The availability of bound nicotinic acid to the rat

2.* The effect of treating maize and other materials with sodium hydroxide

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It has been shown that almost all the nicotinic acid in maize is present in a bound form unavailable to pig, rat, dog or chick and can be rendered available by hydrolysis either with sodium hydroxide (Chaudhuri & Kodicek, 1950*a*; Kodicek, 1951*a*; Kodicek, Braude, Kon & Mitchell, 1956; Harper, Punekar & Elvehjem, 1958; McDaniel & Hundley, 1958) or with lime-water (Laguna & Carpenter, 1951; Massieu, Cravioto, Cravioto, Guzmán & Suarez Soto, 1956; Pearson, Stempfel, Valenzuela, Utley & Darby, 1957; Squibb, Braham, Arroyave & Scrimshaw, 1959; Kodicek *et al.* 1959; Kodicek & Wilson, 1959). The effect of treating certain maize preparations with sodium hydroxide has been briefly reported by Kodicek (1951*a*); a further study covering a larger variety of maize fractions, including gluten feed, zein and maize starch has now been made. For comparison, the effect of hydrolyzing gelatin with alkali has also been studied.

EXPERIMENTAL

Analytical methods

The total nicotinic acid in dietary constituents was estimated chemically and microbiologically, and the free nicotinic acid by paper chromatography, as previously described (Kodicek & Wilson, 1959). The bound nicotinic acid was calculated by difference.

The crude protein of the materials was calculated from their nitrogen content, determined by the Kjeldahl method, the factor 6·25 being used. Tryptophan was estimated chemically by the method of Graham, Smith, Hier & Klein (1947). The pH of the maize preparations, as suspensions of 1 g material in 50 ml distilled water, was measured electrometrically, with a pH meter (Marconi and Co. Ltd).

Feeding trials with rats

General. Weanling male rats weighing between 50 and 60 g were used in three trials. Their management and housing were as described by Kodicek & Wilson (1959). The design of the experiments and the diets were of the type described by Harris & Kodicek (1950).

Trial 1. To render them deficient in nicotinic acid, 146 rats were allotted at random to nineteen groups (nos. 1–19) and given for 17 days (preliminary period) basal diet 1

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(Table 1) in which 40% of maize replaced the same quantity of sucrose. They then received for 28 days (experiment proper) one of the experimental diets, which had the general composition of basal diet 1 (3·5% casein) but in which part of the sucrose was replaced by certain preparations of maize meal, gluten feed or zein. Table 2 shows the various dietary modifications, which included addition to some of the diets of NaCl to balance the amount of salt present in samples treated with NaOH and the addition to others of certain amino acids to correct, as described by Kligler & Krehl (1950), the amino-acid deficiency in zein. Rats in group 11 were given daily in a palette dish 1 g gluten feed and those in group 12 were dosed with gluten feed treated with NaOH.

Table 1. *Percentage composition of basal diets*

Constituent	Basal diet 1	Basal diet 2
Casein, 'vitamin-free' (Genatosan Ltd)	3·5	10·0
Sucrose	91·4	84·9
Cottonseed oil	2·0	2·0
L-Cystine	0·1	0·1
Minerals*	3·0	3·0
Vitamins*†	+	+

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

Trial 2. Sixty-two animals were allotted at random to ten groups (nos. 20–29) and received the same treatment as those in trial 1 except that preparations of gluten feed and maize starch were used in the experimental diets as shown in Table 3.

Trial 3. Thirty-three weanling animals were allotted at random to nine groups (nos. 30–38) and were given, from the beginning of the experiment for 28 days, diets derived from basal diet 2 (Table 1) in which part of the sucrose was replaced by zein preparations or gelatin as shown in Table 4 and, as in trial 1, NaCl was added to some of the diets.

Materials

Maize meal, yellow. This meal was a commercial sample of whole-maize flour.

Gluten feed. This preparation was obtained from Corn Products Ltd, London. It was made from Russian yellow maize, and is the nearest product to maize bran that can be produced in the process of wet milling of the cereal. Its proximate percentage composition was, according to Mr A. L. Gaisford: moisture 9·3, crude protein 25·5, starch 24·4, oil 2·1, crude fibre 6·7, ash 3·5 and soluble carbohydrates (by difference) 28·5.

