LXXXIX. THE PHYSIOLOGICAL RÔLE OF VITAMIN B. PART IV.

THE RELATION OF CERTAIN DIETARY FACTORS IN YEAST TO GROWTH OF RATS ON DIETS RICH IN PROTEINS.

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INTRODUCTION.

DRUMMOND, CROWDEN and HILL [1922] reported that rats and cats showed a subnormal rate of growth when fed on diets composed largely of caseinogen, but containing what were considered to be adequate amounts of the inorganic salts and vitamins. These observations seemed definitely related to those of Hartwell [1921, 1924, 1925] made during an investigation of mammary secretion in rats, in which she traced a quantitative relation between the protein level of the diet and some factor or factors showing a similar distribution in foodstuffs to vitamin B. A somewhat similar conclusion was reached by Reader and Drummond [1926] who obtained satisfactory growth in rats only when the ratio of protein to the yeast extract used in the diet was about 5 or less.

A consideration of these and other results did not lend support to the opinions that have been expressed to the effect that the vitamin requirement stands in relation to the amount of carbohydrate ingested or metabolised, and provisional support was given to Hartwell's view that it is concerned with the fate of the proteins or their constituent units as a whole rather than with the metabolism of the nitrogen-free residue as was at one time suggested. Another suggestion has been advanced by McCollum and Simmonds [1925] who ventured the view that the subnormal rate of growth noted in our experiments was due to an improper balance between the calcium and phosphorus in the diet, leading to "high-phosphorus" rickets. Such a possibility had suggested itself to us, especially as in the first set of experiments some lameness was noted in the experimental cats, but Reader and Drummond were unable to detect any failure of calcification on X-ray examination of the rats reported in the paper published in 1926. Furthermore, it seemed

unlikely that supplementing the protein-rich diet with additional yeast extract itself rich in phosphorus and poor in calcium could improve the balance of these elements.

It is, therefore, not by any means clear what the action of the yeast extract is in these experiments and further tests were made in order to throw some light on the matter.

Some of Hartwell's results point to the yeast factors operating in her experiments being organic in nature. She was unable to reproduce the effect of the yeast extract by adding salts; the failure of calcium being significant in the light of McCollum's suggestions. Nevertheless, it seemed desirable to ascertain whether the phenomenon observed by us had the same origin as that studied by her, and to determine whether the yeast extract served to adjust the salt or acid-base balance of the diet; it must be remembered that the metabolism of large quantities of caseinogen calls for the neutralisation of considerable quantities of sulphuric and phosphoric acids.

Another point to be studied was obviously the nitrogen metabolism of animals failing to grow satisfactorily on a diet inadequately balanced as regards protein and the yeast factor, in order to find out whether there was any failure to digest and absorb proteins or a disturbance of metabolism reflected in changes in the distribution of nitrogen in the urine.

It will be convenient to deal with this part of the investigation first.

EXPERIMENTAL.

Nitrogen balance. Three young male rats of the same litter were placed in metabolism cages of the Hopkins pattern. Analyses of the excreta were made every few days, decomposition of the urine having been prevented by collection in dilute sulphuric acid and a small quantity of toluene. The

Table I.

	No. Days in period	Total food con- sumption	Average daily con- sumption	Nitrogen- intake g.	Nitrogen-output		Nitrogen balance g.	Change in wt. of rat g.
		Dry wt. g.	Dry wt. g.		Faeces g.	Urine g.		
Control rat on	4	45.85	11.46	1.9890	0.1364	1.2110	+0.6416	+ 5
Diet A (low	4	53.00	13.25	1.9867	0.1073	1.3020	+0.5774	+ 8
protein, low	4	59.99	14.99	1.9378	0.1635	1.3720	+0.4023	+ 7
yeast)	4	68.03	17.01	2.0651	0.1521	1.3685	+0.5445	+10
	5	76.24	15.24	2.4551	0.1786	1.6660	+0.6104	+ 9
Rat on Diet E	4	32.18	8.04	3.3051	0.1258	2.7895	+0.3898	+10
(high protein,	4	34.94	8.73	3.5891	0.1416	3.840	-0.4265	-12
low yeast)	4	41.97	10.49	3.6682	0.1330	3.2340	+0.3012	+ 7
	4	44.59	11.14	3.9796	0.1455	3.4577	+0.3764	+13
	4	43.26	10.81	3.8808	0.1489	3.6855	+0.0464	+ 1
Rat on Diet C	4	36.01	9.00	3.9287	0.1335	3.052	+0.7432	+15
(high protein,	4	39.31	9.82	4.0695	0.1910	3.780	+0.0785	+ 5
high yeast)	5	46.25	9.25	4.4527	0.2119	4.0135	+0.2273	+ 4
	4	46.36	11.59	4.4569	0.1741	4.284	-0.0012	+ 3
	4	47.05	11.76	4.5234	0.1650	3.983	+0.3754	+ 5

faeces were removed daily, dried at 100°, powdered, and a sample weighed out for analysis.

