CLIII. THE B-VITAMINS IN HUMAN URINE.

By MARGARET HONORA ROSCOE.¹

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Mass.

(Received April 9th, 1936.)

IN recent years much attention has been directed to the part played by lack of the B-vitamins in the production of a number of diseases of obscure origin, and the use of one or more constituents of the vitamin B complex has been advocated for the cure of a variety of conditions. A review of this subject will be found in the "Discussion on avitaminosis (B group) in tropical and temperate countries" at the Royal Society of Medicine, in 1934.

In particular, the occurrence in a number of diseases of a multiple peripheral neuritis, similar to that of beriberi, has suggested a causal relationship of the B-vitamins. Cobb and Coggeshall [1934] have summarised the causes of neuritis and given a list of conditions in which it occurs as the possible result of dietary deficiency; among these are included combined system disease, occurring in pernicious anaemia [Gildea *et al.*, 1930], and "alcoholic" polyneuritis [Minot *et al.*, 1933; Strauss, 1935]. The deficiency in the former state has been thought possibly to be due in some cases to defective absorption of the vitamins, in the latter state to an insufficient intake, since advanced alcoholic addicts consume alcohol at the expense of more normal, vitamin-containing constituents of the diet.

Unfortunately, most of the evidence connecting the B-vitamins with these conditions has been of a curative nature, and as such does not throw much light on their causes, since the fact that a substance cures a disease does not necessarily prove that its lack has caused the disease. A further disadvantage of human curative observations of a dietetic nature is the difficulty of ensuring that only one constituent of the diet is altered at a time. This is particularly true when additional vitamin B₁ is fed, since it has been shown [Cowgill *et al.*, 1925] that this increases the appetite, so that there is a concurrent increase in the intake of all other constituents of the diet. But perhaps the most serious disadvantage till recently has been that no pure sources of the B-vitamins have been available for clinical use, so that therapeutic effects obtained from feeding yeast or even liver have often been attributed solely to the B-vitamins contained in these substances. However, as the knowledge of the composition of the various B-vitamins advances, it is becoming possible to investigate their separate effects. Some therapeutic effects of crystalline vitamin B₁ have already been examined [Vorhaus *et al.*, 1935].

The assumption that conditions such as "alcoholic polyneuritis" and combined system disease are caused by lack of the B-vitamins has therefore rested on the fact that they occur in patients who have, it is thought, been living on diets deficient in the vitamins. It is, however, seldom easy to decide whether a diet has contained adequate B-vitamins; for the distribution of the B-vitamins in

¹ Working with a Rockefeller Fellowship for Medical Research, awarded by the Medical Research Council.

foods is widespread and variable, the quantities consumed are always difficult to ascertain, the amounts needed by man have not been definitely settled, and there is no certainty that the amount consumed will always be absorbed.

A method of discovering whether a patient is in a deficient condition is therefore badly needed. By adopting the method used with vitamin C [Harris *et al.*, 1933; Johnson and Zilva, 1934], a study was made of the vitamin B₁ and B₂ content of human urine, in the hope that the amount of these vitamins excreted might give some indication of a surplus or deficiency of them in the body. The urine of three normal subjects was examined and also that of a patient with "alcoholic" polyneuritis.

HISTORICAL.

The presence of an antineuritic factor in human and animal urine was shown by Muckenfuss [1918], Gaglio [1919] and Van de Walle [1922], the last two also showing that the amount was reduced in starvation and in vitamin B₁ deficiency. More recently, Gulick and Daniels [1931] investigated the vitamin B (B₁) content of the milk feeds, urine and faeces of eleven infants. They found a fairly uniform retention of vitamin B, increasing with the size of the child.

Helmer [1935] investigated the amount of vitamins B₁ and B₂ in urine from three normal subjects receiving a weighed, well balanced diet and in the urine from one patient with untreated pellagra. He found that whilst the urine from the normal subjects contained appreciable amounts of both vitamins B₁ and B₂, neither of these vitamins could be detected in the urine from the pellagrin.

Thus there are already indications that the amount of the B-vitamins in the urine is affected by the oral intake of vitamins, but it is not known whether any quantitative relationship exists.

