Effect of Processing on Distribution and In Vitro Availability of Niacin of Corn (Zea mays) [a, b]

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SUMMARY

Niacin distribution was studied in one "starchy" and one "flint" sample of yellow corn. On the basis of 10% moisture, the seed coat averaged 3.2 mg, and the endosperm and the germ 2.0 mg per 100 g each. The endosperm contributed 80% of the total niacin, whereas the germ and the seed coat respectively supplied 11 and 9%. Of the niacin in the endosperm, germ, and seed coat, respectively 55, 73, and 42% were extractable with water at room temperature. During the lime cooking, the endosperm lost an average of 19% of its niacin, and the germ, 56%. The cooking liquor contained 0.43 mg per 100 g of niacin, equivalent to a total loss of around 22%. Of the niacin in raw corn, nixtamal (masa), and tortillas, respectively about 68, 76, and 73% were extractable with water at room temperature. Hydrolysis with pepsin yielded about 69% of the niacin of all samples. After trypsin hydrolysis, and final digestion with pancreatin, values of 78 and 100% of the niacin, respectively, were found compared with sulfuric acid hydrolysis of the same material. Differences in amino acid balance rather than in "bound" niacin thus appear responsible for the differences between raw and processed corn in biological activity and pellagragenic action.

ALTHOUGH PEOPLE who eat large amounts of corn *(Zea mays)* traditionally have an increased susceptibility to pellagra (Anon. 1954), this disease is not common in the rural populations of Latin American countries despite large consumption of this cereal (Bressani *et al.*, 1958). The relationship between corn diets and pellagra, as it formerly occurred in the United States or is found today in countries like Egypt, Yugoslavia, and Basutoland, is still not fully understood.

The alkaline treatment of corn has been reported to destroy its pellagragenic factor (Borrow *et al.*, 1948; Woolley, 1946). Subsequent evidence suggests (Elvehjem and Krehl, 1955; Koeppe and Henderson, 1955; Krehl *et al.*, 1944, 1945a, b, 1946a, b) that pellagra is due to an imbalance of the essential amino acids, increasing the niacin requirement of the animal. Lime treatment of the corn, as practiced in Mexico and Central America (Cravioto *et al.*, 1952), appears to correct this amino acid imbalance partly; at least, rats and other experimental animals grow better and are less subject to pellagra when fed lime-treated rather than raw corn (Cravioto *et al.*, 1952; Fiorentini *et al.*, 1956; Laguna and Carpenter, 1951; Massieu *et al.*, 1956; Squibb *et al.*, 1959).

Bressani and Scrimshaw (1958) demonstrated that lime treatment of corn significantly lowers the solubility of zein, so that, at least in the rat, digestive enzymes make it easier for the blood to absorb amino acids than when untreated corn is fed. No significant differences are found in the amino acid composition of raw and lime-treated corn after acid hydrolysis (Bressani and Scrimshaw, 1958).

Kodicek (1960) and Kodicek *et al.* (1956) found, from both swine growth studies and paper chromatography, that part of the niacin in corn occurs in a "bound" form. They conclude that the pellagragenic action of corn is due to the fact that this "bound" niacin is unavailable to the animal organism. More recently, Pearson *et al.* (1957) showed that simple boiling of corn has the same effect as lime treatment on "bound" niacin or amino acid availability.

The present study represents a further effort to determine the effect of processing on the distribution and availability of niacin in corn.

MATERIALS AND METHODS

One sample each of a yellow, starchy type of "highland corn" and a "lowland corn" with a yellow, flint-type kernel was subjected to the following treatments: 1) *cooking in lime water* by procedures described previously (Bressani and Scrimshaw, 1958; Bressani *et al.* 1958), 2) *germination* for four days at room temperature after sterilizing the raw kernels 2 min with 0.2% $HgCl_2$ solution and washing three times with distilled water, 3) *pressure cooking* of a mixture of 100 g of corn and 80 ml of water for 30 min at 15 lb pressure and 121.5°C, 4) *toasting* 20 min on an electric hot-plate, 5) *steaming* raw corn 10 min in an autoclave with no water added at 15 lb pressure and 121°C. Part of the material resulting from each of these treatments was dried and ground to pass 40 mesh.

The remaining kernels, in lots of 100, were manually separated into seed coat, endosperm, and germ fractions. If not already cooked, softening 30 min in water at room temperature facilitated separation. This dissected material was air-dried to constant weight, pooled, ground to 40 mesh, and stored at 4°C.

The niacin content of the raw corn, anatomical fractions and products of the different treatments, was estimated microbiologically in duplicate samples after hydrolysis for 30 min with 1*N* sulfuric acid in the autoclave at 15 lb pressure. The pH of the hydrolysate was adjusted to 6.8, and 5 aliquots of different volume were distributed in tubes replicating each aliquot three times. Inoculation was carried out with *Lactobacillus arabinosus 17-5*, using Difco media (Difco Laboratories, Detroit, Michigan).

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Table 1. Distribution of niacin in whole corn, and anatomical fractions of the grain before and after cooking (values adjusted to 10% moisture).