Three diets of the composition used by Reader and Drummond were employed.

	Low protein, low yeast (A)	High protein, high yeast (C)	High protein, low yeast (E)
Caseinogen	20	70	70
Rice starch	70	20	20
McCollum's salt mixture	5	5	5
Yeast extract	4	16	4
Cod-liver oil	2	2	2

Fig. 1.

The growth of the three animals is shown in Fig. 1, the curves of which are similar in general features to those recorded previously from this laboratory. The analyses and figures in Table I indicate clearly that there is no appreciable disturbance of the digestion and absorption of protein in the case of rats showing unsatisfactory growth on diets rich in protein but with an inadequate amount of the related yeast factor (Diet *E*). The average faecal excretion of nitrogen daily in each group is—Diet *A*, 0.0351; Diet *C*, 0.0417; Diet *E*, 0.0297 g.; figures well within the normal range for animals of this weight.

Distribution of nitrogen in the urine. In this experiment groups of several animals were placed in metabolism cages in order to obtain sufficient urine for a full examination. Four groups were employed, three of which were fed on the diets already described whilst a fourth was given a modified diet containing a normal proportion of protein (20 parts) but the larger amount (16 parts) of yeast extract. This was necessary in order to control the effect of the extra yeast with its high content of nitrogenous substances, purines, etc., on the composition of the urine.

The rats were all males of about 150 g. at the beginning of the experiment and the urines were usually collected and analysed every second day. In some

later experiments only two rats were kept in each cage and the urines were collected over a period of four days. The urinary analyses were made by the following methods. Total nitrogen by Kjeldahl, urea by van Slyke's urease method, ammonia by Folin's aeration process, creatinine and uric acid by the Folin colorimetric methods, amino-acids by the formalin method, allantoin by Handovsky's [1914] modification of Wiechowski's technique; it being found more satisfactory to determine nitrogen in the mercury precipitate than to estimate the excess of that element in the final filtrate by volumetric means. Duplicate analyses were made in practically all cases.

Table II. *Diet A. (Control 1.)*

No. of rats	Period Hours	Volume of urine cc.	Total nitrogen g.	Urea-nitrogen		Ammonia-N		Creatinine-N		Uric acid-N		Allantoin-N		Undetermined N	
				g.	% of total N	g.	% of total N	g.	% of total N	g.	% of total N	g.	% of total N	g.	% of total N
4	48	80	2.061	1.801	87.39	0.093	4.5	0.0149	0.7	0.0054	0.28	—	—	0.144	7.13
8	—	267	6.845	6.110	89.69	0.197	4.1	0.0416	0.6	0.0145	0.20	—	—	0.482	5.41
4	96	218	4.922	4.576	92.96	0.105	2.14	0.0446	0.9	0.0125	0.25	0.143	2.9	0.0409	0.85
4	72	185	3.942	3.538	89.74	0.0897	2.21	0.0312	0.8	0.0107	0.27	0.164	4.2	0.1084	2.78
4	96	245	5.995	5.258	87.70	0.1612	2.60	0.0405	0.7	0.0121	0.20	0.100	1.7	0.4232	7.1
Av. daily output per 100 g. rat weight (for last 3 experiments only)		8.54	0.1963	0.1767		0.0047		0.00145		0.00046		0.0054		0.0131	

Table III. *Diet E.*

9	48	365	14.460	13.540	93.63	0.4772	3.3	0.0382	0.26	0.0150	0.1	—	—	0.3896	2.71
4	96	410	14.920	14.17	94.95	0.364	2.4	0.0443	0.30	0.0130	0.09	0.138	0.90	0.1907	1.36
4	72	298	10.690	10.01	93.69	0.3142	2.9	0.0274	0.26	0.01	0.09	0.113	1.06	0.2154	2.00
4	96	355	14.01	12.86	91.81	0.4055	2.9	0.035	0.25	0.0125	0.1	0.108	1.15	0.6862	3.79
Av. daily output per 100 g. rat weight (for last 3 experiments only)		15.38	0.5735	0.536		0.0157		0.00155		0.0005		0.0052		0.0199	

