CCLXXXIV. THE ESTIMATION OF THE VITAMIN B₂ COMPLEX IN HUMAN URINE

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By estimating the amounts of vitamins C and B₁ excreted in the urine, it has been possible to obtain an estimate as to the nutritional condition of a human being with regard to these vitamins [Harris & Ray, 1935; Abbasy *et al.* 1935; Harris & Leong, 1936].

In recent years work has been done by several investigators, including Helmer [1935] and Roscoe [1936], to determine the amount of the vitamin B₂ complex secreted in human urine.

Helmer [1935] estimated the amount of vitamin B₂ in the urine of three normal persons and of one patient suffering from pellagra. He found appreciable amounts of the vitamin in the normal urines but none in the urine of the pellagra patient.

Roscoe [1936] determined the excretion of vitamin B₂ in the urine of three normal subjects and of one case of alcoholic polyneuritis. She found appreciable amounts of the vitamin in all the urines. The amount excreted by the patient with alcoholic polyneuritis was similar to that excreted by the normal subjects. On administration of larger doses of vitamin B₂ the retention of the vitamin by the patient was, however, slightly greater than that of the normal controls.

As in the cases of vitamins C and B₁, if it is presumed that the rate of excretion in the urine is dependent on the amount already stored in the body, then by comparing the quantities of vitamin B₂ excreted in the same period by different individuals a relative measure of their reserves of the vitamin should be obtained.

In patients with achlorhydria—a condition known to be not uncommon—a low urinary excretion of vitamins B₁ and C, indicating a low reserve has been observed (Private communication from Dr L. J. Harris). It was thought of interest to investigate whether in achlorhydric patients a corresponding deficiency of the vitamin B₂ complex existed.

EXPERIMENTAL

The urines of two adult patients at Addenbrooke's Hospital, Cambridge, were examined and compared with those of three normal adults on ordinary diets.

The first patient had achlorhydria and suffered from intermittent diarrhoea for several years. His symptoms had started while on active service in Mesopotamia more than 18 years ago. It was thought probable that the initial illness had been bacillary dysentery although his agglutination reactions were negative at the time of admission to the hospital. This patient had been found "below normal" in vitamin B₁ and low also in vitamin C.

The second was a case of idiopathic hypochromic anaemia with complete achlorhydria. Reserves of both vitamins B₁ and C had been found to be low.

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Measurements of vitamin B₂ were made by feeding rats kept on vitamin B₂-free diet with suitable daily doses of the urines and determining their weight gains over a period of 5 weeks.

Technique of assay

The method adopted was, with slight modifications, the same as described by Roscoe [1936].

Young rats about 3 weeks old and weighing 35–40 g. were kept in separate cages. The diet consisted of:

Sucrose	...	...	...	60 %
Casein	...	...	...	20 %
Arachis oil	...	...	...	15 %
Salt mixture	...	...	...	5 %
Cod liver oil (1 drop per rat daily)				

Care was taken to prevent coprophagy.

The rats were weighed twice weekly. One week after being placed in separate cages, they were given doses of a solution containing vitamin B₁ as a daily supplement. The vitamin B₁ solution was prepared by diluting the contents of a 1 ml. ampoule (containing 500 I.U. vitamin B₁) of "Benerva" (Hoffmann-La Roche) with water to 100 ml. 1 ml. of this aqueous solution, containing 5 I.U., was given to each rat daily.

Ten days after the first administration of the vitamin B₁, when the weights of the rats had remained constant on two consecutive biweekly weighings, doses of urine or autoclaved "marmite" were given to them daily.¹

The urine was collected for a period of 24 hr. in a large, dark-coloured bottle containing 150 ml. alcohol and 5 ml. hydrochloric acid. Three 24 hr. collections of urine were mixed together and concentrated by distillation *in vacuo* to a volume of 300 ml. Thus, a day's output was roughly represented by 100 ml. The concentrated urine was made up to pH 4.

The autoclaved "marmite" was prepared by heating a specimen of special "laboratory marmite" to 120° for 1 hr. at about pH 9 so as to destroy the heat-labile portion of the vitamin B complex. 1 ml. of the solution represented 0.5 g. of the original "marmite".

The urine was measured out with a pipette on to a small dish and mixed with sugar. The daily dose of vitamin B₁ was also added to the contents of the dish to make it more acceptable. The rats refused to take more than 1 ml. of the concentrated urine of the two patients, representing 1/100th of the day's output. With normal urine the rats could be persuaded to take 2 ml. or more of the concentrated urine. The "marmite" given to some of the rats was also measured out each day on small dishes and given separately.

