	—	—	—	96.7	104.2	38.1	40.5	8.2	9.1	—	—	—			
"	VI	305	345	68.8	139.7	50.0	45.3	45.3	7.1	16.6	—	—	—	—	—	—	84.1	103.0	43.1	47.5	11.7	11.7	—	—	—			
Urea	I	334	387	87.1	76.5	56.6	138.1	138.1	9.0	11.6	—	—	—	—	—	—	85.2	84.9	47.6	98.7	11.9	12.6	—	—	—			
"	II	291	317	72.1	68.4	44.9	115.1	115.1	8.6	9.4	—	—	—	—	—	—	87.8	89.0	44.1	93.0	12.4	12.7	—	—	—			
Average for the whole		292	311	76.3	93.1	41.2	53.6	53.6	8.0	15.5	—	—	—	—	—	—	78.7	79.3	38.6	46.3	10.8	12.3	—	—	—			

The figures of Experiment ammonia VIII referring to the goat have not been included in the various averages.

the muscle this condition is reversed in 20 per cent. of the individual experiments. A relatively high or low value in the liver is associated with a correspondingly high or low value in the muscle in 55 per cent. of the experiments.

The average ratio of the protein nitrogen to the total nitrogen in the liver is 90.5 per cent. with the same total variation as in the case of the ratio

TABLE VI.

Giving insoluble or protein nitrogen as a percentage of the total nitrogen before and after injection.

Experiments		Liver		Muscle	
		Before	After	Before	After
Early ammonia	*	88.0	89.26	90.0	90.0
"	A	92.90	91.80	92	89.6
"	B	91.88	89.00	88.6	89.0
"	C	91.67	90.97	88.4	88.4
"	D	—	—	—	—
"	E	92.62	93.32	88.8	88.6
"	F	92.27	91.90	89.7	89.2
"	G	93.60	93.53	90.6	90.2
Last ammonia	I	92.22	91.82	87.9	88
"	II	87.52	87.82	87.6	87.6
Sodium chloride	I	91.20	91.24	89	89
"	II	89.95	90.28	—	—
"	III	88.20	87.10	90.1	89.6
"	IV	88.85	88.23	88.3	89.1
"	V	91.16	90.20	89.8	89.5
Glycocoll	I	87.19	88.04	89.4	89
"	II	91.79	90.69	89.0	89.6
"	III	88.25	88.18	88.1	88.3
"	IV	90.78	88.18	89	90.5
"	V	89.08	87.50	89.4	89.1
"	VI	91.22	89.03	—	—
Urea	I	90.23	89.18	87.3	87.9
"	II	91.12	90.46	88.5	87.2
Average for whole series		90.5	89.7	88.9	88.8

of non-protein to total nitrogen, which however only amounts to ± 3.5 per cent., when expressed as the percentage variation; this shows in a striking fashion how much more liable to variation is the non-protein nitrogen than the protein nitrogen. The average value for the muscle is 88.9 per cent. with a total variation of ± 2.01 or of ± 2.2 per cent.

6. *The amino acid nitrogen* (see Tables V and XIII).

The average value for 100 g. fresh liver is 76.3 mg., the total variation being ± 23.3 mg. or ± 30.5 per cent.; a study of the initial values

in van Slyke's [van Slyke and Meyer, 1913, 1] experiments reveals a total variation of ± 13.5 mg. or of ± 31 per cent. (calculated as a percentage of the mean (42.5 mg.) of the maximum and minimum values). In one case the amino acid content of two different lobes of the same liver gave a total

TABLE VII.

Giving total soluble or non-protein nitrogen as a percentage of the total nitrogen before and after injection.

Experiments		Liver		Muscle	
		Before	After	Before	After
Early ammonia	*	12.01	10.92	10.02	10.11
"	A	7.11	8.23	8.67	10.46
"	B	8.13	11.01	11.44	10.91
"	C	8.34	12.42	11.65	11.57
"	D	—	—	—	—
"	E	7.35	6.67	11.13	11.45
"	F	7.74	8.13	10.3	10.77
"	G	6.42	6.47	9.34	9.79
Last ammonia	I	7.76	8.16	12.16	12.04
"	II	11.91	11.63	12.4	12.41
Sodium chloride	I	8.79	8.48	10.97	11.03
"	II	10.05	9.72	—	—
"	III	11.83	12.9	9.96	10.41
"	IV	11.15	11.76	11.65	10.9
"	V	8.83	9.79	10.2	10.5
Glycocoll	I	12.80	11.9	10.51	10.85
"	II	8.21	9.39	11.0	10.4
"	III	11.42	11.79	11.94	11.73
"	IV	9.22	{ 10.93 } { 12.7 }	10.94	9.51
"	V	10.93	{ 11.62 } { 13.41 }	10.61	10.99
"	VI	8.77	{ 10.45 } { 11.79 }	—	—
Urea	I	9.76	10.8	12.72	14.16
"	II	8.89	9.55	11.53	12.81
Average for whole series		9.5	10.3	11.1	11.2