Gluten feed treated with 0·5 *N-NaOH.* This sample was prepared by heating 300 g gluten feed with 1 l. of 0·5 N-NaOH for 30 min at 100°. The mixture was then cooled and its pH (10·4) was adjusted with conc. HCl to the original acidity of gluten feed (pH 4·5). The material was dried at 70°, and ground.

Gluten feed treated with 0·14 *N-NaOH.* The preparation of this sample was similar to that of the previous one, except that more dilute NaOH (0·14 N) was used. The pH of the material after treatment was 6·2, and it was brought to 4·5 with conc. HCl before drying and grinding.

Table 2. *Trial* 1, *experiment proper. Mean values, with their standard errors, for gain in weight, food intake and intake of nicotinic acid and tryptophan of deficient and treated rats given the low-casein diet* 1 *during the experiment proper*

Group no.	No. of rats*	Modification of diet 1: Maize meal (%)	Modification of diet 1: Other maize preparations	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 and amide (μg/day): Free	Bound	Total	Intake of tryptophan (mg/day)
1	16 (3)	40	—	—	−2·2±0·5	6·3±0·2	−5·5	2	47	49	4·5
2	8	40	—	25	2·8±1·3	7·8±0·7	4·2	27	59	86	5·6
3	16	40	—	50	8·4±2·9	9·8±0·5	11·9	52	74	126	7·1
4	4	40	—	250	17·4±1·4	13·8±0·8	18·0	253	105	358	9·9
5	18 (5)	35	G.F. (10%)	—	2·0±0·4	6·2±0·2	−4·9	2	68	70	4·8
6	18	35	G.F. (10%)	50	11·9±0·2	10·1±0·3	16·7	53	112	165	7·9
7	4	35	G.F. (10%)	250	21·6±2·8	13·8±1·3	22·2	254	153	407	10·8
8	18	35	G.F. treated with 0·5 N-NaOH (11%)†	—	6·0±0·7	8·4±0·3	9·7	43	55	99	6·6
9	4 (1)	35	G.F. treated with 0·14 N-NaOH (10%)	—	−2·1±0·7	6·8±0·3	−4·5	2	73	75	5·3
10	4 (3)	35	G.F. enzyme-digested (10%)	—	−0·9±0·6	6·2±0·1	−2·1	2	70	72	4·8
11	4 (1)	25	G.F. (1 g/day)	—	0±1·8	8·0±0·4‡	−0·4	2	77	79	5·3
12	4	25	G.F. treated with 0·5 N-NaOH (1·2 g/day)†	—	6·1±0·6	10·3±0·7‡	8·3	47	42	89	6·6
13	4 (3)	35+A.A.§	Zein (Corn Products) (5%)	—	−3·0±0·8	5·8±0·4	−7·9	1	40	41	4·1
14	4	35+A.A.§	Zein (Corn Products) (5%)	50	12·4±1·1	10·5±0·6	16·9	52	72	124	7·5
15	4 (2)	35+A.A.§	Zein (Corn Products) treated with N-NaOH (5·7%)†	—	−1·7±1·0	6·2±0·3	−3·8	2	42	44	4·4
16	4	35	Zein (Corn Products) treated with N-NaOH (5·7%)†	—	−2·7±0·3	6·8±0·4	−5·7	2	46	48	4·8
17	4 (1)	35	Zein (Glaxo) (5%)	—	−2·3±0·3	6·1±0·1	−5·3	1	71	72	4·4
18	4	35	Zein (Glaxo) (5%)	50	11·1±0·4	10·7±0·4	15·0	52	124	176	7·7
19	4	35	Zein (Glaxo) treated with N-NaOH (5·7%)†	—	4·0±0·6	8·0±0·4	7·1	37	54	91	5·8

G.F., gluten feed; A.A., amino acids.

* Numbers in parentheses denote the number of rats that died.

† Contained NaCl from neutralization of NaOH with HCl; the same amount of NaCl was added to the diets of the control groups.

‡ Included the daily dose of gluten feed.

§ The following amino acids were added to the diet: DL-histidine 0·4, DL-valine 0·7, DL-threonine 0·5, L-arginine 0·2, L-lysine 1·0%.

