STUDIES IN THE CHEMICAL DYNAMICS OF ANIMAL NUTRITION

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CONTENTS

	PAGE
Part I. Problems Relating to the Functions of Nitrogenous Foodstuffs in the General Economy of Nutrition	123
Part II. The Methods for Estimating Residual Nitrogen in the Serum and Tissues	129
Part III. The Distribution of Nitrogen in the Serum, Liver, and Small Intestine during Digestion and during a Fast	134
Part IV. The Question of Albumen Synthesis in the Mucous Membrane of the Stomach	143
Part V. Further Experiments on Autolysis; the Relation of Autolysis to Nutrition	144
Part VI. A Theory of Nitrogenous Metabolism	157

PART I

INTRODUCTION. PROBLEMS RELATING TO THE FUNCTION OF ALBUMENOUS FOOD-STUFFS IN THE GENERAL ECONOMY OF NUTRITION

In spite of the fact that metabolism occupies a very important place in the literature of modern physiology, but little is known with certainty of the fate of the products of tryptic digestion of the albumens after leaving the alimentary tract. Various theories have been propounded to explain the rôle of the nitrogenous food-stuffs in the general economy of nutrition, of which that suggested originally by Liebig and modified by Pflüger has been, perhaps, the dominant. According to this theory the products of digestion of the albumens are incorporated into the protoplasm molecules of the living cells, and there in a readily oxidisable form, held available for the supply of the energy needs of the organism.

In quite recent years however, another, and almost diametrically opposed hypothesis, has been regarded with much favour, and has found an admirable exponent, amongst others, in the person of Speck.¹ According to this hypothesis, the nitrogen is rapidly eliminated from the products of digestion of the albumens, and excreted in the form of urea, whilst the carbohydrate part of the molecule forms, on oxidation, the main source of the energy supply of the organism. The tissues themselves undergo degradation only under exceptional circumstances, as, for example, when the food supply is insufficient for the energy needs, or when the conditions of metabolism are abnormal, as is the case during fever, or when the oxygen supply is insufficient, or after phosphorus poisoning. These abnormal conditions will be discussed again later. According to Speck, therefore, we must distinguish between the metabolism of energy and the metabolism of tissue; and as the main source of energy is derived from the oxidation of the non-nitrogenous part of the molecule, the albumens should play a comparatively subordinate rôle in the general economy of nutrition. Some support is given to this conception by the recent researches of Sivéⁿ² and of Chittenden,³ both of whom find that equilibrium can be maintained on diets that contain very much less nitrogen than was supposed to be necessary by the earlier investigators.

An important new view of the matter has been advanced recently by Folin,⁴ which does not differ very materially from that advocated by Speck. Folin has shown that the change from a nitrogen-rich to a nitrogen-poor diet is followed by marked changes in the composition of the urine. Not only does the urea output diminish in actual quantity; its relative quantity in comparison with the other nitrogenous constituents of the urine also diminishes. On the other hand, the creatinine output remains always constant, whether the diet be nitrogen-rich or nitrogen-poor. The neutral sulphur of the urine maintains a similar constancy. From these facts Folin concludes that metabolism can be considered under two heads, viz. :—as *endogenous* metabolism, which is due to a constant tissue waste (and is analogous, therefore, to the tissue metabolism of Speck), which is responsible for the constituents of the urine (creatinine and neutral sulphur) which do not

vary with the diet, and *exogenous* metabolism, which is responsible for the greater part of the urea, and other varying constituents. According to Folin then, the only nitrogenous matter necessary for the maintenance of equilibrium is that required to supply the waste represented by the endogenous metabolism. Any superfluous nitrogen is eliminated as rapidly as possible in the form of urea.

We have, therefore, widely different views as to the function of the nitrogenous constituents of the food-stuffs; on the one hand we have the view that the products of digestion are built up into the tissues, and that, relatively, large amounts of albumen are necessary to maintain the organism in full bodily vigour; on the other hand we have the view that only small quantities are necessary—just the amount that is required for the repair of the tissue waste which is continually taking place, and that the main part of the bodily energy is derivable from the non-nitrogenous constituents of foods.

Which of these views, if either, is correct? What is the true function of nitrogen in the economy of nutrition? With the object of obtaining an answer to these questions, the following researches were undertaken.

METHODS OF RESEARCH

Our ignorance is to be largely ascribed to the paucity of our methods of research. Most of the older theories are based on experiments which had for their object the determination of the balance between ingesta and egesta, either by the analysis of inspired and expired air (Zuntz, Speck, etc.), or by the estimation of nitrogen in food and excreta (Pflüger, Voit, etc.), or by the estimation of caloric values (Rubner, etc.), or by a combination of all these methods of experiment (Atwater).

The systematic determination of the distribution of nitrogen amongst the various nitrogenous constituents of urine by the method of Folin already referred to marks a distinct advance in the study of metabolism.

An attempt has been made in the following researches to throw some light on the mechanism of nutrition by an entirely different method. It was assumed that if the products of albumen degradation

be built up into the bioplasm, and held there in a form in which they could be readily eliminated for the supply of the energy needs of the organism, as demanded by the hypothesis of Pflüger, it should be possible to detect variations in the *immediate* post-mortem changes in the tissues, according to whether such tissues are derived from animals during digestion, or during a period of fasting. It was assumed that tissue of an animal with nitrogenous matter stored ready for elimination for the supply of energy needs, should show a more rapid post-mortem change than tissues derived from an animal during a period of fasting.

With the exception of the small intestine, which will be discussed in detail below, it was found, in the researches carried out in conjunction with Miss J. E. Lane-Claypon,⁵ that but small change took place, as far as the nitrogen at any rate was concerned, during the first four hours of incubation with water ; but that, in the case of the liver, after this so-called 'latent period,' a rapid degradation of the albumen set in (lasting generally from 4-6 hours), the rate of which depended on the state of nutrition of the animal at the time of death. This autolysis was more rapid in the case of the fasting than of the fed animal—the reverse of what might be expected if the hypothesis of Pflüger be correct. From the results obtained it appeared that the autolytic enzyme functioned by acting when the energy needs of metabolism were not satisfied by the food-stuff ingested. The results also furthered the hope that this method of research might be available as a general method for the study of metabolism. It was tested⁶ by being applied to a special case of abnormal metabolism, viz., when animals are fed with thyroid glands, and results parallel in every respect with those got by other methods were obtained.

What is the mechanism now by means of which this autolytic enzyme acts ? Does it exist in the tissue as a zymogen, from which the enzyme is liberated by the means of a kinase-like body, as in the case of trypsinogen, or is it always present in the tissue, but is prevented from acting during periods of full nutrition by the presence of inhibitory bodies derived from metabolites, which gradually disappear after ingestion of food ?

The latter of these hypotheses seemed the more likely, and in the second paper on the subject of autolysis, it was suggested that the tissue stability of the liver was the resultant of the mass action of three sets of bodies, viz., the tissue itself, the metabolites, or bodies derived from metabolites, and the autolytic enzyme.

The following immediate questions then arose:—(i) In what way do the metabolites act? Do the products of tryptic digestion themselves inhibit the action of the autolytic enzyme, or is it the products derived therefrom which exert this inhibitory action? (ii) If such metabolites, or bodies derived therefrom, exert an action in the manner suggested, are they continually circulating in the blood-stream, or are they taken up in the tissues and acting intracellularly?

To obtain an answer to question (i) the influence of glucose, and of the products of tryptic digestion of caseine on the rate of autolysis of the liver were determined. For an answer to question (ii) the relative inhibitory action of sera from fed and fasting animals on autolysis was investigated. As a result of these experiments it was found that glucose exerted no action on the rate of autolysis, whereas the products of tryptic digestion exerted a marked inhibitory effect.

In the experiments on the action of sera, it was found that in one series no difference could be detected between the influence exerted by the serum of a fasting animal and that of a fed animal; in a second series, however, the inhibitory action of the serum of a fed animal was slightly greater. In this second series, the serum contained a larger amount of uncoagulable nitrogenous bodies. All experiments were carried out with sera diluted with a triple volume of normal saline.

As the result of the above-mentioned preliminary experiments, the question as to whether the products of tryptic digestion circulate in the organism acquires additional importance. That they inhibit autolysis there is little doubt: on the other hand experiments carried out with sera do not conclusively prove that the serum of a fed animal contains these products in quantity sufficient to shew a greater inhibitory power than the serum of a fasting animal.

A series of researches was, therefore, undertaken with the object of investigating the distribution of nitrogen in the tissues in the different stages of nutrition, and of determining the ratio of the nitrogen of the coagulable albumen to the total nitrogen. The difference between the total nitrogen and the nitrogen of the coagulable albumen has, throughout these researches, been termed 'residual nitrogen.' (Ger. Reststickstoff). This expression is more convenient than the older term 'extractives nitrogen,' for, as will be seen in the sequel, the latter, in part at least, appear to form an essential part of the tissue.

The problem of the residual nitrogen is no new one, and has been attacked by many investigators who have obtained varying results; their work will be referred to when discussing the experimental part of these researches. The contradictory results obtained are due, without doubt, to the faulty technique employed; for this reason a comparative investigation has been made of the value of two or three different methods: as a result a new method has been employed, which obviates the fallacies of the other methods. These researches are described in Part II.

Part III deals with the application of the method to the examination of serum, liver, and small intestine. As a result it was found that the tissues of fed animals do not contain more residual nitrogen than those of fasting animals. Other interesting results were obtained, the discussion of which is deferred to Part VI.

