XXXII. FEEDING EXPERIMENTS WITH A DIETARY IN WHICH TYROSINE IS REDUCED TO A MINIMUM.

By GINSABURO TOTANI.

From the Biochemical Laboratory, Cambridge.

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Knoop's discovery [1910] that the α-amino-acids can be synthesised in vivo from non-nitrogenous organic compounds and ammonia is one of the most significant contributions to the physiology of metabolism. He and Kertess [1911] isolated the *d*-acetyl derivative of γ-phenyl-α-aminobutyric acid from the urine of a dog to which the sodium salt of the corresponding α-ketonic acid had been given subcutaneously, demonstrating the fact that the synthesis of the amino-acids from ketonic acids can be effected in the animal body.

As a result of the administration of γ-phenyl-α-hydroxybutyric acid to a dog, there was a rise in the excretion of the corresponding amino-acid, showing that the α-hydroxy-acids can be converted into the α-amino-acids.

Applying the method of liver perfusion to the problem, Emden and Schmitz [1910, 1912] have demonstrated that an analogous process may take place in connection with the synthesis of the normal protein constituents, such as tyrosine, phenylalanine and alanine from the corresponding ketonic acids, thus showing that the phenomenon of amino-acid synthesis is a general reaction.

The well-known observations of Loewi [1902] followed by the elaborate experiments of Abderhalden, Henriques and others, have conclusively proved that an animal can maintain itself and exhibit growth when free amino-acids replace the protein of the food.

It was the original intention of the present research to decide whether tyrosine can be replaced by its corresponding ketonic acid in a dietary in which the mixture of the hydrolysis products of caseinogen were the sole

source of nitrogen supply; it being assumed from the work of Abderhalden [1913] that, with a deficiency of this aromatic amino-acid, an animal is unable to exhibit normal nutrition. My preliminary experiments led, however, to the unexpected result that in the case of rats most of the individuals fed upon a dietary which contained at most a minimal supply of tyrosine, not only maintained their body weight, but also exhibited growth. The original intention of my research could not therefore be carried out, but the experiments which show the apparently unessential nature of tyrosine seem to be of interest, and are discussed in this paper.

On the quantitative isolation of Tyrosine from Caseinogen.

Before attempting the feeding experiments, an endeavour was made to isolate tyrosine quantitatively from the hydrolysis products of protein. By applying the method of recrystallisation to the amino-acid mixture from caseinogen hydrolysed by acid, in spite of adopting the details of the method of Abderhalden and Fuchs [1913], it was found to be extremely difficult to get rid of all substance yielding the Millon's reaction from the last filtrate, though no more crops of the characteristic crystals of tyrosine could be obtained therefrom by further crystallisation. In three experiments, in two of which hydrolysis was effected with sulphuric acid and in one with hydrochloric acid, the amounts of tyrosine obtained were respectively 3·45, 3·70 and 3·23 %. In comparison with the figure 4·5 %, which is provisionally accepted as the probably correct proportion of tyrosine in caseinogen, the above figures are evidently low, but, on the other hand, they are closely similar to those obtained by Osborne and Guest [1911], viz. 3·9, 3·2 and 3·4 %. These latter authors, in spite of careful work, were also unable to isolate the whole of the tyrosine present in caseinogen by direct crystallisation. It is evident that the solubility of tyrosine is profoundly affected by other amino-acids present in the mixture. In the hope of eliminating this influence, the following method was used. Caseinogen was boiled with sulphuric acid of 25 % strength for 72 hours, so that complete hydrolysis might be effected. After the removal of sulphuric acid, the solution was submitted to repeated crystallisation, the process of evaporation being always performed in vacuo. Even when the tyrosine had been separated as completely as possible, the filtrates still gave a Millon's reaction with apparently undiminished intensity. Starting with 30 g. of caseinogen, 1·02 g. of tyrosine were obtained in characteristic needles. To the filtrate an aqueous solution of mercuric