variation of 8 per cent. It will be seen that the Sørensen method in my hands gave a rather higher figure than van Slyke found by his method.

The average value for the muscle is 78.7 mg. with a total variation of ± 20.6 mg. or of ± 26.1 per cent. In the case of the gracilis, van Slyke's figures show a total variation of ± 15.5 mg. or of ± 30 per cent.; the extremely interesting fact that the amino acid content of the triceps is usually 10 to 20 mg. higher than that of the gracilis has already been referred to. Buglia

and Constantino using the Sørensen method found an amino acid content in the muscle of two fed dogs of 84 mg. and 77 mg. respectively.

In 64 per cent. of the experiments a relatively high or low value in the liver is associated with a correspondingly high or low value in the muscle but neither maximum nor minimum values occur in the same animal. Although the average value for the muscle is slightly higher than that of the liver this condition is reversed in 35 per cent. of the experiments and a similar variability is present in van Slyke's experiments, the amino acid content of the liver being sometimes higher and at other times lower than that of the muscle.

In comparing the values of the amino acid nitrogen with those of the soluble nitrogen, 73 per cent. of the experiments show a relatively high or low value for both forms of nitrogen in the liver although neither the maximum nor the minimum values occur in the same dog; 47 per cent. of the experiments show a relatively high or low value for both forms of nitrogen in the case of the muscle.

7. *Amide nitrogen* (see Tables V and XIII).

The average value for the amide nitrogen in 100 g. fresh liver is 41.2 mg. and the total variation is ± 16.8 mg. or ± 40.7 per cent.; for the muscle the average is slightly smaller, namely, 38.6 mg. with a total variation of ± 19.6 mg. or ± 50.7 per cent. The maximum values for both tissues occur in the same dog and the same holds good for the minimum values; in only 28 per cent. of the experiments is a relatively high value in the one tissue associated with a relatively low value in the other. In Folin's first paper there is a total variation in the urea values for the muscle of ± 11.5 mg. or of ± 29 per cent. (calculated as a percentage of the mean of the maximum and minimum values), in his third paper the percentage amounts to ± 44 per cent. but here also as in the case of the non-protein nitrogen the influence of diet is unmistakable, the minimum values occurring in cats fed on a rice and cream diet. In Fiske and Sumner's [1914] experiments the urea values for the muscle range from 14 mg. to 82 mg. which gives a total variation of ± 70 per cent.

Although the average figure for the liver is slightly higher than for the muscle the reverse condition is present in 39 per cent. of the experiments. In the liver there is quite a close general correspondence between the values for the total soluble nitrogen and the amide nitrogen; although neither maximum nor minimum values are found in the same animal, in 83 per cent. of the cases a relatively high value for the one form of nitrogen is associated

with a correspondingly high value for the other form and *vice versa*. In the muscle on the other hand such a correspondence is only present in 55 per cent. of the experiments.

8. *Ammonia nitrogen* (see Tables V and XIII).

The average amount of ammonia nitrogen present in 100 g. fresh liver is 8.0 mg., the total variation is ± 4.6 mg. or ± 57.9 per cent.; the average for the muscle is rather higher, 10.8 mg. with a total variation of ± 5.5 mg. or of ± 51.2 per cent. The minimum values occur in the same dog but the maximum values do not coincide; in 57 per cent. of the animals the values are correspondingly high or low in both liver and muscle.

With regard to the relation of ammonia to the total soluble nitrogen, the value is correspondingly high or low in 56 per cent. of the experiments in the case of the liver and in 66 per cent. in that of the muscle.

Percentage distribution of the amino, amide and ammonia nitrogen in the total soluble nitrogen.

