

immediate nor a late paralytic effect. When, however, 0.1 cc. of a combination made up of equal parts of a 2% suspension of virus and P. C. B. filtrate was introduced into the same area in 3 other animals, an immediate paresis or paralysis occurred in the leg of the homolateral side. There was no quiet period during the interval between the injection of the virus and the production of paralysis. The paralysis of the homolateral leg spread gradually and without a lag to the opposite leg, until it finally involved all the muscles usually affected in quadriplegia.

8023 P

Vitamin B₁ and B₂ Content of Human Urine.

O. M. HELMER. (Introduced by K. K. Chen.)

(With the assistance of Mary Rickards Richardson and Marian Wheeler).

From the Lilly Laboratory for Clinical Research, Indianapolis City Hospital, and the Department of Medicine, Indiana University School of Medicine.

With the hope of throwing some light on the fate of vitamins B₁ and B₂ in the body, preliminary experiments have been made to determine whether human urine contains demonstrable amounts of vitamins B₁ and B₂. As yet there are no satisfactory chemical methods available for these vitamins such as are now in use for determining vitamin C. Therefore biological assays for the vitamin B₁ and B₂ content of the urine were made by the rat feeding technique.

Since it was impossible to fractionate the urine in any way without loss of vitamin, the 24 hour urine samples were concentrated by vacuum distillation and dried *in vacuo* over sulphuric acid. The dried and powdered urine was thoroughly mixed with one part by weight of sucrose and one-half part by weight of Crisco, and fed to rats on a basal diet in quantities equivalent to 1/25 of the daily 24 hour urine output. In the test for vitamin B₁ the basal diet was supplemented with 500 mg. of autoclaved yeast. For the vitamin B₂ experiments the source of the vitamin B₁ was an extract of rice polishings made according to Rosedale.¹ The rats were fed the urine preparations after a depletion period in which the weight had become stationary for 3 weighing periods of 2 days each.

¹ Rosedale, J. L., *Biochem. J.*, 1927, **21**, 1266.

TABLE I.

Experimental Animal	Supplement	No. of Rats	Days Fed	Aver. Total Gain in Wt. gm.	Aver. Weekly Gain in Wt. gm.
+ control	500 mg. yeast	4	28	74.5	18.7
+ “	1 cc. B ₁ extract + 500 mg. auto- claved yeast	2	24	34.0	10.5
— “	500 mg. auto- claved yeast	8	43	7.4	1.2
— “	1 cc. B ₁ extract	4	49	1.0	0
B ₁ normal urine	500 mg. autoclaved yeast + urine	8	27	20.8	6.1
B ₂ “ “	1 cc. B ₁ extract + urine	4	28	28.2	7.1
B ₁ pellagra urine	500 mg. autoclaved yeast + urine	1	24	20.0*	5.8
B ₁ “ “	500 mg. autoclaved yeast + urine	1	24	—2.0	—1.0
B ₂ “ “	1 cc. B ₁ extract	1	20	1.0	0
B ₂ “ “	1 cc. B ₁ extract	1	24	9.0	2.6

*In dying condition in spite of gain.

The urine used for these experiments was collected from 3 normal subjects who ate a weighed amount of a well-balanced diet of 2750 calories per day and which, according to present knowledge, contained adequate amounts of vitamins B₁ and B₂. The urine from one patient with untreated pellagra was also collected.

The results (Table I) indicate that the amount of urine equivalent to 1/25 of the daily output of a normal subject contains demonstrable amounts of vitamins B₁ and B₂. These values are minimal values, for the data on rats which failed to eat all of the urine supplement were not excluded from the table. At the end of the experimental period the rats fed the normal urine were in good condition, whereas the negative controls and the rats fed urine from a patient with pellagra were dead or in a dying condition.

We feel these experiments demonstrate that it is possible to determine the vitamin B₁ and B₂ content of human urine by rat feeding experiments, thereby possibly providing a means of studying the physiology of these vitamins and a method of determining the dietary requirements of these vitamins for man under normal and pathological conditions. Balance studies in normal and diseased conditions are now in progress.