CLX. THE NON-ESSENTIAL NATURE OF TYROSINE.

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THE text-book statement that tyrosine is an indispensable amino-acid rests upon the observations of Abderhalden [1913] who showed that a dog, while unable to maintain weight on a diet from which the tyrosine had been almost completely removed, was able to do so when a supplement of tyrosine was given. The same author [1915] extended the work to rats and came to the conclusion that tyrosine is essential to growth and maintenance.

The work on the dog has never been repeated, but both Totani [1916] and Lightbody and Kenyon [1928] have failed to substantiate the findings in the second paper. The former used as nitrogen source acid-hydrolysed caseinogen, freed from tyrosine by crystallisation and supplemented with tryptophan. He found that rats grew well on such a diet and that tyrosine supplements had no effect upon the growth rate. Totani also came to the conclusion, which has not been supported by other workers [see Jackson *et al.*, 1928], that hydrolysed gelatin, with a supplement of tryptophan alone, will enable the rat to maintain itself and even gain in weight. The addition of tyrosine to such a diet had no effect, but the results are too erratic to permit of any conclusions being drawn.

Lightbody and Kenyon used a mixture of hydrolysed hair and gelatin—the choice of proteins being governed by a desire to keep the phenylalanine content low. Their rats grew—not normally—but the growth was in no way affected by tyrosine supplements. They came to the conclusion that tyrosine is not an essential amino-acid.

There are several difficulties in this problem. The first is that of removing tyrosine quantitatively from a hydrolysate; the second is the impossibility of removing phenylalanine; and a third is the unsatisfactory nature, both of gelatin, and of hydrolysed proteins, however supplemented, from a nutritional point of view. In the present work the first two of these difficulties have been overcome, and the failure to settle the question finally is due to the last.

A tyrosine-free amino-acid mixture was prepared as follows: 800 g. of caseinogen were digested for 3 weeks with pig's pancreas. The digest was then adjusted to p_H 4.6 with hydrochloric acid and allowed to stand in the ice-chest for 2 days. The tyrosine was filtered off (26 g. crude product), the filtrate concentrated *in vacuo* to 4 litres and the tyrosine again crystallised out. The filtrate from this was diluted to 10 litres, adjusted to p_H 7.6 and treated, in two lots of 5 litres, with 2 g. of catechol and the juice from 1.5 kg. of potatoes, added in 50 ml. doses at intervals, for some 18 hours. The fluid was kept well stirred, and air was passed in continuously. Further 2 g. of catechol were added during the time. The Millon reaction was followed and also that obtained by adding

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fresh tyrosinase and a trace of catechol to a sample. The former reaction never entirely disappeared—it only became weak and atypical, but the latter at the end of the 18 hours had entirely gone.

The two lots were reunited and boiled with charcoal, and after filtration evaporated to dryness, the first stages in an open pan, and the last in a vacuum-oven. The product was ground to a fine powder, and made up into a diet of the following composition:

Amino-acid mixture	...	...	18 %
Starch	...	...	42 %
Sugar	...	...	20 %
Fat (palm kernel oil)	...	...	15 %
Salt mixture	...	...	5 %

Vitamins were given in the form of 3 drops of cod-liver oil and an alcoholic extract of autolysed yeast, equivalent to 0.75 g. of fresh yeast per rat daily. The latter contains vanishingly small quantities of tyrosine.

Two groups of young black-and-white rats were used four in each group. The diet was fed *ad lib.*, and to the second group 100 mg. tyrosine were given between the four animals daily. The amount used corresponds with the minimum tryptophan requirement of 20 mg. daily. There were two of each sex in each group. The experiment lasted 35 days.

Table I.

	Rat	Sex	Initial weight g.	Final weight g.	Gain g.	Average
Group I	1	♂	69	110	41	43.5
	2	♀	72	122	50	
	3	♂	80	119	39	
	4	♀	75	119	44	
Group II	5	♀	73	111	38	42.0
	6	♀	84	130	46	
	7	♂	62	110	48	
	8	♂	71	109	38	

Rats 5, 6, 7 and 8 received tyrosine.

It will be seen that there is no difference between the growth rates of the two groups.

The experiment was repeated, using caseinogen which had been hydrolysed by means of 25 % sulphuric acid in an autoclave for 3 hours. The hydrolysate, after removal of sulphuric acid with baryta, was treated as above to free it from tyrosine. In this case, the fluid was boiled with charcoal just before treatment with the tyrosinase, and the reaction required only 12 hours. The repetition was made necessary by the possibility of small quantities of tyrosine having been introduced with the pancreas, and of undigested tyrosine peptides, which the tyrosinase will not attack.

