# CCXI. THE VITAMIN $B_1$ CONTENT OF FOODS.

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It was shown by Drury *et al.* [1930] that on a diet deficient in vitamin $B_1$ young rats developed a progressive bradycardia which was cured by the administration of substances containing the vitamin but was unaffected by vitamins A, D, A and D combined and sources of vitamin $B_2$. They also showed that the cure occurred without increased food intake and was therefore not secondary to the restoration of appetite. The bradycardia was sinus in origin and was not cured by the injection of barium or by vagal section.

This specific sign of vitamin $B_1$ deficiency has been further used by Birch and Harris [1934] for the quantitative determination of the vitamin $B_1$ in foods. Results obtained by this method were in line with assays made using other methods, *i.e.* the cure of convulsions in rats and the growth rate.

The bradycardia method has been compared in this laboratory with the growth-rate method of vitamin $B_1$ assay, and the findings of Birch and Harris that the method is reliable for quantitative determinations have been confirmed. The activity of samples of various substances determined by both methods is seen in Table I.

Table I.

| Material | Units per g. (growth rate) | Units per g. (bradycardia) |
|---|---|---|
| Vitamin $B_1$ concentrate. 3310 | 97·0 | 115·0 |
| Wheat germ (proprietary) sample $M_1$ | 13·7 | 13·5 |
| Wheat germ (proprietary) sample $M_2$ | 14·0 | 13·75 |
| Ox liver | 1·4 | 1·5 |

Since there existed no comprehensive study of human foods in terms of the International Standard vitamin $B_1$, and in view of the need for this in studying human requirements, it was decided to use the bradycardia method for assaying common foodstuffs, and with a view to the practical applications, to make the determination where possible on the food in the form in which it is normally eaten. Foods of low vitamin content have, of necessity, been assayed raw only.

The tests have been carried out during several months, throughout which period small groups of rats have from time to time been given graded doses of the International Standard. This has not only enabled a simultaneous assay to be made, but has also permitted a large number of standard readings to be collected (Table II).

Between the dose levels of 20 and 30 mg., the method gives an accuracy within $\pm 20\%$. The probable error at the level of 30 mg. is $\pm 17\%$ when all values are considered, but is reduced to $\pm 14\%$ when the four extreme values (out of 28) are omitted. It has therefore been attempted to give doses calculated to produce a cure of about 3 days' duration but, owing to the small traces of the vitamin in many foods, this has not always been possible. Considerations

Table II. *Duration of cure obtained with International Standard.*

| | 10 mg. Hours | 15 mg. Days | 20 mg. Days | 30 mg. Days | | 40 mg. Days | 50 mg. Days |
|---|---|---|---|---|---|---|---|
| | 15 | 0·0 | 1·85 | 3·0 | 1·7 | 3·55 | 3·75 |
| | 27 | 2·55 | 2·25 | 2·95 | 3·6 | 2·8 | 4·1 |
| | 7 | 1·4 | 1·75 | 3·3 | 4·1 | 4·6 | 4·6 |
| | 24 | 0·75 | 1·25 | 3·1 | 5·0 | 3·3 | 3·9 |
| | 28 | 2·0 | 3·1 | 3·2 | 3·3 | 4·75 | 7·0 |
| | — | — | 2·7 | 1·8 | 3·2 | 2·75 | 5·9 |
| | — | — | 1·5 | 3·6 | 2·6 | 3·15 | 7·0 |
| | — | — | 2·2 | 4·0 | 1·7 | 5·0 | 4·0 |
| | — | — | — | 3·0 | 4·0 | 7·0 | — |
| | — | — | — | 3·0 | 1·8 | 3·0 | — |
| | — | — | — | 3·0 | 4·35 | 3·0 | — |
| | — | — | — | 2·0 | 2·5 | 5·8 | — |
| | — | — | — | 2·8 | 2·45 | — | — |
| | — | — | — | 2·8 | — | — | — |
| | — | — | — | 2·8 | — | — | — |
| Av. | 0·8 | 1·34 | 2·07 | 3·02 | | 4·1 | 5·0 |

of time have not so far permitted the use of several dose levels for each food but these are gradually being worked out, as also are the seasonal foods not included in Table IV.