Table IV. *Diet A "modified." (Control 2.)*

4	120	440	9.363	7.952	84.94	0.282	4.10	0.0493	0.5	0.0201	0.2	0.400	4.3	0.660	5.96
2	96	144	3.377	2.963	87.76	0.131	3.9	0.0226	0.7	0.007	0.2	0.1617	4.8	0.0918	2.64
Av. daily output per 100 g. rat weight		9.9	0.221	0.1913		0.0076		0.0013		0.0005		0.010		0.0093	

Table V. *Diet C.*

4	120	475	15.310	13.73	89.69	0.4543	3.7	0.0297	0.2	0.0139	0.09	0.3144	2.1	0.7677	4.22
2	96	174	7.318	6.674	91.20	0.2606	3.6	0.0195	0.27	0.0067	0.09	0.130	1.8	0.2272	3.04
Av. daily output per 100 g. rat weight		15.65	0.5653	0.511		0.0183		0.0013		0.0005		0.0109		0.0233	

The results are set out in Tables II to V. In the first place it is of interest to note the diuresis that is marked as a result of the high protein diets. The volume of urine passed daily by the rats on these diets was nearly double

that voided by the controls on the low-protein ration. It seems probable that the diuresis is due to the greatly increased urea formation in the former animals.

Apart from this an inspection of the tables reveals very little. On the diets rich in caseinogen there is the expected rise of urea and ammonia nitrogen; in all cases there is the constancy of creatinine and uric acid excretion that one would anticipate, whilst the allantoin excretion reflects the fate of the purine bases that are added to the diet in the form of the yeast extract.

In other words, these analyses reveal no disturbance of the nitrogenous metabolism that could be regarded as a possible cause of the failing nutrition of the rats fed on diets rich in protein and imperfectly balanced as regards yeast.

*Attempts to determine the nature of substances responsible
for the effect of yeast.*

In the first place it seemed essential to ascertain whether the action of the yeast extract was attributable to inorganic or organic constituents. A litter of eight young rats was divided into four groups. Two were fed on the high-protein, high-yeast diet (*C*), whilst two others were fed on the high-protein, low-yeast diet (*E*) to which had been added the ash corresponding to the additional 12 parts of yeast extract that were present in diet *C*. The growth curves of these animals are shown in Charts 1 and 2, Fig. 2, and indicate that the salts present in the ash of the other 12 parts of yeast extract did not serve to permit good growth on the high-protein ration. The question then arose as to how it could be determined whether vitamin B was responsible for the effect we were studying. It is generally accepted that this substance is unstable to alkalis and that the antineuritic and growth-promoting properties of foodstuffs such as yeast are not shown by the usual tests after they have been treated with alkalis. Accordingly a diet was constituted in which 70 parts of caseinogen and 4 parts of yeast extract were supplemented with 12 parts of extract that had been autoclaved at 120° for 1 hour at a strongly alkaline reaction and subsequently adjusted to the original hydrogen ion concentration of the extract by the addition of acid. To our surprise this material was, if anything, even more effective in enabling growth on high protein to be satisfactory than the untreated extract (Chart 3, Fig. 2).

The control tests in which all the yeast extract was given in the form of the material that had been treated with alkali (Chart 4, Fig. 2) demonstrated that no growth was made. The nutritive failure of these two animals was checked and growth resumed when the diet was further supplemented with 5 mg. daily of a concentrate prepared from yeast according to Seidell's method [1926].

These experimental results seemed clearly to indicate that at least two

substances, both organic in character, are responsible for the effect of the yeast and that one is probably identical with the substance that is generally known as vitamin B.

The preparation of the yeast concentrate that showed this effect was placed at our disposal by the kindness of Dr A. Seidell. He has described its preparation [1926] and its power of preventing loss of weight in pigeons in

Fig. 2.

doses of about 10 mg. daily. Tested on rats in the usual manner for testing for the presence of vitamin B in foodstuffs we found that the following increments were registered on administering the doses stated:

Dose of yeast concentrate	Change of weight per week
2 mg.	+ 3.5
5 "	+ 5.3
10 "	+ 7.0
20 "	+ 9.7

On the basis that a diet of normal protein content when supplemented with 20 mg. of this concentrate is adequate for steady growth the attempt was made to determine whether increasing the dose of concentrate in proportion to the increase of yeast that had been found necessary would enable good growth to take place on diets rich in protein.

It was found that such was not the case. Doses as high as 80 mg. daily of this preparation did not supplement a diet containing 70 parts of caseinogen as did the equivalent 16 parts of yeast extract.