As it is obvious from the results obtained in Part III, that the products of tryptic digestion do not themselves directly act *in vivo* in inhibiting autolysis, a further search was made for agencies likely to bring about this result. These researches, with all the others bearing on autolysis, are described in Part V. It was found, finally, that acids cause autolysis of the liver without any previous latent period, whilst alkalis have a marked inhibitory action. Now it is known that ammonia is formed from the products of tryptic digestion in the alimentary tract, and stored up in the liver and other tissues. The quantitative relations of the amounts to the quantity of acid necessary to produce the maximum rate of autolysis have been

investigated. As a result of these researches, certain conclusions have been drawn as to the general chemical mechanism of nutrition, and as to the rôle played by the nitrogenous food-stuffs (Part VI).

Another problem, also bearing directly on the general chemical dynamics of nutrition has been investigated, and certain preliminary results obtained. Hofmeister has stated his belief that coagulable albumens can be synthesized from peptones or albumoses in the mucous membrane of the stomach. Glaessner, working in Hofmeister's laboratory, obtained experimental results tending to confirm this hypothesis. As, for reasons discussed in Part II, there are certain objections to the experimental method employed, and as, furthermore by the analytical processes used in this work, the coagulable albumen can be directly estimated, the researches of Hofmeister and Glaessner have been repeated. The experiments are described in Part IV. They do not confirm the results of Hofmeister and Glaessner.

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PART II

ON THE METHOD FOR ESTIMATING RESIDUAL NITROGEN IN SERUM AND TISSUES

Attention has often been called to the difficulty of precipitating albuminous bodies without at the same time removing from solution substances which are otherwise easily soluble in water. This subject has been investigated recently by Haslam, who has demonstrated certain fallacies in the methods devised for the separation of albumoses by fractional precipitation with salts. S. N. Pinkus (private communication) has obtained similar results.

In the previous work on autolysis in the papers already quoted, the nitrogen of the non-coagulable bodies has been estimated in the

filtrate after precipitating the albumen with trichloroacetic acid (added when the mixture is boiling). As the experiments have throughout been comparative, and carried out under as nearly as possible like conditions, the method has been satisfactory enough.

In the determination of the residual nitrogen in tissues, it was not always possible to carry out the experiments under perfectly identical conditions; it was necessary, furthermore, to determine the absolute quantities of residual nitrogen in each case. Preliminary experiments were therefore undertaken to test the reliability of older methods, and to determine how much the results varied under different conditions. For this purpose a mixture of a solution of Witte's peptone and serum was made, and this mixture was diluted with varying quantities of water; it was found that the amount of residual nitrogen obtained after precipitating with tannic acid (in the cold) or with trichloroacetic acid (boiling), varied quite appreciably with the strength of the solution in which precipitation took place; it was found that more residual nitrogen was obtained in the filtrate when the precipitants were added to a dilute than when added to a concentrated solution. Tannic acid precipitates of course a certain part of the peptone; trichloroacetic acid, on the other hand, precipitates only a minute quantity if added to a sufficiently dilute boiling solution. On the other hand, trichloroacetic acid, when added to the solution of the peptone-serum mixture above a certain dilution, produces a precipitate in such a finely divided state that filtration through filter paper is almost impossible. As a general result it was found that the trichloroacetic acid precipitation method gives fairly accurate results when the strength of the solution was maintained within certain definite limits of concentration.

It was important, therefore, to have a method available which would allow the coagulation to be carried out under perfectly constant conditions, and at the same time give the residual nitrogen in absolute numbers.

For such a method I am indebted to the suggestion of S. N. Pinkus. He has shown that if anhydrous sulphate of soda be added to solutions of albumens or to tissues, the water is abstracted from the same with a very slightly increase of temperature, and without any appreciable

alteration of the albumen. In this way the albumen solution or tissue can be dried (at a temperature not exceeding 38°) and kept without alteration for a considerable time. The tissue dried in this way can be coagulated, and the nitrogen of the coagulum directly estimated. The difference between the number thus obtained and the number obtained by estimating the total nitrogen in the albumen solution or tissue represents the residual nitrogen.

The method has been carried out (in the case of serum) in the following way :—A measured quantity of serum is poured into a flat-bottomed, glazed, porcelain dish. An equal weight (roughly) of anhydrous sulphate of soda is then added. The mixture is then allowed to stand for some time. The anhydrous sulphate of soda is converted into the crystalline hydrated form, and the water is thus abstracted from the serum. The dried mass can be readily removed from the flat-bottomed dish by a knife (this is the reason for using a flat-bottomed dish instead of an evaporating basin) and powdered in a mortar. When finely powdered it is transferred to the Kjehldahl flask in which the incineration with sulphuric acid is carried out (a 700 c.c. round-bottomed Jena flask was always used for this purpose). Any small amount of the powder remaining in the porcelain dish or mortar can be removed by rubbing the vessels with small fresh quantities of anhydrous sulphate of soda, which is then added to the main quantity. The albumen is then coagulated by boiling the mixture in the Kjehldahl flask with absolute alcohol (100 c.c. alcohol for 15 c.c. serum, or 50 c.c. alcohol for 5 gr. tissue) for half-an-hour with reflux condensor. The alcohol is then decanted off on to a filter. Water is then added to the powder remaining in the flask (300-400 c.c.) and the mixture is then heated on a water bath. By this means the sulphate of soda and non-coagulable bodies are dissolved up and the coagulum remains suspended in the solution in the form of a fine powder. The water is decanted off through the same filter as that used for the alcohol, and the greater part of the coagulum remains behind in the Kjehldahl flask. This is again heated with water on a water bath, and the hot water is again decanted off through the filter. When the washings are free from sulphate of soda and have run through the

filter the filter paper containing small quantities of the coagulum is transferred to the Kjehldahl flask. Sulphuric acid is then added, the mixture incinerated and the nitrogen determined in the usual way.

In the experiments with serum described in Part III, 15 c.c. were always used for each experiment. The total nitrogen was estimated in this quantity by incinerating with 30 c.c. concentrated sulphuric acid; a filter paper was added to the mixture, so that the total coagulable albumen nitrogen was always determined under the same conditions. Another 15 c.c. were used for the determination of the coagulable nitrogen in the way just described.

For the examination of the tissues 5 grams of material were used for each determination. The finely divided tissue was weighed on a photographic balance (accurate to about 0.02 of a gram). It was then transferred to a flat-bottomed porcelain dish with a knife, and mixed up by means of the knife with a little more than an equal weight of the sulphate of soda. The glass pan of the balance was carefully cleansed by rubbing up with fresh quantities of sulphate of soda, and the mixture kept until wanted. The mixture was then powdered in a mortar, coagulated with alcohol, washed, and the nitrogen of the coagulum determined in the way already described for serum. The total nitrogen was determined in 5 grams of fresh tissue. Thirty c.c. concentrated sulphuric acid were employed for incineration in each case. A filter paper was added in the determination of the total nitrogen as in the case of the serum.

When working with solutions of albumens the same method can be employed. A little more sulphate of soda is added than the weight of water present.

The following series of experiments illustrate the foregoing remarks. In the last series the coagulable albumen is determined in a serum; the same serum is mixed with a concentrated solution of Witte's peptone, and the coagulable albumen determined in the mixture. These experiments were carried out very rapidly, and although the coagulum was washed only twice by decantation, it will be observed that only a minute quantity of the peptone nitrogen has been carried down. Far larger quantities of water-soluble bodies were present in this case than are ever met with in tissues.

EXPERIMENTS

Determination of non-precipitable nitrogen in a mixture of sheep's serum and 10 per cent. solution of Witte's peptone after precipitating with tannic acid (Hedin's³ solution). The nitrogen estimated in an aliquot portion of the filtrate.

Series I

	Serum c.c.	Water	Strength of serum solution %	Peptone solution c.c.	Hedin's solution c.c.	N in filtrate c.c. $\frac{N}{10}$ H^2SO^4 required
<i>a</i>	25	25	50	5	25	20.1
<i>b</i>	25	75	25	5	25	20.2
<i>c</i>	25	175	$12\frac{1}{2}$	5	25	22.8
<i>d</i>	25	375	$6\frac{1}{4}$	5	25	24.8
<i>e</i>	25	75	25	5	25	21.1

In experiments *a*, *b*, *c*, *d*, the peptone solution was added to the diluted serum before adding the tannic acid. In (*e*) tannic acid was added to the serum before the peptone solution. These experiments shew that the amount of residual nitrogen obtained varies with the concentration of the solution in which the precipitation is carried out.

Series II. Same as I, with another serum, and another 10 per cent. peptone solution. The total N is represented by the following numbers :—(*a*) 24.0, (*b*) 26.0, (*c*) 27.9, (*d*) 28.6.

Series III. With trichloroacetic acid precipitation.

(*a*) 25 c.c. serum + 25 c.c. water + 5 c.c. peptone solution.

The mixture was then boiled. 25 c.c. of 10 per cent. trichloroacetic acid were then added to the mixture whilst still hot. Filtrate was washed with hot water; it was then removed from paper and washed again with hot water. Nitrogen estimated in total washings.

Result :

$$N = 49.0 \text{ c.c. } \frac{N}{10} H^2SO^4$$

(*b*) 25 c.c. serum + 75 c.c. water + 5 c.c. peptone solution.

Boiled. 25 c.c. trichloroacetic acid solution added.

Manipulation as in (*a*).

Result :

$$N = 51.8 \text{ c.c. } \frac{N}{10} H^2SO^4$$

Here again variation with dilution. Greater dilution impracticable owing to difficulty of filtration.

Series IV. Sodium sulphate method. Same serum and peptone solution as in Series III.

