LIII. VITAMIN B_1 IN THE ANIMAL ORGANISM

I. THE MAXIMUM STORAGE OF VITAMIN B_1 IN THE TISSUES OF THE RAT

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THE influence of diet on the vitamin B_1 content of the tissues of rats has been studied by various workers. Osborne & Mendel [1923] found variations in the vitamin B_1 content in the livers of rats depending on the allowance of this vitamin in the food, and Westenbrink [1932] made quantitative measurements of the relative amounts of vitamin B_1 left in organs of rats which had been fed on a vitamin B_1-deficient diet for varying periods. Recently, using the rat-growth method of assay developed by Chase & Sherman [1931], Brodie & MacLeod [1935] studied the distribution of vitamin B_1 in the tissues of rats maintained on three types of diets containing different amounts of this vitamin. These authors reported that tissues from animals which had been kept on a vitamin B_1-free diet for 1 month were practically devoid of the vitamin. In the organs of rats fed on a "normal diet" (one-third whole milk powder, two-thirds whole wheat plus 2% salt mixture) it was found that kidney was half, brain one-third and muscle one-tenth as rich as the heart or liver in vitamin B_1 content. When a supplement of 2% of yeast was added to this diet there appeared to be extra storage of the vitamin in muscle, liver, kidney and brain, but not in the heart, spleen and blood.

In all these studies on the storage of vitamin B_1 in tissues no attempt has been made to express the amount of the vitamin in terms of any standard unit of reference, say, in terms of the International Unit. It is evident that unless such a reference unit is employed, various aspects of the study of the storage and distribution of this vitamin in the animal body must lack precision. Thus, if a suitable unit of measurement be adopted, it will be possible to interpret in precise figures the extent of the vitamin B_1 storage not only from the point of view of the concentration of the vitamin per unit weight of tissue but also of the total amount held in reserve.

Using the International Unit of vitamin B_1 as the basis of reference, the quantitative experiments reported below have been carried out to study (1) the maximum storage of the vitamin in the rat, (2) the distribution of the vitamin in various tissues and (3) the influence of dietary intake on the storage of the vitamin.

EXPERIMENTAL

Estimation of vitamin B_1. The "bradycardia" method for the assay of vitamin B_1 was used in this study [Drury *et al.* 1930; Birch & Harris, 1934; Harris, 1934], graded doses of the International Standard acid clay being tested on groups of rats simultaneously with the tissues to be examined. A dose-response curve was obtained by plotting the duration of cure against the respective doses of the I.S. and was used as a reference curve for the estimation of the amount of vitamin B_1 in the tissues.

Rats and diets used. Adult piebald rats of both sexes were used. They had been reared, until 4 months old, on the laboratory stock diet, consisting of brown bread, milk, meat, liver and cabbage. At the beginning of the experiment the animals were put into separate cages and fed on diets containing varying amounts of vitamin B_1. The source of vitamin B_1 was an accurately standardized acid clay adsorbate, found to contain 125 I.U. per g. This was finely ground up and incorporated in the basal diet, which consisted of 60 % castor sugar, 15 % "light white" caseinogen, 15 % arachis oil, 5 % salt mixture and 5 % autoclaved marmite (autoclaved at *p*H 10, 1½ atmospheres, for 4 hours). Tissues were also examined from rats fed on the laboratory stock diet and on a "10 % yeast diet", the latter consisting of 10 % dried yeast, 55 % castor sugar, 15 % "light white" caseinogen, 15 % arachis oil and 5 % salt mixture. Each rat received 3 drops of cod liver oil daily.

The rats were maintained on their respective diets for stated periods, after which they were chloroformed and killed by decapitation. The tissues were removed and weighed and aliquot portions used for assay at once. On occasions when no rats were available for the "bradycardia" test, the tissues were stored (for not more than 2 days) in stoppered bottles in a refrigerator.

The tissues thus examined for vitamin B_1 content were the liver, muscle, heart, brain, kidney and spleen, while in a few rats the intestine, fat and blood were also assayed.

