48. ASSESSMENT OF THE LEVEL OF NUTRITION. URINARY EXCRETION OF ANEURIN AT VARYING LEVELS OF INTAKE¹

BY Y. L. WANG AND JOHN YUDKIN

*From the Nutritional Laboratory, University of Cambridge
and Medical Research Council*

(Received 1 February 1940)

THE estimation of aneurin in the urine was suggested by Harris & Leong [1936] as a method of assessing the body "reserves" of the vitamin. Using the bradycardia method of Birch & Harris [1934], the urinary output was found to be dependent on the past intake, being low, for example, in beriberi and in conditioned deficiencies [Harris *et al.* 1938]. The procedure involved the determination of the "resting level" of excretion and the response to test doses. Since then, many investigators, using either the bradycardia method, or the thiochrome method of Jansen [1936], have measured the urinary excretion of aneurin and confirmed the fact that it is correlated with the state of nutrition of the body. Westenbrink & Goudsmit [1938] found low excretions in women during pregnancy. Schroeder [1939] investigated many patients suffering from a variety of diseases and in the majority found low excretions of aneurin. Wang & Harris [1939] also found low excretions in conditioned deficiencies attributed to anorexia or faulty absorption of the vitamin.

According to most observers, a diet adequate in aneurin is associated with a daily output of 100 μ g. or more of the vitamin. Wang & Harris [1939] found the daily excretion of most of the normal individuals whom they examined to lie between 150 and 240 μ g. with a maximum of 480 μ g. and a minimum of 90 μ g. The values obtained by Ritsert [1938] lay between 110 and 510 μ g., whilst Schroeder [1939] obtained values of 100–400 μ g. Westenbrink & Goudsmit [1938] are of the opinion that individuals excreting over 100 μ g. of aneurin are saturated with the vitamin. Wang & Harris [1939] suggested, as provisional standards, that excretions below about 90 μ g., combined with delayed response to test doses, could be regarded as evidence of a "sub-average", and probably a "sub-normal", level of nutrition.

For the purpose of assessing the level of nutrition in regard to aneurin, the object to be aimed at is to determine the presumed intake of the vitamin from measurements of the quantity excreted. For this to be possible, it is necessary, as pointed out by Wang & Harris [1939], to have "standard reference curves" relating the amount of aneurin excreted to the intake of the vitamin under standardized conditions, and also to know whether variations between individuals are small enough to allow of the use of such reference values.

The work so far reported on the relation between intake and output has been mainly in the nature of determining the excretions in persons on normal but unspecified diets with and without various supplements of aneurin. For example, the excretion has been determined after oral administration of 5 mg.

¹ Communicated to the Biochemical Society 9 December 1939 [Wang & Yudkin, 1939].

or more of the vitamin by Westenbrink & Goudsmit [1938] and by Seiclounoff [1939], whilst Schroeder [1939] estimated the urinary output after similar large doses injected intravenously. The results of Jolliffe *et al.* [1939], who measured the excretion on a well-controlled deficient diet before and after oral ingestion of aneurin, are discussed in more detail below.

The work to be described was undertaken in order to determine whether it was possible to obtain these "standard reference values" for the excretion of aneurin. We have measured the amounts excreted over a wide range of intake, ranging from definite deficiency (about 100 $\mu\text{g.}$ daily) to distinctly high levels (over 2 mg. daily). A special study was made of the level of excretion with an intake conforming to the reputed daily requirements (300 I.U. or 900 $\mu\text{g.}$). Lastly, the effects were studied of exercise and of variations in the proportions of fat and carbohydrate in the diet.

EXPERIMENTAL

The subjects of the experiments were four healthy men. Three of these—G. G. B., age 23, Y. L. W., age 31 and J. Y., age 29—were research workers and the fourth—S. A. C., age 24—a laboratory technician. Their weights were 58, 65, 67 and 72 kg. respectively. The intake of aneurin was controlled by taking a basal deficient diet and adding at intervals known supplements of pure aneurin hydrochloride ("Benerva" in ampoules).