25 c.c. serum added to 5 c.c. peptone solution.

$$\text{Total N} = 490 \text{ c.c. } \frac{N}{10} \text{ H}^2\text{SO}_4.$$

$$\text{Coagulable N, (Expt. I)} = 435.7 \frac{N}{10} \text{ H}^2\text{SO}_4.$$

$$(\text{Expt. II}) = 434.7 \frac{N}{10} \text{ H}^2\text{SO}_4.$$

$$\text{Residual N} = (490.0 - 435.2) \frac{N}{10} \text{ H}^2\text{SO}_4 = 54.8 \frac{N}{10} \text{ H}^2\text{SO}_4.$$

$$\text{N in peptone solution alone} = 47.4 \frac{N}{10} \text{ H}^2\text{SO}_4.$$

Hence residual N in serum = $7.1 \text{ c.c. } \frac{N}{10} \text{ H}^2\text{SO}_4$; a number which subsequent experiments shows to be a trifle low.

Series V. Sodium sulphate method. Determination of N in coagulum.

$$15 \text{ c.c. of serum. Coagulable N} = 116.2 \frac{N}{10} \text{ H}^2\text{SO}_4.$$

15 c.c. of serum + 10 c.c. 10 per cent. Witte's peptone.

$$\text{Coagulable N} = 118.5 \frac{N}{10} \text{ H}^2\text{SO}_4.$$

Here, in spite of large amount of residual N from the peptone, very little is carried down with the coagulum.

The method gives, therefore, accurate results, and can be made independent of the concentration of a solution, as a dried powder is always used for coagulation.

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PART III

THE DISTRIBUTION OF NITROGEN IN THE SERUM, LIVER, AND MUCOUS MEMBRANE OF THE SMALL INTESTINE DURING DIGESTION OF FOOD AND DURING A FAST

The method of research has been already described.

The liver was put through a fine mincing machine before weighing.

The intestine was cut out from below the pylorus to just above the ileo-coecal valve. It was opened up longitudinally and nailed down on a wooden board. The surface of the mucous membrane was carefully cleansed with cotton wool, and the mucous membrane was scraped off with the blunt edge of a knife. Scrapings were made alternately from the cardiac and the coecal end, and put into a tube and thoroughly mixed with a knife. The tube was stoppered until it was required for weighing out. The weighings were all made as soon as possible after killing the animals.

For the determination of total nitrogen the tissue was mixed with sulphuric acid in a Kjehldahl flask immediately after weighing. The portions of tissue required for estimation of the nitrogen of the coagulable albumen were mixed with sulphate of soda immediately after weighing. This mixture could be kept till wanted.

The animals used were always cats, except when otherwise stated. They were fully anaesthetized by A.C.E. mixture, and bled to death by opening the carotid artery when under the influence of the anaesthetic.

The blood was collected in a tall cylinder and allowed to clot. The serum was then poured off into a narrower cylinder, and the corpuscles, which were decanted off with the serum, allowed to settle. The required quantities of serum were pipetted directly, either into the Kjehdahl flask or into the flat-bottomed dish, according to whether the total N or the N of the coagulum was to be estimated.

Various morphological details were noted, such as weight, weight of liver, length of intestine, etc. In order to know the concentration of residual nitrogenous bodies, the total solids were estimated. In the case of serum this was done by heating about 2 c.c. in porcelain crucibles, first at about 60° and afterwards in a Victor Meyer bath jacketed with toluol at 107° C. The mucous membrane and liver were treated in the same way; instead of using a crucible, however, they were dried after spreading out as much as possible on a watch glass. In this case the ordinary paired watch glasses were used. Heating was continued to constant weight. The fed animals were killed about five hours after the last meal. The fasting animals were killed when the stomach was empty. The animals were killed in pairs, a fed and fasting animal

being killed on the same morning or the same afternoon. This was to obviate any difference that might be caused by differences of hydraemic state of the tissues due to differences of temperature. The general results are given in the accompanying table (Table I). The following abbreviations are employed :—

F = animal fasting.

D = animal killed during active digestion.

N = total nitrogen, expressed in c.c. $\frac{N}{10}$ H²SO⁴ necessary for neutralization of NH³

R = residual nitrogen " " " " "

N - R = nitrogen of coagulable albumen.

The results obtained are summarized below.

TABLE I*

No.	Nutrition	Weight Kilos	SERUM			LIVER				SMALL INTESTINE				
			N	N-R	Solids %	Weight	N	N-R	Solids %	Length	Weight Mucous Membrane	N	N-R	Solids %
1	F	1'95	127'0	115'0	8'7	...	...	...	...	...	...	...	...	...
2	F	2'85	126'0	116'0	8'3	...	...	...	...	...	...	...	...	...
3	D	2'60	131'6	122'0	9'0	...	...	...	...	...	...	...	...	...
4	D	3'10	125'5	112'2	8'8	...	...	...	...	...	...	...	...	...
5	F	3'20	130'0	118'0	8'4	...	...	...	...	...	...	...	...	...
6	F	3'15	124'0	116'0	8'3	...	...	...	...	...	...	...	...	...
7	D	3'50	149'0	135'0	9'9	...	...	...	...	...	...	...	...	...
8	D	3'45	108'0	99'0	7'4	...	...	...	...	...	...	...	...	...
9	F	2'85	135'6	124'0	9'2	45'9	144'0	128'2	31'6	...	...	...	...	...
10	F	3'30	136'5	119'4	9'0	54'1	145'5	127'7	33'3	...	...	...	...	...
11	D	2'90	121'7	109'0	8'4	63'6	130'0	118'2	30'3	...	...	...	...	...
12	D	2'25	125'5	117'2	8'8	61'3	122'5	107'8	29'1	...	...	...	...	...
13	F	3'25	130'4	117'7	9'3	54'8	126'7	107'5	31'0	...	...	...	...	...
14	F	3'60	131'3	127'0	9'6	58'4	131'0	114'5	30'2	...	...	...	...	...
15	D	4'35	125'6	114'0	8'8	98'6	97'5	79'8	37'7	...	...	...	...	...
16	D	4'40	143'6	135'2	9'8	109'8	103'5	94'9	34'3	...	...	...	...	...
17	F	2'60	131'7	113'3	9'1	109'2	99'0	82'0	47'6	4'6"	11'6	111'0	71'3	26'2
18	D	3'50	133'3	120'0	9'4	80'0	109'5	lost	33'4	4'0"	11'7	109'0	71'5	26'1
19	F	3'00	122'8	111'0	8'4	64'4	107'0	86'4	40'1	5'6"	11'2	117	lost	26'1
20	D	2'90	128'2	110'0	8'7	90'3	117'5	101'0(?)	31'2	6'0"	13'6	109'0	74'0	27'3
21	F	3'30	...	...	...	65'7	110'0	96'3	34'1	4'4"	16'0	112'0	80'5	25'9
22	D	3'30	...	...	...	84'3	102'7	87'2	27'7	4'10"	15'1	lost	68'5	23'9
23	F	2'85	...	...	...	56'7	124'0	104'2	30'3	...	14'6	111'0	72'0	23'9
24	D	2'90	...	...	...	79'3	123'1	107'3	28'9	5'0"	16'6	110'0	73'6	25'3

* The nitrogen determinations refer to 15 c.c. of serum, and 5 grams of liver or of mucous membrane of intestine.

Serum. With reference to the influence of nutrition on the composition of the serum there are numerous researches, of which the most recent are those of von Bergmann and Langstein.¹ The question as to whether the products of tryptic digestion can be detected in the serum has, in spite of the number of works on this subject, been by no means definitely settled. Another question, which has given rise to considerable controversy, refers to the presence of albumoses and peptones in blood (bodies giving the biuret reaction). These have been claimed to be found by Emden and Knoop,² by Langstein,³ and by Nolf.⁴ Their existence, on the other hand, has been denied by Abderhalden and Oppenheimer,⁵ and by Neumeister.⁶ Now in Part II of these researches (see p. 129) it has been shown that when albumens are coagulated in concentrated solutions, the coagulum carries down with it a not inappreciable quantity of water soluble substances. On the other hand, when coagulation is carried out in dilute solutions the precipitate is so finely divided that satisfactory filtration is almost impossible. For these reasons it is not surprising that contradictory results have been obtained.

In nearly every case in these researches, etc., the filtrates obtained after coagulation, both alcoholic and aqueous, have been submitted to the biuret reaction. In no single case was a positive result obtained.

Neither the liver nor the serum was found to contain a trace of albumose or peptone. With regard to the residual nitrogen of the sera the results are evident from the following table :—

TABLE II

Fasting animals				Animals killed during digestion			
No.	N	N - R	R	No.	N	N - R	R
1	127.0	115.0	12.0	3	131.6	122.0	9.9
2	125.0	116.0	10.0	4	125.5	112.2	13.3
5	130.0	118.0	12.0	7	149.0	135.0	14.0
6	124.3	116.0	8.0	8	108.0	99.9	9.0
9	135.6	124.0	11.6	11	121.7	109.0	12.7
10	136.5	119.4	17.1	12	125.5	117.2	8.3
13	130.4	117.7	12.7	15	125.6	114.0	11.6
14	131.3	127.0	4.3	16	143.6	135.2	8.3
17	131.7	113.3	18.4	18	133.3	120.0	13.2
19	122.8	111.0	11.8	20	128.2	110.0	18.2
Total	1295.3		117.9		1292.0		118.5
Ratio	$\frac{R}{N}$	$= \frac{9.10}{100}$			$\frac{R}{N}$	$= \frac{9.16}{100}$	

We see from these numbers, that both the total nitrogen and the residual nitrogen in 150 c.c. of serum, taken from ten different animals, are almost absolutely identical in quantity. We are bound, therefore, to conclude that there is *absolutely no evidence that the products of tryptic digestion as such, are circulating in the blood stream.*

The residual nitrogen, except in three or four cases, varies very little from a mean. The variations bear, however, no relation to the state of nutrition.