chloride was added; the first portions of the precipitate thus formed contained histidine [cf. Kossel, 1896], which was identified by means of its picrolonate. On the further addition of the precipitant to the now distinctly acid solution, more precipitate was obtained, which partly consisted of a sulphur-containing compound, probably cystine [cf. Suter, 1895]. These precipitates were filtered off, and the filtrate was so far saturated with mercuric chloride that the solution contained sufficient to give a yellow precipitate with baryta water; then, finely powdered baryta was added with continuous stirring until tyrosine was completely precipitated, as shown by the absence from the filtrate of Millon's reaction and of the modified diazo test for tyrosine [Totani, 1915]. Excess of baryta should be carefully avoided, as the tyrosine compound is apt to be redissolved in excess. In several experiments it was found that 50 g. of baryta were sufficient to precipitate all the tyrosine present in a litre of the solution. The precipitates were filtered off by suction and washed with baryta water; they were then suspended in water containing a slight excess of sulphuric acid, and decomposed with hydrogen sulphide. The filtrate and washings from the mercury sulphide and barium sulphate were concentrated; chlorine removed by means of silver sulphate, and the excess of silver by hydrogen sulphide. The whole solution was now made up to 500 cc. and contained approximately 5 % sulphuric acid. It was then precipitated with 10 % phosphotungstic acid solution. After leaving overnight, the precipitate was filtered off, and washed with 5 % sulphuric acid, until the washings no longer yielded Millon's reaction. The filtrate and washings were freed from an excess of phosphotungstic acid by baryta and subsequently from the latter by sulphuric acid. They were then submitted to repeated crystallisation. In this manner a further quantity of 0·23 g. of pure tyrosine was obtained. The amino-nitrogen content of this sample was estimated by means of van Slyke's method:

0·0554 g. gave 7·8 cc. N at 19° and 758 mm. = 8·03 % N.

Calculated for $C_9H_{11}O_3N$	Found
N 7·73 %	8·03 %

The tyrosine was therefore probably pure.

Since the filtrate from the last crystallisation still gave an intense Millon's reaction, an endeavour was made to crystallise more tyrosine, but without success. The total quantity of tyrosine isolated was 4·16 % of the protein used; considerably more than that obtained by direct crystallisation.

For the purpose of isolating tyrosine, therefore, it is advantageous to

remove other constituents as completely as possible from the hydrolysis mixture. Based on the observation that the more soluble fractions of a hydrolysis mixture will retain tenaciously material yielding a Millon's reaction, Gortner [1911] drew attention to the possible presence of some other aromatic phenolic body in the molecule of keratin. Guggenheim [1913] isolated dihydroxyphenylalanine from *Vicia fava*. This reacts with Millon's reagent, and it is possible that such a compound may exist in the protein molecule.

The above method of isolating tyrosine was beyond doubt more nearly quantitative than direct crystallisation, but it proved to be extremely tedious and unsuitable for preparing material for feeding experiments.

The results of numerous experiments agree in showing that tyrosine is liberated almost completely at a very early stage of tryptic digestion. Abderhalden and Voegtlin [1907] isolated 4·5 % from the tryptic products of caseinogen.

Advantage was taken of the above fact in a further endeavour to separate tyrosine with a greater completeness. Caseinogen was first digested with trypsin, and the liberated tyrosine filtered off. The filtrate was then submitted to acid hydrolysis in the hope of obtaining a further yield.

The tyrosine which separated from a thorough pancreatic digest of 60 g. of caseinogen was filtered off and purified by recrystallisation from water. The filtrate was concentrated on the water-bath and kept cool in a freezing mixture for several hours, and a further quantity of pure tyrosine obtained. Submitting the main solution to further crystallisation, it was found that a little more tyrosine could be isolated, but only after a non-Millon-yielding fraction, probably leucine, was first removed. The amount of tyrosine thus obtained was 2·27 g.

The whole solution was now made up to 400 cc., with the addition of sulphuric acid to the extent of 25 %, and boiled under a reflux condenser for 72 hours. After removal of the sulphuric acid, the solution was submitted to crystallisation until no more tyrosine could be isolated. The quantity of tyrosine isolated as the result of the secondary acid hydrolysis was 0·174 g.; the whole yield obtained amounting therefore to 4·07 % of the protein. In another analysis carried out with 100 g. of caseinogen, 4·02 g. of tyrosine were obtained.

