The availability of bound nicotinic acid to the rat

4.* The effect of treating wheat, rice and barley brans and a purified preparation of bound nicotinic acid with sodium hydroxide

By D. K. CHAUDHURI† and E. KODICEK

Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council

(*Received* 20 *June* 1959—*Revised* 29 *September* 1959)

It has been demonstrated that almost all the nicotinic acid in maize is in bound form unavailable to rat, pig, dog, poultry and, possibly, man (see Kodicek, 1960; Kodicek, Braude, Kon & Mitchell, 1959). Other cereal products, such as wheat, rice and barley brans, also have their nicotinic acid in bound form, which rats are unable to utilize (Chaudhuri & Kodicek, 1950*a*). The bound forms in various cereals are similar in their lack of biological activity and it is at present assumed that they all are of similar, if not identical, composition. From wheat and rice brans, purified preparations of bound nicotinic acid, at various concentrations, have been made by several workers (Krehl & Strong, 1944; Chaudhuri & Kodicek, 1950*b*; Chaudhuri, 1954; Guha & Das, 1957; Das & Guha, 1957).

We report in the present paper further investigations, extending a previous brief communication (Chaudhuri & Kodicek, 1950*a*) on the biological activity of bound nicotinic acid present in wheat, rice and barley brans, and also of a purified preparation from wheat bran.

EXPERIMENTAL

Analytical methods

The determinations of free, bound and total nicotinic acid and tryptophan were made by techniques previously described (Kodicek & Wilson, 1959).

Feeding trials with rats

The management and housing of animals were the same as in previous experiments (Kodicek & Wilson, 1959), and the basal diet (Table 1) was that used by Harris & Kodicek (1950). Two trials were carried out.

Trial 1. One hundred and sixty weanling male rats (50–60 g) were allotted to twenty-one groups and were given the basal diet for a preliminary period of 17 days to render them deficient in nicotinic acid. They were then put on the experiment proper, lasting 28 days, during which they were given daily supplements of the materials described below in the amounts shown in Table 3. This trial consisted of three experiments, in which some of the treatment groups were repeated, so that the number of animals in

* Paper no. 3: *Brit. J. Nutr.* (1960), **14**, 25.

† Present address: Government Quinine Factory, Darjeeling, West Bengal, India.

the groups ranged from 4 to 14. The results were pooled and the standard errors of means were based on pooled within-group estimates of variance.

Trial 2. In this experiment, thirty-two weanling male rats were used (Table 4). The design of experiment was the same as in trial 1, except that rats in group 25 were given in the experiment proper a diet in which the untreated maize meal was replaced by 40% maize meal treated with N-NaOH, as described below. The food intake of individual rats was measured daily.

Table 1. *Percentage composition of the basal diet*

Maize meal, yellow	40
Casein, 'vitamin-free' (Genatosan Ltd)	3·5
Sucrose	51·4
L-Cystine	0·1
Minerals*	3·0
Cottonseed oil	2·0
Vitamins*†	+

* As previously given (Kodicek & Carpenter, 1950).
† Without nicotinic acid.

Materials

Purified preparation of bound nicotinic acid. It was made from wheat bran by the technique previously reported (Chaudhuri & Kodicek, 1950*b*) and contained 10 μg bound nicotinic acid/mg and only 4 μg tryptophan/mg. The aqueous solution (10 mg/ml) was given daily by mouth to rats in groups 10–12 in amounts of 0·25 or 0·5 ml. The rats in group 12 also received 0·75 mg DL-tryptophan/day.

Purified preparation of bound nicotinic acid, treated with N-NaOH. The preparation of bound nicotinic acid (100 mg) was dissolved in 4·5 ml distilled water and 0·5 ml 10 N-NaOH was added. The solution was left standing at room temperature for 15 min, brought to pH 6·8 with conc. HCl and the volume then adjusted to 10 ml with distilled water. After centrifuging, the clear supernatant liquid was given by mouth daily to rats of groups 13 and 14, in amounts of 0·25 and 0·5 ml daily, respectively.

Wheat bran, treated with N-NaOH (for trial 1). A commercial sample of wheat bran, 100 g, was treated with 1 l. N-NaOH for 1 h at 100°, the mixture was cooled, brought to pH 6·5 with conc. HCl, evaporated to dryness on a water-bath and ground for feeding to rats in group 17. The amounts given (400 mg/day) were equivalent to not more than 250 mg untreated wheat bran, because of the high salt content of the material. The untreated wheat bran was given to rats in groups 15 and 16, in daily amounts of 250 and 500 mg, respectively.