An examination of the average initial values for the whole series shows clearly that the percentage of the different forms of non-protein nitrogen in the total soluble nitrogen differs quite definitely in the liver and muscle; the percentage of amino acid and of amide nitrogen is considerably greater in the liver than in the muscle, and on the other hand the percentage of undetermined nitrogen in the latter is higher than in the liver; there is therefore a characteristic distribution of the constituents of the non-protein nitrogen for each tissue.

TABLE VIII.

Average values for the different groups of experiments expressed as a percentage of total soluble or non-protein nitrogen.

Experiments	Liver						Muscle					
	Percentage of Amino acid N		Percentage of Amide N		Percentage of Ammonia N		Percentage of Amino acid N		Percentage of Amide N		Percentage of Ammonia N	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Early ammonia	23.4	22.8	13.3	15.2	3.2	3.7	16.5	15.5	9.3	9.6	3.2	3.4
Last ammonia	24.6	21.8	14.4	16.6	1.7	8.9	22.7	21.7	11.6	12.7	3.4	4.1
Sodium chloride	28.6	27.3	13.7	13.4	2.7	2.8	21.0	21.3	9.4	9.2	2.7	2.9
Glycocoll	24.1	36.3	15.5	14.8	2.8	4.3	23.2	24.2	12.6	12.7	2.5	2.4
Urea	25.5	20.5	16.2	35.9	2.6	4.0	21.5	19.0	11.4	21.1	2.5	2.7
Average value for whole series	26.1	29.9	14.1	17.2	2.7	4.9	20.7	20.7	10.1	12.0	2.8	3.2

In this connection attention may be directed to the influence exerted by the presence of acid or alkali on the characteristic nitrogen distribution of the autolysing liver in vitro [Bostock, 1912, 2]. The addition of alkali causes the production of a higher percentage of ammonia and of a lower percentage of amide and amino acid nitrogen, whereas the addition of acid brings about a diminution in the percentage of ammonia and an increase in the percentage of amide and amino acid nitrogen. Whether the preponderance of bases in muscle tissue is in any way responsible for the characteristic distribution of nitrogen in this tissue would be difficult to determine, but it approximates more closely to the distribution in autolysing liver in presence of alkali than to that of normal autolysing liver, while it differs from autolysing liver in presence of acid more than from normal autolysing liver.

EFFECT OF INJECTION OF VARIOUS SUBSTANCES ON TISSUE COMPOSITION.

Having studied the variations in the constituents of the nitrogenous material of the tissues which occur in the liver and muscle of normal dogs we may pass to a consideration of the effects produced by the introduction of physiological salt solution and of different nitrogen-containing solutions. The same aspects of tissue analysis will be dealt with as in the section on physiological variation and in a similar order with the exception of the total nitrogen, which has not been treated by itself but only in relation to its constituents.

Table IX contains data regarding the amount of the particular substances and the volume of fluid injected together with the amount of nitrogen introduced per kilo. body weight in the different experiments. It will be seen that in all the earlier work, including all the experiments with sodium chloride and the first three with glycocoll and the ammonia experiments except the last three, dilute solutions practically isotonic with physiological salt solution were used because it was thought that this would interfere less with the normal water content of the tissues. In the later experiments much more concentrated solutions were used and with more striking results as both the glycocoll and ammonia experiments show. It should be mentioned that in the last ammonia experiment 90 cc. in all of a 10 per cent. solution of glucose were introduced alternately with the solution of ammonia. With regard to the urine secreted during the operation, as a rule in the ammonia experiments little or no urine was found in the bladder on the death of the animal, so that the amount of ammonia excreted by this

TABLE IX.

*Giving all the experiments and data as to weight of the animal and amount of nitrogen per kilo.
body weight contained in the injected fluid, etc., etc.*