The basal diet consisted of well-washed, autoclaved polished rice, corned beef, herrings (fresh cooked, or in its various cured forms), small amounts of white bread, butter, milk steamed for 2 hr. at pH 9, strawberries, raspberries and chocolate. Supplements of ascorbic acid and of capsules containing vitamins A and D were also taken. The caloric value of the diet was sufficient to meet the bodily requirements of each subject, since their weights did not alter appreciably throughout the period of the experiment. Although the diet was not weighed each day, the amounts of the various items taken daily by each individual were fairly constant and did not differ greatly for the four subjects. The approximate quantities of the items in the daily diet were as follows: rice 150 g., herrings 300 g., white bread 100 g., milk 400 ml., butter 20 g., sugar 50 g., chocolate 100 g., strawberries or raspberries 150 g.

The amount of aneurin in this diet is 100–150 $\mu\text{g.}$, calculated from the tables of Fixsen & Roscoe [1937–8] and also in most cases confirmed by us, using a modification of the method of Wang & Harris [1939]. No attempt has been made to allow for the vitamin-sparing action of the fat in this diet. It appears from experiments to be described below that the excretion of aneurin is little, if at all, affected by the fat content of the diet.

The daily output of aneurin was measured by the method of Wang & Harris [1939]. The estimations were carried out by each of us independently and almost invariably agreement was obtained within 10% for the higher values and 20% for the very low values.

(1) *Variation of excretion with intake*

Three subjects—S. A. C., Y. L. W. and J. Y.—took part in this experiment. Before the deficient diet was begun the "resting" excretion of each subject on his ordinary diet was determined for several days. The deficient diet without addition of aneurin was taken for 10–14 days and then supplements of 600, 1200 and (in 2 cases) 1800 $\mu\text{g.}$ were in turn taken daily for a period of 4–6 days each. The supplement was taken immediately before or after the main (midday) meal.

The effect of a still higher level of intake was studied on a normal diet with a supplement of 2 mg. daily. The daily excretions of aneurin in this experiment are shown in Fig. 1.

Fig. 1. Excretion of aneurin with varying levels of intake.

It will be seen that the excretion during the period of depletion fell for 5 or 6 days and then reached a value which remained fairly constant until the supplements of aneurin were begun. With each level of supplement, the amount excreted at first increased and then appeared to reach a steady value within 2 or 3 days. The figure also shows the rapidity with which the excretion fell when the intake of aneurin was suddenly decreased after a period of high intake. The supplement of 2 mg. daily on a normal diet was taken for about 7 weeks and then the deficient diet with no supplement was resumed. The excretion fell rapidly to a low level—reaching one-third or less of its previous value in 1 or 2 days—and then continued to fall slowly for the remaining 8 or 9 days of the experiment.

The relation between intake and excretion of aneurin under these conditions is shown in Fig. 2. The aneurin content of the basal diet is taken as 100 μg . The aneurin content of the normal diets was calculated roughly by taking the average excretion on this diet and interpolating on the curve. This gave values of approximately 1200 μg . for S. A. C., 800 μg . for J. Y. and 1600 μg . for

Fig. 2.

Y. L. W. The excretion at each level of intake is taken as the average excretion excluding the first day's value at each level. It will be observed that, with increasing quantities of aneurin ingested, the amount excreted at first increases very little. When the intake reaches some 1200 μg . there is a fairly sudden rise in the amount excreted with increasing intake. The interpretation of these results is discussed below.

(2) *Urinary excretion with intakes of aneurin conforming to reputed requirements*

The reputed daily human requirement of aneurin is 300 I.U. or 900 μg . [League of Nations Commission, 1938]. The excretion at this level of intake was studied with four subjects for a somewhat longer period than at any one level in the previous experiment. As a preliminary, the subjects were depleted for 7 days by taking the basal diet without supplement. The intake of 900 μg . was obtained by a supplement of 800 μg . of aneurin, the basal diet being presumed to contain 100 μg . (see above). By an oversight, however, only 600 μg . were taken for the first 2 days. The supplement of 800 μg . was taken for 7 days. The results of this experiment are shown in Fig. 3. The average daily excretions with the intake of 900 μg ., excluding the first day, ranged from 137 to 233 μg . (Table 1).