Table 3. *Trial 2, experiment proper. Mean values, with their standard errors, for gain in weight, food intake and intake of nicotinic acid and tryptophan of deficient and treated rats given the low-casein diet 1 during the experiment proper*

Group no.	No. of rats*	Modification of diet 1: Gluten feed (%)	Modification of diet 1: Other maize preparations	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 and amide (μg/day): Free	Bound	Total	Intake of tryptophan (mg/day)
20	14 (1)	40	—	—	2·5 ± 1·7	8·3 ± 0·5	3·0	3	148	151	6·8
21	10	40	—	50	10·7 ± 0·4	10·7 ± 0·4	13·9	53	192	245	8·8
22	10	40	—	250	16·4 ± 5·6	11·2 ± 1·0	16·5	254	200	454	9·2
23	4	40, treated with 0·5 N-NaOH†	—	—	10·9 ± 1·5	12·1 ± 0·5	12·7	207	0	207	9·9
24	4	20	—	—	−2·4 ± 0·7	6·3 ± 0·2	−3·7	1	57	58	3·9
25	4	20	—	50	6·0 ± 0·7	8·6 ± 0·3	10·0	52	77	129	5·3
26	4	20, treated with 0·5 N-NaOH†	—	—	3·3 ± 1·5	9·0 ± 0·6	8·0	82	0	82	5·6
27	4 (2)	15	Maize starch (25 %)	—	−3·3 ± 0·7	7·2 ± 0·3	−6·4	1	49	50	—
28	4	15	Maize starch (25 %)	50	9·0 ± 0·7	10·3 ± 0·2	12·4	52	70	122	—
29	4 (1)	15	Maize starch, treated with 0·5 N-NaOH (29%)‡	—	−3·5 ± 0·9	6·5 ± 0·3	−7·9	2	43	45	—

* Numbers in parentheses denote the number of rats that died.
† Contained NaCl but no NaCl was added to the diets of the control groups.
‡ Contained 4·5 % NaCl; the same amount of NaCl was added to the diets of the control groups 27 and 28.

Table 4. *Trial 3. Mean values, with their standard errors, for gain in weight, food intake and intake of nicotinic acid and tryptophan by deficient and treated rats given the 10% casein diet 2 throughout*

Group no.	No. of rats	Modification of diet 2	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 and amide (μg/day)			Intake of tryptophan (mg/day)
							Free	Bound	Total	
30	3	Zein (Corn Products) (5%)	—	16·4 ± 5·6	11·9 ± 2·5	18·5	4	2	6	14·6
31	3	Zein (Corn Products) (5%)	50	25·6 ± 0·2	16·7 ± 2·0	22·4	56	3	59	20·5
32	3	Zein (Corn Products) treated with N-NaOH (5·7%)†	—	9·9 ± 5·8	9·9 ± 2·1	12·5	5	0	5	11·9
33	4*	Zein (Glaxo) (5%)	—	2·0 ± 1·8	7·9 ± 0·1	3·5	3	39	42	9·8
34	4	Zein (Glaxo) (5%)	50	25·2 ± 1·3	15·5 ± 0·8	23·4	55	77	132	19·2
35	4	Zein (Glaxo) treated with N-NaOH (5·7%)†	—	22·0 ± 0·2	16·3 ± 0·8	19·5	79	0	79	20·2
36	4	Gelatin (5%)	—	13·5 ± 5·3	13·3 ± 2·0	12·4	5	0	5	16·0
37	4	Gelatin (5%)	50	21·4 ± 1·0	15·8 ± 0·5	19·4	56	0	56	19·0
38	4	Gelatin, treated with N-NaOH (5·7%)†	—	10·0 ± 3·2	11·2 ± 2·0	11·8	4	0	4	13·4

* One rat died.
† Contained 0·7% NaCl; the same amount of NaCl was added to the diets of the control groups.

Gluten feed, enzyme-digested. This material was prepared by incubating, for 36 h at 37°, 300 g gluten feed suspended in 800 ml distilled water, with 2 g each of papain (British Drug Houses Ltd) and Takadiastase (Parke, Davis and Co. Ltd). The pH of the mixture was 4·5. The material was dried at 70°.

