LIV. VITAMIN B₁ IN THE ANIMAL ORGANISM

II. A QUANTITATIVE STUDY OF THE METABOLISM OF VITAMIN B₁ IN RATS

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LITTLE is known concerning the quantitative aspects of the metabolism of vitamin B₁. The presence of vitamin B₁ in the excreta was reported by Cooper in 1914; and some later workers have suggested that the output of the vitamin in urine is probably diminished when the intake is low [Muckenfuss, 1918; Gaglio, 1919; Van der Walle, 1922; Helmer, 1935]. Recently, in experiments both with human subjects and rats [Harris & Leong, 1936], it has been shown that the urinary excretion of vitamin B₁ depends on (1) the vitamin B₁ reserves of the subject, and (2) his immediate vitamin B₁ intake [cf. also Roscoe, 1936]. Quantitative data were obtained which suggested that the amount excreted in the urine might serve as an indication of the state of vitamin B₁ nutrition in experimental subjects. One interesting point which emerged was that, with the subjects studied, the daily urinary output of vitamin B₁ amounted to only about 10 % of that ingested. The remaining 90 % might be accounted for in several ways: (1) storage in the tissues, (2) excretion with the faeces, (3) destruction in the alimentary tract or (4) conversion into end-products during metabolism. The experiments reported in this paper have been carried out in order to throw more light on these points.

In making a balance sheet with regard to vitamin B₁ metabolism, difficulty arises concerning the extent of bacterial synthesis of the vitamin in the gut. Guerrant *et al.* [1935] have shown that this synthesis occurs in the caecum of the rat and that the amount that can be synthesized depends on the type of carbohydrate in the diet. Whipple & Church [1935] have reported that the presence of fat in the alimentary canal is essential for the elaboration of this vitamin by micro-organisms. No attempt, however, has been made to study the extent of this bacterial synthesis. This aspect of the problem is also made more obscure by the uncertainty as to how much of the faecal vitamin B₁ is derived from unabsorbed food. In the quantitative study of the relationship between vitamin B₁ intake and its faecal output which is reported below, evidence has been obtained to show that the amount of bacterial synthesis is relatively small and may readily be allowed for, so that a reliable study of the vitamin B₁ balance can accordingly be made.

Method of assay of vitamin B₁

The "bradycardia" method for the estimation of vitamin B₁ was used [Drury *et al.* 1930; Harris, 1934; Birch & Harris, 1934] and the standards taken for comparison consisted of a series of graded doses of International Standard acid clay. During the past 18 months a large number of assays of the i.s. have been made in this laboratory, using piebald rats of 45–60 g. weight, and the results obtained on separate occasions have been quite consistent. Four levels of dosage

were tested (with an average of 45 observations at each level) and the means were used to construct a dose-effect curve. The curve was fitted to the data by the method of least squares (Fig. 1). The standard error (ϵ) of each mean was about $\pm 3.8\%$, where $\epsilon = \pm \sqrt{\frac{\Sigma d^2}{n(n-1)}}$. A detailed statistical discussion of the accuracy of the "bradycardia" method will be given in a subsequent communication. All these data have been collected and used to construct a dose-effect curve (Fig. 1) which is used as the reference curve in this study.

Fig. 1. "Bradycardia" method: dose-effect curve.

1. THE SOURCE OF VITAMIN B_1 IN FAECES

The results of a quantitative study of the daily faecal production of vitamin B_1 by rats (both normal and vitamin B_1 -deficient) are given in Table I. Adult

Table I. *Excretion of vitamin B_1 in the faeces of normal and vitamin B_1 -deficient rats*

Normal rats														
Rat no.	...	...	...	1	2	3	4	5	6	7	8	9		
Days on vitamin B ₁ -free diet				1	1	1	3	4	0	0	0	0		
Single test-dose given (I.U.)				0	0	0	0	0	10*	20*	20*	20*		
Output during next 24 hours (I.U.)				0.9	0.9	1.4	0.9	1.4	0.9	1.4	0.9	1.8		
Output on 2nd day (I.U.)				0.9	1.4	1.4	1.4	—	0.9	0.7	0.9	1.0		
„ 3rd „				0.5	—	0.9	0.9	—	—	1.4	0.9	1.4		
„ 4th „				0.9	—	1.4	—	0.9	—	—	—	0.5		
„ 5th „				—	—	0.9	—	—	—	—	—	—		
Vitamin B ₁ -deficient rats														
Rat no.	...	...	...	1	2	3	4	5	6	7	8	9	10	11
Single test-dose given (I.U.)				0	0	0	0	0	1.4*	1.4*	10*	10*	20*	20*
Output during next 24 hours (I.U.)				1.4	1.4	1.4	0.5	1.4	1.4	1.4	0.5	0.5	0.5	0.5
Output on 2nd day (I.U.)				—	0.5	0.5	0.5	0.9	1.4	0.7	1.3	0.5	1.4	1.2
„ 3rd „				—	—	—	1.0	0.5	—	0.6	0.5	—	0.7	1.2
„ 4th „				—	—	—	—	0.9	—	0.7	—	—	—	0.7