Wheat bran, treated with 0·5 N-NaOH (for trial 2). The same commercial sample of wheat bran, 100 g, was treated with 500 ml of 0·5 N-NaOH for 30 min at 100°, brought to pH 6·8 with conc. HCl, dried at 70° and ground for feeding to rats in group 27, at a level of 290 mg/day. This amount was equivalent to 252 mg untreated wheat bran. The amount of untreated wheat bran given to control rats (group 26) was 250 mg/day.

Rice bran, treated with N-NaOH. Rice bran (100 g), obtained from Dr T. Moran,

Research Association of British Flour-Millers, was treated with N-NaOH in the same manner as the wheat bran. The dried ground material was given to rats in group 19, in daily amounts of 240 mg, equivalent to 151 mg of untreated rice bran, which was given to control rats (group 18) in daily amounts of 170 mg.

Barley bran, treated with N-NaOH. Barley bran (100 g), obtained from Dr T. Moran, was treated in the same way as wheat bran. The dried, ground material was given to rats in group 21, in daily amounts of 430 mg, equivalent to 270 mg of untreated barley bran, which was given to control rats (group 20) in daily amounts of 250 mg.

Maize meal, treated with N-NaOH. One kg of yellow maize meal, a commercial sample used for the preparation of the basal diet, was treated with 5 l. N-NaOH for 30 min at 100°. The mixture was then cooled, brought to pH 6·8, dried at 70° and ground for feeding to rats. It contained 0·3 parts salt in 1·3 parts treated maize meal. During the experimental period proper this material replaced the untreated maize meal for rats in group 25.

Nicotinamide for dosing. Nicotinamide, 10 mg dissolved in 100 ml distilled water, was given daily by mouth to control rats in groups 2–5, 23 and 24, in the amounts shown in Tables 3 and 4.

DL-Tryptophan for dosing. DL-Tryptophan, 400 mg dissolved in 100 ml distilled water, was given daily by mouth to rats in groups 6–9 and 12, in the amounts shown in Table 3.

RESULTS

Nicotinic acid and tryptophan in dietary constituents

The contents of free and bound nicotinic acid and of tryptophan in dietary constituents are given in Table 2. The untreated samples of the maize meal, the preparation of bound nicotinic acid and the wheat, rice and barley brans contained all or almost all

Table 2. *Amounts of free and bound nicotinic acid and of tryptophan in diet constituents*

Constituent*	Nicotinic acid† (μg/g) Free	Nicotinic acid† (μg/g) Bound	Tryptophan (mg/g)
Maize meal, yellow	0·3	18·9	0·7
Maize meal, treated with N-NaOH	15·1	0	0·5
Preparation of bound nicotinic acid	0	10000	4·0
Preparation of bound nicotinic acid, treated with N-NaOH	10000	0	4·0
Wheat bran	0	203	2·9
Wheat bran, treated with N-NaOH (for trial 1)	136	0	1·9
Wheat bran, treated with 0·5 N-NaOH (for trial 2)	177	0	2·5
Rice bran	0	253	1·8
Rice bran, treated with N-NaOH	210	0	1·2
Barley bran	0	211	1·9
Barley bran, treated with N-NaOH	125	0	1·2
Casein	3·5	0	12·0

* The pH of the samples treated with NaOH was adjusted to 6·5–6·8 with conc. HCl; they contained, therefore, NaCl at various concentrations (see p. 36).

† The content of free nicotinic acid was estimated by paper chromatography (Kodicek & Wilson, 1959) and the content of bound nicotinic acid was calculated by difference from the content of total nicotinic acid, estimated both chemically and microbiologically.

the nicotinic acid in bound form. Paper chromatography and microbiological assay each showed that nicotinic acid was released from its bound form after treatment with alkali.

Feeding trials with rats

Trial 1 (*Table* 3). The mean growth curves of the rats are shown in Fig. 1 and the mean gains in weight of rats in the various groups are listed in Table 3. It can be seen that the growth response corresponded to the graded doses of nicotinamide or tryptophan given to rats in the control groups 1–9. Addition of the purified preparation in amounts of 2·5 or 5 mg (containing 25 or 50 μg bound nicotinic acid) had no effect on

Table 3. *Trial* 1, *experiment proper. Mean values with their standard errors for weight gains of rats given various supplements after a preliminary deficiency period*