Maize starch. This commercial sample was of unknown origin.

Maize starch, treated with 0·5 N-NaOH. This material was prepared by treating 600 g maize starch with 3 l. of 0·5 N-NaOH for 60 min at 100°. The pH of the material was then adjusted with conc. HCl to 6·8 and it was dried at 70°.

Zein (Corn Products). This sample was obtained from Corn Products Ltd, London. It contained, according to Dr M. W. Rees, 16·2% nitrogen, 19% of which was amide nitrogen.

Zein (Corn Products) treated with N-NaOH. The zein, 150 g, was treated with 300 ml N-NaOH for 30 min at 100°. Its pH (13·0) was then adjusted to 6·5 with conc. HCl and it was dried at 70°.

Zein (Glaxo). This sample was prepared by Glaxo Laboratories Ltd, Greenford. It contained, according to Dr Rees, 15·9% nitrogen, of which 19·3% was amide nitrogen.

Zein (Glaxo) treated with N-NaOH. This material was prepared from the Glaxo zein, which was treated in the same way as the zein sample from Corn Products Ltd.

Gelatin. It was a commercial sample.

Gelatin treated with N-NaOH. Gelatin (100 g) was treated with 100 ml N-NaOH for 30 min at 100°. The material was then cooled and its pH brought to 6·8; it was dried at 70°, and ground.

RESULTS

Nutrients in dietary constituents

Values for free and bound nicotinic acid, tryptophan and crude protein in the various dietary constituents are given in Table 5. It can be seen that, in confirmation of previous results (Kodicek & Wilson, 1959), the untreated maize meal and gluten feed had very little free, available nicotinic acid; gluten feed had only about 1% of the total nicotinic acid in the free form. Treatment of gluten feed with 0·5 N-NaOH liberated all the bound nicotinic acid, but more dilute NaOH (0·14 N) failed to do so, since the pH of 6·2, obtained during treatment, would not favour the splitting of the bound form. The gluten feed digested with papain and Takadiastase had no liberated nicotinic acid.

Zein supplied by Corn Products Ltd had only traces of nicotinic acid, all in the bound form, which after treatment with N-NaOH was found to be free. The high content of nicotinic acid, all in the bound form, of the zein supplied by Glaxo Laboratories Ltd was entirely unexpected and is thought to have resulted from co-precipitation of bound nicotinic acid during preparation of the crude zein (see below). After hydrolysis with N-NaOH, this zein had a high content of free nicotinic acid, 72·9 μg/g.

I found that bound nicotinic acid in maize can be co-precipitated with zein. Gluten feed (200 g), containing 44·7 μg bound nicotinic acid/g, was refluxed for 2 h on

a boiling water-bath with 600 ml 80% ethanol and 0·5 ml conc. HCl; the mixture was then cooled and centrifuged. The residue was washed with 200 ml 80% ethanol acidified with conc. HCl. The combined supernatant liquids (385 ml) were analyzed chemically for nicotinic acid. They contained 3·74 mg nicotinic acid, a recovery of 42% of bound nicotinic acid. A portion of the supernatant liquids (150 ml) was reduced at 70° under reduced pressure to 10 ml, and 20 ml distilled water were added. The precipitate was centrifuged and dried at 70°; the yield was 7·6 g. The nicotinic acid, all in the bound form, in this crude zein amounted to 119·5 μg/g, a recovery of 61% of the bound nicotinic acid in the total supernatant liquid. In another experiment, 150 ml of the supernatant liquids were directly mixed with 650 ml distilled water, a precipitate of crude zein being thus formed. When dried, this precipitate (5·0 g) contained 58 μg bound nicotinic acid/g, a recovery of 20% of the bound nicotinic acid present in the combined supernatant liquids.