* Single test-dose given in the form of the International Standard acid clay.

— These specimens were not assayed.

normal rats were placed on a vitamin B₁-free diet, and single test-doses of the vitamin were given in the form of the i.s. The amount given varied from 10 to 50 i.u. of vitamin B₁, and several animals were given no dose. The daily faecal matter was collected and assayed for its vitamin B₁ content. It will be seen from Table I that in spite of large variations of vitamin B₁ intake (0–50 i.u.) the amount excreted in the faeces daily is relatively constant. With the basal diet used in these experiments the daily faecal output was 0.5–1.8 i.u., depending largely upon the weight of faeces. Similar results were also obtained with polyneuritic rats whose body stores of the vitamin had been depleted after 4–5 weeks on a vitamin B₁-free diet. These polyneuritic rats continued to excrete 0.5–1.4 i.u. per day in the faeces even when no vitamin B₁ was given. From these findings it is clear that the faecal vitamin B₁ is not derived from the food. This finding is subject, however, to the limitation that when the intake of vitamin B₁ is very large, i.e. at levels higher than 80 i.u. per day, appreciable amounts may escape absorption from the gut and be excreted in the faeces (see below).

The various possible sources of vitamin B₁ in faeces have been discussed by many workers [Dutcher & Francis, 1923; Roscoe, 1931; Guerrant *et al.* 1935]. All the available evidence, together with the observations presented above, seems to indicate that bacterial synthesis in the lower part of the intestine probably accounts for most of the vitamin B₁ in the faeces. This synthesized vitamin is presumably retained in the bacterial cells and is therefore not absorbed through the intestinal wall but excreted with the faeces.

It is realized that conclusive proof of the responsibility of bacterial synthesis for all the vitamin B₁ activity in the faeces would entail a separation of the micro-organisms from the non-bacterial matter and a quantitative demonstration of the vitamin B₁ potency in the bacterial portion. Since this aspect of the study would constitute a separate problem in itself, it is proposed, for the purpose of this vitamin B₁ balance study, to apply a correction of 1.5 i.u. per day for bacterial synthesis. This amount is deducted from the daily faecal output of vitamin B₁ and the difference is then taken to represent the amount derived from any unabsorbed vitamin in the food.

2. URINARY AND FAECAL OUTPUT OF VITAMIN B₁ WITH VARIED DIETARY INTAKE

Experimental animals and diets used. Twenty piebald rats which had been reared on the laboratory stock diet were used in these experiments. When 3 months old they were divided into 10 groups of 2 rats each and placed in separate wire cages with $\frac{1}{8}$ in. mesh and elevated bottoms. These groups were placed on a series of diets containing graded amounts of vitamin B₁. The vitamin B₁ was in the form of an acid clay adsorbate¹ (125 i.u./g.) which was finely ground up and carefully incorporated in the basal diet. The composition of the various diets is given in Table II. Each rat was given 3 drops of cod liver oil daily. With the exception of group I, which received no vitamin B₁, all the other groups were kept on their respective diets for a preliminary period of 4–10 weeks so as to ensure that a steady state of vitamin B₁ retention and excretion had been attained.

After this period the rats were transferred to metabolism cages and a few days later, when the rats had become accustomed to their new environment, the

¹ I am indebted to Dr A. Z. Baker of Vitamins Ltd. for generous supplies of the activated acid clay.