Bioch. x	Experiments	Kilos. body weight	g. of substance injected	g. N in amount injected	g. N per kilo. body wt.	Duration of injection in minutes	Strength of solution	cc. fluid injected	Diet previous to experiment	Remarks
	Early ammonia	I 21	7	1.673	0.079	5	isotonic	—	—	—
	"	II 7	2.25	0.537	0.076	25	"	—	milk and tapioca.	died during injection
	"	III 20	4.28	1.022	0.051	33	"	—	meat	—
	"	IV 6.7	2.98	0.712	0.106	30	"	—	milk and tapioca	died during injection
	"	V 7	4.79	1.114	—	120	"	—	milk, lard, tapioca	killed 10 min. after injection
	"	VI 11	2.66	0.635	—	48	"	—	meat	"
	" (goat)	VIII —	—	—	—	—	—	—	—	—
	"	* —	1.97	0.47	—	47	isotonic	197	tapioca, lard	died during injection
	"	A —	5.4	1.323	—	60	"	545	—	—
	"	B 15	6	1.47	0.098	75	"	600	tapioca, lard	—
	"	C 6.65	2	0.49	0.073	30	"	20.2	meat, dog biscuit	died during injection
	"	D 7.2	3.7	0.906	0.125	50	"	378	"	"
	"	E 8.3	5	1.225	0.147	75	"	500	"	—
	"	F 6.6	3.24	0.793	0.120	80	"	—	"	—
	"	G 9.7	4.02	0.929	0.095	100	"	402	"	—
	"	I 8.3	5.05	0.901	0.106	72	5 %	101	porridge and milk	died during injection
	Last ammonia	II 6	4.15	0.74	0.123	63	5 %	83	"	"
	"	III 7.2	7.15	2.085	0.28	56	5 %	143	"	"
	"	I 11.25	—	—	—	80	0.9 % NaCl	710	"	—
	Sodium chloride	II —	—	—	—	—	"	—	"	—
	"	III 7.65	—	—	—	40	"	1000	"	—
	"	IV 12.7	—	—	—	—	—	—	"	—
	"	V 4.73	—	—	—	30	0.9 % NaCl	550	"	died before injection
	"	I 6.22	8	1.49	0.24	60	1.6 %	500	"	—
	Glycocoll	II 6.62	12.8	2.39	0.36	90	"	800	"	—
	"	III 23.5	16	2.98	0.12	46	"	1000	"	—
	"	IV 31.9	30	5.6	0.17	50	6 %	500	"	—
	"	V 13.2	15	2.9	0.21	31	6 %	250	"	—
	"	VI 6.5	13.3	2.48	0.38	49	10 %	133	"	—
	"	I 8.9	12.55	5.85	0.65	50	5 %	251	"	—
	Urea	II 6.9	8.05	3.75	0.52	75	5 %	161	"	—

channel must have been very small. In the glycocoll experiments on the other hand the bladder was quite frequently full at the close of the experiment and the amount of amino acid excreted can be seen in Table X; in no case does it amount to more than 20 per cent. of the total amount injected. A considerable amount of amide nitrogen was excreted in the second urea experiment; unfortunately the urine in the case of the first urea experiment was lost.

TABLE X.

Giving the amount of total nitrogen, amino acid, amide and ammonia nitrogen in mg. excreted in the urine secreted during the period of the injection.

Experiments	Total Nitrogen	Amino acid Nitrogen	Amide Nitrogen	NH ₃ Nitrogen
Glycocoll I	602	304 = amino acid + NH ₃ nitrogen		
„ II	1062	312.6	—	51.8
„ III	267	14.3	—	11.5
„ IV	611	355	—	28.5
„ V	—	456.9	—	23.2
„ VI	215	158.5	—	6.1
Urea II	730	—	670	28

If one wishes to produce the maximum fixation by the tissues of the various nitrogen-containing substances introduced into the blood stream, then undoubtedly this is most readily brought about by having a high concentration of the substance in the blood. This requisite concentration is best obtained by the rapid introduction of a concentrated solution of the substance under examination. This is well exemplified in the case of the experiments with urea (see Table V) when the amount injected corresponded to 0.6 and 0.5 g. nitrogen respectively per kilo. body weight.

TABLE XI.

Giving average percentage of dried tissue in 100 g. moist tissue in the different groups of experiments.

Experiments	Liver		Muscle	
	Before	After	Before	After
Early ammonia ...	27.26	25.58	25.71	25.02
Last ammonia ...	24.3	23.1	22.7	23.0
Sodium chloride ...	27.39	25.98	25.56	25.95
Glycocoll ...	26.18	23.91	24.40	24.27
Urea ...	26.0	26.4	23.9	24.06
Average for whole series	26.64	25.05	24.89	24.72