EXPERIMENTAL.

1. *The subjects studied and their vitamin intake.*

A study was made of the excretion of vitamins B₁ and B₂ in three control male subjects (I, II and IV), admitted to hospital for reasons unconnected with diet or metabolism, and in one male patient (III) with "alcoholic" polyneuritis.

This patient, 53 years of age, had for years consumed moderate amounts of alcohol, at times as much as 1 pint of whisky a day but usually not more than 2 or 3 pints a week. For years he had eaten irregularly, sometimes taking practically nothing but 1 pint of whisky daily, during several days, as his apparent source of calories. It was impossible to determine any regular dietary habits. Recently his appetite had been very poor. There had been no prominent gastro-intestinal symptoms but there was marked decrease of hydrochloric acid in the stomach contents.

A month before entry he noted tenderness of the calves of the legs, sensations as of pins and needles in the feet. At that time progressive "weakness" of the legs began. Examination on entry to the hospital showed definite but not severe signs of "alcoholic" polyneuritis. Strength was diminished in the legs and feet. The gait was ataxic on a slightly broad base. Knee jerks were diminished and ankle jerks were absent. Babinski's sign was not present. The calves were markedly tender on pressure. There was slight hyperaesthesia of the soles of the feet. Vibration sense was moderately diminished, but position sense was apparently normal. By the end of the observations, which included a period (3) during which a high vitamin diet was fed, diminished ankle jerks were the only residual sign.

Since it was not possible to feed diets of known vitamin content, the excretions at different levels of vitamin intake were compared. In each case the subjects received the different diets for 12 days and the vitamins in the total

urine excreted during this period were estimated. By this procedure it was hoped to eliminate variations caused by day to day changes in the food and by any lag in excretion when the diet was changed.

The control "normal" subjects were observed for two successive periods of increasing vitamin intake, periods 1 and 2 below. The patient with "alcoholic" polyneuritis was observed for a third period, 3, with a still higher vitamin intake. This patient received 1 quart of whisky daily throughout the experiment. The diets given were as follows:

Period 1. The ordinary hospital diet.

Period 2. The same diet with daily additions of 15 g. (10 tablets) of "Harris Yeast Vitamin" and 18 g. (3 level teaspoonfuls) of "Vegex" ("Marmite").

Period 3. A high vitamin diet as used for the treatment of cases of "alcoholic" polyneuritis by Minot *et al.* [1933] and by Strauss [1935], together with "Harris Yeast Vitamin" and "Vegex" in the same amounts as in Period 2.

"Harris Yeast Vitamin" (12.5 g.) and "Vegex" (15 g.) in the proportions fed to the patients in Periods 2 and 3, were mixed with water, made up to 100 ml. with water, and the B-vitamins estimated in this preparation, which will be referred to as the "B-vitamin mixture". The amount of additional vitamins fed in Period 2, as compared with Period 1, was thus roughly ascertained.

The vitamin excretion was estimated in the urine only. The amount excreted in the faeces was not taken into account, since it would almost certainly be affected by bacterial action in the gastro-intestinal tract and therefore be no guide to the true excretion. At the same time it must be remembered that, especially when large amounts of the vitamins are being fed, it is possible that absorption may be incomplete, so that any balance sheet constructed without consideration of exogenous excretion of the vitamins can only be approximate.

The total urine for each 12-day period was collected, 10 % of 95 % alcohol containing 3 % of HCl, being used as preservative. It was then concentrated at about 50° *in vacuo* to a volume of 1 litre. The concentration could be carried to this point without appreciable sedimentation occurring. The concentrated material was brought to a p_H of about 4.0 with NaOH and stored in the cold till used. 83 ml. of the final preparation then represented a day's output of urine.

It has recently been shown that vitamin B₂ can be resolved into two factors [Kuhn *et al.*, 1933, 1, 2, *etc.*], one factor being a lyochrome (flavin) and the second known as supplement or vitamin B₆. In this work, however, the vitamin B complex was estimated as a whole, so that there is no indication whether the urines tested would not, on further work, be found to contain large amounts of either flavin or vitamin B₆. That they did contain both these factors to some extent was however demonstrated, for in the absence of either factor, no "vitamin B₂ complex" activity could have been observed by the growth method employed.