Group no.	No. of rats*	Daily supplement	Nicotinic acid or amide from supplement (μg/day) Free	Bound	Trypto-phan from supple-ment (mg/day)	Gain in weight (g/week)
1	13 (4)	None	0	0	0	−1·4±0·8
2	13	Nicotinamide, 25 μg	25	0	0	2·2±0·8
3	13	Nicotinamide, 50 μg	50	0	0	8·8±0·8
4	5	Nicotinamide, 75 μg	75	0	0	9·7±1·5
5	4	Nicotinamide, 100 μg	100	0	0	13·7±1·3
6	4 (1)	DL-Tryptophan, 0·5 mg	0	0	0·5	−2·5±1·3
7	4	DL-Tryptophan, 1 mg	0	0	1·0	0·6±1·3
8	4	DL-Tryptophan, 2 mg	0	0	2·0	7·2±1·3
9	4	DL-Tryptophan, 4 mg	0	0	4·0	17·4±1·3
10	9 (2)	BNA, 2·5 mg	0	25	0·01	−1·6±1·0
11	14 (4)	BNA, 5 mg	0	50	0·02	−1·7±0·8
12	4	BNA, 5 mg + DL-tryptophan, 0·75 mg	0	50	0·77	2·6±1·3
13	9 (1)	NaOH-treated BNA, 2·5 mg	25	0	0·01	2·1±1·0
14	9	NaOH-treated BNA, 5 mg	50	0	0·02	9·5±1·0
15	14	Wheat bran, 250 mg	0	51	0·73	1·6±0·8
16	5	Wheat bran, 500 mg	0	102	1·46	7·4±1·5
17	8	NaOH-treated wheat bran, 400 mg†	54	0	0·75	10·9±1·0
18	8	Rice bran, 170 mg	0	43	0·31	0·3±1·0
19	8	NaOH-treated rice bran, 240 mg†	50	0	0·29	8·9±1·0
20	4 (2)	Barley bran, 250 mg	0	53	0·48	−1·4±1·3
21	4	NaOH-treated barley bran, 430 mg†	54	0	0·52	9·8±1·3

BNA, purified preparation of bound nicotinic acid.
* Figures in parentheses are the numbers that died.
† Treated with N-NaOH (see p. 36). Contains 0·59 parts salt in 1·59 parts.

the deficiency (groups 10 and 11). Only when the preparation was hydrolysed with N-NaOH was it fully active in accordance with its nicotinic-acid content (groups 13 and 14). Similar results were obtained with untreated and NaOH-treated rice and barley brans (groups 18–21). When untreated wheat bran was given as a supplement, the rats given the dose of 250 mg/day showed a slight, but statistically significant, improvement in growth (group 15) ($P \sim 0{\cdot}01$). This effect was most likely due to the relatively high tryptophan content of the cereal. The performance of these rats

compared well with that of rats given an equivalent amount of tryptophan, namely 0·75 mg/day, together with 5 mg untreated preparation of bound nicotinic acid (group 12). When the deficient rats were given daily 500 mg wheat bran, the amount of tryptophan in the supplement was sufficient to effect a cure (group 16). However, rats in group 17 given a daily supplement of wheat bran treated with NaOH, equivalent to 250 mg of the untreated sample, grew significantly better than rats in group 15 receiving the untreated supplement.

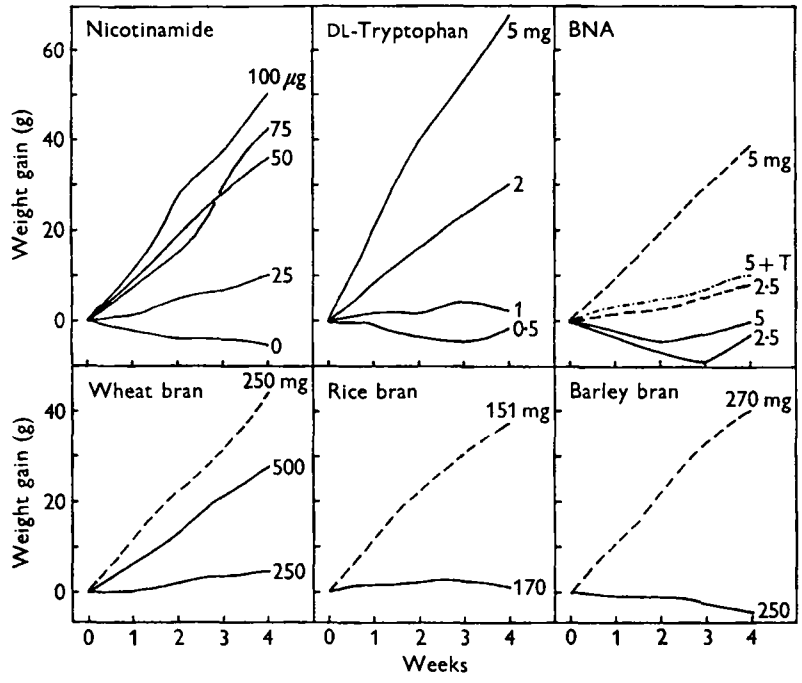

Fig. 1. Mean growth curves of rats given various dietary supplements in experiment proper of trial 1. BNA = purified preparation of bound nicotinic acid. ——, untreated supplements; —·—·—, 5 mg untreated BNA + 0·75 mg DL-tryptophan (5+T); — — —, alkali-treated supplements. The amounts of alkali-treated supplements are given in terms of original material/rat/day.