In connection with the above analysis, the following bromination experiments were carried out. Millar [1903] first worked out a method for estimating tyrosine by measuring the quantity of bromine necessary to form the dibromo-substitution compound. Plimmer and Eaves [1913] modified the

method so that the estimation of even small amounts of tyrosine could be effected with accuracy; they applied the method in an endeavour to determine the tyrosine content of protein. It was found, however, that the presence of histidine and tryptophane, both capable of absorbing bromine, was a disturbing factor. The former could be removed by phosphotungstic acid, but, with regard to tryptophane, it was found that although this amino-acid was destroyed by boiling with acid, its decomposition products still reacted with bromine; accordingly, tyrosine could not be estimated by the bromine method in a solution containing the whole of the products of acid hydrolysis. Plimmer and Eaves therefore utilised a tryptic digest of the protein in which the tyrosine had been liberated at an early stage of the hydrolysis, while tryptophane was assumed to be still in combination.

It is clear that the bromination method cannot be applied for the purpose of determining how much tyrosine remains in solution after its isolation by direct crystallisation has been attempted. The amount of tyrosine separated may be measured, however, by determining the bromine absorbed by the whole hydrolysis mixture, and that absorbed after the tyrosine has been crystallised out; the difference should correspond to the amount of tyrosine obtained. This method I have applied. In a preliminary experiment the quantity of bromine absorbed by pure tyrosine was determined; this gave 1·803 g. of bromine for 1 g. of tyrosine. The figure is rather higher than the calculated value, 1·765 g., but agrees with the results obtained by Millar and by Plimmer.

The solution of hydrolysis products from 60 g. of caseinogen freed as completely as possible from tyrosine in the former experiment (solution I) was submitted to bromination. As control material the same quantity of caseinogen was treated in a precisely similar manner, but the tyrosine not separated (solution II). Each solution was made up to 500 cc. with the addition of a small quantity of hydrochloric acid to complete the solution of the amino-acids. 25 cc. of each solution were brominated.

	Bromine absorbed (in g.) by	
	Solution I	Solution II
	0·4200	0·6349
	0·4176	0·6334
Mean	0·4188	0·6341

Difference = 0·2153 for 3 g. protein,

$\therefore$ 7·176 for 100 g. protein.

Since 1 g. of tyrosine absorbs 1·803 g. of bromine, 7·176 g. correspond to 3·98 g. of tyrosine. From the solution I, tyrosine was, as previously stated, isolated to the extent of 4·07 % by the crystallisation method; the figures obtained agreed, therefore, within the limits of experimental error. It is clear, therefore, that tyrosine was removed from the amino-acid mixture to the extent of 4 % of the protein used. No attempt to estimate with accuracy the amount of tyrosine left in solution was successful.

Feeding Experiments with Caseinogen.

Method. All the experiments described in what follows were carried out on the lines adopted by Hopkins [1912] in his investigations dealing with the accessory growth factors. Young albino rats were employed; they were taken from a stock which had been fed upon oats and bread and milk. The origin and previous history of the animals were known. The experiments were carried out on strictly comparative lines. The experimental and control animals were so chosen that the groups compared were uniform in origin, sex and weight. They were fed and treated in an exactly similar manner, except with regard to the essential factor with which the investigation was concerned. Rats of the same sex were kept two together, bucks with bucks and does with does, in wire cages which were placed in a cellar of uniform temperature. The food was given in excess of consumption, and water was administered freely. Having regard to the results obtained by Hopkins and others, it is clear that when synthetic diets are fed, it is necessary to supply the accessory growth factors. In my experiments these were administered in the form of an alcoholic extract of milk, in a quantity such that the two rats received an amount corresponding with 1·5 cc. of fresh milk per diem. Observations were made on the change of weight and on the general well-being of the animals. In one experiment the survival period was determined. Nitrogen equilibrium was not determined. Henriques [1909] observed that the body weight of a rat might be maintained for some days, even when nitrogenous equilibrium no longer existed. Experiments carried out in this laboratory show, however, that such a relation is only temporary. The rats were weighed daily at a specified time, and the amount of food consumed during the previous 24 hours determined.

Experiment I.

In this the sole nitrogenous supply to the animal was an amino-acid mixture from which the tyrosine had been removed as completely as possible on the lines discussed in previous sections.