The experiment was carried out as before, but the results were slightly less regular (Table II). In addition to the vitamins, a supplement of 20 mg. of tryptophan per rat was given daily, to compensate for that destroyed in the acid hydrolysis. The experiment was carried on for 30 days.

It thus appears that the addition of tyrosine to diets deficient therein is without effect on the growth rate of rats, and tyrosine must accordingly be classed as a non-essential amino-acid. But Embden and Baldes [1913] showed that in the surviving liver of the dog tyrosine is probably formed from phenyl-alanine, and there is no doubt that the two amino-acids may follow similar lines

Table II.

	Rat	Sex	Initial weight g.	Final weight g.	Gain g.	Average
Group I	1	$\text{O}_3 + \text{H}_2\text{O} + \text{O}_3$	82	120	38	(of 3) 30.3
	2		80	108	28	
	3		78	111	33	
	4		66	75	9	
Group II	5	$\text{O}_3 + \text{H}_2\text{O} + \text{O}_3$	75	108	33	30.1
	6		70	107	37	
	7		70	100	30	
	8		68	91	23	

Rats 5, 6, 7 and 8 received tyrosine.

Rat 4 was abnormal, and has been ignored in the calculation of the average gain in weight.

of metabolism. They ought therefore to be treated as a group, with regard to indispensability, and indeed phenylalanine may well be the more important, for while it is possible for tyrosine to be produced from phenylalanine, the reverse change is less probable.

There is no method for removing phenylalanine from amino-acid mixtures, as there is for tyrosine, or for destroying it, as for tryptophan. A phenylalanine-free diet requires a phenylalanine-free protein, and the only one described in the literature is the keratin of goose-feathers [Abderhalden and le Conte, 1905]. It was decided therefore to attempt to feed rats on a hydrolysate of goose-feathers, and to test the effect of the addition of tyrosine and of phenylalanine to such a diet.

In order to render the keratin digestible, as well as for the removal of the tyrosine, it is necessary to hydrolyse it, and this is not possible with enzymes. Three different hydrolysates were prepared. For the first, 500 g. of goose-feathers were boiled for 18 hours with 2 litres of 25 % sulphuric acid, and after neutralisation with baryta and filtration, the mixture was freed from tyrosine as above. The resulting powder was an evil-smelling, black, deliquescent substance, and when made up into a diet, proved so unpalatable that the rats would not touch it. A further 500 g. were hydrolysed, this time for 3 hours in an autoclave, also with 25 % sulphuric acid. The material was slightly less repulsive, but even so, the animals would not consume sufficient to maintain balance.

The diet was made up as with the caseinogen, and a supplement of tryptophan was given. Two groups of four were used as before, Nos. 5, 6, 7 and 8 being given tyrosine, and 6 and 7 a similar quantity of phenylalanine in addition for a part of the experiment. Duration of experiment: 30 days.

Table III.

	Rat	Sex	Initial weight g.	Final weight g.	Loss g.	Average
Group I	1	$\text{O}_3 + \text{H}_2\text{O} + \text{O}_3$	108	88	20	16.5
	2		109	92	17	
	3		80	62	18	
	4		82	71	11	
Group II	5	$\text{H}_2\text{O} + \text{O}_3 + \text{H}_2\text{O} + \text{O}_3$	111	87	24	15.75
	6		100	92	8	
	7		71	56	15	
	8		82	66	16	

These two efforts having failed, a third method of hydrolysis was attempted. The feathers in this case were boiled with concentrated hydrochloric acid for

10 hours, and the acid was subsequently removed as far as possible by repeated vacuum-distillation, and most of the remainder, after neutralisation with sodium hydroxide, by treating the hydrolysate with its own volume of alcohol and leaving it in the ice-chest for a day. The filtrate was freed from tyrosine as before and the mixture dried out.

It is unnecessary to give the results; they repeat the finding of the other two hydrolysates, and the experiment was abandoned at this stage.

The only conclusion that can be drawn, therefore, is that the rat continues to grow, even when all the tyrosine is excluded from the diet. Whether this represents a real ability to synthesise the amino-acid for all purposes, or simply a power to use dietary phenylalanine, remains an open question.

SUMMARY.

Caseinogen hydrolysates, completely freed from tyrosine by means of tyrosinase, will support growth in young rats, whether supplemented with tyrosine or not.

Goose-feather keratin, however hydrolysed, and with the addition of tryptophan, tyrosine and phenylalanine, would not maintain young rats.

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