2. Details of the vitamin tests employed.

Vitamin B₁ estimations. The method of estimating this vitamin was a modification of that described by Burn [1932] and Coward *et al.* [1933]. Determination was made of the percentage of animals, in the original work pigeons, in the present study rats, which were cured of the neuritis which develops, when vitamin B₁ is absent from the diet, after a given dose of vitamin B₁-containing material. A curve showing the percentage of animals cured by different amounts of a standard vitamin B₁ preparation was constructed at the same time, and by

comparison with this, the amount of the vitamin in the tested dose of each material could be found.

This curative method was employed for two reasons. Since only one dose of vitamin-containing material was used for each animal, the total quantity needed for a test was small, whereas in growth tests doses are fed daily over a period of weeks to each animal. Also, difficulty was experienced in persuading animals deprived of vitamin B₁ to consume daily doses of concentrated urine, whereas a single dose could be introduced artificially.

The rats used weighed 100–200 g. and had previously received a synthetic ration with adequate, but not superabundant, B-vitamins. They were given a diet containing vitamin B₂ but no vitamin B₁, this being constituted as follows: washed caseinogen (R. H. Thomas) 20 g.; corn starch 60 g.; salt mixture (McCollum No. 61) [Itter *et al.*, 1935] 5 g.; and "Crisco" 15 g.; mixed with 100 ml. of a watery solution of autoclaved "Vegex" (1 hour at p_H 9.0) containing the equivalent of 50 g. of "Vegex", to supply vitamin B₂. The diet was cooked for 2–3 hours in a steam steriliser. Cod liver oil was fed as a separate daily dose.

After 3 or more weeks, during which time the rats lost about one-third of their weight, acute neuritis developed. Three degrees of severity of symptoms were recognised, slight, definite and severe, and rats with each degree were used to titrate each dose, but no correlation was found between ease of cure and the severity of symptoms. Similarly it was not found that the weight of the rats when dosed influenced the cures.

As soon as the nervous symptoms were apparent, a single dose of the material to be tested was introduced into the stomach, by means of a pump made from a graduated 5 ml. syringe to which was attached a length of small size rubber catheter. In this way it was possible to ensure that the animal received the whole of its dose. The rat was then observed for 2 days, at the end of which time the effect of the dose was always clear.

The results of the tests are shown in Table I. The curative effects of the following materials were investigated.

Table I. *The curative effect of various vitamin B₁-containing materials on rat neuritis.*

Material		Doses tested (ml.) and number of rats cured. In brackets the number of rats observed						Amounts needed to cure 50–70% of animals
Urines*	Subject	Period	0.06	0.125	0.25	0.5	1.0	
I	(1)	—	—	0 (1)	0 (1)	0 (1)	0 (1)	>2.0 ml.
	(2)	—	—	0 (1)	0 (2)	4 (6)	—	0.5 ml.
II	(1)	—	—	—	—	—	—	>2.0 ml.
	(2)	—	—	—	0 (2)	4 (6)	—	0.5 ml.
IV	(1)	—	—	—	—	—	0 (3)	>1.0 ml.
	(2)	—	—	—	—	0 (4)	3 (6)	1.0 ml.
III	(1)	—	—	—	—	—	0 (1)	>2.0 ml.
	(2)	—	—	0 (2)	3 (6)	—	—	0.25 ml.
	(3)	—	0 (2)	3 (6)	—	—	—	0.125 ml.
B-vitamin mixture (ml.)			—	0.125	0.25	0.5	—	0.25–0.5 ml.
			—	0 (1)	0 (4)	6 (7)	—	—
Acid clay, international standard (mg.)			—	10	20	30	—	20 mg.
			—	3 (10)	6 (10)	8 (10)	—	—

* Concentrated so that 1 litre≡12 days' output.

(a) *Standard.* With the international standard acid clay adsorbate of vitamin B₁, and with 10 rats receiving each dose, 10 mg. (1 International Unit) produced cures in 30% of the animals, 20 mg. (2 i.u.) in 60% and 30 mg. (3 i.u.) in 80%.