We can certainly conclude that it is not the products of tryptic digestion in the serum which exert any influence on the rate of autolysis of the liver.

The variations in the total nitrogen are influenced chiefly by the hydraemic condition of the blood. When the percentage of total solids is high, the total nitrogen is high, and *vice versa*.

The Liver.

TABLE III

Fasting animals				Animals killed during digestion			
No.	N	N - R	R	No.	N	N - R	R
9	144.0	128.2	15.8	11	130.0	118.2	11.8
10	145.5	127.7	17.8	12	122.5	107.8	14.7
13	126.7	107.5	19.2	15	97.5	79.8	17.7
14	131.0	114.5	16.5	16	103.5	94.9	8.6
17	99.0	82.0	17.0	22	102.7	87.2	15.5
19	107.0	86.4	20.6	24	123.1	107.3	15.8
21	110.0	96.3	13.7	...	...	...	...
23	124.0	104.2	19.8	...	...	...	...
Total	987.2		140.4	Total	679.3		84.1
Average	123.4		17.5	Average	113.2		14.0
Ratio $\frac{R}{N} = \frac{14.2}{100}$				Ratio $\frac{R}{N} = \frac{12.3}{100}$			

From this table, it is evident, that so far from the ratio of residual nitrogen to total nitrogen being larger in the case of animals during digestion than in fasting animals, it is smaller. The total nitrogen is also generally larger in fasting animals. There are two or three exceptions, as, for example, in the case of animals 17 and 19. It will be noticed, however, that in these two cases, the percentage of total

solids is very high (47.6 and 40.1 respectively). Furthermore, animal No. 17 weighs only 2.6 kilos, whereas the liver weighs 109.2 grams. These figures seem to indicate a very fatty liver, and probably this accounts for the abnormality. The numbers seem to indicate that in the fasting animals autolysis is already taking place, and that the tissue of the liver is degrading in order to supply the energy needs of the organism.

Here, again, there is no evidence that the products of tryptic digestion directly inhibit autolysis *in vivo*.

The Mucous Membrane of the Small Intestine.

TABLE IV

Fasting animals				Animals killed during digestion			
No.	N	N - R	R	No.	N	N - R	R
17	111.0	71.3	38.7	18	109.0	71.5	37.5
21	112.0	80.5	31.5	20	109.0	74.0	35.0
23	110.0	72.0	39.0	21	110.0	73.6	36.4
Total 334.0				Total 328.0			
Ratio $\frac{R}{N} = \frac{33.0}{100}$				Ratio $\frac{R}{N} = \frac{33.2}{100}$			

It will be noticed in the above table that the ratio of residual to total nitrogen is a constant, and independent of the state of nutrition of the animal. The concordance of numbers obtained from different animals is so close, that in five out of the six cases the analyses would have been satisfactory if they had referred to a pure homogeneous product.

Another point of considerable interest is the fact, that the percentage of residual nitrogen is considerably higher than that found in any of the other tissues examined. As the mucous membrane of the small intestine plays an important part in the general nitrogenous metabolism, this point is of great significance, and as will be shown below, the ratio is higher in the carnivora, in which the mucous membrane of the intestine has been examined, than in the herbivora. It appears to be a constant for each species of animal.

Only a part of this residual nitrogen is eliminated on simple coagulation by boiling the fresh tissue; if, however, the tissue be

incubated with water, a gradual elimination takes place in four hours ; the degradation changes then cease. This phenomenon has been already described in the first paper. (*Journ. Physiol.* 32, p. 159) ; it is to be distinguished from ordinary autolysis.

The physical constants determined show that very little change takes place on coagulating the moist fresh tissue ; no more, in fact than takes place in the case of the liver, which does not contain a large quantity of residual nitrogen, and which shews very small change during the first four hours of incubation with water. All these facts justify the conclusion that the greater part, at least, of the residual nitrogen of the small intestine is not mere 'extractive' nitrogen, but represents a part of the protoplasm molecule which is held in loose combination, and which is intimately connected with the processes of nitrogenous metabolism, as is evidenced from the fact that it is greater in quantity in the case of carnivorous animals than in that of herbivora. This point will be referred to again in the summary, (part VI).

Comparison of the ratio residual nitrogen : total nitrogen in different animals.

Dog—No. 1.	Fasting	...	41.2	} 38.8 (average).
" "	2. Fed	...	36.5	
Pig	...	...	...	32.3
Rabbit—No. 1...	...	...	29.7	} 28.8
" "	2...	...	28.2	
Sheep	...	...	...	27.6
Ox	...	...	...	19.2

In the case of the ox, the sample for analysis was not taken from the whole intestine.

In the rabbit, only small quantities could be obtained for each analysis ; these were accurately weighed on a fine balance.

SOME PHYSICAL CONSTANTS OF TISSUE EXTRACTS

The following determinations of physical constants were made. The results throw but little light on the method of combination of the residual nitrogen with the main part of the bioplasm. It might be

expected from the generalisations made below that changes other than those affecting the nitrogen take place in the tissues ; the results obtained show no parallelism between the physical and the nitrogenous changes.

Five grams of tissue were allowed to stand for a short time with 50 c.c. of cold water, made just acid with one drop of acetic acid. A part of the extract was, in each case, heated rapidly to 100° , in a closed vessel (so that no evaporation took place), and maintained at this temperature in a boiling water bath for 15 minutes. The determinations were made with boiled and unboiled extracts.

Liver. Electrolytic conductivity

Unboiled extract	R = 495.2, c = 651.670, k = 1317
Boiled extract	R = 443.5, c = 651.670, k = 1469
	<hr/>
Difference	... 152

Small Intestine. Electrolytic conductivity

Unboiled extract	R = 320.9, c = 651.670, k = 2031
Boiled extract	R = 290.6, c = 651.670, k = 2242
	<hr/>
Difference	... 211

Freezing point

Unboiled extract	... $\Delta = 0.08^{\circ}$
Boiled extract	... $\Delta = 0.09^{\circ}$

Another experiment was made. Five Grams of tissue were boiled for half hour with 50 c.c. alcohol. The latter was decanted off, and the precipitate washed several times with hot water. The washings, both alcoholic and aqueous, were then evaporated to a small bulk in *vacuô*, and dissolved in water. This solution was made up to 32 c.c. The freezing point of this solution was then compared with the freezing point of an extract of 5 grs. tissue in 30 c.c. cold water. Result :

Δ For tissue extract	... = 0.17°
Δ For extractives solution	= 0.17°

Attention has been called in an earlier paper to the fact that a considerable degradation of the small intestine takes place in the first four hours of incubation with water, which is followed by a period of small change. This phenomenon is discussed in detail in Part VI. The following experiment shows that the changes of electrical conductivity are not parallel to this change; there is no sudden increase in the first four hours, followed by a period of no change.

The experiment was carried out with a suspension of 5 grs. tissue in 50 c.c. water; temperature 40°. The conductivity of the boiled extract was also determined.

Time	Resistance
11'10	... Incubation commenced.
11'18	... 267.6
11'33	... 257.1
11'48	... 252.0
12'15	... 242.5
12'30	... 236.0
1'27	... 199.0
2'5	.. 170.5
2'40	... 145.1
3'13	... 126.0
4'20	... 94.4
5'0	... 82.5
Boiled extract. Resistance 171.8	

These results show a continuous decrease, which is due probably to changes other than nitrogenous degradation.

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PART IV

THE QUESTION OF THE SYNTHESIS OF ALBUMENS IN THE MUCOUS
MEMBRANE OF THE STOMACH

This question has already been referred to in the introduction.

The latest experiments on the subject, viz., those of Glaessner, conducted in Hofmeister's laboratory, were carried out in the following manner:—The mucous membrane of the stomach was stripped off and divided into two symmetrical halves. The one half was coagulated, and the amount of peptones and albumoses determined in the filtrate of the coagulum by the method of Pick. The other half was incubated in a moist chamber at 40° for three hours, and then coagulated, and the albumoses and peptones determined in the same way as in the unincubated half. Glaessner showed that there was a diminution of these bodies which reached a maximum when the animal was killed five hours after a meal.

The method employed in this research was similar; instead however, of using Pick's method, the coagulable albumen was determined directly by the procedure described in Part II.

Results :

Dog A. Killed four hours after a heavy meat meal.

Non-incubated half.	Total N.	103·0	N - R	94·0	Ratio	$\frac{N-R}{N} \times 100$	91·2
Incubated half.	„	105·0	„	92·2	„	„	87·7

Dog B. Killed five hours after a meal.

Non-incubated half.	Total N.	108·5	N - R	96·8	Ratio	$\frac{N-R}{N} \times 100$	91·2
Incubated half.	„	113·0	„	100·7	„	„	89·1

The residual nitrogen was also determined in a fasting dog and found to be higher than in digesting dogs, *i.e.* as 23 : 97 (total N).

The changes taking place in a tissue during secretory activity are now forming the subject of another research. It is hoped to deal with these in a subsequent paper.

The above experiments give no evidence of a synthesis of albumens.

PART V

FURTHER RESEARCHES ON AUTOLYSIS: THE RELATION OF
AUTOLYSIS TO NUTRITION*Section A. Autolysis of the Liver*

The method employed in the following researches is essentially that described in the first paper¹ (Lane-Claypon and Schryver, *loc. cit.*)

One or two minor modifications were made. After precipitating with trichloroacetic acid, the mixture was allowed to cool. It was filtered when cold, after standing for several hours; the filtrate was washed with hot water and, in the event of the bulk not being too great, directly evaporated down with sulphuric acid in the Kjeldahl flask.