Results

The maximum storage of vitamin B_1 in rats' tissues. Table I gives the vitamin B_1 contents of the tissues of rats reared on the laboratory stock diet. It will be seen that on this diet, which is adequate although not very rich in vitamin B_1, the tissues showed no large variations in vitamin B_1 concentration. The main sites of storage from the point of view of total reserves are the liver and muscle. The concentrations of vitamin B_1 in the tissues of rats receiving large amounts of the vitamin are shown in Table II. A "saturation" limit appeared to have been reached when the liver had a vitamin B_1 concentration of about 1·9–3·2 I.U./g. (av. 2·6 I.U./g.). The only other organ holding a comparably high reserve was heart (av. 2·7 I.U./g.), while the maximum concentrations in the other tissues examined were: kidney 1·2 I.U./g., spleen 1·0 I.U./g., brain 1·0 I.U./g., lung

Table I. *Vitamin B_1 reserves in tissues of rats reared on the laboratory stock diet**

	Concentration of vitamin B_1 (I.U./g.)				Total reserves of vitamin B_1 (I.U.)		
	Rat A	Rat B	Rat C	Av.	Rat A	Rat B	Rat C
Body wt. (g.) and sex ...	152 ♂	160 ♀	240 ♂		152 ♂	160 ♀	240 ♂
Liver	1·5	1·7	1·4	1·5	7·5	8·2	11·9
Heart	1·3	1·3	1·6	1·4	0·9	0·9	1·0
Kidney	0·4	0·2	0·6	0·4	0·6	0·3	0·8
Brain	1·2	0·9	2·0	1·4	1·6	1·4	2·4
Spleen	1·2	1·0	1·8	1·3	1·8	1·2	1·8
Lung	0·5	0·6	1·2	0·8	0·4	0·6	1·2
Muscle	0·4	0·4	0·5	0·4	19·2	20·4	24·0
Body fat	0·5	0·2	–	0·4	2·5	1·2	–
Blood	–	–	0·3	–	–	–	2·7
			Total (I.U.)		34·5	34·1	45·8

* The precise vitamin B_1 content of this diet is unknown, it consists of brown bread, milk, meat, liver and cabbage.

(–) These tissues were not assayed.

Table II. *Maximum vitamin B_1 contents of rats' tissues*

No. and sex	1 ♂	2 ♀	3 ♂	4 ♂	5 ♀	6 ♂	7 ♂	
Body weight (g.) ...	167	180	320	294	286	190	263	
Nature of diet ...	10% yeast	10% yeast	10% yeast	5% acid clay	5% acid clay	10% acid clay	20% acid clay	Average for rats nos. 1–7
I.U. vitamin B_1/g. diet	4	4	4	6	6	12·5	25	
Period on diet (weeks)	1	4	12	7	11	8	4	
I.U./g. liver	2·9	2·8	3·2	3·2	2·1	1·9	1·9	2·6
„ heart	2·0	3·0	2·8	3·0	1·9	3·0	3·1	2·7
„ kidney	1·0	0·4	1·4	1·6	1·3	1·5	1·4	1·2
„ brain	0·8	1·0	1·3	1·2	0·8	1·1	1·8	1·1
„ spleen	1·9	1·0	0·9	0·8	0·9	1·0	0·6	1·0
„ lung	0·5	0·5	0·8	0·4	0·6	0·7	0·8	0·6
„ muscle	0·6	0·5	0·7	0·7	0·5	0·7	0·6	0·6
„ intestine	–	0·7	<0·1	–	–	0·5	–	0·4
„ body fat	–	0·2	0·1	0·8	0·3	0·6	–	0·4
„ blood	–	–	<0·1	–	–	<0·5	–	–

(–) These tissues were not assayed.

0·6 I.U./g. and muscle 0·6 I.U./g. Unlike that in the other tissues, the concentrations of the vitamin in the intestines and body fat seemed to vary greatly from one animal to another (<0·1–0·7 I.U./g. in the case of the intestine and 0·1–0·8 I.U./g. in body fat). With the exception of the liver and heart, the organs of rats A, B and C, which received the laboratory stock diet, were found to be nearly as rich in vitamin B_1 as those from rats which had presumably higher vitamin B_1 intakes. For the reserves of rats receiving smaller allowances of vitamin B_1 and whose tissues were therefore less "saturated", see Table IV.

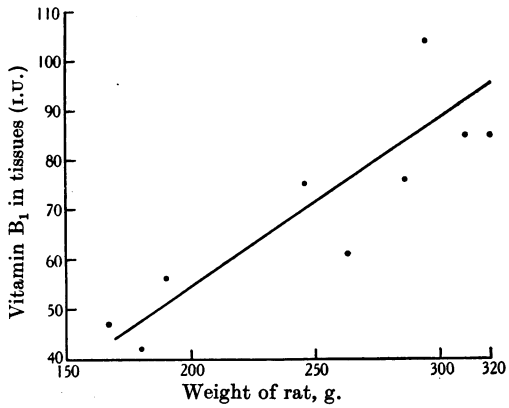

Fig. 1. Amount of vitamin B_1 stored in the tissues of "saturated" rats, showing the relationship between the amount stored and the weight of rat. The tissues examined were liver, heart, kidney, brain, spleen, lung and muscle.