Trial 2 (*Table* 4). In this experiment, the food intake was measured, which permitted calculation of the gain in weight/100 g food eaten and of the total intake of nicotinic acid and of tryptophan. The performance of rats given wheat bran treated with NaOH (group 27) was similar to that of those in group 17 receiving a similar treatment. The gain in weight was significantly greater than that of rats given untreated wheat bran (group 26). It can be seen, in agreement with this finding, that the former rats had daily 54 μg of free nicotinic acid whereas the latter consumed only traces. Rats in group 25, given the diet with the maize meal treated with NaOH, grew significantly better than those in group 22, given the basal diet with untreated maize meal.

Table 4. *Trial 2, experiment proper. Mean values with their standard errors for weight gains and food intakes and mean values for intake of free, bound and total nicotinic acid and tryptophan of rats given various supplements after a preliminary deficiency period*

Group no.	No. of rats	Modification of the basal diet or supplement	Dose of nicotinamide (μg/day)	Gain in weight (g/week)	Food intake (g/day)	Gain in weight (g)/100 g food eaten	Intake of nicotinic acid or amide (μg/day) Free	Bound	Total	Intake of tryptophan (mg/day)
22	8†	None	—	−2·5 ± 1·0	6·3 ± 0·4	−6·4 ± 1·7	2	47	49	4·4
23	4	None	25	4·1 ± 1·4	9·0 ± 0·6	5·5 ± 2·4	27	68	95	6·3
24	8	None	50	7·0 ± 1·0	9·2 ± 0·4	10·6 ± 1·7	52	70	122	6·4
25	4	NaOH-treated maize meal, 40%‡	—	5·9 ± 1·4	11·1 ± 0·6	8·1 ± 2·4	68	0	68	4·3
26	4	+wheat bran, 250 mg/day	—	0·8 ± 1·4	6·9 ± 0·6§	1·6 ± 2·4	2	103	105	5·6
27	4	+NaOH-treated wheat bran, 290 mg/day‡	—	13·1 ± 1·4	11·3 ± 0·6§	17·0 ± 2·4	54	85	139	8·6
Significance of differences (t test). Estimates of P between groups			22 and 23	***	***	***				
			22 and 24	***	***	***				
			22 and 25	***	***	***				
			22 and 26	**	*	***				
			26 and 27	***	***	***				

• $P > 0{\cdot}05$; ** $0{\cdot}02 < P < 0{\cdot}05$; *** $P < 0{\cdot}02$; the standard errors of means were based on pooled within-group estimates of variance.

† Two rats died.

‡ NaOH-treated maize meal and NaOH-treated wheat bran contained 0·3 parts salt in 1·3 parts and 0·15 parts in 1·15 parts, respectively. The NaOH-treated maize replaced maize meal in the basal diet.

§ Does not include the dose of wheat bran.

DISCUSSION

Though untreated wheat, rice and barley brans failed to cure nicotinic acid-deficient rats, samples treated with alkali, by means of which the bound nicotinic acid was released, improved their growth. The bran supplements were such that the additional tryptophan derived from them was too small in amount to have an effect, except with the supplement of 250 mg wheat bran which supplied as much as 0·73 mg tryptophan/day. There was with it a slightly larger, statistically significant, gain in weight compared with that of deficient control rats not receiving such a supplement. The improved performance was similar to that shown by rats given a daily dose of 1 mg tryptophan or of 0·75 mg together with an untreated preparation of bound nicotinic acid. When the daily supplement of wheat bran was increased to 500 mg, the tryptophan content was sufficiently high (1·46 mg) to overcome in part the deficiency of nicotinic acid.

The results with the purified preparation of bound nicotinic acid clearly indicate that it had no biological activity unless the nicotinic acid had been released by treatment with alkali. The small amounts of the preparation used (2·5–5 mg) preclude the

possibility that substances other than nicotinic acid were responsible for the curative effect.