The effect of intravenous injection on the percentage of dried tissue in fresh liver and muscle (see Tables I and XI). The percentage of dried tissue in the fresh liver after injection is 25.05 and is rather smaller than the initial value, the total variation is ± 3.36 or ± 13.4 per cent., a rather wider range than was found before the injection. In 77 per cent. of the experiments the percentage is smaller after injection than in the initial values, showing that in these cases the injection has increased the water content of the tissue. The average for the muscle is 24.7 per cent. and is practically the same as the initial value, the total variation is ± 2.9 or ± 11.7 per cent. In half the experiments the initial value is greater than the value after injection, in the remaining half this condition is reversed. A study of the individual experiments or groups of experiments shows that the water content of the muscle varies as the result of the injection much less than that of the liver. It is quite evident, however, in both cases that the changes in the water content of these tissues offer a totally inadequate explanation for all the variations in the different forms of nitrogen which have been observed. This is particularly the case in those experiments where definite changes were produced in the various nitrogenous constituents of the total soluble nitrogen by the injection of urea, glycocoll and ammonia.

TABLE XII.

- (a) *Giving total insoluble or protein nitrogen in g. per 100 g. fresh tissue, calculated as the average value for each group of experiments.*

Experiments	Liver		Muscle	
	Before	After	Before	After
8 early ammonia ...	2.636	2.700	3.120	2.935
Last ammonia ...	2.719	2.716	2.761	2.837
Sodium chloride ...	2.729	2.765	3.049	3.036
Glycocoll ...	2.885	2.748	3.046	3.263
Urea ...	3.032	3.088	2.909	2.921
Mean for whole series	2.772	2.701	3.018	3.013

- (b) *Giving total insoluble or protein nitrogen in g. per 100 g. dried tissue, calculated as the average value for each group of experiments.*

Experiments	Liver		Muscle	
	Before	After	Before	After
Early ammonia ...	9.669	10.55	12.13	11.73
Last ammonia ...	11.19	11.75	12.16	12.33
Sodium chloride ...	9.965	10.64	11.93	11.70
Glycocoll ...	11.01	11.49	12.48	12.57
Urea ...	11.66	11.69	12.17	12.14
Mean for whole series	10.4	10.8	12.0	12.1

The effect of intravenous injection on the protein nitrogen (see Tables IV and XII). A consideration of the amount of nitrogen injected in the form of ammonia, amide or of amino acid shows how useless it would be to expect to find signs of its influence on the protein nitrogen; the maximum amount injected was 0.065 g. per 100 g. of tissue in the case of the first urea dog.

If the assumption be made that the injected nitrogen is converted into protein and that it is fairly evenly distributed throughout the body, as the protein nitrogen content of the liver under normal conditions is about 2.70 per cent. this would only give a possible increase to 2.76 per cent., a change small enough to be within the limits of experimental error. It will be seen from Table XII that the average value for the whole series in the case of both liver and muscle whether calculated on the fresh or the dried tissue is practically identical before and after injection, with the exception of a slight diminution in the fresh liver compared with the initial value. In 57 per cent. of the individual experiments however, the value after injection is greater than the initial value in the liver while in the remaining percentage the reverse is true; the range of variation is ± 21.3 per cent. The initial value in the muscle is slightly less than the subsequent value in 52 per cent. of the cases and the range of the variation is ± 16.7 per cent.

In 90 per cent. of the experiments a high or low initial value in the liver is associated with a correspondingly high or low value after injection, and this holds good in 84 per cent. of the experiments in the case of the muscle.

The effect of injection on the total soluble nitrogen (see Tables V, XIII and XIV). The results of the injection of the various solutions can best be studied in Table XIII where the average values for each different group are gathered together, for, as might have been expected from the study of the physiological variation in the normal tissues, individual variations after injection are very considerable; the general trend of the experiments becomes quite clear when the average of the group of similar experiments is examined. Starting with a consideration of the changes effected in the soluble nitrogen, it will be seen that in the liver there is an increase in every group, not excluding the sodium chloride group, but the only significant increase is in the urea and glycocoll groups, where it amounts to 12 and 10 per cent. respectively calculated as a percentage of the initial value. In the individual experiments there are exceptions to the general rule about the relation of the initial to the subsequent values, 23 per cent. of these show a diminished value after injection. Attention must be directed to the glycocoll experiments IV, V and

TABLE XIII.

Giving the average values for the different groups of experiments.