Fig. 1 shows the relationship between the vitamin B_1 content and the weights of rats whose tissues had been "saturated" by a large intake of the vitamin. For these rats the vitamin B_1 reserve was roughly 0·3 I.U./g. body weight.

Distribution of vitamin B_1 in various tissues. The total vitamin B_1 reserves in the organs of these "saturated" rats are given in Table III. It will be observed that the combined deposits in liver and muscle represented 80–90% of the total storage in the organs examined. The remaining 10–20% were distributed

Table III. *Distribution of vitamin B_1 in tissues of rats fed on diets rich in vitamin B_1*

Rat no.	1	2	3	4	5	6	7
Nature of diet	10% yeast	10% yeast	10% yeast	5% acid clay	5% acid clay	10% acid clay	20% acid clay
I.U. vitamin B_1/g. diet	4	4	4	6	6	12·5	25
Av. daily vitamin B_1 intake (I.U.)	40*	40*	40*	90	110	180	375
Period on diet (weeks)	1	4	12	7	11	8	4
Weight of rat (g.)	167	180	320	294	286	190	263
Total vitamin B_1 in liver (I.U.)	16·4	15·4	32·0	35·2	22·0	14·3	22·8
,, heart ,,	1·4	3·2	2·8	3·2	1·9	1·9	2·3
,, kidney ,,	1·2	0·5	2·8	3·2	3·8	2·4	1·4
,, brain ,,	1·2	1·4	1·9	1·9	0·7	1·4	2·8
,, spleen ,,	1·9	0·5	0·9	0·9	0·9	0·8	0·5
,, lung ,,	0·5	0·5	1·4	0·5	0·7	1·0	1·4
,, muscle ,,	24·6	17·0	42·7	58·8	39·6	31·5	31·0
,, intestine ,,	–	1·4	–	–	–	2·3	–
,, body fat ,,	–	2·2	–	–	6·3	–	–
,, blood ,,	–	–	<0·1	1·2	–	<0·5	–
,, above tissues ,,	47	42	85	104	76	56	62
,, liver, as % of total	35	37	38	34	29	26	37
,, muscle, as % of total	52	41	50	57	52	56	50
Combined reserves in liver and muscle, as % of total	87	78	88	91	81	82	87

* Calculated on the assumption that 10 g. food were eaten daily. (–) These tissues were not assayed.

Table IV. *Vitamin B_1 storage in liver and muscle as influenced by diet*

Rat no. and sex	Nature of diet	I.U. vitamin B_1/g. diet	Av. daily vitamin B_1 intake (I.U.)	Period on diet (weeks)	I.U. vitamin B_1/g. liver	I.U. vitamin B_1/g. muscle	
1 ♂	Vitamin B_1-free diet	0	0	4	<0·1	<0·1	Low reserves
2 ♀	,, ,,	0	0	4	<0·2	<0·1	
3 ♂	0·1% acid clay diet	0·13	0·9	8	0·1	0·4	
4 ♂	0·1% ,,	0·13	0·9	8	0·3	0·1	
5 ♂	0·25% ,,	0·3	3·0	8	1·4	0·2	
6 ♂	0·25% ,,	0·3	3·0	12	0·8	0·3	
			Av. for rats 3–6		0·6	0·3	
7 ♂	0·5% ,,	0·6	7·0	9	1·4	0·3	Average reserves
8 ♂	0·5% ,,	0·6	8·0	12	1·9	0·3	
9 ♂	1% ,,	1·2	13·0	9	1·6	0·4	
10 ♂	1% ,,	1·2	13·0	10	1·9	0·4	
11 ♂	Laboratory stock diet	*	*	12	1·4	0·5	
12 ♀	,, ,,	*	*	12	1·7	0·4	
			Av. for rats 7–12		1·7	0·4	
13 ♂	2% acid clay diet	2·5	30	9	2·8	0·5	Maximum reserves
14 ♂	2% ,,	2·5	35	9	2·8	0·5	
15 ♂	10% yeast diet	4·0	40†	1	2·9	0·6	
16 ♀	10% ,,	4·0	40†	8	2·8	0·5	
17 ♂	10% ,,	4·0	40†	12	3·2	0·7	
18 ♂	5% acid clay diet	6·0	90	7	3·2	0·5	
19 ♂	5% ,,	6·0	110	11	2·1	0·7	
20 ♂	10% ,,	12·5	180	6	1·9	0·7	
21 ♂	10% ,,	12·5	200	7	1·9	0·8	
22 ♂	20% ,,	25·0	300	6	1·9	0·6	
23 ♂	20% ,,	25·0	500	7	2·8	0·6	
			Av. for rats 13–23		2·6	0·6	