PROCEDURE.

Rats of 130–140 g. wt. and of either sex are kept on a diet consisting of:

| | | |
|---|---|---|
| Castor sugar ... ... ... ... | 60 | 100 parts. |
| Arachis oil ... ... ... ... | 15 | |
| "Light white casein" ... ... ... | 20 | |
| McCollum's salt mixture ... ... | 5 | |

Autoclaved dried brewer's yeast 15 % of basal diet[1].
Cod-liver oil, one drop daily per rat.

The rats are housed 3 or 4 in a cage on screens, in rooms kept at 65° F. by a thermostatically controlled heating system. They are fed twice daily to minimise errors arising from wastage and subsequent lack of food. After about 4 weeks, the depleting rats are put into single cages ($8 \times 8 \times 10$ in.) with $\frac{1}{2}$ in. mesh floors, standing 2 in. above deep trays, and daily readings are taken on the Matthews electrocardiograph. Readings are usually begun when the body weight is about 110–125 g. By using depleted rats at this weight, each animal can be used for 8 or more tests before its usefulness is ended by convulsions, severe inanition or a premortal fall of temperature. Fig. 1 shows a typical series of responses for one animal, compared with progressive bradycardia of a negative control.

Readings, except in the case of low levels of standard, have been taken once in 24 hours, but a definite sequence has been followed and each animal examined at approximately the same hour each day. Those suitable for use have received a test dose as soon as their heart rate is known. In attempting to give doses

[1] Autoclaved for 6 hours at 120° in layers 1 in. deep.

During the early part of this work a number of rats developed ulcers and oedema of the feet and tail, loss of hair and staining of the abdominal fur. As a result of this the autoclaved yeast, which had been used as 10% of the diet, was increased to 15% and the subsequent disappearance of the lesions led to the conclusion that the diet as originally used had been inadequate in vitamin $B_2$. The alteration was without effect on the heart-rate records.

which would produce an approximate 3-day cure, the large amounts required in many cases have so far deterred us from testing at a second and higher level. Doses of 2 or 4 g. have been used for most foods, and occasionally 6 g. in two equal parts at 6–8 hours' interval. Bulky materials have been chopped or ground and observation made to prevent wastage of dose.

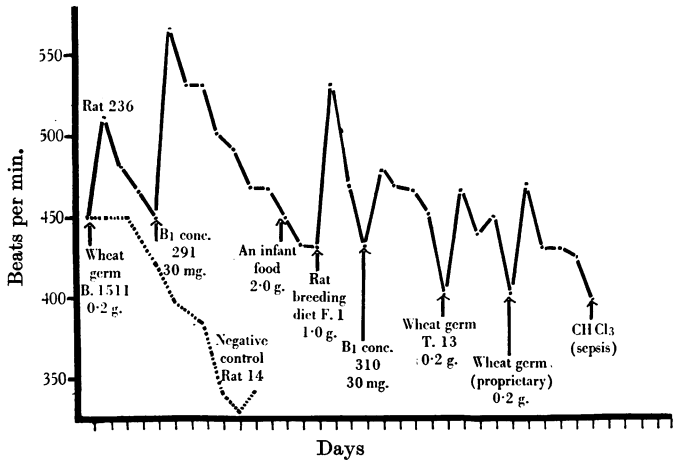


Fig. 1.

Most foods have been given when the heart-rate is between 350 and 450 per minute, and tests of each substance have been made at both the higher and lower rates. Between these limits duration of the response does not depend upon the initial heart rate (see Table III).

Table III. *Relation between initial heart rate and duration of response.*

| Material | Initial heart rate | Duration of response Days | Material | Initial heart rate | Duration of response Days |
|---|---|---|---|---|---|
| Egg yolk | 430 | 3·0 | Mushroom (*cont.*) | 390 | 2·5 |
| | 420 | 2·6 | | 374 | 1·2 |
| | 420 | 2·8 | | 354 | 2·1 |
| | 354 | 3·75 | Grape fruit | 450 | 1·5 |
| | 340 | 2·45 | | 420 | 2·3 |
| Mushroom | 462 | 2·1 | | 400 | 1·2 |
| | 450 | 2·1 | | 370 | 1·8 |

When rats with heart rates above 450 have been used, many of the responses have tended to be prolonged or irregular. This applies particularly to the first dose given after the depletion period and is shown in Fig. 2 which illustrates an abnormal initial response followed by eight normal responses.