Table II. *Composition of diets*

Rats (group) ...	I	II	III	IV	V	VI	VII	VIII	IX	X
Diet no. ...	1	2	3	4	5	6	7	8	9	10
Acid clay absorbate (g.)	0	0.1	0.25	0.5	1	2	5	10	20	30
Sucrose (g.)	60	60	60	59.5	59	58	57	55	48	42
Arachis oil (g.)	15	15	15	15	15	15	14	14	13	11
Light-white caseinogen (g.)	15	15	15	15	15	15	14	13	13	11
Autoclaved marmite* (g.)	5	5	5	5	5	5	5	4	3	3
Salt mixture (g.)	5	5	5	5	5	5	5	4	3	3
Vitamin B ₁ (i.v.)/g. food	0	0.125	0.313	0.625	1.25	2.5	6.25	12.5	25.0	37.5

In addition each rat received 3 drops cod liver oil daily.

* Autoclaved at pH 10, 1½ atmospheres, 4 hours.

actual experimental period was started. During this time measurements of food intake and vitamin B₁ excretion were made daily. Precautions were taken in order to eliminate as far as possible any contamination of the excreta with the particles of food which might be dropped while the animals were feeding, the food pots being placed inside larger vessels so that any spilled food might be deposited in the latter.

Collection of excreta. The faeces were collected daily and dried *in vacuo* over H₂SO₄. After the experimental period the combined faecal matter of each rat was weighed, ground up in a mortar, and aliquot samples used for the estimation of vitamin B₁. The daily urines were collected over 5 ml. of 5% HCl to which had been added a few drops of toluene. The bottoms of the metabolism cages were rinsed out daily with a few ml. of water, a glass rod being used with a rubber ring attached to one end. The washings were added to the day's urine. In the case of urines of low vitamin B₁ content, several days' collection were pooled in order to get a sufficient amount for an assay. The technique used for the concentration of the vitamin B₁ before it could be assayed was essentially the same as that previously described by Harris & Leong [1936]. The only modification was that 4, instead of 2, successive adsorptions with acid clay were made in order to obtain a more complete separation of the vitamin. Such adsorptions were carried out with the urines of groups I–V where the urinary excretion of vitamin B₁ was very small. In the cases of rats with larger intakes of vitamin B₁, and therefore larger excretion, no such concentration of the vitamin was necessary and aliquot portions of the daily urine were used direct for assay (groups VI–X).

Results. The results of the balance experiments are given in Tables III–V. The duration of the actual experimental period, i.e. during which the daily food intake was noted and the assay of vitamin B₁ in urine and faeces was made, varied somewhat from group to group. This in turn depended on the level of vitamin B₁ intake: those rats getting little or no vitamin B₁ in their food excreted so little of it in the urine that a 5–10 days' collection had to be combined for one single estimation, whereas animals with higher intake (and therefore larger output) needed to be observed for a period of only about a week in order to obtain a fairly reliable balance sheet. In order to minimize errors due to variations in animal response in the "bradycardia" tests duplicate assays were carried out whenever possible on the urines, while in the case of the faeces the average values of the results of 3–8 tests were taken.

Table III. *Intake and output of vitamin B₁ at inadequate levels of intake, i.e. 0-3.1 I.U. daily*

Group and rat no.	Experi-mental days	Intake of food g.	Intake of vitamin B ₁ (I.U.)	Output in urine I.U. of vitamin B ₁	Total wt. of faeces g.	Output in faeces		
						Vitamin B ₁ present (I.U.)	Amount unabsorbed from food (after correction for bacterial synthesis)	Balance between intake and output of vitamin B ₁ (I.U.)
I, 1	1- 3	35	0	0.5	7.0	9.1	—	—
	4- 6	28	0					
	7- 9	25	0	0.7				
	10-11	16	0					
	12-14	24	0					
	15-16	13	0					
	Av. per day	8.1	0	0.1	0.4	0.5	0	-0.1
I, 2	1- 3	38	0	0.9	7.5	10.5	—	—
	4- 6	30	0					
	7- 9	29	0	1.8				
	10-11	17	0					
	12-14	22	0					
	15-16	13	0					
	Av. per day	9.3	0	0.2	0.4	0.6	0	-0.2
II, 3	1- 5	32	4.1	1.4	7.0	9.1	—	—
	6-10	33	4.2					
	11-15	36	4.6	1.4				
	16-20	33	4.2					
	Av. per day	7.2	0.9	0.1	0.35	0.5	0	+0.8
II, 4	1- 5	38	4.8	0.9	8.0	10.4	—	—
	6-10	36	4.6					
	11-15	33	4.3	1.4				
	16-20	29	3.6					
	Av. per day	6.8	0.9	0.1	0.4	0.6	0	+0.8
III, 5	1- 2	18	5.6	1.4	6.0	14.4	—	—
	3- 5	29	9.1					
	6- 7	21	6.6	1.4				
	8-10	32	9.9					
	11-12	15	4.7	1.9				
	13-15	26	8.2					
	Av. per day	9.4	2.9	0.3	0.4	1.0	0	+2.6
III, 6	1- 2	19	6.0	0.5	8.2	2.3	—	—
	3- 6	38	11.8					
	7- 9	28	8.8	2.8				
	10-12	35	10.8					
	13-14	17	5.4	2.3				
	Av. per day	9.8	3.1	0.4	0.6	1.7	0	+2.7