Except when otherwise stated, the numbers given under the heading 'autolysis' refer to the number of c.c. $\frac{N}{16}$ H_2SO_4 necessary for neutralisation of NH_3 obtained in the Kjeldahl analysis. Four grams of tissue were used for each experiment.

Series I. Effect of Serum.—In all the experiments with serum, a portion was incubated with saline as a control (same volume as liquid in actual experiment). After incubation, serum in same dilution (also incubated for the same time) was added to the control; this mixture was then rapidly heated to boiling, and trichloroacetic acid in definite quantity added. The same amount of saline as was used for the control was added to the portion incubated with serum. The mixture was then boiled, and the same amount of trichloroacetic acid added as was used in the control. By this means coagulation in the actual experiment and the control were carried out under absolutely identical conditions.

Sheep's Serum on Cat's Liver.—20 c.c. liquid to 4 grams liver.

Time of Autolysis	Serum %	Autolysis *	Control *	Difference
7 hours	100	18.8	31.2	12.4
7 "	50	15.5	26.0	10.5
9½ "	100	21.2	36.0	14.8
9½ "	25	17.7	28.5	10.8

A star indicates total N in filtrate from coagulum (includes residual N, N from products of tryptic digestion, etc.)

From these numbers it is evident that, whereas serum (even 100 per cent.) strongly inhibits the rate of autolysis, it does not entirely stop it.

Series II.—Effect of sera of fed and fasting animals (cats) on autolysis of liver of a fasting animal.

Series (a). Twenty c.c. of 25 per cent. solution of serum to 4 grams liver. Same liver used for experiment with fed as with fasting animal.

Time	Serum of fed animal			Serum of fasting animal		
	With serum*	Control*	Difference	With serum*	Control*	Difference
7 hours	16.3	20.0	3.7	16.5	16.2	0
9 hours	19.3	27.8	8.5	21.0	24.0	3
		Total 12.2			Total 3.0	

Here the serum of fed animal exerts an inhibitory action. Note, however, that the numbers under the heading 'control' are higher. This indicates that the serum of the fed animal contains a larger quantity of extractives.

Series (b).—Same as *Series (a)*. Liver and sera from different animals.

Time	Serum of fed animal			Serum of fasting animal		
	With serum*	Control*	Difference	With serum*	Control*	Difference
6½ hours	18.2	24.0	5.8	19.5	26.6	7.1
9¾ hours	19.5	25.2	5.7	22.0	25.0	3.0
		Total 11.5			Total 10.1	

Here the same difference of the inhibitory action is not observed as in *Series II (a)*. From experiments on the composition of serum in Part III, one would not expect to find any very definite relationship between the inhibitory action of the serum and the state of the nutrition of the animal from which it has been obtained. The factors influencing autolysis will be demonstrated later.

Series III.—Effect of the action of the products of tryptic digestion of caseine on autolysis.

Series III (a).—Caseine in with twenty times its weight of water was digested in alkaline solution with trypsin. The mixture was then boiled and made just acid with acetic acid. Ammonia was then added until the reaction was plainly alkaline, and the whole was then concentrated by gently warming on a water bath.

The control was made by incubating with saline, and then adding the same quantity of products of tryptic digestion as was used in the actual experiment. The portion incubated with the products of digestion was diluted before coagulation with the same amount of saline as was used for the control. The filtrate in which N was estimated includes, of course, the products of digestion as well as those of autolysis.

$$5 \text{ c.c. trypsin solution} = 36.0 \text{ c.c. } \frac{N}{10} \text{ H}_2\text{SO}_4 \text{ (Kjehldahl)}$$

20 c.c. saline + 5 c.c. to 4 grams of liver; control in 25 c.c. saline.

Time	With products of digestion	Control	Difference
7 hours	48.0	58.0	10.0
8 "	57.7	65.0	7.3
9 "	62.7	66.3	3.9

Series III (b).—To test the effect of the quantity of the products of digestion on autolysis rate. The control in this case represents the N of the filtrate obtained by boiling the tissue with the products of tryptic digestion, adding trichloroacetic acid, etc., without previous autolysis.

T.D. = products of tryptic digestion. (5 c.c. = $36 \text{ c.c. } \frac{N}{10} \text{ H}_2\text{SO}_4$)

S = Saline

Solution used	Time	* N in filtrate	Autolysis
20 c.c. T.D. No saline.	0 hours (control)	147.5	...
	7 hours	153.2	5.7
	9 hours	157.5	10.0
10 c.c. T.D. + 10 c.c. S.	0 hours (control)	77.2	...
	7 hours	87.0	9.8
	9 hours	89.4	12.2
5 c.c. T.D. + 15 c.c. S.	0 hours (control)	38.7	...
	7 hours	53.3	14.6
	9 hours	57.2	18.5
5 c.c. T.D. + 35 c.c. S.	0 hours (control)	40.4	...
	7 hours	54.3	13.9
	9 hours	57.2	16.8

These figures indicate that the rate of autolysis is influenced by the quantity of the products of tryptic digestion, but little by the dilution.

Series III (d).—With another sample of the products of digestion of caseine the following results were obtained :—

Products of digestion alone $N = 111.7 \text{ c.c. } \frac{N}{10} \text{H}_2\text{SO}_4 \text{ (20 c.c.)}$

Time	With T.D. *	Control *	Difference
8 hours	128.5	138.0	9.5
24 „	140.0	135.2	4.8

Experiments were also made to determine whether previous treatment of the product of tryptic digestion with mucous membrane of the small intestine exerted any influence on the inhibitory power. No difference could, however, be detected.

Series III (e).—It will be shown below that acids accelerate autolysis, whereas alkalis inhibit it ; it was, therefore, of interest to determine whether the products of tryptic digestion inhibit autolysis in distinctly acid solutions, especially as it is somewhat difficult to be sure of the reaction to litmus of the products of digestion.

The sample of digestion products used had been prepared by digesting an alkaline solution (5 per cent.) of caseine for three months with *Rhenania trypsin*.

Amount of acid used for 4 grams liver = 20 c.c. $\frac{N}{10}$ acetic acid

To this was added 10 c.c. T.D. solution = 95.5 $\frac{N}{10} \text{H}_2\text{SO}_4$

Time	With T.D. *	Control A *	Control B *
2 hours	106.0	116.3	115.5
4 „	115.5	129.8	131.0
6 „	123.3	144.5	144.5

Control A was made by boiling the mixture incubated with water before adding the T.D. solution ; then adding trichloroacetic acid. In control B the T.D. was added first ; then the mixture boiled and trichloroacetic acid added. These results clearly indicate the marked inhibitory action of the products of tryptic digestion.

Series III (f).—*Attempts to reverse autolysis.*

4 grams of liver in each case were incubated with 20 c.c. $\frac{N}{10}$ lactic acid. After $2\frac{1}{4}$ and $3\frac{1}{4}$ hours respectively, the following mixtures were added :—

- (a) 20 c.c. T.D. (of which N = about 190 c.c. $\frac{N}{10}$ H_2SO_4)
 (b) 20 c.c. T.D. + 20 c.c. $\frac{N}{10}$ NaOH, so as to neutralize the lactic acid added.
 (c) 20 c.c. T.D. + 25 c.c. $\frac{N}{10}$ NaOH, so as to have a distinctly alkaline solution.

The effects of these solutions on the autolysed tissue, after acting for one and for two hours in the incubator, were investigated. In each case, instead of estimating the N in the filtrate, the N of the coagulable albumen was determined (by the method given in Part II), sulphate of soda being added in sufficient quantity to absorb all the water (*i.e.*, somewhat more than the weight of the whole mixture):

N of coagulum after autolysis with acid for $2\frac{1}{4}$ hours = 66 c.c. $\frac{N}{10}$ H_2SO_4

				N of coagulum	
Product of $2\frac{1}{4}$ hours autolysis + T. D.			after 1 hour		65.5
" $2\frac{1}{4}$ " " + T. D. + 25 c.c. NaOH	"	1	"		64.2
" $2\frac{1}{4}$ " " + T. D.	"	2	hours		65.0
" $2\frac{1}{4}$ " " + T. D. + 20 c.c. NaOH	"	2	"		65.2
" $3\frac{1}{4}$ " " + T. D.	"	1	hour		63.0
" $3\frac{1}{4}$ " " + T. D. + 25 c.c. NaOH	"	1	"		63.0
" $3\frac{1}{4}$ " " + T. D.	"	2	hours		54.5
" $3\frac{1}{4}$ " " + T. D. + 25 c.c. NaOH	"	2	"		56.0

N of coagulum after autolysing with acid for $4\frac{1}{4}$ hours = 57.5 $\frac{N}{10}$ H_2SO_4

Although these results do not give any evidence of the reversal of the autolytic process, such an action is by no means improbable; there is no doubt of the powerful inhibitory action, which indicates that the reaction is probably reversible.

Series IV.—Effects of products of peptic digestion. In the following experiments, the autolysis was investigated in 20 c.c. saline to which were added 5 c.c. of a carefully neutralized 10 per cent. solution of Witte's peptone.

Time	With peptone *	Control *
0	67.3	67.3
2 hours	72.3	72.8
4 "	74.0	76.3
6 "	86.5	86.0
8 "	92.3	94.0
24 "	103.0	113.2

The peptone exerts, therefore, an inhibitory action only in the later stages.

Series V.—The effects of acids and alkalis on autolysis. When the main facts in the following series had been already discovered, a paper by Hugo Wiener appeared in the *Physiologische Centralblatt* (August, 1905) in which he suggested that the latent period of autolysis described in the first paper, was due to the fact that the tissues were still alkaline, and that the autolytic enzyme did not act until the tissues became acid (Cf. Hedin).

Series V (a).