The curve expressing these results is shown in Fig. 1, together with the curve given by Coward *et al.* [1933] for pigeons. It will be seen that the two are exactly

comparable in form, but that the pigeon appears to need twice as much vitamin B₁ as the rat. Thus to cure 50 % of the pigeons a dose of 30 mg. was required, while apparently 15 mg. would have been sufficient for rats; and to cure 80 % of the pigeons 60 mg. were required, while for rats only 30 mg. were needed.

Fig. 1. Response to graded doses of acid clay adsorbate (international vitamin B₁ standard) of pigeons and rats with neuritis due to vitamin B₁ deficiency.

This result contradicts that obtained by Kinnersley *et al.* [1930], who found that the vitamin B₁ requirements of pigeons and rats were equal, as judged by the number of days after a single dose of test material during which cures of neuritis lasted. It agrees, however, with the statement made by Plimmer *et al.* [1927], that whilst pigeons need 4 % dry yeast in the diet to maintain weight, rats only need 2 %. It is probable that the different amounts of vitamin B₁ needed by the pigeon and the rat are related to the different body weights of these species, thus the pigeon may be considered to be roughly twice as large as the rat. This matter has been investigated fully by Cowgill [1932], who took restoration of appetite in depleted animals as the criterion of vitamin B₁ activity. At the same time it may be pointed out that Cowgill's other hypothesis, that the need of animals of a given species for the vitamin increases with increasing weight, was not supported in these observations, where cure of neuritis was the criterion, for the weight of the rats, within the limits observed, 88–179 g., did not influence the cures.

(b) *Urines.* When testing the urines it was found that if the doses tested were of the order 1, 2, 4, 8 *etc.*, the minimum dose which had any effect cured 50–60 % of the rats. Such minimum effective doses, irrespective of the exact proportion of the animals cured, were taken to contain equal quantities of vitamin B₁ and to be equivalent to 20 mg. of acid clay, which promoted cures in 60 % of the animals, that is equivalent to 2 I.U. No doubt, if the number of animals observed with doses of half these amounts had been larger, a small proportion would have been cured, comparable with the proportion, 30 %, cured by 10 mg. of acid clay.

It will be seen that no vitamin B₁ could be detected in the urine from patients receiving the ordinary hospital diet without additional vitamins, Period 1, although as much as 2.0 ml. of the concentrated urine was tested. Larger amounts than this could not be given as they appeared to be toxic, causing the death of the animals before the effect on the nervous symptoms could

be observed. Whether these urines did in fact contain small amounts of vitamin B₁ remains therefore in doubt, and it can only be said that 2 ml. of concentrated urine contained less than 2 i.u. of vitamin B₁, so that 83 ml. concentrated urine, or 1 day's output, contained less than 83 i.u. The urines from the patients receiving additional B-vitamins, Periods 2 and 3, all contained appreciable amounts of vitamin B₁, from 0.125 to 2.0 ml. containing 2 i.u., or, 1 day's output (83 ml.) containing 167–1333 i.u. of vitamin B₁.

(c) *B-vitamin mixture, given to subjects during Periods 2 and 3.* The minimum amount of this material found to cure (0.5 ml.) relieved the symptoms of 86 % of the rats observed; 0.5 ml. would thus seem to be equivalent in effect to 30 mg. of acid clay (80 % cures) and so to contain 3 i.u. of vitamin B₁. It was therefore calculated that $\frac{0.5}{3}$ ml., that is 0.17 ml. contained 1 i.u. of vitamin B₁. It would have been expected that some of the animals receiving 0.25 ml. (1.5 i.u.) would have been cured; actually none of the 4 rats observed was cured.

The equivalent of 120 ml. of this B-vitamin mixture was fed daily to the patients during Periods 2 and 3 of the experiments. They therefore received 720 i.u. of vitamin B₁ in addition to the amount present in their food.

Vitamin B₂ estimations. The growth method of Chick and Roscoe [1928] was employed for the estimation of this vitamin.