As suggested above, there appeared to be a synthesis by bacteria of about 0.5-1.5 I.U. of vitamin B₁ in the faeces of rats placed on the vitamin B₁-free diet No. 1. Therefore in cases where the daily faecal output of vitamin B₁ amounted to less than 1.5 I.U. it was regarded as due solely to bacterial formation and it was concluded that there had been no excretion derived from unabsorbed vitamin B₁

in the ingested food. When the excretion of vitamin B₁ in the faeces was more than 1.5 I.U. per day (see groups VI–X), this figure was deducted from the amount found in the faeces, the difference representing the vitamin B₁ unabsorbed from the food.

Table IV. *Intake and output of vitamin B₁ with moderate levels of intake, i.e. 7.5–31 I.U. daily*

Group and rat no.	Experimental days	Intake of food g.	Intake of vitamin B ₁ (I.U.)	Output in urine I.U. of vitamin B ₁	Total wt. of faeces g.	Output in faeces		
						Vitamin B ₁ present (I.U.)	Amount unabsorbed from food (after correction for bacterial synthesis)	Balance between intake and output of vitamin B ₁ (I.U.)
IV, 7	1–3	39	24.4	2.8	9.0	16.2	—	—
	4–5	19	11.9	3.2				
	6–8	28	17.5	3.2				
	9–10	23	14.4	2.8				
	11–13	30	18.9	2.8				
	Av. per day	10.7	6.7	0.7	0.7	1.3	0	+6.0
IV, 8	1–3	41	25.7	2.8	12.0	26.4	—	—
	4–6	39	24.2	2.8				
	7–8	27	16.7	2.8				
	9–11	35	22.0	2.8				
	12–13	25	15.7	3.8				
	Av. per day	12.7	7.9	0.5	0.7	1.5	0	+7.4
V, 9	1	9	11.3	2.8	3.0	5.4	—	—
	2	9	11.3	3.8				
	3	12	14.5	2.8				
	4	12	14.5	3.8				
	5	11	13.8	3.2				
	Av. per day	10.6	13.1	3.5	0.6	1.1	0	+9.6
V, 10	1–2	20	25.1	4.2	7.5	27.0	—	—
	3–4	23	28.8	4.2				
	5–6	20	25.1	3.8				
	7	12	14.5	2.8				
	8	12	14.5	3.2				
	9	12	14.5	2.8				
	10	12	14.5	3.2				
	11	12	14.5	3.2				
	Av. per day	11.2	13.8	2.5	0.7	2.5	1.0	+10.3
VI, 11	1	11	27.5	15.2	5.0	18.0	—	—
	2	9	22.5	11.2				
	3	9	22.5	16.8				
	4	12	30.0	9.5				
	5	13	32.5	10.5				
	6	12	30.0	16.0				
	Av. per day	11	27.5	13.2	0.8	3.0	1.5	+12.8
VI, 12	1	17	42.5	13.0	5.5	15.4	—	—
	2	14	35.0	10.8				
	3	13	32.5	13.8				
	4	11	27.5	8.4				
	5	14	35.0	16.2				
	Av. per day	13.8	34.5	12.4	1.1	3.1	1.6	+20.5

Table V. *Intake and output of vitamin B₁ with high levels of intake, i.e. 87-704 I.U. daily*

