FEEDING EXPERIMENTS WITH A DIET LOW IN TYROSINE

Thesis

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by

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Introduction

Abderhalden (Z. Physiol. Chem. 83,444,1913) fed an adult dog a diet in which the nitrogen was supplied by fully hydrolyzed casein which had been freed from tyrosine, as completely as possible, by crystallization. He found that the dog rapidly lost weight. However, when tyrosine was restored to the diet, the dog gained in weight and recovered in health.

Abderhalden (Z. Physiol. Chem. 96,1,1925) found that when an amino-acid mixture free from l-tyrosine was fed to rats the nitrogen balance immediately became negative. Upon the addition of tyrosine or phenylalanine to the diet the nitrogen balance rose, although equilibrium was not reached. Apparently either tyrosine or phenylalanine are capable of supplying some homocylic compound which is required by the animal. Unsuccessful attempts were made to replace tyrosine and phenylalanine with their oxidation products, p-hydroxyphenylpyruvic and phenylpyruvic acids, indicating that the animal cannot synthesize the homocylic amino-acids.

Totani (Biochem. J. 10,382,1916) fed rats several diets consisting of a basal mixture of acid-hydrolyzed gelatin, fat, sugar, salts in the form of ashed dog biscuit, and vitamins which were supplied by small portions of dried alcoholic extract of milk, to which was added different amino-acids. He found that all of the rats fed on this diet plus tryptophane, could maintain themselves for the experimental period of thirty-one days. Two rats even gained in weight. The addition of tyrosine to the basal mixture plus tryptophane had scarcely any effect. Totani states that this must be explained in one of two ways, either phenylalanine is capable of replacing tyrosine or the animal has the power to synthesize the benzene ring. Becuase of the small amount of phenylalanine in gelatine, Totani thought the latter explanation the more probable.

Shambaugh and Lewis (J. Biol. Chem. 67,XXX,1926) administered phenylalanine to rabbits subcutaneously in doses of one gram per kilo and studied the changes which took place in the blood and urine. After the administration of phenylalanine the phenols of the

blood doubled and those of the urine increased so that ten percent of the aromatic nucleus of the phenylalanine could be accounted for as eliminated in the urine as phenols. There was no increase in the urinary amino-nitrogen. The increase in phenols was partly due to the presence of an o-diphenol derivative which gave the typical o-diphenol reaction with ferric chloride. The administration of tyrosine increased the blood and urine phenols, but gave no traces of o-diphenol. This, then, is evidence that phenylalanine and tyrosine do not follow the same path of intermediary metabolism in the rabbit.

The indefiniteness of proof and the lack of agreement made it desirable to attempt to obtain further information regarding the effects of a diet low in tyrosine. With an improved method of tyrosine determination (Folin and Ciocalteu, J. Biol. Chem. 73,627,1927) which permits a more accurate knowledge of tyrosine in food components, we hoped to obtain a protein mixture for our diet which would be even lower in tyrosine and phenylalanine than is hydrolyzed casein or gelatin, and to supply adequate salts and vitamins from undoubted sources. This was accomplished by using a mixture of hair hydrolysate, digested gelatin, trypto-

phane, histidine, and cystine. By feeding such a diet to a larger number of rats, for a longer period of time, we hoped to obtain more definite information regarding the well-being of a rat on a diet extremely low in tyrosine.

Experimental

Two diets IIa and IIb having the following composition were prepared:

	IIa	IIb
Hydrolyzed gelatin	8.80%	9.06%
Hair hydrolysate	4.50	4.50
Tryptophane	.20	.20
Histidine	.20	.20
Cystine	.10	.10
Tyrosine	.20	.00
Yeast	3.00	3.00
Salt mixture (Osborn & Mendel)	4.00	4.00
Butter	15.00	15.00
Dextrine	64.00	64.00

Preparation of the Protein Constituents of the Food

One kilo of gelatin was dissolved in five liters of solution buffered at Ph 7 by the addition of di-potassium phosphate and potassium dihydrogen phosphate. Ten grams of pancreatin (Digestive Ferments) were added and the mixture digested for four days at 38° C. The fourth day, more pancreatin was added and the digestion

allowed to proceed for four more days. It was then removed to the refrigerator for two days, at the end of which time, it was concentrated in vacuum to a thick paste and then dried in a vacuum oven at 60° C until brittle.

Hair was used as a source of nitrogen because, while it is low in tyrosine, it does contain some amino-acids that would appear to complement gelatin as arginine, and to be very low in phenylalanine content, (Kossel, Z. Physiol. Chem. 44,347,1905). In the preparation of the hydrolysate, one kilo of human hair was washed in gasoline, dried, washed in warm water and again dried. It was then hydrolyzed four hours under a reflux condenser, with two liters of concentrated hydrochloric acid, filtered, and evaporated to a thick rubberlike mass in vacco at 50° C, thus removing most of the acid. The residue was then dissolved in 500 cc of water by warming in a water bath at a temperature not to exceed 60° C. The solution was cooled in a freezing mixture and adjusted to a Ph of 5.4 with 50% sodium hydroxide. This point was determined when five drops of the solution in 5 cc of water plus five drops of methyl red gave the same color as 5 cc of the Ph 5.4 buffer and five drops of methyl red when compared in a Coolege comparator. The

solution was allowed to stand in the refrigerator forty-eight hours and then filtered on a Buchner to remove the tyrosine and a large amount of sodium chloride which precipitated. The precipitate was washed with small quantities of ice water. All washings that did not give a positive test with Millon's reagent were added to the original filtrate. This solution was concentrated in vacco nearly to dryness and finally dried to brittleness in a vacuum oven at 60° C.