	Liver								Muscle								
	Total soluble N		Amino acid N		Amide N		Ammonia N		Total soluble N		Amino acid N		Amide N		Ammonia N		
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	
Experiments	274	280	64.3	64.0	36.7	42.6	8.8	10.5		376	369	62.3	57.4	35.2	35.7	12.1	12.6
Early ammonia	303	308	74.6	66.1	43.8	51.3	5.2	27.6		386	394	87.8	85.8	45	50.4	13.2	16.4
Last ammonia	306	314	87.6	85.8	42	42.2	8.4	8.9		368	365	77.5	77.8	34.7	33.6	10.3	10.7
Sodium chloride	326	360	78.6	130.9	50.7	53.5	9.2	15.6		380	396	88.2	96	47.9	50.6	9.7	9.9
Glycocoll	312	352	79.6	72.4	50.7	126.6	8.3	14.4		401	455	86.5	86.9	45.8	96.0	12.1	12.6
Urea																	
Average value for the whole series	292	311	76.3	93.1	41.2	53.6	8.0	15.5		379	383	78.7	79.3	38.6	46.3	10.8	12.3

TABLE XIV.

Giving the average values for the different groups of experiments.

Experiments	Liver						Muscle									
	Total soluble N		Amino acid N		Amide N		Ammonia N		Total soluble N		Amino acid N		Amide N		Ammonia N	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Early ammonia	10.0	10.9	2.35	2.50	1.35	1.67	0.323	0.42	14.6	14.7	2.42	2.38	1.36	1.43	0.489	0.505
Last ammonia	13.6	16.4	3.30	2.86	1.80	2.21	0.220	1.18	17.0	17.1	3.84	3.72	1.98	2.18	0.58	0.715
Sodium chloride	11.2	12.2	3.03	2.95	1.54	1.62	0.306	0.342	14.4	14.1	3.04	3.00	1.36	1.29	0.400	0.41
Glycocoll	12.4	15.0	3.00	5.80	1.94	2.24	0.352	0.650	15.6	16.3	3.60	3.96	1.95	2.06	0.396	0.405
Urea	12.1	13.3	3.03	2.74	1.93	4.75	0.317	0.545	16.7	18.9	3.61	3.60	1.91	4.00	0.425	0.535
Average for whole series	10.9	12.4	2.86	3.65	1.52	2.10	0.300	0.618	15.2	15.4	3.16	3.20	1.55	18.7	0.440	0.495

VI, where after the injection two separate portions of liver were analysed, there is a considerable difference in the figures obtained for the different portions, but this is not surprising in the light of vanSlyke's discovery, already referred to, that the amino acid content of different lobes may vary in a very marked degree. The soluble nitrogen of the muscle only shows a definite increase, amounting to 13 per cent. in the urea group; in the sodium chloride and early ammonia groups it is slightly less than the initial value, and this condition is present in 40 per cent. of the individual experiments. It may be observed that when the values are calculated on the dried muscle instead of on the fresh tissue it is only in the sodium chloride group that the initial is greater than the subsequent value, and even here the difference is really negligible. The total variation in the muscle after injection is ± 19 per cent. and is actually smaller than the variation in the initial values.

The effect of intravenous injection on the ratios of non-protein and protein nitrogen respectively to the total nitrogen (see Tables VI and XV). The average value for the ratio of the non-protein nitrogen in the liver after injection is rather higher than the initial value and amounts to 10.3 per cent., the total variation is ± 3.47 or ± 33.6 per cent. In 27 per cent. of the experiments, however, the initial value is the greater of the two and in 4 per cent. it is unaltered, only in the urea and glycocoll groups is the average value for the group definitely increased by the injection.

The average for the muscle is 11.2 per cent. with a total variation of ± 2.32 or of ± 20.7 per cent. although the average after injection is slightly greater than that of the initial value; in 27 per cent. of the experiments this condition is reversed and in 5 per cent. it is unaltered, only in the urea group is there a definite increase due to the injection.

The total variations of the ratios of the protein nitrogen to total nitrogen are of course the same as those of the ratio of the non-protein to total nitrogen, but expressed as percentage variations they only amount to ± 3.8 per cent. for the liver and ± 2.2 per cent. for the muscle and are practically the same as those of the initial values.

Effect of the injections on the amino acid nitrogen (see Tables V and XIII). Turning now to the amino acid nitrogen, it appears that only in the amino acid group is the value for the liver increased after injection, in all the other groups and in 52 per cent. of the individual experiments the initial value is slightly the greater of the two. The increase in the case of the glycocoll group is very marked and amounts to 66 per cent. In the muscle the effect of the injection of glycocoll is much less pronounced and there is a

comparatively slight increase of 8.8 per cent.; in 33 per cent. of the individual cases the initial value is greater than the subsequent value.