Group and rat no.	Experimental days	Intake of food g.	Intake of vitamin B ₁ (I.U.)	Output in urine I.U. of vitamin B ₁	Total wt. of faeces g.	Output in faeces		
						Vitamin B ₁ present (I.U.)	Amount unabsorbed from food (after correction for bacterial synthesis)	Balance between intake and output of vitamin B ₁ (I.U.)
VII, 13	1	17	106	52.0	7.0	49.0	—	—
	2	14	88	55.2				
	3	13	81	33.6				
	4	13	81	38.4				
	5	13	81	63.0				
Av. per day		14	87	48.4	1.4	9.8	8.3	+31
VII, 14	1	19	119	91.2	9.0	72.0	—	—
	2	19	119	76.8				
	3	19	119	90.0				
	4	15	94	76.0				
	5	17	106	52.0				
Av. per day		17.8	111	77.2	1.8	14.4	12.9	+21
VIII, 15	1	15	188	63	9.0	405	—	—
	2	16	200	75				
	3	14	175	90				
	4	10	125	84				
	5	18	225	100				
	6	14	175	76				
Av. per day		14.5	181	81	1.5	67.5	66.0	+34
VIII, 16	1	19	238	84	10.0	510	—	—
	2	17	213	92				
	3	14	176	104				
	4	15	188	76				
	5	15	188	76				
	6	14	176	112				
Av. per day		16.7	197	91	1.7	85	83.5	+23
IX, 17	1	14	350	76	11.0	1120	—	—
	2	18	450	104				
	3	9	225	76				
	4	9	225	84				
	5	8	200	88				
	6	13	325	112				
	7	12	300	76				
Av. per day		11.9	297	88	1.6	160	158.5	+51
IX, 18	1	18	450	130	15.0	1398	—	—
	2	20	500	110				
	3	21	525	115				
	4	19	475	115				
Av. per day		19.5	488	118	3.8	350	348.5	+22
X, 19	1	16	600	78	15.4	1617	—	—
	2	10	375	42				
	3	13	488	96				
	4	13	488	72				
Av. per day		13	488	72	3.8	404	402.5	+14
X, 20	1	19	713	108	21.6	2268	—	—
	2	18	675	64				
	3	19	713	96				
	4	19	713	84				
Av. per day		18.8	704	88	5.4	567	565.5	+51

The results giving the urinary and faecal outputs of vitamin B₁ at different levels of intake are readily seen by inspection of Figs. 2 and 3.

When the amount of vitamin B₁ ingested was less than 3 I.U. per day, its output in the urine was very low, i.e. 0.1–0.4 I.U. per day; but the quantity excreted increased with increasing intake. It will be observed that at very high levels of intake (i.e. about 100–700 I.U. daily) the urinary excretion of vitamin B₁ remained more or less constant at about 90 I.U. per day.

Fig. 2.

Fig. 2. Relationship between intake and output of vitamin B₁.

• Urine. + Faeces.

Fig. 3.

Fig. 3. Relationship between intake and output of vitamin B₁.

• Urine. + Faeces.

There was no faecal excretion of vitamin B₁ when the amount given was below 10 I.U. per day. When this level was exceeded, there was incomplete absorption from the digestive tract and the faecal output was found to increase as the intake became larger.

The amount of vitamin B₁ that can be absorbed daily from the gastrointestinal tract of the rat appears to be rather limited when the vitamin is in the form of an acid clay adsorbate. It must be realized however that the 90 I.U. that were excreted in the urine (and therefore the amount that was absorbed) represented about 50 times the daily requirement of the rat. Preliminary experiments have also been carried out by administering large doses of vitamin B₁ in the form of a concentrated solution and the results seem to indicate that the amount of the vitamin that could be absorbed daily from the gut was not appreciably increased.

3. EXCRETION OF VITAMIN B₁ IN URINE AND FAECES AFTER INJECTION OF LARGE AMOUNTS OF VITAMIN B₁

In order to find out whether there was any excretion of vitamin B₁ into the intestinal canal, the following experiment was performed. Two adult normal rats were placed on a vitamin B₁-free diet for 10 days and their urinary and faecal outputs of vitamin B₁ during the last 3 days were estimated. After this preliminary period, large doses of vitamin B₁ (250 I.U. per day) were given to

these rats by subcutaneous injection and their urine and faeces (marked with carmine) were assayed for vitamin B₁. The results are shown in Table VI.

Table VI. *Excretion of vitamin B₁ in urine and faeces after subcutaneous injections of large amounts of the vitamin*

Experi- mental days	Amount of vitamin B ₁ injected (i.u.)	Rat A		Rat B	
		Excretion of vitamin B ₁ (i.u./day)		Excretion of vitamin B ₁ (i.u./day)	
		Urine	Faeces*	Urine	Faeces*
1	0	—	—	—	—
8	0	—	—	—	—
9	0	<0.2	0	<0.2	0
10	0	<0.2	0	<0.2	0
11	250	<0.2	0	<0.2	0
12	250	170	0.4	130	0.2
13	250	220	1.4	220	1.9
14	0	220	1.9	190	1.9
15	0	5.2	0.9	—	—
16	0	—	0.7	—	—

* Amount excreted after correction for bacterial synthesis.