In Table I are shown the total gains in weight in 5 weeks of rats fed on different samples of urine and on graded doses of autoclaved "marmite".

In Fig. 1 are represented the average weekly gains in weight of rats on: (a) normal urine, (b) urine from a patient suffering from achlorhydria, (c) autoclaved "marmite", (d) unsupplemented vitamin B₂-free diet.

¹ Experience gained in this work indicates that the experiments should be carried out with young rats and the feeding of the vitamin B₂-containing materials should be started shortly after the rats have ceased growing. With a batch of six rats which were on the vitamin B₂-free diet for 6 weeks after they ceased to grow, a much lower gain in weight was obtained with supplements of 0.4 ml. autoclaved "marmite" daily for 5 weeks, as compared with the weight gain observed with rats which had been only a little over 2 weeks on the deficient diet.

Table I. *Total gain in weight in g. of rats in 5 weeks*

Three days' output of urine concentrated to 300 ml., so that 1 ml. was equivalent to 1/100th of an average day's excretion

	0.5 ml. urine daily Av.	1 ml. urine daily Av.
Achlorhydric patients		
A	0 } 2 } 1 0 }	2 } 0 } 1 One rat died after 32 days
B	3 } 3 } 4 5 }	8 } 5 } 5 2 }
Normal subjects		
(1)	26 } 19 } 21 18 }	36 } 43 } 40 41 }
(2)	26 } 23 } 23 19 }	38 } 28 } 36 42 }
(3)	11 } 7 } 11 14 }	16 } 26 } 19 14 }
Controls	0.2 ml. daily Av.	0.4 ml. daily Av.
Autoclaved "marmite" solution (0.5 ml. "marmite" made up to 1 ml. solution)	10 } 15 } 11 9 }	40 } 40 } 40
		0.8 ml. daily Av.
		61 } 54 } 55 49 }

It will be seen that whereas there was a distinct gain in the weight of the rats fed on urines from normal individuals, there was no appreciable gain of weight in the rats fed on urines of the two patients with achlorhydria. A significant gain in weight was also observed with rats fed on autoclaved "marmite", even when the daily dose was only equivalent to 0.2 g.

The gains in weight were not strictly proportional to the quantities of the different samples which were administered. Also, with one of the three samples of urine from the normal individuals there was a definitely lower response in growth of the rats, as compared with the other two. This might, perhaps, have been partly due to the fact that the feeding of urine was commenced in this case when the rats had already been on the vitamin B₂-free diet for over 5 weeks instead of just over 2 weeks, as in the other experiments. But notwithstanding these minor discrepancies the growth responses of the rats with normal urine were, as a whole, in sharp contrast to those obtained with the urines of the two patients. This indicates that the urines of the two patients with achlorhydria contain far less vitamin B₂ than did those of the normal individuals.

Comparing the growth of the group of rats fed on the urines of the two patients with the growth of a batch of 12 control rats on vitamin B₂-free diet, it is apparent that the rats fed on the urines fared better. Out of the 12 control rats 3 were dead before the end of 5 weeks; and many of the others showed distinct loss of weight. Amongst rats fed on the urines of the two patients, there was only one death. The others appeared to be in better condition than the control rats at the end of the experiment. This indicates that although the two urines contained too little vitamin B₂ to help materially the growth of the rats, there was none the less a small amount of the vitamin complex (or perhaps one of the constituents of the complex) present.

Obviously, more cases will have to be investigated before it can be stated that the achlorhydric condition is causally related with the vitamin B₂ deficiency, but the experiments show that, at least in the two cases examined, a relative deficiency of vitamin B₂ complex existed, together with other vitamin deficiencies.

Fig. 1. ○=Daily doses of urine of patient A. □=Daily doses of urine of normal individual (1). ●=Daily doses of autoclaved "marmite". △=Negative controls on vitamin B₂-free diet.

It appears also that the rat-growth method of determining the vitamin B₂ content of urine is suitable for comparing different samples of urine, and for judging whether a particular specimen is rich or poor in the vitamin. The results gained, however, suggest that unless the method can be made much more refined it is not sensitive enough for ascertaining quantitatively the actual vitamin content of urine.

SUMMARY

The vitamin B₂ complex in the urines of three normal individuals and of two patients with achlorhydria has been estimated by the rat-growth method.

The growth responses resulting from the feeding of urine have been compared with the effects of graded doses of autoclaved "marmite".

With the same daily doses, a much larger growth response was obtained with the urines of the normal individuals than with the urines of the two patients.

It is concluded that the two achlorhydric patients were deficient in vitamin B₂ complex.

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