Rats of 35–40 g. weight were given a diet consisting of washed caseinogen (R. H. Thomas) 20 g.; corn starch 60 g.; salt mixture (McCullum No. 51) [Itter *et al.*, 1935] 5 g.; and "Crisco" 15 g.; mixed with 100 ml. of water and cooked for 2–3 hours in a steam steriliser. After 1 week the rats were also given 0.1 ml. daily of a vitamin B₁ concentrate, prepared from brewer's yeast [Kinnersley *et al.*, 1933], an amount equivalent to 0.6 g. dry weight of the original yeast and containing sufficient vitamin B₁ for normal growth. Cod liver oil was fed as a separate daily dose. The animals were observed for a further week or until they were no longer growing, and the doses of the vitamin B₂ containing materials were then fed. The feeding of these materials was continued for 5 weeks, 6 days a week, and the growth during this period observed. In order to increase the palatability, sugar was mixed with the doses of urine.

In Table II are shown the results obtained when the vitamin B₂ in the B-vitamin mixture and in the concentrated urines was tested by this method.

Table II. *The growth promoting effect of various vitamin B₂-containing materials for rats.*

Amounts of urine* and B-vitamin mixture fed to young rats (40–60 g. weight) as source of vitamin B ₂ , together with the resulting weight increase in 5 weeks. In brackets the number of rats observed										
Material		Average weight increase		Average weight increase		Average weight increase		Average weight increase		
Urines*	Subject	Period	Dose ml.	g.	Dose ml.	g.	Dose ml.	g.	Dose ml.	g.
I	(1)	0.5	45 (4)	1.0	59 (4)	—	—	—	—	—
		(2)	—	—	—	—	0.25	62 (6)	0.5	67 (2)
II	(1)	0.25	43 (4)	0.5	57 (2)	—	—	—	—	—
		(2)	—	—	0.25	56 (6)	—	—	—	—
IV	(1)	—	—	0.125	56 (4)	0.25	59 (5)	0.5	64 (1)	—
		(2)	—	—	—	—	0.125	61 (4)	0.25	58 (5)
III	(1)	—	—	0.25	56 (4)	0.5	64 (2)	—	—	—
		(2)	—	—	0.25	53 (4)	0.5	61 (2)	—	—
		(3)	—	—	0.06	50 (4)	0.125	63 (5)	0.25	68 (3)
B-vitamin mixture		—	—	0.125	56 (4)	0.25	61 (4)	—	—	—

* Concentrated so that 1 litre = 12 days' output.

It will be seen that large variations in the amount of vitamin B₂-containing materials fed produced only small differences in the growth response. Thus the concentrated urine from subject IV, during Period 1, was tested in three different doses, 0.125, 0.25 and 0.5 ml. and the average growth during 5 weeks observed in rats receiving these amounts was 56, 59, and 64 g. This result was entirely different from the results obtained previously with this method, when rats bred at the Lister Institute, London, were used. As an example of these results the test for vitamin B₂ in dried banana may be quoted [Roscoe, 1931, Table III]; 1 g. of banana promoted 35 g. growth in 5 weeks, 2 g. promoted 62 and 4 g. promoted 102 g.

It was at first thought that the inability of large amounts of the concentrated urines to stimulate a greater weight increase might be due to a toxic material in the urines which inhibited growth. Two facts were opposed to this theory however, the first being that growth did not appear to be affected by the actual size of the dose, irrespective of the vitamin content, and the second that when other rats received the B-vitamin mixture as source of vitamin B₂, the difference between the responses to 0.125 and 0.25 ml. was also very slight, the average weight increase with these two doses being 56 and 61 g. respectively. It seems, therefore, that for some unexplained reason, the rats did not respond to graded doses of vitamin B₂ in the same way as those employed in previous experiments.

This fact emphasises the necessity for employing in all vitamin B₂ tests some material as standard with which others may be compared, and of not relying on a standard growth response. Only in this way can comparisons be made between the results obtained in different laboratories, for it is apparent that growth response is an uncertain factor, varying according to the strain of animals employed, and even, it is possible, in the same strain according to the time of year.