* The precise vitamin B_1 content of this diet is unknown; it consists of brown bread, milk, meat and cabbage.

† Calculated on the assumption that 10 g. food were eaten daily.

between the following: heart, kidney, brain, spleen and lung, intestine and body fat.

The influence of dietary intake on the storage of vitamin B_1. In this study it was decided to assay only the liver and muscle for their vitamin B_1 reserves as these tissues were found to contain as much as 80–90 % of the total. The results are shown in Table IV. Rats kept for 1 month on a vitamin B_1-free diet had their tissue reserves depleted to such a great extent that the concentration in the liver was less than 0·2 I.U./g., and that in muscle was less than 0·1 I.U./g. The storage rises with increasing levels of vitamin B_1, maximum concentration being attained when the daily intake was 30 I.U. of vitamin B_1. There was little additional storage of the vitamin at levels of intake higher than 30 I.U. per day (see Table IV).

Discussion

Table II shows that the richest stores (i.e. the highest vitamin B_1 reserves/g. weight of tissue) are the liver and heart, which have an average value of 2·6 I.U./g. With the exception of intestine, body fat and blood, which appeared to vary greatly in vitamin B_1 content from one animal to another, the concentrations of the vitamin in all other tissues of "saturated" animals were of about the same order, i.e. 0·6–1·2 I.U./g. It is likely that the amount in the blood will be higher immediately after feeding, and this point is being investigated.

The fact that the bradycardia in these tests is cured by the body fat of rats seems to suggest that the antineuritic vitamin may be stored in appreciable amounts in fat. In view of the work by Evans & Lepkovsky [1932] on the sparing action of fat on vitamin B_1 the question arises as to whether it was this fat-sparing action, and not the vitamin B_1 stored in the fat, that had been responsible for the cure of the bradycardia. Later work by Evans *et al.* [1934], however, showed that in order to obtain this sparing action the percentages of fat, protein and vitamin B_2 complex in the diet had to be very high (50, 36 and 10 % respectively). The basal diet given to the rats used in the bradycardia tests contained these factors only to the extent of 15, 15 and 6 % respectively, which amounts, according to the work of Evans *et al.* [1934], would be too low to produce fat-sparing action on vitamin B_1. Therefore it seems possible that the cure of bradycardia obtained by feeding the fat from these rats (in small test-doses of 1–2 g.) may be due to the presence of some form of vitamin B_1 in the fat.

In the rats whose tissues were "saturated" with vitamin B_1, its concentration in the liver was about four times that in muscle. (Brodie & MacLeod [1935] reported a tenfold difference for these two tissues in rats fed on a "normal" diet.) In spite of this small concentration in muscle it will be seen (Table III) that collectively the muscles hold more vitamin B_1 than the liver and actually contain between 40–60 % of the total body reserves, inasmuch as the musculature accounts for 20–45 % of the body weight. (The weight of the muscles of the piebald rats kept on the diets used in this study was found to be about 30 % of the gross body weight.) The liver is responsible for 30–40 % of the total reserves.

Summary

The influence of the intake of vitamin B_1 on the amount of the vitamin that can be stored in the tissues of the rat has been studied. Maximum storage was attained when the intake of vitamin B_1 was about 30 I.U. per day, a further increase above this level giving rise to no appreciable additional accumulation of reserves.

The highest local concentrations of vitamin B_1 in rats' tissues were observed in the heart (2·7 I.U./g.) and liver (2·6 I.U./g.). The total storage in muscle, where the concentration was 0·6 I.U./g., accounted for about 50 % of the total reserves in the body of the "saturated" rat, while that in liver amounted to about 35 %. The maximum vitamin B_1 content which could be obtained was roughly 0·3 I.U./g. body weight.

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