(3) *The effects of carbohydrate, fat and exercise on aneurin excretion*

The effects of an increase in the amounts of carbohydrate or fat in the diet and of moderate exercise were studied in the four subjects on the basal diet with 800 μg . supplement of aneurin (Fig. 3). Additional carbohydrate was taken as glucose, 100 g. being consumed for 1 day and 200 g. daily for a further 4 days. After an interim period of 3 days on the basal diet with 800 μg . of supplement, 200 g. of fat were added daily to the diet for a period of 4 days. This was taken in the form of butter, made into synthetic cream with a portion of the daily allowance of milk. After a further interim period of 1 day, the subjects were

exercised on a bicycle ergometer for 2 days. The exercise performed by each subject amounted to an equivalent of approximately 500 Cal. each day. The results (Table 1) indicate a slight decrease in excretion with the carbohydrate and with exercise and a less definite decrease with the high fat diet.

Fig. 3. Excretion of aneurin with intake corresponding to reputed requirement. Effects of carbohydrate, fat and exercise.

Table 1. *Average daily excretion of aneurin with reputed minimum intake (900 µg.)*

	Aneurin excreted per day, µg.				% decrease, average
	S. A. C.	J. Y.	Y. L. W.	G. G. B.	
Basal diet + 800 µg. aneurin	137	219	233	184	—
Basal diet + 800 µg. aneurin + 200 g. glucose daily	109	180	199	174	15
Basal diet + 800 µg. aneurin + 200 g. fat daily	124	198	211	167	10
Basal diet + 800 µg. aneurin + exercise	105	190	180	180	17

(4) *Clinical manifestations of aneurin deficiency*

Although the main object of this experiment was, as we have indicated, to determine the factors concerned in the excretion of the vitamin, a few observations of a clinical nature were carried out during the course of our investigations. They were made with the three subjects who took part in the first experiment, at the beginning and end of the first depletion period of 10–14 days.

We record these mainly because, while our work was nearing completion, a communication appeared in which it was reported that definite subjective and objective manifestations of aneurin deficiency were observed within a few days on a diet deficient in this vitamin [Jolliffe *et al.* 1939]. The subjective symptoms, some of which began to appear 4 days after the diet was started, were said to include anorexia, fatigue, lassitude, precordial pain, dyspnoea on exertion, palpitations, burning of the feet and muscle cramps. On the 5th day, objective signs, such as changes in the electrocardiogram, hyperaesthesia of the legs and tenderness of the calf muscles were observed. It was stated that these symptoms and signs rapidly resolved when large doses of aneurin were taken.

In our experiments, the majority of these manifestations were not observed. Constipation was present in three of the four subjects (excluding J. Y.). Lassitude, fatigue and anorexia were well defined in all subjects. However, these symptoms persisted throughout the periods in which the experimental diet was taken, even when supplements amounting to 1800 μ g. were being taken. The symptoms disappeared within 1 or 2 days when our normal diets, which certainly contained less than 1800 μ g. of aneurin, were resumed. It is clear therefore that the symptoms were not due to aneurin deficiency, although at present we are unable to account for their appearance. No other symptoms were observed. Objectively, in the few investigations conducted by us we did not encounter the changes described by the American workers. The exercise tolerance was found to be unaltered and the electrocardiogram (in the one case studied) was not affected. The upper limit of audible pitch, which Selfridge [1937] suggests is a sign of aneurin deficiency, was completely unaffected by the lack of the vitamin.