The tryptophane was prepared from casein as described by Cole, (Practical Physiological Chemistry, 135) and extracted with butyl alcohol using Onslow's method, (Biochem. J. 15,397,1921). A much better yield was obtained when fresh gland was used to digest the casein. Histidine dichloride was obtained from fresh corpuscle paste by a method outlined by Morrow, (Biochem. Lab. Methods, 142). Morrow's method (Biochem. Lab. Methods, 140) was used in preparing l-tyrosine from raw silk.

Preparation of the Salt Mixture

Because of the high sodium and chlorine content of the hydrolysate, Osborn and Mendel's salt mixture (J. Biol. Chem. 37,572,1919) was modified, in that the sodium carbonate and hydrochloric acid was omitted. This made the sodium chloride content of the food practically that fed by Sherman (J. Biol. Chem. 62,331, 1925)

Tables of Analysis

Gelatin

	Method of Analysis	mil g/g
Tyrosine	Folin & Ciocalteu J. Biol. Chem. 73,627,1927	.48
Total nitrogen	Seales & Harrison J. Ind. & Eng. Chem. 12,350,1920	162.4
Amino nitrogen	Northrop, J. Biol. Chem. 9,767,1926	43.3

Hair Hydrolysate

Tyrosine	Folin & Ciocalteu J. Biol. Chem. 73,627,1927	.22
Cystine	Folin & Loony J. Biol. Chem. 51,427,1922	44.5
Sulphur	Benedict J. Biol. Chem. 6,363,1909	13.5
Total nitrogen	Kjeldahl, Seales & Harrison J. Ind. & Eng. Chem. 12,350,1920	95.6
Amino nitrogen	Northrop J. Biol. Chem. 9,767,1926	88.9
Sodium	Hawk Practical Physiol. Chem. 786	356.0
Chlorine	Official & Tentative Methods of Analysis of A. D. A. C.	418.5

	Tyrosine		Cystine		Sulphur		Total N		Amion N		Sodium		Chlorine	
	mil g/g	mil g/g food	mil g/g	mil g/g food	mil g/g	mil g/g food	mil g/g	mil g/g food	mil g/g	mil g/g food	mil g/g	mil g/g food	mil g/g	mil g/g food
Hair Hydrolysate	.22	.009	44.5	2.2	13.5	.61	95.6	4.3	88.9	4.0	356.0	16.0	418.0	18.0
Hydrolyzed gelatin	.48	.042					162.4	14.3	43.3	3.8				
Yeast	7.70	.346												
Vegex	.38													

Feeding

Seventeen rats at twenty-eight days of age were divided into two groups in such a manner that litter-mates were equally divided between the two groups. These groups were fed diets IIa and IIb for a period of eight weeks.

The yeast as a source of vitamin B appeared to be insufficient, as indicated by the food intake the first three weeks, so one milligram of Vegex was fed daily beginning the fourth week.

The growth of the rats is shown in tables I and II and by the average growth curve I.

Discussion of Results

From these experiments it is evident that rats can survive and grow slowly for a period of eight weeks on a diet containing less than .04% tyrosine. This is in agreement with the results obtained by Totani. We believe the conditions under which our experiment was conducted afford better control of the following experimental factors: (1) less