I have shown above that tyrosine can be more completely separated by making use of a combined tryptic and acid hydrolysis, followed by repeated crystallisation, than by the usual method. From 1 kilogram of the commercial caseinogen preparation known as "protene" 38·52 g. of tryosine were obtained in an apparently pure state. This figure is lower, of course, than that obtained as above from pure caseinogen, but making allowances for the impurities in "protene," the separation is, as a matter of fact, equally complete. The amino-acid mixture from which the tyrosine had been thus removed was used in preparing the diet for the experiment now to be described. The solution was evaporated nearly to dryness, and then a known quantity of potato starch was added. The whole mass was so far dried that the material could be easily powdered by means of a coffee mill. Fat and cane sugar were subsequently mixed in, and tryptophane to the extent of about 1·5 % of the protein which yielded the amino-acid mixture.

In the control diet, tyrosine was added to the extent of 3 % of the original protein.

As regards nitrogen content, the food was prepared in two forms: one containing 3 and the other 2 %. The actual composition of the diets was as follows:

	Food of 3 % N content	Food of 2 % N content
Products from hydrolysed protein and starch mixed (N 7·0 %)	43·0 %	28·6 %
Tryptophane	0·5	0·4
Starch	20·1	34·6
Cane sugar	21·2	21·2
Fat	12·5	12·5
Salts (ashes of oats and dog biscuit in equal proportion) ...	2·7	2·7

Before feeding, a small quantity of water was added, and rubbed in by hand, so that the food mixture was obtained in the form of lumps of adequate size and consistence for consumption by the rats. The dried alcoholic extract of milk was administered in a small daily ration as mentioned above.

Eight healthy male animals were employed: of these four were fed upon the basal diet only (set A), the other four received the tyrosine addition (set B). During the first 24 days all received the food containing 3 % of nitrogen; later, that containing 2 % was given with the intention of diminishing the tyrosine supply.

The result of the experiment may be summarised in the following figures:

Set	Rat No.	Duration of feeding on 3 % N food in days	Gain in weight in %	Duration of successive feeding on 2 % N food in days		Further gain in weight in %	
				First	Following	First	Following
A	1	24	38·6	10	7	0	7·5
	2	24	36·6	10	7	5·0	5·5
	3	24	34·6	10	—	9·5	—
	4	24	22·1	10	—	9·7	—
			Mean 32·9			6·0	
B	5	24	26·0	10	—	9·9	—
	6	24	30·9	10	—	7·4	—
	7	24	37·0	10	—	4·2	—
	8	24	26·1	10	—	8·5	—
			Mean 30·1			7·5	

The behaviour of individual animals was closely similar in all cases, and no marked difference between the two sets could be established. All the animals consumed the food equally well, and remained in good condition. Wet filter paper was given from time to time as a precaution, but no intestinal trouble was any time observed. From this experiment it may be concluded that rats may exhibit almost normal growth when the supply of tyrosine is diminished to an extremely small amount. This result, however, differs from that obtained by Abderhalden. In one of his experiments [Abderhalden, 1913] a dog was used; a preparation of the digestive products of caseinogen freed from tyrosine as completely as possible, by direct crystallisation alone, was given to the animal, which lost body weight to the extent of 750 g. in nine days. There was a further loss in the four following days, though this was partly caused by an insufficient intake of food. The loss of weight, however, was regained almost entirely when tyrosine was added to the previous dietary. Abderhalden therefore came to the conclusion that tyrosine is an essential factor in nutrition. As already mentioned, the original intention of the present research was to discover if tyrosine could be replaced in nutrition by the corresponding ketonic acid. When the work began there was, to the best of my belief, no suggestion in the literature for the plan of testing the possible nature of intermediary metabolic products by replacement of the original amino-acid in a diet. Since then, however, in the third edition of his Lehrbuch, Abderhalden [1915, 1] has made brief mention of an experiment carried out on rats by himself for the purpose of investigating the replaceability of tyrosine by its ketonic acid. In this experiment the quantitative separation of tyrosine was unsuccessful; the rat, however, was unable to

maintain its nitrogenous equilibrium on the reduced supply of tyrosine. The description of this research, mentioned incidentally in the text-book, is so brief that the details are not clear. It is obvious, at any rate, that Abderhalden's results were entirely different from mine[1].