A consideration of the experimental method adopted by Jolliffe and his co-workers makes their results difficult to interpret. During a control period of 5–15 days prior to depletion, each of their subjects took daily supplements of approximately 2.5 mg. of aneurin. The appearance of symptoms of deficiency within so short a time after these large doses seems surprising. More important, however, is the fact that their deficient diet contains relatively large amounts of aneurin. According to these authors their diet contained 474 μ g. of aneurin calculated from the tables of Cowgill [1934], or 360 μ g. according to Williams & Spies [1938]. These figures seem low when it is observed that their diets included 100 g. beef, 250 g. beet or other vegetable and 200 g. of apple or banana. We have thought it of interest to recalculate the aneurin content of their diet from the tables of Fixsen & Roscoe [1937–8] and are led to assume an aneurin content of some 750–1000 μ g. If we accept the latter figures, it would appear that their diet contained approximately the quantity of aneurin accepted as the minimum requirements and probably little below the average normal intake of these subjects. Lastly, the daily excretions of aneurin as measured by Jolliffe and co-workers showed quite large fluctuations, amounting at times to several hundred %, although the diet was constant. The results obtained by us on a steady diet, excluding the first day or two after a change in aneurin intake, were relatively very constant.

DISCUSSION

Variation of excretion with intake

It has been pointed out that, with increasing intake of aneurin, the quantity excreted at first increases only slightly; the curve becomes steeper when the intake exceeds approximately 1200 μ g. Extrapolation of the curves to zero intake suggests that some 50–80 μ g. of aneurin would still be excreted daily on a

diet completely free from the vitamin. It is of course clear that these values are not equilibrium values; it is certain, for example, that on the deficient diet excretions which are only slightly less than the intake cannot be continued indefinitely. It is known too that a prolonged low intake or decreased absorption of the vitamin, as seen in beriberi or in conditioned deficiencies, is associated with an output approaching zero [e.g. Harris *et al.* 1938; Yudkin, 1938]. But the fact that the quantity of the vitamin excreted at these low intakes approaches a steady value, at any rate for several days, and that small increments in the intake do not produce great changes in the excretion, indicates that, at these low levels, the body is excreting part of its reserve of aneurin. The sharper rise in the excretion values when the intake exceeds some 1200 μg . suggests that above this level the intake is more than sufficient to make up for the daily loss from the body's reserves. The inflexion in the curve is more clearly seen if the excretion is plotted as a percentage of the intake (Fig. 4). In each case, a

Fig. 4. Excretion of aneurin as percentage of intake.

minimum percentage occurs with an intake between 900 and 1200 μg . Again, the larger percentage excretions below this level are to be explained by more of the excretion being at the expense of reserves as less is ingested. It should be emphasized again that the points on the curve do not represent equilibrium values of excretion.

If this interpretation of our results is correct, then the level of aneurin intake above which the body reserves are no longer being depleted in maintaining the excretion represents the minimal requirement of the body in respect of the vitamin. For the three subjects of this experiment then, the requirements lie between 900 and 1200 μg . or 300–400 i.u., which is in good agreement with the reputed requirement.

In view of the relatively few levels of intake which we have investigated and the difficulties inherent in prolonging or repeating an experiment of this nature with human subjects, we do not wish to attach undue significance to the foregoing suggestions in interpreting our results. At the present juncture we feel that they furnish the most reasonable explanation of the results and, if the interpretation we have put forward is confirmed, they offer an independent method of determining the body's requirements of the vitamin.

The animal experiments of Leong [1937] are of significance in this connexion. With rats fed at different levels of aneurin varying from deficiency to moderately high levels, Leong found that the excretions were related to the intake in a curve very similar to ours for human subjects. A sharp increase in the excretion of aneurin occurred with daily intakes exceeding some 20–25 μg . Estimation of

the vitamin in the tissues showed that intakes below this were associated with a steady depletion of the body stores, whilst on intakes above 20–25 μg . there was an increase in the stores. These results with animals therefore are in agreement with our suggestion that the point of inflexion in the curve relating intake and output of aneurin with human subjects is a measure of their requirements.

The actual values for the excretion of aneurin by the deficient subjects during the course of depletion, viz. 89, 75 and 66 μg . per day, agree well with the provisional conclusion given by Wang & Harris [1939], that a value of less than 30 I.U. (= 90 μg .) per day for the "resting level" represents certainly a "sub-average" and probably also a "sub-normal" state of nutrition.