— These specimens were not assayed.

It will be seen that subcutaneous injections of 250 i.u. of vitamin B₁ daily were followed by an average daily urinary excretion of 200 i.u. by rat A and 180 i.u. by rat B. The average daily faecal outputs of vitamin B₁ in these 2 rats were 0.9 and 1.8 i.u. respectively (after correction for bacterial synthesis). These results show that large injections of vitamin B₁ could be absorbed readily into the blood stream and that about 75 % of the amount absorbed was excreted in the urine. The amount passed out in the faeces was insignificant, while the difference between the intake and total excretion of this vitamin (av. about 60 i.u./day) might be attributed to (1) destruction during metabolism, and (2) storage in the rat's body. Since the maximum storage of vitamin B₁ in an adult rat weighing 250 g. is about 70 i.u. [Leong, 1937], it is clear that only a small fraction of the amount unaccounted for could have been due to storage in the rat's tissues, and that destruction in the tissues was responsible for the greater part of the loss. It is also evident that the amount of vitamin B₁ excreted into the gut is very small even when large amounts of the vitamin are present in the tissues.

4. DETERMINATION OF THE BODY STORES OF VITAMIN B₁ AT THE END OF THE EXPERIMENT

The summarized results in Table VII show that there was a positive balance of vitamin B₁ (i.e. excess of intake over total output in urine and faeces) in all the groups with the exception of group I which received no vitamin B₁. At higher levels of intake (e.g. in groups VII–X) there was an average daily balance of about +31 i.u. and the question was to decide if this amount represented storage in the tissues of the rats.

It seems clear that a rough approximation of the extent of storage during the experimental period can be made if the initial and final reserves of the vitamin be known. In a previous paper [Leong, 1937] it was shown that the amount of vitamin B₁ stored in a rat of about 150 g. weight and fed on the Laboratory stock diet is about 35 i.u. As the rats used in the present study were reared on the same diet (and are also of the same weight), this value can be taken to represent

the content of vitamin B₁ in their tissues at the beginning of the experiment. The extent of the reserves in these rats at the end of the experimental period was estimated by assay on their tissues. In rats 1-4, estimations of vitamin B₁ were made in the main sites of storage, i.e. liver, muscle, heart, lung, brain, kidney and spleen. As regards rats 5-20, only the liver and muscle were assayed and the total amount in each rat was calculated on the assumption that the combined reserves in liver and muscle of "saturated" rats amounted to 80% of the total storage [Leong, 1937]. The results are given in Table VII.

Table VII. *Vitamin B₁ stores in the rats' tissues at the end of the experiment*

Group	Rat	Intake of vitamin B ₁ (I.U./day)	Vitamin B ₁ balance, i.e. intake minus output (I.U./day)	Days on experi- mental diet	Loss or gain in weight during experimental period g.	Vitamin B ₁ stores in rat at end of experimental period (I.U.)	Loss or gain in body stores during experimental period (I.U.)*
I	1	0	- 0.1	20	- 52	1.3†	- 34
	2	0	- 0.2	20	- 37	1.3†	- 34
II	3	0.9	+ 0.8	57	- 31	4.5†	- 31
	4	0.9	+ 0.8	57	- 38	2.5†	- 33
III	5	2.9	+ 2.6	80	+ 28	24‡	- 11
	6	3.1	+ 2.7	58	+ 23	24‡	- 11
IV	7	6.7	+ 6.0	66	+ 55	30‡	- 5
	8	7.8	+ 7.4	80	+ 117	42‡	+ 7
V	9	13.1	+ 9.6	68	+ 66	41‡	+ 6
	10	13.8	+ 10.3	63	+ 40	38‡	+ 3
VI	11	27.5	+ 12.8	63	+ 55	59‡	+ 24
	12	34.5	+ 20.5	67	+ 121	89‡	+ 54
VII	13	87	+ 31	63	+ 125	118‡	+ 83
	14	111	+ 21	76	+ 139	77‡	+ 42
VIII	15	181	+ 34	44	+ 107	86‡	+ 54
	16	197	+ 23	50	+ 99	84‡	+ 49
IX	17	297	+ 51	46	+ 57	81‡	+ 46
	18	488	+ 22	40	+ 78	86‡	+ 51
X	19	488	+ 14	32	+ 66	80‡	+ 45
	20	704	+ 51	32	+ 33	77‡	+ 42

* Calculated on the assumption that the initial vitamin B₁ store in each rat was 35 I.U.