It is, of course, an objection to my experiments that the actual amount of tyrosine left in the diet is uncertain. Abderhalden claims that in his amino-acid mixture not more than 0·2 % of tyrosine was left behind. It will be understood from what has gone before that to give an actual figure for this is impossible. It is extremely improbable, however, having regard to the methods that I used, that there was more tyrosine in the dietary used by me than in that employed by Abderhalden.

In the endeavour to avoid this difficulty, further experiments were carried out on a gelatin diet. In connection with the effect of tyrosine deficiency, it is important to remember the clear evidence obtained by Emden and Baldes [1913] that phenylalanine can be converted into tyrosine in the body. This was shown conclusively during the perfusion of the surviving liver with the former substance. An explanation, therefore, for the normal growth of animals with tyrosine deficiency may be sought in this conversion of phenyl-alanine, which is present in a considerable amount in protein, into the missing phenolic amino-acid. Such a rôle of phenylalanine in replacing tyrosine was suggested in a research of Abderhalden's [1914].

Feeding Experiments with Gelatin.

It has become so clear from recent researches that the efficiency of a dietary is not determined by the amount, but rather by the quality, of its nitrogenous constituents, that earlier researches upon the protein-sparing power of gelatin need not be referred to. So far back as 1876 Escher observed an improvement in the nutritive value of gelatin by the addition of tyrosine alone; but now that it is known that tryptophane, which is entirely absent from gelatin, is an essential factor for maintenance, any investigation made without the addition of this need not be considered. Kaufmann's [1905] paper is the first that needs attention. He found that protein could be replaced by gelatin, when tyrosine and tryptophane were added, to the extent of from one-third to one-half of the total nitrogen of the diet. Under these circumstances, the nitrogen balance was maintained. This experiment

[1] Abderhalden's full publication (1915, 2) was not seen until after this paper was written.

was carried out on a dog. In a further experiment performed upon himself, he reached the striking result that there was nitrogenous equilibrium when gelatin, with the addition of the absent amino-acids, replaced the whole of the protein of the diet. Kaufmann's experiment was repeated by Rona and Müller [1906] upon dogs. They could replace the protein by gelatin plus tryptophane and tyrosine, up to the extent of two-fifths of the total nitrogen, but not beyond this. They concluded, therefore, that the protein-sparing power of gelatin could not be enhanced by the addition of the missing aromatic amino-acid. Later on the problem was again attacked by Abderhalden and Manolin [1910], whose experiments confirmed Kaufmann's result. Because of the possible indigestibility of normal gelatin, these last observers employed predigested gelatin, to which several amino-acids, comprising not only those actually missing, but also those which are present in relatively small amount in the gelatin molecule, were added. In the dog, they found that this mixture could replace from three-fifths to two-thirds of the protein nitrogen in the food. Moreover, in a still later experiment, Abderhalden [1912] claims to have maintained a dog practically in nitrogenous equilibrium even on complete replacement of protein by the above mixture of gelatin and amino-acids. As a result of this experiment, Abderhalden assumes that a supply of the aromatic amino-acids is essential to life. As a matter of fact, however, until Abderhalden made the further reference in the text-book as mentioned above, to his study of tyrosine deficiency, there was really no evidence for the indispensability of an aromatic grouping, except in the case of tryptophane.

If a diet free from tyrosine is to be prepared from gelatin, it is essential that the gelatin should be pure. Among the many samples tested by me, the "gold label" gelatin of the French firm Coignet Père & Fils & Cie, with the mark "extra" was found to be practically free from other protein. Millon's reaction, however, was fairly distinct, when the gelatin was dissolved in water, with the addition of a little sulphuric acid, and excess of the reagent carefully avoided. Plimmer and Eaves [1913] submitted a gelatin[1] digest, previously freed from histidine, to bromination, and found that a certain amount of bromine was absorbed. They considered this due to the presence of tyrosine, and calculated its amount as 0·24 %. Recently, however, Siegfried and Reppin [1915] observed a marked difference in the amounts of halogen absorbed by gelatin according to the conditions under

[1] Plimmer and Eaves used also "gold label" gelatin as material, but did not mention the variety.

which bromination was performed. Intact gelatin absorbed much more than hydrolysed. They held that in the gelatin molecule some complex capable of combining with bromine might be present, which disappeared during acid hydrolysis. If this evidence be taken into consideration, the bromine value of gelatin, as found by Plimmer and Eaves, ought not to be regarded as due exclusively to tyrosine. Mörner [1899] states that even the purest gelatin gives a marked colour with Millon's reagent. Murlin [1907] endeavoured to purify gelatin by Kirchmann's method, but Millon's reaction remained undiminished in his final product. It is clear that at present we have no knowledge as to the nature of the Millon-yielding substance in gelatin. I had to be content, therefore, to use gelatin which, while certainly free from other protein, still yielded at least a slight colour with Millon's reagent. I shall now describe two feeding experiments, in one of which intact gelatin was used, and in the other the hydrolysed product.