Table 5. *Content of free, bound and total nicotinic acid, tryptophan and crude protein of dietary constituents*

Constituent*	Nicotinic acid† (μg/g) Free	Bound	Total	Trypto-phan (mg/g)	Crude protein (mg/g)
Maize meal, yellow	0·3	18·9	19·2	0·75	85
Gluten feed	0·5	44·7	45·2	1·00	255
Gluten feed, treated with 0·5 N-NaOH	44·9	0	44·9	1·00	255
Gluten feed, treated with 0·14 N-NaOH	0·4	41·8	42·2	1·00	255
Gluten feed, enzyme-digested	0·5	46·6	47·1	1·00	255
Zein (Corn Products)	0	3·9	3·9	0·60	1000
Zein (Corn Products), treated with N-NaOH	3·4	0	3·4	0·60	1000
Zein (Glaxo)	0	99·5	99·5	0·70	1000
Zein (Glaxo), treated with N-NaOH	72·9	0	72·9	0·60	1000
Maize starch	0	0·3	0·3	—	71
Maize starch, treated with N-NaOH	0·2	0	0·2	—	71
Gelatin	0	0	0	0	842
Gelatin, treated with N-NaOH	0	0	0	0	842
Casein, 'vitamin-free' (Genatosan Ltd)	3·5	0	3·5	12·00	915

* The pH of the samples treated with NaOH was adjusted with conc. HCl; they contained, therefore, NaCl in varying concentration (see pp. 14 and 18).

† Values for total nicotinic acid are means of chemical and microbiological estimations. 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.

The precipitated material could be further purified by the procedure of Chaudhuri & Kodicek (1950*b*), used for the preparation of concentrates of bound nicotinic acid from wheat bran. It appeared therefore to contain bound nicotinic acid like that in wheat bran. It is evident that it is possible to prepare, under certain conditions, crude zein containing significant amounts of nicotinic acid, all in the bound form.

Maize starch contained only traces of nicotinic acid, too small for accurate determination of the amount of the bound form, but there appeared to be no free nicotinic acid. After treatment with N-NaOH all the nicotinic acid was found in the free form.

Gelatin did not contain any detectable amount of nicotinic acid.

Tests with rats

Trial 1 (*Table* 2). The average performance of the rats during the experiment proper was in keeping with that found previously (Kodicek & Wilson, 1959). Diets containing untreated maize meal alone (40%), or untreated maize meal (35%) with 10% gluten feed, did not support growth unless nicotinamide was supplied. Replacing gluten feed by a sample treated with 0·5 N-NaOH resulted in improved weight gain (group 8), significantly better ($P < 0·001$) than that of deficient control rats (group 5) and corresponding to the amount of free, available nicotinic acid consumed. A similar situation was observed in rats given daily doses of untreated or NaOH-treated gluten feed (groups 11 and 12). The rats in the former group did not thrive, but those in the latter showed a significantly improved weight gain ($0·01 < P < 0·02$) concurrently with the greater consumption of free nicotinic acid. On the other hand, diets containing gluten feed treated with 0·14 N-NaOH, or with enzymes, had no growth-promoting effect, in line with their low content of free nicotinic acid, despite the fact that relatively large amounts of total nicotinic acid were being consumed by the treated rats (groups 9 and 10).

Addition of 5% zein to an already pellagragenic diet (with 35% maize meal) resulted only in the usual continuation of the deficiency produced in the preliminary period. The deficiency was possibly more severe, since three rats out of four died during the experiment proper. Supplementation with nicotinamide corrected the deficiency (group 14). Treatment with N-NaOH of this zein, deficient in both nicotinic acid and tryptophan, did not result in any improvement of the deficient rats. Addition of five amino acids, reported to correct the imbalanced protein (Kligler & Krehl, 1950), had no effect on the results. A different response was elicited with the zein (Glaxo) that contained significant amounts of co-precipitated bound nicotinic acid. Whereas the deficient and positive controls responded in the same way (groups 17 and 18) as those given diets with the zein not containing nicotinic acid, the rats given this zein (Glaxo), treated with N-NaOH, grew significantly better than the negative controls ($P < 0·001$). The daily intake of free nicotinic acid by the former was 37 μg, compared with 2 μg consumed by the deficient rats in group 17.