After the first dose the rhythm of response appears to become established and further abnormalities are unusual until the final or penultimate test. Abnormal responses have occurred to 40% of the initial doses given to 130 rats and have led us to adopt the practice of giving, when the heart rate is between 440 and 460, a preliminary dose of 300 mg. wheat germ (proprietary), not to be considered for assay purposes, and beginning the routine tests when the heart rate is falling daily after the maximum response. Certain other atypical readings have been discarded. Most of these are from the last dose given, when

the rat has been well enough to take the dose, and has succumbed to, or been chloroformed for, rapidly developing inanition before the response was completed. Disregarding the initial steadying dose which is no longer given for purposes of assay, the discarded responses amount to 10·7 % of 1200.

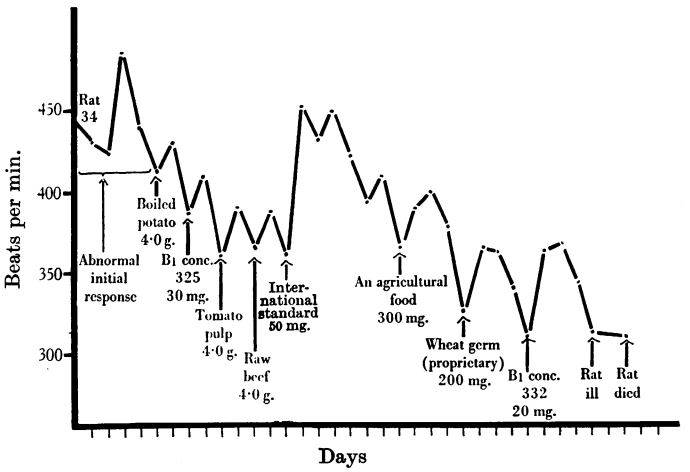


Fig. 2. Initial abnormal response followed by eight normal responses.

It will be seen in Table IV that the substances showing a high probable error have given in most cases one or more negative responses. Some of these may be due to the test substance not being homogeneous, as in sardine, but in other instances no such explanation suggests itself. A negative response has been included in the table only when examination of the previous and subsequent history of the animal concerned has shown it to give normal responses to other substances.

Table IV.

* indicates that the probable error for the dose used is over 50%.

| Material | Dose | Responses | Average duration in days | Units per g. |
|---|---|---|---|---|
| MEAT AND OFFALS: | | | | |
| Lean raw beef | 2 g. | 0, 1·8, 0, 0·8, 1·3 | 0·8 | 0·5* |
| Ox liver cooked | 2 | 2·3, 3·25, 3·3, 3·4 | 3·1 | 1·5 |
| Cooked veal (from pie) | 4 | 3·0, 1·9, 1·5, d., d. | 2·1 | 0·5 |
| Lean raw mutton | 4 | 3·5, 2·1, 1·7, 2·0, 3·0 | 2·46 | 0·6 |
| Roast lamb (lean) | 4 | 2·0, 1·35, 2·0, 2·25, 2·8 | 2·1 | 0·5 |
| Braised sheep's tongue | 4 | 0, 0, 0, 1·2, 1·2 | 0·5 | 0·2* |
| Raw sheep's kidney | 2 | 4·75, 3·0, 3·85, 4·0, 4·0 | 3·9 | 1·9 |
| Roast leg of chicken | 4 | 2·5, 0, 2·0, 0, 3·5 | 1·6 | 0·4 |
| Roast pork (lean) | 1·5 | 6·0, 4·0, 4·0, 6·0, 4·0 | 4·8 | 3·2 |
| Boiled ham (lean) | 2 | 4·8, 4·25, 4·6, 4·0 | 4·4 | 2·2 |
| Raw pig brain | 4 | 2·0, 3·0, 1·6, 2·5 | 2·3 | 0·6 |
| Raw pig kidney | 1 | 3·4, 5·8, 2·1, 3·0, 3·2 | 3·5 | 3·4 |
| FISH: | | | | |
| Raw cod muscle | 4 | 1·7, 2·5, 0, 3·0 | 1·8 | 0·4 |
| Raw whiting | 4 | 0, 3·0, 0, 1·0 | 1·0 | 0·3* |
| Soft herring roe | 2 | 1·9, 0, 3·0, 0 | 1·2 | 0·6* |
| Fried halibut | 4 | 1·9, 2·9, 1·9, 2·9 | 2·4 | 0·6 |
| Sardine (tinned) | 4 | 0, 1·9, 1·3, d., d. | 1·1 | 0·3* |
| Prawn (fresh boiled) | 4 | 2·2, 1·4, 0, 0, 0 | 0·7 | 0·3* |