Experiment (II) with non-hydrolysed gelatin.

A basal dietary was prepared in the following way. A jelly composed of 250 g. of pure gelatin and 420 g. of starch was dried to such a consistence that it could be passed through a mincing machine. This treatment was repeated until the whole could be sifted through a sieve of 1·5 mm. mesh, and the product was then completely dried. The other constituents, fat, sugar and salts, were finally mixed in the same proportion as in the diet of the previous experiment. The total nitrogen content of the basal dietary so prepared was 3·4 %. The calculated energy value was just over 5 calories per gram. The protein-free milk extract was given in a small ration as before. Three sets of animals were compared: the first set (C), were fed on the basal dietary alone; the second (D) received in addition tryptophane to the extent of 1·5 % of the gelatin used; the third set (E) were given, in addition to the tryptophane, tyrosine to the extent of 3 % of the gelatin used. The food was consumed equally well by all the rats, until a few days before their death. In all the sets, a steady loss of weight occurred from the beginning of the special feeding. The experiment was continued till all the animals had succumbed.

If set C be compared with set D, it will be seen that there was no difference either in the rate of loss of weight, or in the survival period. Even in the case of the rats fed upon the gelatin plus tryptophane and tyrosine, the

difference was but slight. An extension of the survival period by one week, is, I think, too short to be of much significance[1].

Set C N in food supplied by gelatin			Set D N in food supplied by gelatin + tryptophane			Set E N in food supplied by gelatin + tryptophane + tyrosine		
Rat No.	Survival period in days	Loss in weight in %	Rat No.	Survival period in days	Loss in weight in %	Rat No.	Survival period in days	Loss in weight in %
9	17	45·0	15	16	45·7	21	37	44·8
10	30	44·8	16	23	46·1	22	37	47·2
11	32	46·0	17	38	58·1	23	36	42·9
12	30	45·0	18	39	51·8	24	44	47·8
13	30	40·8	19	27	45·5	25	33	44·2
14	28	48·0	20	24	44·8	26	32	47·3
Mean	27·8	44		27·8	48·6		34·8	45·7

Average loss of weight 46·4 %.

As the following experiment shows, the failure to obtain any improvement by the addition of the missing amino-acids was not due to the lack of their significance in metabolism, but to the fact that the intact gelatin itself is very badly absorbed.

Experiment (III) with hydrolysed gelatin.

In this experiment, the products of the acid hydrolysis of gelatin, were employed as the source of nitrogen supply in the basal dietary. The food was prepared exactly as in the case of the caseinogen preparation of Experiment I. The total nitrogen content was brought to the same value as that of the non-hydrolysed gelatin diet of Experiment II.

Six sets of animals were compared; in each set four rats (two bucks and two does) were employed. The rats of the first set (F) were for comparison fed upon the non-hydrolysed gelatin diet, as used in Experiment II. All the other sets were put upon the hydrolysed gelatin food. The second set (G) had this basal diet alone; the third (H) the same with the addition of tryptophane; the fourth (I) with tryptophane and tyrosine. To the last two sets (J) and (K) histidine and cystine were given in addition to the above, in the hope of still further enhancing the nutritive power of the gelatin. Cystine, of course, is missing from gelatin, and histidine occurs in a very small amount only. In set (K) tyrosine was replaced by synthetic phenylalanine.

[1] It may be mentioned that three of the rats from set E displayed peculiar symptoms some 12–24 hours before death. Paralysis began in the hind-legs, and extended rapidly over the trunk to the fore-legs, so that the animal could no longer move. Clonic and tonic spasms then followed and death occurred in a few hours. A post-mortem examination revealed nothing remarkable. As to the cause of these symptoms, I have no suggestion to offer, but it does not seem probable that they were accidental. They certainly did not suggest a tetanus infection.