Convinced from these results that at least two dietary factors derived from the yeast were concerned, we conducted a series of experiments on rats fed on the usual vitamin B-deficient diet employed in the laboratory in order further to trace the relationship between those stable to alkali, and those destroyed by that agent.

The following changes in weight were recorded for a series of pairs of rats of nearly the same initial weight fed on the standard vitamin B-deficient diet with various supplements.

Average weekly alterations in weight of rats fed on vitamin B-deficient ration plus supplements.

Alkali-treated % in diet	Supplement		Change in g.
	yeast extract mg. per day	Seidell's concentrate mg. per day	
0	0	0	- 9.0
0	2	2	+ 3.5
0	5	5	+ 5.5
2	0	0	- 7.5
4	0	0	- 7.5
2	2	2	+ 5.7
2	5	5	+ 5.0
4	2	2	+ 10.5
4	5	5	+ 26.0

The average weekly increment of rats of this age on the basal diet supplemented with 4 % of untreated yeast extract is about 20 g., so that the group receiving 4 % of alkali-treated extract plus 5 mg. of Seidell's concentrate can be regarded as showing a normal rate of growth.

DISCUSSION¹.

It seems clear from these results that definite indication has been obtained of the existence of two factors in the yeast extract we employed (marmite) that play an important part in regulating growth in young rats. These factors may be differentiated by the action of hot alkali which destroys one leaving the other still physiologically active. It is seemingly the latter that is primarily concerned in the relation between yeast extract and the protein content of the diet that has been traced.

It is possible that our observations provide an explanation of the good growth on diets rich in protein shown by Osborne and Mendel's rats [1921] which received a ration containing two sources of vitamin B, namely yeast and alfalfa.

¹ When this paper was in the press our attention was called by Dr Seidell to the publications of Goldberger and his colleagues [1926] with which we were unfortunately not familiar. In these evidence is presented of the existence of more than one dietary factor of the vitamin type in yeast. The relation between Goldberger's P-P factor, "bios" and our alkali-stable unit awaits investigation.

They are also obviously closely related to those reported by Seidell [1926] who found that autoclaved yeast, in which, presumably, the antineuritic factor had been largely destroyed, supplemented the growth-promoting effect of his preparation of yeast concentrate in a curious manner and also to those published by Smith and Hendrick [1926].

The question of the identity of the so-called antineuritic vitamin with the water-soluble growth-promoting factor has been many times discussed without anything approaching a definite answer having been obtained.

Perhaps the most convincing work recorded in the literature is that of Funk and Dubin [1922] and Funk and Paton [1922] who drew attention to the fact that the rat requires more than one accessory substance of the vitamin B type for growth and well-being. This conclusion was reached as a result of experiments on the fractional adsorption of substances in yeast extracts on the surface of fuller's earth and norit. They believed these substances to be firstly that usually described as the antineuritic factor, and secondly a substance or group of substances responsible for the so-called "bios" effect observed in the growth of yeasts and other micro-organisms¹.

It is not proposed in this paper to review the extensive literature on the question of the number and characteristics of the water-soluble vitamins as in our opinion this can only be done adequately when the results of further investigations are to hand.

SUMMARY.

1. Evidence is presented that the failure to grow at a normal rate shown by rats fed on diets consisting largely of protein (caseinogen) but containing the usual proportion of yeast serving as a source of vitamin B is not due to an impairment of the digestion or absorption of the ingested protein.

2. No signs of an abnormal course of protein metabolism could be detected in such animals by a study of the distribution of the chief nitrogenous constituents in the urine. The increased urea production on the protein-rich diets causes a marked diuresis.

3. The subnormal rate of growth alluded to is apparently due neither to deficiencies in the inorganic constitution of the ration nor to disturbances of the acid-base exchange.

4. The restoration of a normal rate of growth that follows the addition of extra yeast extract is also seen when the extract has previously been treated with hot alkalis so as to inactivate the factor generally known as vitamin B.

5. For a normal rate of growth to be shown by rats fed on diets consisting mainly of protein two factors, which can be differentiated by their behaviour on treatment with alkalis, seem to be necessary.

¹ Funk gave this factor the designation "vitamin D." Unfortunately at about the same time McCollum suggested the title of vitamin D for the antirachitic vitamin. Some slight confusion followed but has now disappeared as a result of the almost general adoption of McCollum's choice. A satisfactory nomenclature of these water-soluble vitamins will have to be elaborated.

6. One of these would appear to be the substance known as vitamin B or the antineuritic vitamin. It is rapidly destroyed by alkalis. The other resists treatment with alkali and would seem to be the factor chiefly concerned in the action of the yeast supplements in rendering high-protein diets adequate for growth.

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