Table IV (*cont.*).

| Material | Dose | Responses | Average duration in days | Units per g. |
|---|---|---|---|---|
| DAIRY PRODUCE: | | | | |
| Milk | 10 ml. | 1·45, 3·0, 1·2, 3·0, 2·6 | 2·25 | 0·23 |
| Cheese, Gorgonzola | 4 g. | 1·9, 1·5, 0, 3·2, 1·4 | 1·6 | 0·3 |
| ,, Cheddar | 4 | 0, 0, 0, d., d. | 0 | 0 |
| ,, Cheshire | 4 | 3·1, 0, 0, 2·5, 0 | 1·1 | 0·3* |
| Crustless cream Cheddar | 4 | 0, 0, 0, 0 | 0 | 0 |
| Egg yolk boiled | 2 | 3, 3·75, 2·6, 2·8, 2·45 | 2·9 | 1·4 |
| Egg white boiled | 4 | 1·3, 1·1, 0, 1·2, 2·1, 0, 1·4 | 1·0 | Trace* |
| VEGETABLES: | | | | |
| Beans, dry haricot | 2 | 2·8, 4·0, 0, 2·5, 3·0 | 2·5 | 1·2* |
| ,, dry butter | 2 | 2·0, 3·0, 2·95, 5·0 | 3·2 | 1·6 |
| Baked beans (canned) | 2 | 0, 0, 0, 0, 1·7 | 0·3 | Trace* |
| Beetroot, boiled | 4 | 4·0, 4·0, 1, 2·0, 3·0 | 2·8 | 0·7 |
| Savoy raw, etiolated | 4 | 1·8, 2·4, 1·2, 4·0 | 2·35 | 0·6 |
| ,, green | 4 | 3·8, 1·6, 5·0, 2·55 | 3·24 | 0·8 |
| Sprouts, raw | 4 | 2·8, 1·8, 2·0, 1·3, 4·0 | 2·4 | 0·6 |
| Carrot, raw | 4 | 3·3, 1·7, 3·9, 2·2, 1·5 | 2·5 | 0·6 |
| Celery, raw | 4 | 0, 2·3, 0, 0 | 0·6 | Trace |
| Cress, raw | 4 | 2·9, 1·3, 0, 4·45 | 2·2 | 0·5* |
| Cauliflower, raw | 4 | 6·0, 5·0, 4·7, 2·0 | 4·4 | 1·1 |
| ,, cooked | 4 | 0, 1·1, 3·8, 0 | 1·2 | 0·3* |
| Cucumber | 4 | 1·5, 0, 1·2, 1·8, 0, 0·9 | 0·9 | 0·3* |
| Lentils, raw | 2 | 4·0, 2·2, 4·1, 4·9, 6·0 | 4·2 | 2·1 |
| Lettuce | 4 | 3·4, 4·6, 2·7, 3·1, 3·9 | 3·5 | 0·9 |
| Mushroom, raw | 4 | 2·1, 2·1, 2·1, 2·5, 1·2 | 2·0 | 0·5 |
| Onion, raw | 4 | 2·2, 0, 4·3, 0, 2·15 | 1·7 | 0·4* (badly eaten) |
| Peas, canned | 1 | 1·3, 1·6, 2·45, 0, 0 | 1·1 | 1·2* |
| Potato, raw | 4 | 1·9, 3·0, 1·15, 0, 1·1 | 1·4 | 0·4* |