Trial 2 (*Table* 3). The mean gain in weight of rats given a diet with 40% gluten feed (group 20) was only 2·5 g/week during the experiment proper. The positive controls (groups 21 and 22) gained weight according to their intake of free nicotinic acid. The rats given diets with alkali-treated gluten feed (group 23) recovered from the deficiency and grew at a significantly greater rate (10·9 g/week; $0·02 < P < 0·05$) than the deficient rats. A similar pattern of response was found in rats given diets with only 20% gluten feed (groups 24–26). Rats given the alkali-treated gluten feed (group 26) had a significantly better gain in weight than the deficient controls ($0·01 < P < 0·02$).

Treatment of maize starch with 0·5 N-NaOH had no effect on the pellagragenic activity of the maize diet; the rats in group 29 remained deficient and one died during the experiment proper.

Trial 3 (*Table* 4). In these experiments a 10% casein diet (basal diet 2) was used,

and zein or gelatin was added, at the 5% level, to induce a nicotinic-acid deficiency as described by Krehl, Henderson, de la Huerga & Elvehjem (1946). The addition of zein (Corn Products) to the diet led to a small retardation in the mean gain in weight (group 30), which was corrected by supplements of nicotinamide. Alkaline hydrolysis of this zein had no effect on the pellagragenic activity of the diet. The zein (Glaxo) that contained 99·5 μg bound nicotinic acid/g also retarded growth when given to rats in a diet containing 10% casein (group 33). Supplements of nicotinamide cured the deficiency. Rats in group 35, given the diet with alkali-treated zein (Glaxo), gained weight at almost the same rate as the positive controls. Gelatin, untreated or hydrolyzed with NaOH, retarded growth, and the low growth rate was corrected by supplements of nicotinamide.

DISCUSSION

In agreement with previous findings (see Kodicek & Wilson, 1959), maize and gluten feed had only traces of their nicotinic acid in free, available form. Treatment with NaOH, at a concentration sufficient to produce a distinctly alkaline medium, liberated the bound nicotinic acid entirely. Confirming the findings of Melnick (1942) and Coates, Ford, Harrison, Kon, Shepheard & Wilby (1952), I found that digestion with papain and Takadiastase did not release any bound nicotinic acid.

Depending on their content of free nicotinic acid, these foodstuffs were, without exception, able to cure or improve the nicotinic-acid deficiency of rats. Materials in which no bound nicotinic acid had been released, either because they were left untreated or were treated by ineffective procedures, or contained no nicotinic acid originally, such as gluten feed treated with enzymes or with 0·14 N-NaOH, alkali-treated maize starch, purified zein and gelatin, had no curative effect. This observation applies not only to dietary régimes in which a low-protein diet was given to the animals, but also to diets relatively high in protein (14%) in which an amino-acid imbalance was produced by the addition of an imbalanced protein, such as zein.

The experiments with zein, contaminated with, or free from, bound nicotinic acid, indicate that alkali treatment of this protein, by itself, is not sufficient to cure the deficiency and that the liberation of bound nicotinic acid is the important factor in the change of the pellagragenic foodstuff into a material curing pellagra. These findings do not support the view expressed by Bressani & Scrimshaw (1958) that correction of an imbalance of amino acids by alkali hydrolysis of zein is the explanation of the curative effect of maize treated with lime-water. In any event, this group of workers (Squibb *et al.* 1959) appears to have recently accepted the thesis put forward in this and previous papers that the lime-water treatment of maize increases the availability of nicotinic acid.

Squibb *et al.* (1959) suggest that, despite this release of bound nicotinic acid in *tortilla* (maize treated with lime-water), the effect of consumption of this maize product on the low incidence of pellagra in Central America is only a minor one and that the consumption of beans plays a more important part. It is, indeed, a fact that beans, as well as other legumes, have all their nicotinic acid in free, available form (Kodicek, 1951*b*; Kodicek *et al.* 1956). However, the consumption of maize products