ANIMAL I			ANIMAL II		
Time	With acid		Time	With acid	
	$\text{H}^2\text{SO}_4 \frac{\text{N}}{40}$ 20 c.c.	Control in Saline 20 c.c.		$\text{H}^2\text{SO}_4 \frac{\text{N}}{40}$ 20 c.c.	Control in Saline 20 c.c.
0.	8.7	8.7	0	11.7	11.7
4 hours	23.3	11.5	4 hours	25.4	15.6
6 "	40.0	22.2	6 "	33.5	21.1
8 "	44.7	29.2	8 "	41.0	31.7
10 "	50.7	37.0	10 "	45.8	38.5
24 "	61.8	55.8	24 "	52.5	49.5

Series V (b).

Effect of $\text{NaOH} \frac{\text{N}}{40}$ 20 c.c.		
Time	With alkali	Control
0	7.7	7.7
4 hours	9.1	10.5
6 "	9.7	12.0
8 "	13.8	18.3
10 "	15.4	25.2
24 "	26.3	34.2

Series V (c).

Effect of $\text{Na}^2\text{CO}_3 \frac{\text{N}}{40}$ 20 c.c.		
Time	With alkali	Control
0	10.6	10.6
4 hours	12.0	14.2
6 "	13.7	19.0
8 "	16.4	27.5
10 "	22.1	34.6
24 "	41.4	50.8

Series V (d).

Effect of $\text{NaHCO}_3 \frac{\text{N}}{40}$ 20 c.c.

Time	With bicarbonate	Control
0	9.8	9.8
4 hours	11.2	10.9
6 "	11.8	14.5
8 "	19.5	22.5
10 "	32.0	31.0
24 "	43.0	50.7

As the result of these experiments it will be observed that acid causes a very marked acceleration of autolysis, whereas alkalis have a strong inhibitory action. Bicarbonate of soda acts as a weak alkali in this respect. The curves representing Series V (a) and V (c) are given in the accompanying diagram.

Series V (e).—The effects of sulphuric acid with varying solutions or varying quantities on the rate of autolysis.

In the following experiment 20 c.c. of H_2SO_4 solution were used in every case :—

FIG. 1.

Time in hours.

Broken line : control in saline.

Effect of 20 c.c. $\frac{\text{N}}{40}$ H_2SO_4 solution on autolysis of 4 grams of liver tissue.

FIG. 2.

Time in hours.

Broken line : control in saline.

Effect of 20 c.c. $\frac{N}{40}$ Na_2CO_3 on autolysis of 4 grams liver tissue.

Strength of solution	Amount of acid in 20 c.c.	Fasting animal		Fed animal	
		Autolysis 4 hours	Autolysis 6 hours	Autolysis 4 hours	Autolysis 6 hours ¹
0	0.0	10.0	22.0	10.5	12.3
$\frac{N}{320}$	0.0030 gr.	9.7	22.3	11.8	12.8
$\frac{N}{160}$	0.0061 gr.	13.4	25.4	13.3	16.2
$\frac{N}{80}$	0.01225 gr.	19.5	33.3	17.5	26.2
$\frac{N}{40}$	0.0245 gr.	lost	38.5	22.7	32.5
$\frac{N}{20}$	0.049 gr.	26.4	40.7	24.8	29.5
$\frac{N}{10}$	0.098 gr.	22.8	31.0	21.5	27.2

We see from the above table a progressing rate of autolysis with an increasing strength of acid up to the concentration of $\frac{N}{20}$. Concentrations above this strength appear to be destructive to the enzyme.

1. By autolysis in this and the succeeding experiments, up to Series V (g), is meant the N in the filtrate after coagulation.

Series V (f).—Is the rate of autolysis influenced principally by the strength of solution, or by the absolute quantity of acid present?

To determine this point varying quantities of acids of varying strengths were employed.

Amount of acid	In c.c. solution	Strength	Autolysis 4 hours	Autolysis 6 hours
0	20	0	16.0	17.8
0	40	0	16.7	21.0
0.0245 gr.	20	$\frac{N}{40}$	26.3	...
0.0245 gr.	40	$\frac{N}{80}$	25.0	...
0.0245 gr.	60	$\frac{N}{120}$	25.0	35.3
0.049 gr.	20	$\frac{N}{20}$	24.8	...
0.049 gr.	40	$\frac{N}{40}$	24.9	37.9
0.049 gr.	60	$\frac{N}{60}$	24.5	38.3
0.98 gr.	20	$\frac{N}{10}$	20.0	35.0
0.98 gr.	40	$\frac{N}{20}$	22.0	39.0
0.98 gr.	60	$\frac{N}{30}$	25.3	42.2

These results indicate that provided sufficient acid is present (and it has been already shown in *Series V (e)* that 20 c.c. of $\frac{N}{40}$ acid produces nearly the maximum result), the dilution in the case of a strong acid, like sulphuric acid, has but little effect. The action of the acid is akin, therefore, to the neutralization of a base, or the setting free of a weak acid from a salt, rather than to a phenomenon of hydrolysis. It is the amount of acid, therefore, that exerts the influence on the rate of autolysis rather than the concentration. There is, however, an inhibition with sulphuric acid when the concentration reaches $\frac{N}{10}$. This inhibition is due, as will be seen in the above table, entirely to the concentration and not to the quantity. It is probably, as already mentioned, due to hydrolysis of the enzyme.

Series V (g).—The influence of organic acids on autolysis.

In the experiments with acetic and lactic acids, the rate of autolysis with varying strengths and quantities has been compared with the rate in 20 c.c. $\frac{N}{10}$ sulphuric acid.

The following experiments have been carried out with 20 c.c. liquid.

Acetic acid.

Amount of acid	Strength of acid	Autolysis in 4 hours	Autolysis in 6 hours
0	0	11.7	13.5
0.015 gr.	$\frac{N}{80}$	...	28.6 (?)
0.03 gr.	$\frac{N}{40}$	25.6	27.5
0.06 gr.	$\frac{N}{20}$	28.2	33.6
0.12 gr.	$\frac{N}{10}$	...	36.0
0.24 gr.	$\frac{N}{5}$	38.2	41.6
20 c.c. $\frac{N}{40}$ H ₂ SO ₄		27.8	28.2

Lactic acid.

Amount of acid	Strength of acid	Autolysis in 4 hours	Autolysis in 6 hours
0	0	15.7	23.3
0.0225 gr.	$\frac{N}{80}$	31.2	37.0
0.045 gr.	$\frac{N}{40}$	...	47.8
0.09 gr.	$\frac{N}{20}$	39.6	51.0
0.18 gr.	$\frac{N}{10}$	...	58.2
0.36 gr.	$\frac{N}{5}$	44.7	55.0
20 c.c. $\frac{N}{40}$ H ₂ SO ₄		27.3	41.0

We see from these figures that both acetic and lactic acid exerts a very powerful accelerating influence on the rate of autolysis, especially the latter acid. Lactic acid accelerates the autolysis more than does sulphuric acid in the corresponding strength of solution. This is important in view of the fact that lactic acid is known to be formed at times within the organism.

Series V (h).—Influence of ammonia and trimethylamine on autolysis.

In the following experiments the liver was incubated with the alkaline solutions in corked flasks. The autolysis was determined by estimating the nitrogen in the filtrate from coagulum in a portion before

incubation and a corresponding portion after incubation. Trichloroacetic acid was added before boiling to avoid loss of volatile alkali. The following numbers represent the amount of autolysis in $7\frac{1}{2}$ hours :—

Solution used	Amount of Alkali	Autolysis
Without NH_3	0.	15.4
20 c.c. $\frac{\text{N}}{40} \text{NH}_3$	.0085 gr. NH_3	8.3
20 c.c. $\frac{\text{N}}{20} \text{NH}_3$	.017 " "	6.5
20 c.c. $\frac{\text{N}}{10} \text{NH}_3$	.034 " "	5.4
20 c.c. $\frac{\text{N}}{5} \text{NH}_3$	.068 " "	3.4
20 c.c. $\frac{17}{10} \times \frac{\text{N}}{10} \text{N}(\text{CH}_3)_3$	.2008 gr. $\text{N}(\text{CH}_3)_3$	3.9

Here again we have a strong inhibition due to the alkali. This, as will be shown in the sequel, has an important bearing on the mechanism of nutrition.

Influence of acids on the production of acid products of autolysis.

Magnus-Levy has shown that when autolysis is carried out without antiseptics under conditions assuring asepsis, as far as can be determined by cultures, a considerable evolution of gas takes place (both CO_2 and hydrogen), and certain quantities of fatty acids (amongst which is lactic acid) are produced. It has been noticed in the experiments recorded above that when acids (either sulphuric or an organic acid) are present, although nitrogenous degradation products are formed very rapidly, there is no evolution of gas, and no smell of fatty acids, even after 24 hours incubation. It would seem, therefore, that those conditions which favour the formation of nitrogenous bodies inhibit the formation of acid. It was also noticed that when the liver of a well-fed dog was autolysed in the presence of acid, the mixture at the end of 24 hours was still quite turbid with glycogen. The corresponding mixture in the case of a fasting dog was perfectly clear. This subject requires further research.

Physiological action of the products of autolysis.

Attempts were made to prepare a cytotoxic serum by the injection of cats' liver into the peritoneum of a rabbit. If the fresh liver be

injected in the form of an emulsion comparatively large quantities and repeated injections could be tolerated by the rabbits, without evincing any symptoms. On the other hand, when the liver had been incubated for only four hours (*i.e.*, till incipient autolysis), the animals invariably died, even after the interperitoneal injection of very dilute solutions. In one case an animal died within two or three hours of an injection.

These products when injected into the external jugular vein of an anaesthetized animal, of which the carotid artery was connected with a manometer, showed no change in the blood pressure.