| ,, boiled and peeled | 4 | 0, 1·3, 1·6, 1·9 | 1·2 | 0·3 |
| Radish, raw | 4 | 1·7, 3·5, 1·6, 3·4, 2·8 | 2·6 | 0·6 |
| Rhubarb, raw | 4 | 0, 0, 0, 0 | 0 | 0 |
| Spinach, raw | 4 | 4·0, 1·8, 1·8, 4·0 | 2·9 | 0·7 |
| Turnip, raw | 4 | 0, 2·65, 0, 3·6, 2·0 | 1·7 | 0·4* |
| ,, cooked | 6 | d., 1·4, 0, 0, 0 | 0·2 | Trace |
| Watercress, raw | 4 | 2·3, 1·9, 2·4, 2·6 | 2·3 | 0·6 |
| FRUIT: | | | | |
| Apple | 4 | 1·0, 0, 3·4, 2·5, 2·9, 1·9, 0 | 1·7 | 0·4* |
| Banana | 4 | 2·0, 2·45, 1·6, 2·3, 3·3, 0 | 1·9 | 0·5 |
| Date, flesh | 4 | 0, 3·7, 0, 0, 2·1 | 1·2 | 0·3* |
| Fig, dried | 4 | 5·5, d., 4·0, 3·0 | 4·2 | 1·0 |
| Grape, green, pulp | 4 | 0, 0, 2·0, 0, 1·5 | 0·7 | Trace |
| Grape-fruit, pulp | 4 | 1·2, 1·5, 2·3, 1·8 | 1·7 | 0·4 |
| Orange, pulp | 4 | 0, 4·0, 0, 1·7, 2·3 | 1·6 | 0·4* |
| Pear | 4 | 2·55, 0, 0, 2·4 | 1·2 | 0·3* |
| Plum | 4 | 1·9, 0, 1·6, 2·0, 1·3 | 1·4 | 0·4 |
| Prune, dried, raw | 4 | 2·8, 3·25, 3·0, 4·4, 5·0 | 3·7 | 0·9 |
| Raisin | 4 | 2·1, 2·35, 3·0, 5·0, 2·55 | 3·0 | 0·75 |
| Tangerine, pulp | 4 | 3·4, 0, 1·7, 1·3, 1·9, 2·0 | 1·7 | 0·4 |
| Tomato, pulp | 4 | 2·1, 1·7, 2·0, 1·35 | 1·8 | 0·4 |
| NUTS: | | | | |
| Almond | 2 | 3·0, 0, 2·8, 0, 2·7 | 1·7 | 0·8* |
| Chestnut | 2 | 2·0, 2·3, 3·0, 2·4, 0 | 1·9 | 0·9 |
| Coconut, desiccated | 2 | 0, 0, 0, 1·5 | 0·4 | Trace |
| Hazel | 2 | 4·2, 4·0, 3·7, 3·5, 4·5 | 4·0 | 2·0 |
| Walnut | 2 | 2·5, 2·8, 3·2, 3·0, 4·0 | 3·1 | 1·5 |
| CEREALS AND CEREAL PRODUCTS: | | | | |
| Whole wheat A | 1 | 2·1, 3·0, 3·0, 2·0, 1·6 | 2·3 | 2·3 |
| ,, B | 1 | 1·4, 2·4, 2·8, 3·3, 3·3 | 2·6 | 2·6 |
| ,, C | 1 | 2·8, 1·8, 4·0, 5·0 | 3·4 | 3·4 |

Table IV (*cont.*).