Effect of the injections on the amide nitrogen (see Tables V and XIII). The amide figures are particularly interesting; in the sodium chloride group for the liver they are practically identical before and after injection but in all the other groups there is a definite increase, which amounts to 5 per cent. after the injection of glycocoll, 16 per cent. and 17 per cent. after the injection of ammonia in the first and second group respectively, and 149

TABLE XV.

(a) *Giving the average figure for the different groups of experiments of the total soluble or non-protein nitrogen as a percentage of the total nitrogen.*

Experiments	Liver		Muscle	
	Before	After	Before	After
Early ammonia ...	9.4	9.8	10.45	10.93
Last ammonia ...	10.02	10.18	12.26	12.19
Sodium chloride ...	10.2	10.19	10.85	10.75
Glycocoll ...	10.14	11.61	11.10	10.84
Urea ...	9.32	10.2	12.11	13.47
Mean for whole series	9.5	10.3	11.1	11.2

(b) *Giving the average figure for the different groups of experiments of the protein nitrogen as a percentage of the total nitrogen.*

Experiments	Liver		Muscle	
	Before	After	Before	After
Early ammonia ...	90.5	90.0	89.5	89
Last ammonia ...	89.9	89.8	87.7	87.8
Sodium chloride ...	89.7	89.8	89.1	89.2
Glycocoll ...	89.9	88.2	88.9	89
Urea ...	91.6	88.8	87.8	86.5
Mean for whole series	90.5	89.7	88.9	88.8

per cent. in the case of the injection of urea itself. The heaping up of urea is very striking and is obviously determined by the concentration in the blood, whereas in the case of the other groups the increase of amide nitrogen is presumably due to the conversion of some of the injected ammonia or glycocoll. In 11 per cent. of the individual experiments the amide nitrogen is diminished instead of being increased after injection. Fiske and Karsner [1913] have already been mentioned as having perfused the livers of cats and rabbits with defibrinated blood to which additions of ammonium carbonate or of glycocoll were made. They found a definite increase of urea in the blood

from the ammonium salt but none in the case of glycocoll, but as has already been pointed out the conditions of the experiment were not the most favourable for urea formation.

Jansen [1915] perfused the livers of cats and dogs with ammonium carbonate and various amino acids and found an increase of urea in the blood.

In some experiments [Bostock, 1912, 2] which were carried out on the formation of amide nitrogen from glycocoll by the liver pulp in vitro no deamination of the glycocoll could be observed; the evidence on the whole, however, was in favour of a small amount of urea formation, for both actual and percentage amounts of amide nitrogen were increased, but the percentage was smaller than the actual increase.

The effect of injection on the ammonia (see Tables V and XIII). The total amount of ammonia in fresh tissue is very small only amounting to 2 or 3 per cent. of the total soluble nitrogen; this amount is practically unchanged after the injection of physiological salt solution but all the other groups show a definite actual and percentage increase as a result of injection. There is an increase of 19 per cent. in the early ammonia experiments, 69 per cent. in the glycocoll group, 73 per cent. in the urea and 430 per cent. in the later ammonia group. The amount of ammonia that can be injected is strictly limited by its toxicity and it would appear that some animals are more susceptible than others; the rate of injection, as well as the amount, exercises an influence on the heaping up in the tissues, the best result being obtained when the organism is flooded with the salt in question. The operation of one or other of these factors probably accounts for the greater accumulation of ammonia found in the later ammonia experiments. In 8 per cent. of the whole series of experiments the ammonia is diminished, in 8 per cent. it is unaltered and in the remaining 84 per cent. it is increased after injection.

It is very interesting to note that there has been a deamination of glycocoll, for Lang [1904] claimed that the same thing occurred in vitro, and some earlier experiments of my own with liver pulp seemed to indicate a slight deamination; the result of later work however failed to confirm this and showed the necessity of determining the soluble nitrogen as well as the ammonia in all autolysis experiments. An increase of ammonia is not necessarily derived from the amino acid added but may be due to a more rapid autolysis in the case of a particular digest or even to the presence of putrefactive organisms. In every case the percentage relationship of the ammonia to the soluble nitrogen is the important factor, and this relationship

could not be determined in Lang's or in my own early work because the sole estimations were those of ammonia.