† The total reserves in the following tissues: liver, muscle, heart, lung, brain, kidney and spleen.

‡ Calculated on the assumption that the combined reserves in liver and muscle represented 80% of the total storage in the rat.

The results obtained in this study permit a reliable interpretation of these balance experiments at different levels of vitamin B₁ intake.

Daily intake of 0-1 I.U. On a vitamin B₁-free diet, rats 1 and 2 lost practically all their body stores during the experimental period of 20 days. If the rate of elimination of the vitamin from the tissue reserves is assumed to be constant, the amount lost is about 2 I.U. per day. As the daily output in the urine was about 0.2 I.U. (none in faeces), it seems reasonable to conclude that the difference (about 1.8 I.U.) had been metabolized into end-products. Similar findings are met with in group II where the daily intake of vitamin B₁ is about 1 I.U.

Daily intake of 3-30 I.U. It will also be seen from Table VII that a daily ingestion of about 3-13 I.U. did not produce additional storage of vitamin B₁ (at 3 I.U. per day there was a loss of 11 I.U. from body stores during the experiment). Therefore the excess of intake over output given in Table VII did not represent storage but rather destruction in the rat's body. It was found that better

growth (accompanied by larger body stores of the vitamin) was obtained with intakes of about 30 I.U. per day.

Daily intake of 100–700 I.U. At these high levels of intake the average increase in vitamin B₁ storage during the experimental period was found to be 42 I.U. (rats 13–20, Table VII). This would represent a daily storage of about 1 I.U., assuming that the amount stored had been increasing at a constant rate during the experiment. This quantity stored represents only a very small fraction of the excess of intake over output, which is about 30 I.U. per day (see Table VII). These observations suggest that at these high levels of intake as much as about 30 I.U. of vitamin B₁ are destroyed in the rat's body per day. The results of the injection experiments cited earlier indicate that considerable amounts of vitamin B₁ can be metabolized in the tissues of the rat. It is quite possible, however, that there may also have been some destruction of vitamin B₁ during the passage of the food through the intestinal tract.

SUMMARY

Vitamin B₁ balance experiments have been carried out with adult piebald rats maintained on varying doses of vitamin B₁, ranging from 0 to about 700 I.U. daily, and the daily output of the vitamin in the urine and faeces has been assayed by the "bradycardia" method.

Bacterial synthesis of vitamin B₁ in the intestine has been found to be an unimportant factor with the basal diet used, the quantity excreted in faeces amounting to about 0.5–1.5 I.U. daily (depending on the bulk of the faeces). Data for faecal outputs have been corrected for this small bacterial synthesis.

The principal conclusions may be summarized as follows:

(1) The urinary excretion of vitamin B₁ was found to increase with increased dietary intake. On vitamin B₁-deficient diets the amount excreted was about 0.1–0.4 I.U. daily (part of which had been derived from the body reserves). With adequate intakes, i.e. 7.5–31 I.U. daily, the urinary outputs increased from 0.6–12.8 I.U. With activated acid clay as the source of vitamin B₁, the maximum daily output observed was in the region of 90 I.U.

(2) The faecal excretion of vitamin B₁, apart from the small constant output attributable to bacterial synthesis, was insignificant with intakes of less than 30 I.U. daily. Incomplete absorption of the vitamin occurred when this level of intake was exceeded, the faecal output increasing as the intake became higher.

(3) The amount of vitamin B₁ withdrawn from the body reserves on vitamin B₁-deficient diets was about 2 I.U. daily, of which about 90 % was destroyed in metabolism and 10 % excreted in the urine.

(4) When the vitamin was injected subcutaneously in large amounts (250 I.U.) about 75 % was excreted in the urine and there appeared to be little excretion of the vitamin into the intestinal tract.

(5) At high levels of intake (about 100–700 I.U. per day), about 30 I.U. were unaccounted for in the urinary and faecal excretion per day most of which was probably destroyed in metabolism.

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