The absence of a marked difference in weight increase as a result of feeding widely different amounts of vitamin B₂ considerably reduced the accuracy of the tests, but by comparing two or three different doses of each material it was found possible to assess their relative vitamin B₂ contents. In Table II the doses of the materials tested are arranged so that amounts promoting equal weight increases fall in line. The amounts in each column may then be considered to have contained equal quantities of vitamin B₂.

Since the materials tested in this work were not compared with a standard material, it must be understood that no conclusions can be drawn as to the absolute amounts of vitamin B₂ present. But since the B-vitamin mixture, fed to the patients as source of vitamin B₂ and the urines excreted were tested at the same time and by the same method, a rough estimation of the proportion of the additional intake of the vitamin which was excreted could be made. For the purpose of this work, the vitamin B₂ in the urines was expressed in terms of the amount present in 1 ml. of the B-vitamin mixture, this being called 1 ml. equivalent of vitamin B₂. Then since "Vegex" and "Harris Yeast Vitamin" were fed to the subjects, during Periods 2 and 3, in amounts equal to those present in 120 ml. of the B-vitamin mixture, the additional vitamin B₂ intake, during these periods, was 120 ml. equivalents.

From 0.06 to 1.0 ml. of the different concentrated urines was found to be equal in vitamin B₂ content to 0.125 ml. of the B-vitamin mixture, or 0.5–8.0 ml. contained 1 ml. equivalent of the vitamin. Since 83 ml. of concentrated urine represented in each case 1 day's output of urine, the daily output of vitamin B₂ in different urines varied from approximately 160 to 10 ml. equivalents daily.

3. The vitamin B_1 and B_2 contents of the urine of different subjects.

In Table III the intake and urinary output of vitamins B_1 and B_2 are given for the four subjects. The results relating to the two vitamins will be considered separately.

Table III. *Intake and urinary output of vitamins B_1 and B_2 in four subjects.*

		Periods (12 days each)			
Intake of vitamins B_1 and B_2 :		(1)	(2)	(3)	
All subjects		Hospital diet	Hospital diet + vitamin B_1 720 i.u. and vitamin B_2 120 ml. equivalents* daily	High vitamin diet + vitamin B_1 720 i.u. and vitamin B_2 120 ml. equivalents* daily	
Output and retention of vitamin B_1 (i.u. daily):		Output	Output	Increased retention	Output
I } Controls		< 83	333	387-470	—
II }		< 83	333	387-470	—
IV }		< 167	167	553-720	—
III "Alcoholic" polyneuritis		< 83	667	53-136	1333
Output and retention of vitamin B_2 (ml. equivalents* daily):					
I } Controls		10	80	50	—
II }		20	40	100	—
IV }		80	160	40	—
III "Alcoholic" polyneuritis		40	40	120	160

* Arbitrary units adopted for this work only, see p. 1059.

Vitamin B_1 . As already stated, the amount of this vitamin present in the urine of the subjects receiving the ordinary hospital diet (Period 1) was so small that it could not be estimated by the method here used. This might mean that this diet contained so little of the vitamin that it was all utilised by the patients. The diet was, however, a liberal one, so that this does not seem probable.

The increased intake of vitamin B_1 during Period 2 was reflected in an increased excretion in the case of all the control subjects (I, II and IV). The amounts excreted in the urine were 333, 333 and 167 i.u. daily. Assuming that the vitamin B_1 in the hospital diet remained constant, so that the additional intake during this period was that provided by the "Harris Yeast Vitamin" and the "Vegex", that is 720 i.u. daily, the increased retention during Period 2 could be calculated. It is shown in Table III, and will be seen to have been between 387 and 470 i.u. daily in two cases and between 553 and 720 i.u. in one case. Thus an increased consumption of vitamin B_1 resulted in increased excretion and increased retention.

It was expected that patient III with "alcoholic" polyneuritis, who, it was thought, had been suffering from a deficiency of vitamin B_1 , might, when the intake was high, retain more of the ingested vitamin than the other subjects, to replenish depleted stores. This was not so. During Period 1, again no vitamin B_1 was detected in the urine, so no comparison could be made. During Period 2, the patient actually excreted more vitamin B_1 (667 i.u. daily) than did any of the controls (maximum 333 i.u. daily), and retained less (increased retention of between 53 and 136 i.u. daily). During Period 3, the output of this vitamin was

still further increased, 1333 I.U. daily being excreted, or twice as much as during Period 2.