The increase of ammonia after the injection of urea is an indication of a certain amount of deamination. Wakeman and Dakin [1911] perfused dogs' livers with urea, but under the conditions of their experiments they were unable to detect any increase of ammonia in the blood at the expense of the urea. Janney [1911] investigated the question of whether the conversion of ammonia into urea was a reversible reaction, and after reducing the excretion of ammonia to a minimum by giving large doses of sodium citrate he administered urea but it was not followed by any increase of ammonia in the urine. Lichtwitz [1912] on the other hand criticised Janney's work adversely. He claimed to have found an increase of ammonia and of amino acid with a retention of nitrogen following on the administration of urea to one dog and to four diabetics, but his data are wholly inadequate to establish his conclusions. Marshall and Davies [1914] in their experiments on the distribution and elimination of urea found no evidence of the conversion of urea into any other body but this conclusion is apparently based on indirect evidence as there is no mention of any ammonia estimations.

The changes in the ammonia content of the muscle are much less marked than in the liver; there is an increase in the majority of individual cases, but the amount is unaltered in 18 per cent. and diminished in 9 per cent. of the experiments. In the latest ammonia experiments there is an increase of 24 per cent. but in the other groups the changes are insignificant.

When the values for the non-protein nitrogen and its constituents are calculated on the dried instead of on the fresh tissue they do not materially affect the results, as can be seen from the Tables XIII and XIV, the essential features remain the same and in some cases are even emphasised, as in the urea and glycocoll groups.

When the amino, amide and ammonia nitrogen are calculated as percentages of the non-protein nitrogen it will be seen from Table VIII that the percentage relationships reflect the changes found in the absolute values. Owing to the considerable increase in the value of the non-protein nitrogen itself after the injection of urea and glycocoll, however, the percentage increase in the amide and the amino acid nitrogen in these two groups is less marked than the absolute increase.

As Table XIII is the most important of the whole series it will be well to sum up the results it contains. The sodium chloride group is the only one in which all the values non-protein, amino, amide and ammonia nitrogen,

both total and percentage, remain practically unchanged. Both the ammonia groups show a total and percentage increase in the ammonia and amide nitrogen of the liver after injection, which can only be interpreted as due to the fixation of ammonia and the conversion of some of it into urea. When the organism is flooded with ammonia, to use van Slyke's phrase, it desaturates itself by working it up into the normal product, but whether this is the only way in which desaturation takes place, the data available do not indicate. Another point of interest is that there is no indication of an amination of fatty acids occurring; possibly suitable conditions are not present, but at any rate we are justified in assuming that if amination occurs it is a much more specialised process than the synthesis of urea. Turning to the muscle in the last ammonia group the same changes are observed in a less marked degree than in the liver, but they are scarcely perceptible in the group of earlier experiments. Undoubtedly there is a fixation of ammonia by the muscle and it seems probable that the muscle possesses the power of converting this into urea without the intervention of the liver.

The glycocoll group shows a very marked fixation of amino acid by the liver and quite a marked increase in the ammonia due to a deamination of the glycocoll; there is also a definite but less well marked increase in the amide nitrogen due to a conversion of some of the ammonia into urea. An increase of urea in the blood after the intravenous injection or absorption of large quantities of amino acid has been abundantly shown by van Slyke and by Folin, but I am not aware of the same changes having been demonstrated in the liver, although Folin and his pupils have shown a very marked increase in the urea of the muscle under these conditions. The fixation of amino acid by the muscle is much smaller than by the liver; van Slyke has shown that it does not reach its saturation point as soon as the liver. It is to be noted that although the ammonia has not increased there is a definite increase in the amide nitrogen.

The urea group reveals changes in the ammonia and amide nitrogen, the latter is enormously increased and the former shows a very definite increase, and there can be no doubt of the deamination of some of the urea. The muscle also exhibits a high degree of urea fixation and a slight increase in the ammonia nitrogen.

CONCLUSIONS.

Variation is the rule in those aspects of tissue analysis which form the subject of the present communication, there is not a single instance of a constant value or relation although the range of variation is much greater in some relations than in others.

The intravenous injection of ammonia, urea and glycocoll, respectively, produces an accumulation of these bodies as such in the liver and to a less extent in the muscle. There is no evidence of the synthesis of any of these substances into protein but there is abundant proof of their further metabolism particularly in the liver; ammonia is converted into amide nitrogen in both liver and muscle, urea is deaminised in the liver and muscle while glycocoll is deaminised in the liver and is partially converted into amide nitrogen in both liver and muscle.

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