The products of autolysis or the protoplasm of tissue in its unstable condition are evidently highly toxic. This subject is of considerable interest in connection with the toxæmic symptoms observed in atrophy of the liver. It is proposed to investigate this subject more completely.

Section B. The autolysis of some tissues other than the liver.

Series VI.—Effect of acids on autolysis of kidney and muscular tissue.

A. Muscular tissue. (Quadriceps extensor of cat).

Solution used	Water 20 c.c.	H ₂ SO ₄ $\frac{N}{40}$ 20 c.c.	Acetic acid $\frac{N}{5}$ 20 c.c.	Lactic acid $\frac{N}{5}$ 20 c.c.
4 hours	17.2	21.2	24.0	28.0
6 hours	16.8	21.2	24.0	27.0 (slight loss)

B Kidney

4 hours	16.7	43.7	55.0	53.4
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These results indicate that acid excites the autolytic enzyme in the case of the kidney. The influence of the acid in the case of the muscle is slight; as there is no degradation between 4 and 6 hours the action appears to be a solvent one, rather than one exciting the enzyme.

Series VII.—The influence of muscular activity on autolysis.

In this experiment, the sciatic nerves of a cat were divided on each side as near the crural sciatic plexus as possible. The sciatic nerve on one side was tetanized for 25 minutes, and the animal was killed during tetanization (anaesthetic A.C.E.). The autolysis of the corresponding resting and acting muscles from the two legs was then determined.

Time	Resting muscle	Tetanized muscle
0	12.6	12.2
2 hours	13.2	12.7
4 "	15.8	14.1
6 "	16.3	...
8 "	17.4	18.0
10 "	22.4	23.4
24 "	34.2	32.8

The rate is practically identical for the two sides. The rate of autolysis does not appear, therefore, to be influenced by activity.

Experiments were also made on the rate of autolysis of fresh and frozen meat. No difference could be detected.

In the case of frogs' muscles, tetanization appeared to exert an inhibitory influence on autolysis. The difference was, however, but slight. No precautions were taken in these cases for asepsis, so that no definite conclusions could be drawn.

Series IX. The comparative rate of autolysis in different tissues.

The blood does not autolyse to any appreciable extent in 24 hours, either when incubated with ten times its volume of water, or in 0.5 per cent. ammonium oxalate solution. The thyroid gland also autolyses but to a very slight extent in 24 hours.

In the following experiments, the relative rate of autolysis of 4 grams from each organ in 24 hours was determined. Toluol was employed as an antiseptic.

Organs taken from two different dogs, given in table under columns I and II. In animal I, autolysis lasted 24 hours; in animal II, 20 hours.

Organ	Total N in 4 grams.		N ^I		N ^{II}		N ^{II} - N ^I		N ^{II} - N ^I Total N × 100	
			Nitrogen in filtrate before autolysis		Nitrogen in filtrate after autolysis					
	Animal I 24 hrs.	II 20 hrs.	I	II	I	II	I	II	I	II
Spleen	82.4	89.5	14.0	13.5	49.7	38.5	35.7	25.0	43.3	28.0
Liver	97.6	...	8.7	...	47.8	...	39.1	...	40.0	...
Kidney	76.5	...	14.5	...	32.8	...	18.3	...	23.9	...
Striated Muscle }	95.3	99.5	9.8	12.0	25.0	26.2	15.2	14.2	15.9	14.3
Heart	80.0	87.4	10.2	11.2	27.6	33.7	17.4	12.5	21.7	14.3

It will be observed from this table that the maximum amount of autolysis occurs in the liver and spleen, the two organs which lose the greatest amount of weight during starvation. This affords a striking confirmation of the suggestion made in the first paper, viz., that the autolytic enzyme has the function of protecting the organism from starvation.

PART VI

A THEORY OF NITROGENOUS METABOLISM, DEDUCED FROM THE RESULTS OF THE FOREGOING RESEARCHES

Attention has already been called to the controversy as to whether the products of tryptic digestion of albumens can be detected in the blood ; it has been claimed, furthermore, that the methods hitherto employed for investigating this problem have not been entirely satisfactory. As it was imperative to arrive at a decisive settlement of this question, if possible, in order to gain a clear view as to the function of the albumens in nutrition, researches were undertaken with the object of obtaining a reliable method of chemical analysis, by means of which the nitrogen of the coagulable albumen could be accurately estimated ; such a method is described in Part II. The difference between the total nitrogen and the nitrogen of the coagulable albumen, designated throughout as 'residual' nitrogen, should, if the products of tryptic digestion circulate in the blood-stream, be greater in the case of animals killed during active digestion than it is in that of fasting animals. The method of research was also applied to a similar investigation of the liver and the mucous membrane of the small intestine. A large number of animals were used, and it was found then in no case was the average amount of residual nitrogen greater in the case of fed animal than in that of fasting animals.

The general conclusions drawn from the researches on residual nitrogen may be summarized under the following heads :—

I. The residual nitrogen, as deduced from the analyses of ten sera of fasting animals was found to be 9·10 per cent. of the total nitrogen ; corresponding analyses of the sera of ten fed animals gave the number 9·16. These figures are so close that they

may be regarded as practically identical ; the difference lies well within the limits of experimental error. The individual sera give numbers which do not differ very greatly from this mean.

Furthermore—*In no case was any substance yielding the biuret reaction detected in the filtrate from the coagulum.*

II. In the liver, the percentage of residual nitrogen is larger (14.2) in the case of the fasting than of the fed animals (12.3). This result is probably due to the fact that autolysis was already taking place in the livers of the fasting animals at the time of death.

In no case again were any bodies yielding the biuret reaction to be found in the filtrates from the coagulum.

III. In the case of the mucous membrane of the small intestine, the following results were arrived at :

- (a) The residual nitrogen varies within very narrow limits, if indeed, at all. The analysis made of six samples taken from six different animals gave in five cases numbers that agree so closely that had they referred to a single homogeneous body they could hardly have been more satisfactory. From this we may conclude that the percentage of residual nitrogen in the mucous membrane of the intestine is a constant for a particular species of animal.
- (b) It is not influenced by the state of nutrition.
- (c) It varies with different animals. It was found to be 38.8 in the case of the dog (two samples), 33.2 in the case of the cat (six samples), 32.3 in the case of a pig, 28.8 in a rabbit (two samples), 27.6 for a sheep, and 19.2 for an ox. It will be noticed therefore, that the percentage is higher in the case of carnivora than of herbivora.
- (d) It is also higher in the case of the mucous membrane of the intestine than in any of the other tissues investigated.
- (e) In addition, the mucous membrane shews certain other peculiarities. Attention has already been called to the fact that, unlike other tissues, when incubated with water it *immediately* commences to undergo disintegration.

This disintegration proceeds for three or four hours, after which in most cases it entirely ceases, at any rate for some time (Lane-Claypon and Schryver, *Journ. Physiol.* 31, pp. 173-4). In the researches just quoted, the residual nitrogen was determined in the filtrate from the coagulum, after precipitating with trichloroacetic acid in boiling solution. The preliminary researches of Part II shew that when this method is used, nearly all the residual nitrogen passes into the filtrate, at any rate in fairly dilute solutions. Now the amount found by this method of analysis in the mucous membrane of intestine of five animals was equivalent to $17.8 \text{ c.c. } \frac{\text{N}}{10} \text{ H}_2\text{SO}_4$ for 5 grams of tissue if the residual nitrogen be determined before incubation. After incubation for 4 hours, however (that is to say after the first rapid disintegration had come to a standstill), this number rose to about $32 \text{ c.c. } \frac{\text{N}}{10} \text{ H}_2\text{SO}_4$. This is very nearly the same amount as that found by coagulating the tissue, when dried by sodium sulphate according to the method described in Part II. This seems to indicate that part, at least, of the bodies represented by the residual nitrogen is in some form of chemical combination with the bioplasm.

What conclusions are to be drawn now from the above results ?

In the first place, there is no evidence that the products of tryptic digestion as such can circulate in indefinite quantities in the blood-stream. How, then, are we to explain their fate after leaving the alimentary tract ? What light do the above results throw on this subject ?

Four sets of facts are of special significance in connection with these questions ; they are :—

- (i) The percentage of residual nitrogen is very high in the mucous membrane of the intestine, a tissue most intimately connected with the nitrogenous metabolism.
- (ii) In the limited number of cases examined, it is higher in carnivora than in the herbivora.

- (iii) It is independent of the state of nutrition, and is the same in the fasting as in the fed animal.
- (iv) There is a certain amount of evidence that the bodies represented by the residual nitrogen are in a state of loose chemical combination with the bioplasm.

With these facts to guide us, the following explanation of the mechanism does not seem unreasonable :—

The bodies represented by the residual nitrogen may be regarded as in the same kind of chemical combination, such as exists between an enzyme and its substrate (or, perhaps, between a toxin and antitoxin) ; that in this state they undergo certain chemical changes like hydrolysis or oxidation, such as would take place through the action of an enzyme ; the products of the change would be eliminated and carried in the blood-stream to other parts of the organism ; after chemical change and elimination they would be replaced by other similar side chains, which would, in their turn, undergo the same kind of changes. The more rapid the blood-stream through the organ, the more rapid will the changes be.

According to this conception, then, the passage of the products of tryptic digestion through the mucous membrane is analogous to a continuous chemical process. The bioplasm acts as an enzyme or collection of enzymes, to specific points of which side chains are anchored ; it keeps, furthermore, always saturated with side-chains, as is shown by the fact that the residual nitrogen is the same during digestion as during a fast.

The theory is analogous to that suggested by Verworn¹ to account for the utilisation of carbohydrates.