The rapidity of adjustment of excretion to intake

We have seen that any changes due to intake are reflected fairly promptly in the excretion. Even after the daily ingestion of 2 mg. of the vitamin for several weeks, the cessation of this high intake led to some decrease in the quantity excreted within the first 24 hr. The actual level of excretion therefore depends to a great extent on the diet being consumed within the day or two preceding the test. This confirms the conclusion given in earlier papers from this laboratory. It also emphasizes once again the contention that any attempt to estimate the state of past nutrition in respect of aneurin solely by determinations of the current "resting" excretion and without the administration of test doses, is liable to lead to false conclusions.

Individual variation in aneurin excretion

For the three subjects of the first experiment, the quantities of the vitamin excreted at the same low levels of intake were very similar. This suggests that the rate of mobilization of the body's stores of aneurin was similar in these three individuals. With higher intakes the differences between the subjects became more noticeable. For example, with supplements of 800 or 1200 μg . on the deficient diet, or of 2 mg. on a normal diet, subject S. A. C. excreted only about half the quantities excreted by J. Y. On the deficient diet with a supplement of 800 μg ., G. G. B. showed an excretion about half-way between that of S. A. C. and those of Y. L. W. and J. Y.

If our suggestions regarding the determination of the body's requirements are correct, these differences in percentage excretion in the three subjects on the higher intakes cannot be due entirely to their different requirements, since, as we have seen, these requirements are all of the same order of magnitude. Possibly the different degrees of excretion represent differences in the rate of absorption, or the kidney threshold, or both. From a practical point of view, the differences in percentage excretion mean that, for subjects whose intake of aneurin is adequate, it is possible only to make an approximate estimate of the vitamin ingested from determinations of the quantity excreted. For example, an excretion of 145 μg . occurred with Y. L. W. on a diet containing 700 μg . of aneurin, whereas the same quantity was excreted by S. A. C. when his diet contained 1300 μg .—nearly twice the amount taken by Y. L. W. It is of course clear that these results with three, or on one level four, individuals cannot be taken as indicating the whole range of normal variation. They do however show that, for the higher levels of intake, this range is fairly wide. On the other hand, daily excretions of 100 μg . or under were in all three cases associated with low intakes—less than about 500 μg .

The assessment of aneurin deficiency

We have seen that a number of workers have attempted to assess the state of nutrition of an individual in respect of aneurin by estimation of the "resting level" of excretion of the vitamin on his ordinary diet. It is evident, however, from the above discussion that this gives only a rough guide. In the first place, owing to the variation existing amongst individuals, the excretion gives only an approximate indication of the intake. Secondly, and more important, the excretion varies so rapidly with the intake that it reflects to a large extent the diet during the day or two preceding the time of urinary excretion. It is clear that other data, such as may be obtained by the administration of "test doses" as suggested by Harris and co-workers [Harris & Leong, 1936; Harris *et al.* 1938; Wang & Harris, 1939], are essential for the detection of the presence and degree of deficiency. As pointed out by Wang & Harris [1939], the deficient case differs from the normal in showing one or more days' delay before the plateau of excretion is reached when the standard test dose is given.

Excretion of aneurin with an intake of the reputed requirement of the vitamin

The quantity of aneurin excreted daily with an intake of 900 μg . (that is, on a deficient diet with a supplement of 800 μg .) varied from 137 to 233 μg . This range is very similar to that of 150–240 μg . found by Wang & Harris [1939], within which lay the excretion values of the majority of normal individuals.

The effect of carbohydrate, fat and exercise

We have seen that quite large increases in the quantity of carbohydrate or of fat in the diet, or moderate increases in the amount of exercise performed, resulted in small decreases in the output of aneurin. The small magnitude of these changes, together with the fact that the observations were conducted only over short periods, do not allow any great significance to be placed on this decrease. However, since moderate deviation from this level of aneurin intake—900 μg .—causes a definite change in the quantity excreted within 1 or 2 days, it is legitimate to conclude that the effect of these decided changes in the diet, and of more moderate increase in exercise, affect very little, if at all, the quantity of aneurin excreted.