| Material | Dose | Responses | Average duration in days | Units per g. |
|---|---|---|---|---|
| CEREALS AND CEREAL PRODUCTS (*cont.*): | | | | |
| Crude wheat germ A | 0·4 g. | 2·3, 3·0, 3·0, 1·8, 1·7 | 2·36 | 5·9 |
| ,, B | 0·2 | 2·35, 1·85, 1·6, 2·5, 2·5 | 2·2 | 10·5 |
| ,, C | 0·2 | 1·4, 1·7, 1·6, 2·2 | 1·7 | 8·5 |
| ,, D | 0·2 | 2·4, 2·2, 2·8, 3·8, 1·2 | 2·5 | 12·0 |
| ,, E | 0·2 | 4·3, 5·0, 5·0, 1·8, 3·0 | 3·8 | 18·75 |
| Wheat germ (proprietary) | | | | |
| Sample A | 0·2 | 2·0, 4·0, 3·0, 2·5, 3·0 | 2·9 | 14·25 |
| ,, B | 0·2 | 3·8, 2·8, 2·8, 2·7 | 3·0 | 15·0 |
| ,, C | 0·2 | 2·8, 3·5, 3·0, 2·6 | 3·0 | 15·0 |
| ,, D | 0·2 | 3·0, 3·5, 3·0, 2·55 | 3·0 | 15·0 |
| Wheat bran | 1 | 1·8, 2·8, 0, 0 | 1·2 | 1·3* |
| Oatmeal (dry) | 1 | 2·0, 3·0, 3·8, 3·8, 4·0 | 3·3 | 3·25 |
| Breakfast oatmeal, dry | 1 | 2·3, 3·0, 0, 0, d. | 1·3 | 1·4* |
| Rye germ | 0·4 | 1·3, 2·5, 3·0, 3·0, 5·0 | 3·0 | 7·5 |
| Barley germ | 0·2 | 2·45, 2·0, 2·0, 3·0, 4·8 | 2·9 | 14·0 |
| Maize germ | 0·5 | 1·4, 0, 4·4, 3·8, 2·2 | 2·4 | 4·6* |
| Rice bran A | 0·4 | 3·3, 3·0, 3·16, 3·0 | 3·1 | 7·6 |
| ,, B | 0·4 | 2·0, 3·0, 2·6, 1·7 | 2·3 | 5·6 |
| CONCENTRATES OF VITAMIN $B_1$: | | | | |
| Liquid concentrate IV | 100 mg. | 2·4, 1·5, 2·9, 2·4 | 2·3 | 22·5 |
| ,, VI | 50 | 2·0, 3·0, 3·0, 3·0, 1·9 | 2·6 | 51 |
| Solid concentrate 3321 | 20 | 2·75, 3·3, 3·0, 5·0, 4·8, 5·8 | 4·11 | 200 |
| ,, 344 | 21 | 3·9, 3·0, 2·75, 3·1 | 3·19 | 158 |
| ,, 31 | 30 | 3·9, 3·1, 2·9, 3·8, 2·9 | 3·3 | 107 |
| UNCLASSIFIED: | | | | |
| Coffee bean, ground | 1·0 g. | 2·0, 0, 0, 1·5, 3·0 | 1·3 | 1·4* |
| Cocoa powder | 0·5 | 0, 0, 0, 0 | 0 | 0 |
| Dried brewer's yeast A | 0·2 | 5·35, 3·2, 4·3, 5·4, 4·8 | 4·6 | 23·0 |
| ,, B | 0·4 | 2·0, 3·0, 2·4, d. | 2·5 | 6·0 |

With regard to substances of high vitamin $B_1$ activity, such as yeast and wheat germ, the variation from sample to sample is considerable. Thus with brewer's yeast some samples have been found four times as active as others. Quinn *et al.* [1930] record a 10-fold variation in yeasts. On several hundred samples of wheat germ drawn from a large number of sources, and examined here, a 5-fold variation has been found. Comparable variations probably occur in other cereal germs, of which we have been able to examine only a small number.

## SUMMARY.

1. The reliability of the cure of bradycardia as a means of estimating the vitamin $B_1$ content of various substances has been confirmed.

2. This method has been used for the estimation, in International Units, of the vitamin $B_1$ content of a number of foods.

We wish to express our thanks to Dr Leslie Harris and his department at the Dunn Nutritional Laboratories for valuable instruction in technique, to Prof. J. C. Drummond for helpful criticism, and to the Directors of Vitamins, Limited, for the facilities they have given to make this work possible.

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