This large excretion of vitamin B₁, by a patient who was receiving alcohol daily throughout the experiment, might seem to support the theory put forward by Gigon and Odermatt [1925], that consumption of alcohol results in a washing of this vitamin out of the body, by way of the urine. However, since only one case was observed, no definite conclusions can be drawn.

Vitamin B₂. The normal subjects (I, II and IV) all excreted definite, though small, amounts of vitamin B₂ in the urine during the period when they received the hospital diet alone (Period 1). The amounts excreted were 10, 20 and 80 ml. equivalents daily. When, in Period 2, the intake was increased by 120 ml. equivalents daily, the output was also increased, being 80, 40 and 160 ml. equivalents daily. The increased retention, assuming a constantly increased consumption of 120 ml. equivalents daily, was then 50, 100 and 40 ml. equivalents daily. Thus, as for vitamin B₁, there was an increase in both excretion and retention following an increased intake. The proportion of the additional vitamin excreted to that retained was very variable, being 70 : 50, 20 : 100 and 80 : 40.

Subject III with "alcoholic" neuritis, excreted 40 ml. equivalents of vitamin B₂ daily during Period 1, an amount not different from that excreted by the controls. However, when the intake was increased by 120 ml. equivalents (Period 2), the excretion was not increased, remaining at 40 ml. equivalents daily. The patient therefore appeared to have retained the additional 120 ml. equivalents of vitamin B₂, suggesting that there had previously been a deficiency. The retention of one of the controls was however, nearly as large; thus Subject II retained 100 ml. equivalents daily. It is therefore doubtful whether the retention can be considered to have been significantly large. When the intake of Subject III was increased still more (Period 3), an increased excretion was observed, 160 ml. equivalents daily, as compared with 40 ml. equivalents daily in Periods 1 and 2.

DISCUSSION.

The absolute amount of either vitamin B₁ or vitamin B₂ present in the urine at a given time would not appear to give any clear indication of the adequacy of the diet, for the subjects, even when receiving an adequate diet, excreted only small amounts of these vitamins. It even appeared that vitamin B₁ might be absent from the urines of subjects receiving a normal mixed diet, though it is more probable, especially in view of previous work on the subject, that some was excreted, but that the quantities were too small to be appreciated by the method used. Thus the maximum amount of concentrated urine that it was found possible to test was 2 ml. or 1/40 of a day's output, whereas Helmer [1935], using urine evaporated to dryness, was able to test doses representing 1/25 of a day's output, and found vitamin B₁ activity in such.

Since considerable variations in urinary output of vitamins B₁ and B₂ were exhibited by different "normal" subjects receiving the same diet, no mean normal excretion on a given diet could be found. This result may, however, have been due in part to variations in the amount of the diets consumed, which, in these experiments, was not controlled.

When the intake of vitamins B₁ and B₂ by control "normal" subjects was increased by a known amount, some of the additional vitamin was excreted in the urine and some retained or disposed of by other means. The amount retained was variable, both in absolute amount and in proportion to the amount excreted.

Such variations occurring in normal subjects showed that extreme caution must be exercised in interpreting the results obtained with patients who are supposedly deficient. The absolute amount excreted would not appear to be significant, and any variations in the retention of the vitamins, from that found in normals, would have to be very large to be considered abnormal. Thus it is doubtful whether the slight variations observed in the one patient with "alcoholic" polyneuritis studied in this work, a decreased retention of vitamin B₁ and an increased retention of vitamin B₂ when additional vitamins were fed, can be considered to have been significant.

The results obtained do not on the whole encourage the belief that the balance sheet method can be applied to the study of the metabolism of the B-vitamins, though this question cannot be finally settled until more extensive experiments are carried out with diets of known vitamin content, and until it can be ensured that all the vitamin administered is absorbed.

SUMMARY.