in Mexico and other countries of Central America contributes as much as 80% of the calories of the rural diet (Anderson, Calvo, Robinson, Serrano & Payne, 1948). According to dietary surveys (Woodbury, 1942), the mean daily consumption in Mexico of maize products, treated with lime-water, amounts to about 600 g per adult male consumption unit and that of beans and peas to only 130 g; if the free nicotinic acid content of the Mexican *tortilla* is taken as 10 μg/g (Kodicek & Wilson, 1959) and that of beans as 24 μg/g (Bressani, Marcucci, Robles & Scrimshaw, 1954), the mean daily intake of available nicotinic acid from *tortilla* per male adult would be of the order of 6 mg and from legumes 3·1 mg. These calculated values agree well with those for Guatemalan Indians reported by Bressani *et al.* (1954), who estimate that 6·2 mg nicotinic acid are consumed daily in 500 g maize products and only 1·6 mg in 75 g beans; the tryptophan intake from these two foodstuffs would be 0·23 and 0·13 g, respectively. It appears thus that the contribution of *tortilla* cannot be considered a minor one and that the amount of free nicotinic acid derived from maize treated with lime-water would be sufficient to prevent or cure pellagra even without that obtained from legumes. It is indeed clear that legumes, as well as coffee and possibly native drinks (*pulque*; Krehl, 1949), may contribute in Central America to a satisfactory intake of nicotinic acid (Bressani *et al.* 1954; Squibb *et al.* 1959; Kodicek & Wilson, 1959). For instance, if one takes the mean daily consumption in Mexico of coffee beans as 16 g/adult male and of *pulque* as 0·2 l. (Woodbury, 1942), then the average daily consumption of nicotinic acid from them, all in available form (Kodicek & Wilson, 1959), would be about 2 and 0·8 mg, respectively.

Henderson, Deodhar, Krehl & Elvehjem (1947) reported that dextrinization of starch decreases the requirement of rats for nicotinic acid. The fact that alkali-treated maize starch failed, under our conditions, to alleviate nicotinic-acid deficiency indicates that mere dextrinization of starch, without the release of bound nicotinic acid, played no part in my experiments. This finding confirms those of Laguna & Carpenter (1951), who used maize starch treated with lime-water.

The design of some of the experiments, in which only part of the maize constituents was hydrolyzed with alkali, permits the conclusion that destruction by alkali of a possible toxic factor in maize is not involved in the curative effect, since a major portion of maize (see Table 3) was left untreated and should still have retained any toxic factor. This study, however, shows the importance of the imbalance of amino acids in causing a deficiency of nicotinic acid (see Harper, 1958). This deficiency can be cured or prevented by supplements of nicotinic acid or by foods in which the vitamin has been made available by alkaline hydrolysis or by those rich in tryptophan.

SUMMARY

1. Various maize preparations—maize meal, gluten feed, zein and maize starch—and gelatin were treated with NaOH. Gluten feed was also digested with papain and Takadiastase. Their contents of free and bound nicotinic acid and those of untreated samples were determined by chemical, microbiological and paper-chromatographic techniques.

2. Only those samples treated with alkali of sufficient strength to produce an alkaline medium had their bound nicotinic acid completely liberated. The same was obtained with a sample of crude zein that contained, as a result of co-precipitation, a relatively high amount of bound nicotinic acid. Gelatin and maize starch contained little or no nicotinic acid. Digestion of gluten feed with enzymes did not liberate any bound nicotinic acid.

3. In two trials, weanling male rats were given for 17 days a diet containing 40% maize, so that they developed a deficiency of nicotinic acid. After this period they were given for 28 days various low-protein diets containing untreated or alkali- or enzyme-treated food constituents. Only those materials in which the bound nicotinic acid had been released produced a curative response. After alkaline hydrolysis, crude zein contaminated with bound nicotinic acid cured the deficient rats, but similarly treated zein, containing no nicotinic acid, was ineffective. Maize starch, treated with alkali, but devoid of nicotinic acid, did not cure the deficiency, hence the biological activity of alkali-treated maize was not related to changes in the starch component of the cereal.

4. In a third trial, weanling male rats were given from the beginning a 10% casein diet to which either zein or gelatin, untreated or treated with alkali, had been added. The resulting deficiency of nicotinic acid was prevented only by diets containing a crude zein preparation contaminated with bound nicotinic acid that had been released by the alkaline treatment.

5. It is concluded that, in agreement with previous results, the beneficial effect of alkali-treated foodstuffs in curing or preventing nicotinic-acid deficiency in rats may be attributed solely to release of nicotinic acid from an unavailable bound form, and not to a correction or prevention of an amino-acid imbalance, destruction of a toxic factor or changes in the carbohydrate component of the treated samples.

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