The histidine was obtained from ox blood by the method of Knoop [1907], the hydrochloride obtained being neutralised in solution with sodium carbonate before mixing with the food. The cystine was obtained from hair; the phenylalanine was prepared by the method of E. Fischer, the product used melting at 263°. The amounts of tryptophane and tyrosine added were the same as in the previous experiment. Histidine and cystine were added to the extent of 2 and 1 % of the gelatin respectively. As regards phenylalanine, though it is known that the *d.l.* compound can be completely consumed in the organism of the dog [Schotten, 1883; Knoop, 1905], and also that it increases the homogentisic acid formation, as much as the active compound [Abderhalden, Bloch and Rona, 1907], yet having regard to the general evidence for the necessity of supplying the natural form of amino-acids, the racemic phenylalanine was employed in quantity double that of the tyrosine it was to replace.

Set F				Set G			
Rat No. and sex	Change in weight in %	Duration of feeding in days	Remarks	Rat No. and sex	Change in weight in %	Duration of feeding in days	Remarks
27 ♂	−34·4	31	alive	31 ♂	−18·2	31	alive
28 ♂	−44·4	26	dead	32 ♂	−19·6	31	,,
29 ♀	−33·8	31	alive	33 ♀	−17·9	31	,,
30 ♀	−35·3	31	,,	34 ♀	−17·5	31	,,
Mean	−36·9				−18·4		
	Set H				Set I		
35 ♂	+ 8·5	31	alive	39 ♂	−17·1	31	alive
36 ♂	−15·0	31	,,	40 ♂	− 8·4	31	,,
37 ♀	+ 0·4	31	,,	41 ♀	+ 6·4	31	,,
38 ♀	− 8·4	31	,,	42 ♀	+ 1·4	31	,,
	Set J				Set K		
43 ♂	−26·2	22	alive	47 ♂	+11·3	22	alive
44 ♂	+ 5·2	22	,,	48 ♂	+ 5·8	22	,,
45 ♀	+ 3·2	31	,,	49 ♀	−14·6	31	,,
46 ♀	− 0·9	31	,,	50 ♀	− 0·9	31	,,

If now set (F) be compared with set (G), it becomes very clear that the previously hydrolysed gelatin can be much more readily employed by the rat than the non-hydrolysed, since the loss of weight for the given period is reduced to a half. The difference does not depend upon the quantity of food consumed. The following figures which give the average consumption per 100 g. body weight show that closely similar amounts of food were eaten by the animals in both sets.

A somewhat diminished consumption in the later periods in the case of the set taking the non-hydrolysed gelatin, may be considered as the effect

and not the cause of the loss of weight, which was much more rapid than in the case of the animals taking the hydrolysed gelatin [cf. Hopkins, 1912]. It seems clear that the intact gelatin is badly digested and absorbed by the rat, and as already claimed, this fact explains the failure to obtain good results upon the addition of the missing amino-acids in the previous experiment. The results in the case of set (H) were remarkable. The treatment of the animals was exactly similar to that of set (G), save that a little tryptophane was given. Of four rats, two were not only able to maintain their weight, but also exhibited some growth. The general condition of these animals also remained satisfactory. The condition of the other two rats of this set was also for a long time much better than that of rats receiving no tryptophane. These two animals, it is true, lost weight; but in such experiments a positive result is clearly of the greater significance. It may be concluded, therefore, that rats can at any rate maintain themselves upon the hydrolysed products of gelatin when tryptophane alone is added.

	Set F			Set G		
	Food* eaten by			Food* eaten by		
Duration of feeding	Rats 27 and 28	Rats 29 and 30	Mean of loss of weight	Rats 31 and 32	Rats 33 and 34	Mean of loss of weight
1–3 day	6·4 g.	7·9 g.		5·7 g.	5·5 g.	
4–6 „	7·4	7·1	12·4 %	8·4	8·8	8·3 %
7–9 „	8·3	7·6		7·9	8·9	
10–12 „	7·1	7·1		7·4	7·6	
	Mean 7·3 g.			7·5 g.		
13–15 day	7·1 g.	5·8 g.		8·4 g.	7·3 g.	
16–18 „	6·7	5·8	22·2 %	7·7	7·3	4·9 %
19–21 „	6·4	5·8		7·9	7·1	
22–24 „	7·2	6·2		7·8	6·4	
	Mean 6·4 g.			7·4 g.		