The question now arises : how does the bioplasm maintain its saturation with the side-chains in the absence of ingested food-stuffs ?

To obtain an answer to this question, reference must be made to the researches on autolysis and on the products of metabolism carried from the digestive tract to the portal vein. The following sets of facts are known :—

- (i) The autolytic enzyme acts more rapidly in the liver of a fasting animal than in that of an animal during active digestion.

- (ii) It is inhibited by the action of ammonia and other alkalis, but accelerated by the presence of acids, especially lactic acid.
- (iii) This acceleration of acids is a function, in the case, at any rate, of sulphuric acid, depending on the *absolute* quantity of acid present, but not, except to a very limited extent on the concentration (Part V, Experiment Series V (f) p. 152).
- (iv) According to the researches of Nencki, Pawlow, and Zaleski², ammonia is formed as a product of nitrogenous metabolism in the alimentary tract; there is more ammonia in the portal vein than in any other part of the vascular system. The liver, furthermore, eliminates ammonia on treatment with weak alkalis, and more of this body is obtainable from the organ of a well fed animal than from that of a fasting animal. The numbers are lower in the case of herbivora than in the carnivora.

These facts offer a ready explanation as to the way in which the supply of products of albumen degradation can be maintained for the saturation of the bioplasm with nitrogenous side-chains in the mucous membrane of the intestine and other organs. Nencki, Pawlow, and Zaleski, show that the liver of a dog on treatment with weak alkalis at 40°C, yields on an average 26 mg. of ammonia per 100 gm. of tissue if the animal be killed shortly after a heavy meat meal, about 7.6 mg. if fed on bread and milk only, and about 7.3 mg. if killed during a fast.

The numbers are equivalent to 1.0 and 0.3 mg. NH_3 for 4 grams of tissue, quantities which require 0.0028 and 0.0008 gm. sulphuric acid for neutralization.

Now, on reference to Experiment Series V (e), Part V, (p. 150) it will be seen that in the case of cats, 20 c.c. $\frac{\text{N}}{320} \text{H}_2\text{SO}_4$ containing 0.0030 gm. acid, a quantity only just sufficient to neutralize 1.0 mg. ammonia, exerts but little influence on the rate of autolysis, whereas the same volume $\frac{\text{N}}{160}$ acid produces a marked acceleration. (There will, of course, be small quantities of sodium carbonate and other

alkalis in the tissues*). Furthermore, it has been shown, that in the case of sulphuric acid, it is the absolute quantity rather than the dilution which increases the rate of autolysis.

From these results we arrive at the following conclusion as to the function of nitrogenous foodstuffs in nutrition :—

In order to maintain nitrogenous equilibrium, nitrogenous food stuffs must be ingested in such quantities, and in such form that the ammonia produced therefrom in the digestive tract is sufficient to maintain the intracellular alkalinity of the liver and probably other tissues.

In order to fully understand this mechanism it is of importance here to consider the products other than nitrogenous bodies which are formed during autolysis. Magnus-Levy³ has shown that the liver on autolysis under the most stringent precautions for asepsis yields carbonic and other organic acids, such as lactic acid ; furthermore, the degradation products of fats and carbohydrates are of acidic nature—all would on hydrolysis or oxidation produce acids ; it matters not, however, for the purposes of the present argument whether the acid products are produced from the stored-up food-stuffs, or from the bioplasm itself.

We have, therefore, two classes of products producible, viz., non-nitrogenous acidic bodies and nitrogenous bodies ; the production of the latter class, it has been shown, is stimulated by the presence of the former. In well-nourished animals there is, however, always an excess of ammonia present, hence in this case the tissues will not become acid *in vivo*. The excess disappears gradually, however, if the animal is deprived of food. A certain stage will then be reached, when the production of acid exceeds the amount of ammonia available for neutralization ; the autolytic enzyme then comes into play, liberates amino-acids, etc., which in their turn pass the alimentary tract, and by means of the metabolic processes there taking place liberate ammonia, which again inhibits the production of nitrogenous degradation products. Degradation of tissue should proceed, therefore, at a definite uniform rate. From the preliminary experiments carried out it seems that the production of acids is inhibited by the presence of acids ; we have,

* Ammonia is also responsible for the production of sodium carbonate $(\text{NH}_4)_2\text{CO}_3 + 2\text{NaCl} = \text{Na}_2\text{CO}_3 + 2\text{NH}_4\text{Cl}$.

therefore, a mechanism which tends to prevent excessive acidity or alkalinity of the liver ; in the presence of acid nitrogenous degradation products are formed, from which ammonia is produced in the digestive tract ; it is possible also that the presence of alkali stimulates the production of the non-nitrogenous acid bodies. Further research is needed on this point.

The production of nitrogenous degradation products proceeds also at such a rate that there is never a dearth of nitrogenous bodies in the blood stream, and the bioplasm of the tissues, especially the mucous membrane of the small intestine, can remain saturated with side chains, for we have seen that the liver of a fasting animal contains more residual nitrogen than does that of a well-fed animal.

From these researches, it follows, that while the animal derives most of its energy from the oxidation of carbohydrates, and rapidly eliminates nitrogen from albumens, the latter, for all that, play an important rôle in nutrition, for the degradation products are needed, not only to satisfy, as Folin has suggested, the needs of endogenous metabolism of the organism representing the wear and tear of the tissues, but also to supply sufficient ammonia to maintain a certain amount of general intracellular alkalinity. In the absence of this amount, nitrogenous equilibrium ceases to be maintained.

It remains, lastly, to consider the bearing of these conclusions in certain abnormal cases, resulting in what Speck has called tissue metabolism (*Nahrungstoffwechsel* as contrasted with *Kraftstoffwechsel*).

Four instances are cited by Speck, in which tissue degradation sets in, viz. : (i) lack of oxygen ; (ii) poisoning with phosphorus, arsenic, etc. ; (iii) withdrawal of water ; (iv) in fever.

The factors regulating the animal heat of the body are too little known to render it profitable to discuss the course of metabolism in fever ; whether high temperature is due to increased oxidation or not, is doubtful, as the results obtained by the investigation of the respiratory quotients are open to doubt. Little is known also of the course of metabolism in the absence of water.

The metabolism with lack of oxygen, and in phosphorus poisoning, have formed the subject of numerous researches, and several facts

have been discovered, which are in accord with the theory of metabolism just propounded.

The cases of deficiency of oxygen have been investigated by Fränkel and Geppert⁴ and v. Terray⁵. The latter has shown that there is not only an increased nitrogenous output, but also an elimination of acids, such as lactic acid, in the urine. The cause of the increased nitrogenous output is here clear. Owing to lack of oxygen, the oxidation of carbohydrate does not proceed to the formation of the final product, carbonic acid, which is rapidly eliminated by the lungs; intermediary bodies, such as lactic acid, are formed, in the presence of which, the autolytic enzyme is brought into play, and so tissue degradation takes place. It is of interest to note, that increased nitrogenous output does not take place directly after a deficiency of oxygen; it generally follows in the succeeding days. This delay corresponds to the latent period of autolysis, and there is sufficient ammonia present to neutralize the lactic acid when first formed.

A similar explanation can be applied to the case of phosphorus poisoning, for Bauer⁶ has shown that administration of phosphorus causes a diminished output of carbonic acid and a decreased oxygen consumption; it acts as a toxin to the oxydases.

We see from the above researches that the autolytic enzyme functions, by setting up tissue degradation when the food supply is insufficient for the energy needs of the organism; in this case, these needs are supplied by the utilization of degradation products of the tissue. *In this case autolysis is a strictly physiological process.*

There are, however, cases in which the process is pathological, as for example, in atrophy of the liver, in phosphorus poisoning, etc. Such conditions can result, as the above researches indicate, from a variety of causes, such, for example, as insufficient respiration, local stasis, etc. The difference between physiological and pathological autolysis is probably quantitative rather than qualitative; in the former case, it always proceeds at such a rate, that the tissue, by the mechanism already demonstrated, never becomes strongly acid; in the latter case, it is possible that the acid is produced at such a rate, that

the liver tissue becomes strongly acid, and degrades so rapidly that its functions are thrown out of gear, as in the case of an Eck fistula, and that instead of taking up ammonia after it is formed in digestion, and converting it into urea, it allows this body to circulate in the blood-stream and be eliminated in the urine. It is in these cases accompanied by lactic acid ; the concurrent production of these two bodies is, in this case, no protective mechanism ; it is a process which is pathological in the strictest sense of the word.

In such cases, the above researches would seem to indicate as a remedy the intravenous infection of carbonate of soda.

Another point of interest is the cause of toxæmia in such cases. Preliminary experiments have shown that the liver in an incipient stage of autolysis is intensely toxic. It remains to be determined whether this toxicity is due to the products of autolysis. Further researches will be undertaken for the elucidation of this point.

It remains also to apply the methods and principles already enunciated to be investigated of pathological cases ; much light might be thrown on the pathogenesis of various diseases of the liver by the investigation of the autolysis, under various conditions, of organs from the *post-mortem* room. Such a field of research must be left to pathologists.

There are also various other problems which await solution. What is the mechanism, *e.g.*, of restitution of tissue after autolysis ? Preliminary researches [Part V, Series III (*f*)] show that the products of tryptic digestion of caseine exert a powerful inhibitory effect on autolysis ; it is possible, as already suggested, that the process may be made reversible.

Light might also be thrown on various problems of metabolism by the production of anti-sera to autolytic enzymes, and the investigation of the physiological properties of these sera.

Lastly, much remains to be ascertained as to the functions of the residual nitrogen ; the investigation of the chemical differences of acting and secreting glands should throw some light on this subject.

It is proposed to continue research in these directions.

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