It can be concluded from these and previous findings (see Kodicek, 1960) that cereals, such as wheat, barley and rice, as well as maize and rye, have almost all their nicotinic acid in unavailable, bound form. It may be assumed, by analogy from the findings on pigs, dogs, poultry and rats, that man is also unable to utilize the bound form.

The calculated daily intake of 13·8 mg/head of nicotinic acid in Great Britain in 1957 (Ministry of Agriculture, Fisheries and Food: National Food Survey Committee, 1959) includes the bound nicotinic acid, present in cereals, which contributes about 17% of the total daily intake of nicotinic acid; this value excludes nicotinic acid added to enriched flour, which amounts to about 15% (D. F. Hollingsworth, private communication). Since potatoes may also have their nicotinic acid in the bound form (Krehl & Strong, 1944; Kodicek & Pepper, 1948), and since they contribute about 15% to the daily intake of nicotinic acid (Ministry of Agriculture, Fisheries and Food: National Food Survey Committee, 1959), the total consumption of the biologically active vitamin amounts to about 9 mg/day. This value agrees well with the calculated content of the quantities of yeast found by Goldberger & Tanner (1925) to be needed to cure pellagrins. However, cereals other than maize, would supply adequate tryptophan, which can be utilized for conversion into nicotinic acid.

SUMMARY

1. Chemical, microbiological and paper-chromatographic analyses indicated that the nicotinic acid in wheat, barley and rice brans was entirely in bound form.

2. A purified preparation from wheat bran, containing 10 mg bound nicotinic acid/g, was made; it did not contain any free nicotinic acid. Hydrolysis with N-NaOH for 15 min at room temperature completely liberated nicotinic acid from the bound form.

3. In two trials, 192 weanling male rats were given, for 17 days, a diet containing 40% maize meal and 3·5% casein, so that they developed a deficiency of nicotinic acid. After this period, fifty-one rats in nine groups were given, for 28 days, daily supplements of untreated or alkali-treated wheat, rice or barley brans, in order to ascertain their curative effects. Another five groups, comprising forty-five rats, were given supplements of the purified preparation of bound nicotinic acid, untreated or treated with alkali. A group of four rats was given a diet in which the original maize meal was replaced by an alkali-treated sample. The remainder of the rats served as deficient or positive controls.

4. All the brans in which the bound nicotinic acid had been released by treatment with alkali cured the deficiency, but the untreated samples failed to do so. Similarly, the alkali-treated purified preparation of bound nicotinic acid proved to be effective.

5. It is concluded that wheat, rice and barley brans contain their nicotinic acid in bound form, which, unless released by alkali treatment, is not available to the rat.

We thank Dr T. Moran, C.B.E., Research Association of British Flour-Millers, Cereals Research Station, St Albans, for the generous supply of barley and rice bran, and Miss D. F. Hollingsworth, O.B.E., Ministry of Agriculture, Fisheries and Food, for detailed information on the intake of nicotinic acid in Great Britain. We also thank Mr S. G. Impey and Mr P. W. Wilson for efficient technical assistance.

REFERENCES

Chaudhuri, D. K. (1954). *Sci. & Cult.* **19**, 409.
Chaudhuri, D. K. & Kodicek, E. (1950*a*). *Biochem. J.* **47**, xxxiv.
Chaudhuri, D. K. & Kodicek, E. (1950*b*). *Nature, Lond.*, **165**, 1022.
Das, M. L. & Guha, B. C. (1957). *Sci. & Cult.* **23**, 156.
Goldberger, J. & Tanner, W. F. (1925). *Publ. Hlth Rep., Wash.*, **40**, 54.
Guha, B. C. & Das, M. L. (1957). *Nature, Lond.*, **180**, 1285.
Harris, L. J. & Kodicek, E. (1950). *Brit. J. Nutr.* **4**, xiii.
Kodicek, E. (1960). *Brit. J. Nutr.* **14**, 13.
Kodicek, E., Braude, R., Kon, S. K. & Mitchell, K. G. (1959). *Brit. J. Nutr.* **13**, 363.
Kodicek, E. & Carpenter, K. J. (1950). *Blood*, **6**, 522.
Kodicek, E. & Pepper, C. R. (1948). *J. gen. Microbiol.* **2**, 306.
Kodicek, E. & Wilson, P. W. (1959). *Brit. J. Nutr.* **13**, 418.
Krehl, W. A. & Strong, F. M. (1944). *J. biol. Chem.* **156**, 1.
Ministry of Agriculture, Fisheries and Food: National Food Survey Committee (1959). *Domestic Food Consumption and Expenditure*, 1957. London: H.M. Stationery Office.

Printed in Great Britain