Table I

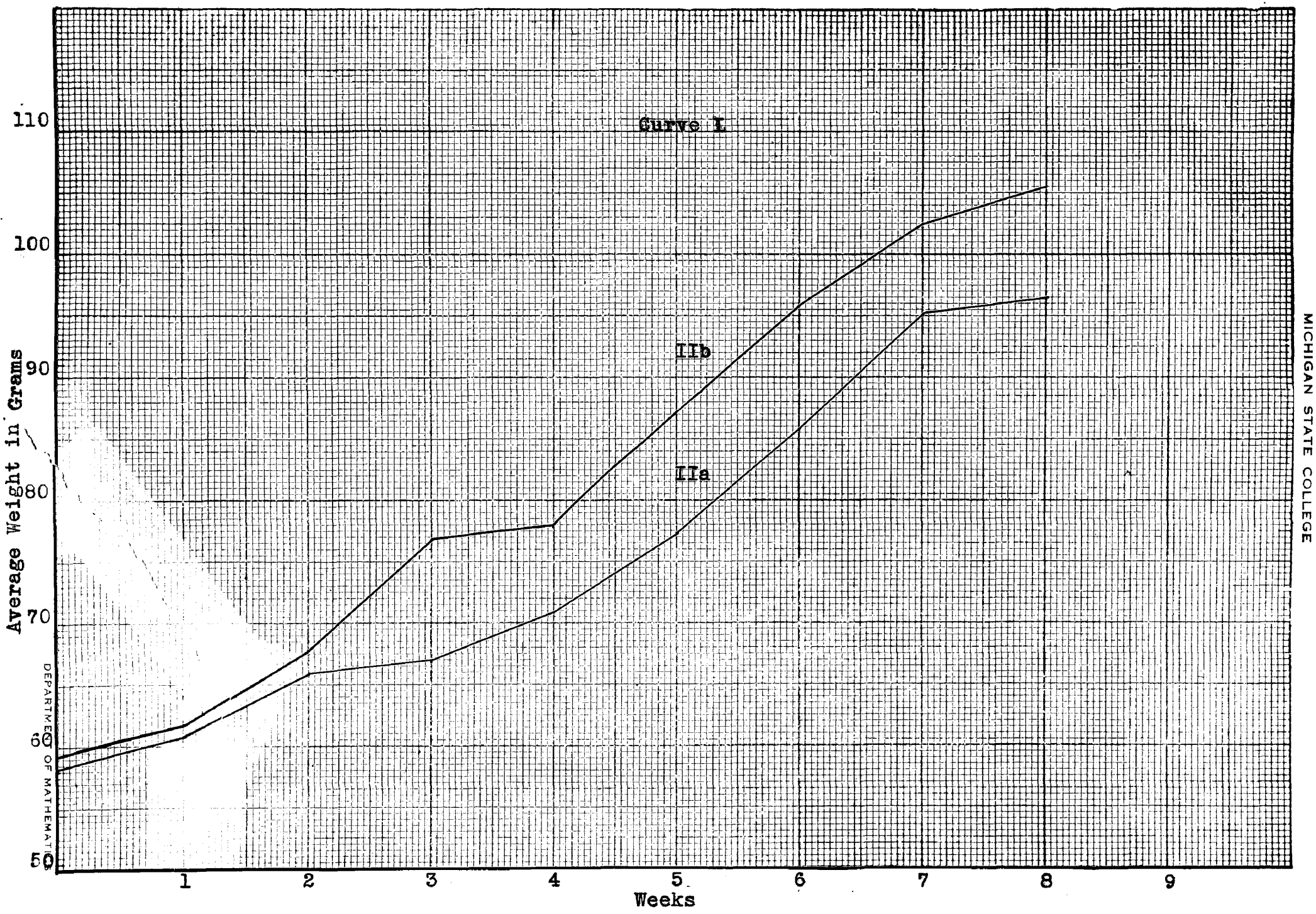
Growth of rats on diet IIa.

Rat no.	Initial weight	Weeks 1	2	3	4	5	6	7	8
2	48.6	51.9	59.5	62.5	62.1	68.9	77.1	86.8	90.6
3	66.7	72.3	77.4	77.1	79.8	84.4	97.8	104.8	107.3
4	65.4	68.0	70.6	65.3	69.0	72.7	80.0	91.0	90.0
5	58.6	58.0	60.0	66.1	59.6	64.4	68.7	81.5	80.0
6	61.9	62.3	68.8	75.8	80.6	85.1	92.7	102.8	101.0
7	53.7	54.6	60.9	66.5	68.5	86.1	99.7	107.9	112.2
8	55.6	57.8	60.9	58.2	66.0	71.7	79.4	89.0	90.5
9	59.9	61.6	66.6	69.6	71.3	73.4	88.8	92.0	92.5
10	55.1	60.1	66.8	71.5	80.7	87.5	88.2	100.0	101.0

Table II

Growth of rats on diet IIb

Rat no.	Initial weight	Weeks 1	2	3	4	5	6	7	8
12	52.0	58.1	52.7	53.0	56.5	62.8	75.9	71.0	68.4
13	65.4	70.0	85.8	101.0	92.5	120.7	135.7	151.7	159.8
14	67.5	72.6	87.5	103.0	113.3	125.0	138.3	149.3	162.0
15	56.1	60.3	65.7	74.2	76.2	78.5	86.1	94.7	95.9
16	58.6	63.2	69.5	76.5	72.2	80.0	80.0	81.0	76.5
17	51.1	45.6	58.5	60.4	70.0	76.7	80.0	85.5	88.7
19	62.5	62.9	69.3	75.2	71.5	77.1	87.2	100.0	105.6
20	62.0	61.3	63.7	71.0	72.0	77.8	81.5	86.4	87.5


Curve I
IIb
IIa
Average Weight in Grams
110
100
90
80
70
60
50
1
2
3
4
5
6
7
8
9
Weeks
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phenylalanine, (2) known amount of tyrosine, (3) salt mixture known to be adequate, (4) vitamins from trustworthy sources, (5) greater number of animals, and (6) a longer feeding period.

Conclusion

For a period of eight weeks, the growth of rats receiving diets as described above is independent of tyrosine content of the food.

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