1. Vitamins B₁ and B₂ were estimated in the urine of four subjects, three normal controls and one case of "alcoholic" polyneuritis. The subjects first received the hospital diet for 12 days, and then, for a further 12 days, the same diet with a known daily addition of B-vitamins. The case of "alcoholic" polyneuritis also received a special high vitamin diet with additional B-vitamins for a third period of 12 days.

2. The total urine excreted during these periods was concentrated and fed to rats deprived of vitamin B₁ or B₂ respectively. The vitamin B₁ activity was estimated by the power of the urines to cure neuritis, the vitamin B₂ activity by their power to promote growth.

3. This method of estimating vitamin B₁, which consisted of finding the proportion of a number of rats that was cured when single doses of the test material were fed, has not previously been used with rats as test animals. The method was standardised by observing the effects obtained with the international standard acid clay adsorbate of vitamin B₁.

4. Vitamin B₁ was not found in the urine of control subjects receiving the hospital diet, though it is probable that small amounts were present. The addition of 720 I.U. of vitamin B₁ daily to this diet was followed by the daily excretion of 167–333 I.U. in the urine, the remainder of the additional intake may be assumed to have been retained.

5. Vitamin B₂ was present in small quantities in the urine of control subjects receiving the hospital diet. Feeding additional vitamin B₂ increased the amount excreted in the urine and at the same time the retention appeared to have been increased.

6. In the one case of "alcoholic" polyneuritis studied, the initial excretion of vitamins B₁ and B₂ was comparable with that of the controls. When additional B-vitamins were fed, the retention of vitamin B₁ was slightly less than that observed in any of the controls, and the retention of vitamin B₂ slightly greater. The differences were, however, probably too small to be significant.

I am indebted to Dr G. R. Minot and Dr W. B. Castle for suggesting the subject of this work and for helpful criticism, and to Miss M. Evans for help with the experimental animals.

REFERENCES.

- Burn (1932). *Quart. J. Pharm. Pharmacol.* **5**, 533.
Chick and Roscoe (1928). *Biochem. J.* **22**, 790.
Cobb and Coggeshall (1934). *J. Amer. Med. Assoc.* **103**, 1608.
Coward, Burn, Ling and Morgan (1933). *Biochem. J.* **27**, 1719.
Cowgill (1932). *Amer. J. Physiol.* **101**, 115.
— Deuell and Smith (1925). *Amer. J. Physiol.* **73**, 106.
“Discussion on avitaminosis (B group).” (1934.) *Proc. Roy. Soc. Med.* **27**, 473.
Gaglio (1919). *Policlinico*, **26**, 1381.
Gigon and Odermatt (1925). *Z. exp. Med.* **47**, 294.
Gildea, Kattwinkel and Castle (1930). *New England J. Med.* **202**, 523.
Gulick and Daniels (1931). *Proc. Soc. Exp. Biol. Med.* **28**, 861.
Harris, Ray and Ward (1933). *Biochem. J.* **27**, 2011.
Helmer (1935). *Proc. Soc. Exp. Biol. Med.* **32**, 1187.
Itter, Orent and McCollum (1935). *J. Biol. Chem.* **108**, 571.
Johnson and Zilva (1934). *Biochem. J.* **28**, 1409.
Kinnorsley, Peters and Reader (1930). *Biochem. J.* **24**, 1820.
— O'Brien, Peters and Reader (1933). *Biochem. J.* **27**, 225.
Kuhn, György and Wagner-Jauregg (1933, 1). *Ber. deutsch. chem. Ges.* **66**, 317.
— — — (1933, 2). *Ber. deutsch. chem. Ges.* **66**, 576.
Minot, Strauss and Cobb (1933). *New England J. Med.* **208**, 1244.
Muckenfuss (1918). *J. Amer. Chem. Soc.* **40**, 1606.
Plimmer, Rosedale, Raymond and Lowndes (1927). *Biochem. J.* **21**, 1141.
Roscoe (1931). *Biochem. J.* **25**, 2050.
Strauss (1935). *Amer. J. Med. Sci.* **189**, 378.
Van de Walle (1922). *Biochem. J.* **16**, 713.
Vorhaus, Williams and Waterman (1935). *J. Amer. Med. Assoc.* **105**, 1580.