Schroeder [1939] claims that a consumption of 250 g. of glucose daily by two patients reduced their aneurin excretion to nil in a fortnight. A study of his results, however, shows that, in both patients, the aneurin excretion did not steadily decrease but fluctuated widely throughout the experiment. On the 10th or 11th day, for example, the excretions of each patient were actually greater than those on the 1st day of the experiment.

Fat has been shown to have an "aneurin-sparing" action in animals, which may be protected from signs of deficiency on a sub-minimal intake of the vitamin if the fat content of the diet is increased. Because of this, it might have been thought that the excretion of aneurin on a steady intake would be increased with a high fat diet. As we have seen, there was in our experiment no appreciable change in the excretion of the vitamin when an additional 200 g. of fat were taken daily. On the other hand, these findings were not unexpected in view of the results of the animal experiments of Kemmerer & Steenbock [1933]. These authors found that a great increase in the amount of fat in the diet did not influence the quantity of aneurin in the tissues of rats, chickens and pigs.

SUMMARY

Three volunteers were placed on a controlled diet deficient in vitamin B₁, graded supplements of the vitamin were administered and the excretion was measured by the method of Wang & Harris. The results may be summarized as follows:

(1) On the basal deficient diet the excretion fell rapidly to a steady value which was maintained for some time.

(2) A constant level of excretion was rapidly attained with each fresh level of intake.

(3) With increasing intake the excretion at first increased only slightly but, when the intake exceeded some 1200 $\mu\text{g.}$, the excretion increased more rapidly. With the reputed requirement of 900 $\mu\text{g.}$ the average excretion (for four subjects) was from 137 to 233 $\mu\text{g.}$

(4) It is suggested that the excretion at lower levels is at the expense of the body's reserves, and that the level at which the excretion begins rapidly to increase may give an indication of the requirements of the body.

(5) The rapidity with which the excretion adjusts itself to changes in the intake must be taken into account in assessing the "level of nutrition". Additional data, such as the results of test doses, are necessary in order to determine this level.

(6) The percentage excretion at different levels varied somewhat from subject to subject, owing possibly to differences in the rate of absorption or in kidney threshold.

(7) The effects on the excretion of exercise and of variation in the amount of carbohydrate and of fat in the diet have been studied.

We are glad to have this opportunity to express our gratitude to Dr Leslie J. Harris for his invaluable advice and criticism. Our thanks are also due to Mr S. A. Cresswell for his whole-hearted cooperation in these experiments.

REFERENCES

- Birch & Harris (1934). *Biochem. J.* **28**, 602.
 Cowgill (1934). *The Vitamin B Requirements of Man*. New Haven.
 Fixsen & Roscoe (1937-8). *Nutr. Abstr. Rev.* **7**, 823.
 Harris & Leong (1936). *Lancet*, **1**, 866.
 ——— & Ungley (1938). *Lancet*, **1**, 539.
 Jansen (1936). *Rec. Trav. chim. Pays-Bas*, **55**, 1046.
 Jolliffe, Goodhart, Gennis & Cline (1939). *Amer. J. med. Sci.* **198**, 198.
 Kemmerer & Steenbock (1933). *J. biol. Chem.* **103**, 353.
 League of Nations Commission (1938). *Bull. Hlth Org.* **7**, 474.
 Leong (1937). *Biochem. J.* **31**, 373.
 Ritser (1938). *Klin. Wschr.* **17**, 1397.
 Schroeder (1939). *Klin. Wschr.* **18**, 148.
 Sciclounoff (1939). *C.R. Soc. biol., Paris*, **131**, 53.
 Selfridge (1937). *Ann. Otol., etc., St. Louis*, **46**, 93.
 Wang & Harris (1939). *Biochem. J.* **33**, 1356.
 ——— & Yudkin (1939). *Chem. Ind.* **58**, 1088.
 Westenbrink & Goudsmit (1938). *Arch. néerl. Physiol.* **23**, 79.
 Williams & Spies (1938). *Vitamin B, and its Use in Medicine*. New York.
 Yudkin (1938). *Lancet*, **2**, 1347.