* The average daily food consumption per 100 g. weight during the experimental period.

In set (I) tyrosine was given in addition to tryptophane, with scarcely any effect. Sets (H) and (I) closely agree. If tyrosine improves the nutritive value of gelatin, its effect is certainly much less than that of tryptophane.

In the last two sets (J) and (K), which received histidine and cystine in addition to tryptophane and tyrosine (or phenylalanine), the condition of the rats was somewhat more satisfactory than in the other sets. I think this experiment may be taken as showing that the complete replacement of protein by hydrolysed gelatin plus the missing amino-acids is quite possible. If tyrosine be necessary for maintenance and growth, phenylalanine seems able to act as raw material for its formation.

Discussion of Results.

Perhaps the most important point brought out in the above experiments is the fact that the rat can maintain itself and even gain weight on a hydrolysed gelatin diet, with the addition of the single amino-acid, tryptophane. Based upon the feeding experiments carried out with Miss Willcock, Hopkins [Willcock and Hopkins, 1906] has suggested that the protein constituents may be utilised not only for tissue formation, or structural maintenance, but in a more specific and direct manner, as, for instance, in the elaboration of essential substances such as adrenaline or other hormones. We have no direct evidence at present to show that adrenaline is derived from tyrosine, though the close resemblance in the chemical constitution of these two substances makes it very tempting to believe that the former is the precursor of the latter.

When animals are observed to grow normally upon food containing most minute quantities of tyrosine (Experiment I, set (A)), it is almost certainly because phenylalanine is capable of replacing tyrosine and serving as its precursor. The amount of phenylalanine in protein is sufficiently great to justify this belief. As regards the result of the experiment in which there was maintenance with tryptophane only added to the gelatin hydrolysis products, some further explanation seems necessary. In this case the total amount of the aromatic groups in the diet was very small. The minimal requirement of the animal in this respect is of course not yet known. It appears probable, however, that the amount of phenylalanine in the gelatin dietary is too small to cover the requirement, and one has to think of the possibility of the synthetic formation of the benzene ring in the body. Recent advances in the physiology of intermediary metabolism have suggested that the synthetic power of the body is much greater than was previously thought. With respect to the simpler aliphatic amino-acids, such as glycine and alanine, it may be taken as already proved that they can be built up in the animal body. As regards the synthetic formation of the more complex amino-acids, such as histidine and phenylalanine, proof is yet lacking. As is well known, Knoop and Windhaus [1905] have suggested that histidine may be formed in the organism from methyliminazole and glycine, by means of an oxidative synthesis, the former substance arising from glucose and ammonia. As regards the synthesis in the organism of amino-compounds derived from benzene or phenol there is, so far as I know, nothing yet known. Nevertheless, the above-mentioned fact that the animal can subsist without suffering

tissue breakdown on hydrolysed gelatin and tryptophane alone, points, I think, to the possibility of a synthesis of a benzene nucleus. On the other hand the indole ring, as many experiments in this laboratory seem to show, cannot be dispensed with from the diet.

SUMMARY.

(1) Tyrosine could not be isolated quantitatively from the hydrolysis products of caseinogen.

(2) When the removal of tyrosine from the amino-acid mixture is made as complete as possible, and is effected to an extent which certainly leaves only minimal quantities of this constituent, there appears to be no effect upon the nutritive value of the amino-acid mixture.

(3) In the case of the rat, the nutritive efficiency of gelatin is greatly increased by previous hydrolysis. Gelatin when fed intact appears to be badly digested and absorbed.

(4) The possibility of completely replacing the protein of a diet by hydrolysed gelatin, plus certain amino-acids, is confirmed.

(5) Some evidence is offered that the addition of tryptophane alone to the hydrolysis products obtained from pure gelatin made these efficient in maintaining the nutrition of animals.

The subject of the present investigation was suggested to me by Professor F. G. Hopkins who is responsible for the feeding experiments and to whom I am greatly indebted for valuable advice.

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[1] Owing to the present circumstance, this book was only obtained at the end of October, 1915, when the present investigation was nearly finished.

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