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Difficulties in the Use of Brachycardia Method of Assaying Vitamin B_1.

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(Introduced by F. F. Tisdall.)

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Using the brachycardia method as described by Harris and Leong,[1] we have attempted to assay vitamin B_1 hydrochloride (Merck),* and the international standard of vitamin B_1. Young piebald rats from our own rat colony, when 45 to 50 gm. in weight, were put on a vitamin B_1-free diet made up of crude casein 18%, McCollum's Salt Mixture (No. 51) 4.5%, sugar 61.5%, cod liver oil 1%, and autoclaved yeast 15%. The latter was strongly alkalinized, autoclaved at 15 lb. pressure in thin layers for 5 hours, and then neutralized and ground. The rats were all kept in wire cages, having meshes $\frac{5}{8}$ in. square, in order to prevent possible coprophagy. After about 12 days on the diet, the rats were weighed daily. When the body weights were constant, electrocardiograph records, using a standard Cambridge electrocardiograph, were taken each day. When the heart rate came down to 370 to 390 per minute, the rat was given a single dose of vitamin B_1. A solution of the vitamin B_1 hydrochloride was made up daily in distilled water, and the international standard was weighed dry. The dose was mixed with a little autoclaved yeast and then fed to the rat. At least 5 rats received each dose, and they usually ate it within one to $1\frac{1}{2}$ hours. If they took longer than 4 hours, the results were often unsatisfactory. Daily electrocardiograms were taken on each rat at about the same time each morning. The rats were held gently by hand while the records were being made and very rarely struggled or squealed during the process. Great care was taken not to hold the skin tightly, and there was no possibility of asphyxia complicating the results. At least 40 beats were counted to get the heart rate, and duplicate readings were frequently made on the tracings.

Even in one tracing, it was often found that the rate was at least 40 per minute faster in one part than another, and 2 tracings from one rat taken within half an hour sometimes showed differences of 100 beats a minute.

† Under the direction of Alan Brown, M.D., F.R.C.P.(C).

[1] Harris, L. J., and Leong, P. C., *Lancet*, 1936, **1**, 886; *Biochem. J.*, 1937, **31**, 672. See also Baker, A. Z., and Wright, M. D., *Biochem. J.*, 1935, **29**, 1803.

* We are indebted to Merck & Co. for supplies of this material.

We have not been able to get graded responses with graded doses. For example, when the following doses of vitamin B_1 hydrochloride were fed, the irregular results shown in Table I were obtained.

TABLE I.

Experiment	Dose	Interval before back to original rate, days
1	3γ	4
	6γ	2.6
	9γ	3.6
	12γ	3.2
2	3γ	2.6
	6γ	6
	9γ	4.5

Anomalous results such as the following were not infrequently encountered. When the rate was 389 per minute, the rat was fed 6 γ. On the next 5 days the rates were 480, 345, 389, 476, 326.

Several variations in the technique were tried. For instance, smaller rats were used which weighed about 30 gm. when they were put on the deficient diet. Also the vitamin B_1 was given when the heart rates were from 400 to 420 per minute. Also larger doses ranging from 12 γ to 30 γ, and from 2.5 to 7.5 I. U. were used. None of these alterations improved the results.

In all, we have studied the responses of 179 rats to single doses of vitamin B_1, and in our hands this method gave inconsistent results.

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Dietary Calcium and pH of the Lower Intestine.*

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Shortly after weaning, litters of young rats were divided into 5 groups and fed the 5 following diets for 7 to 11 weeks, when they were killed and the pH at various levels of their intestinal tract was determined, using the B.D.H. capillator. The capillator was checked

* An excerpt from a thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy in the University of Toronto.

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