Sample	Before lime cooking: Weight (% of whole grain)	Before lime cooking: Niacin (mg/100 g)	Before lime cooking: Niacin (% of total in grain)	After lime cooking: Niacin (mg/100 g)	After lime cooking: Niacin (% of total in grain)	Losses: Niacin loss in fraction	Losses: Niacin loss or gain as % in whole grain
Highland corn							
Whole grain	100.0	2.21	100.0	1.66	100.0	24.9	—26.2
Endosperm	84.0	1.99	79.5	1.72	70.2	13.6	—11.7
Germ	11.0	2.01	10.5	0.84	4.5	58.2	— 6.4
Seed coat	5.0	4.22	10.0	......	......	100.0	......
Cooking water	......	......	......	0.52	25.3	......	+10.0
Lowland corn							
Whole grain	100.0	2.14	100.0	1.63	100.0	23.8	—25.6
Endosperm	82.0	1.93	81.2	1.47	66.5	23.8	—19.5
Germ	11.0	1.92	10.8	1.07	6.4	44.3	— 4.9
Seed coat	7.0	2.21	7.9	......	......	100.0	......
Cooking water	......	......	......	0.49	27.1	......	+10.8

The water-soluble niacin was estimated in water extracts of two or three 1-g samples. These extracts were prepared by continuous shaking for 30 min with 100 ml of distilled water at room temperature; the hydrolysis was omitted for the aqueous extract samples. The *in vitro* niacin hydrolysis studies were carried out with 5-g samples, using enzymatic digestion with pepsin, trypsin, and pancreatin for 24 or 72 hours, as previously described (Bressani and Scrimshaw, 1958).

RESULTS

Table 1 shows the niacin distribution within the kernel of each of the two corn samples, before and after lime treatment. The seed coat of the grains contained more niacin than did the endosperm or the germ. Because the endosperm is large it contained about 80% of the total niacin, whereas the germ contributed only 11%. Although the seed coat contained proportionately more niacin than the other two anatomical fractions, it represented only about 6% of its weight, and therefore contributed only about 9% of the total niacin content for the corn grain.

Table 1 also shows the effect of lime cooking on the niacin distribution in the component parts of the corn kernel. The endosperm of the highland corn lost 13.6% of its niacin, and the lowland corn sample lost 23.8%. These same samples showed a niacin decrease in the germ of 58.2% in the former and 44.3% in the latter. The cooking water of the highland corn had 25.3% of the original total niacin, the lowland sample, 27.1%. The amount of niacin in the cooking water resulted from individual niacin losses of: in highland corn, 11.7% from the endosperm, 6.4% from the germ, and 10.0% from the seed coat removed by the cooking liquor; and in lowland corn, 19.5% from the endosperm, 4.9% from the germ, and 10.8% from the seed coat.

Table 2. Distribution and water-extractable niacin in fractions and products obtained from corn (values adjusted to 10% moisture).

	Niacin concentration (mg/100 g)	Niacin in water extract (mg/100 g)	Niacin in residue (mg/100 g)	% niacin extracted in water
Highland corn				
Raw	2.23	1.54	0.70	68.7
Endosperm	1.99	1.04	0.98	51.5
Germ	2.01	1.60	0.60	72.8
Seed coat	4.22	1.66	2.61	38.8
Nixtamal	1.59	1.22	0.35	77.8
Tortilla	1.72	1.22	0.34	73.5
Cooking water	......	0.37	......	17.7
Lowland corn				
Raw	2.15	1.42	0.68	67.7
Endosperm	1.93	1.09	0.81	57.4
Germ	1.92	1.46	0.51	74.1
Seed coat	2.21	1.08	1.36	44.3
Nixtamal	1.62	1.18	0.41	74.3
Tortilla	1.64	1.16	0.43	73.0
Cooking water	......	0.35	......	16.9

Table 2 shows the quantity of niacin extracted with water from the corn samples. Results were similar with the two whole corns and their respective fractions. About 55% of the niacin from the endosperm and 73% from the germ were extractable with water at room temperature. An average of only 42% of the niacin content of the seed coat and 68% of the total niacin from the two whole ground grains was extracted. Water-extractable niacin averaged 68% from raw corn, and respectively 76 and 73% from nixtamal (masa) and tortilla.

The water-extractable niacin from the other corn preparations is shown in Table 3. In this experiment an average of 71% of the niacin was extracted from raw corn, 66% from toasted grain, 68% from the steamed corn, and 63% from boiled corn. Thus, none of the treatments applied to the raw corn had any significant effect on the proportion of water-extractable niacin.

Table 3. Water-extractable niacin from raw and processed corn (averages of four duplicate samples, values adjusted to 10% moisture).

Niacin	Corn preparation: Raw	Roasted	Steamed	Boiled
Highland corn				
Total (mg/100 g)	2.05	2.14	2.06	2.09
Water extract (mg/100 g)	1.50	1.42	1.41	1.32
Residue (mg/100 g)	0.55	0.72	0.65	0.77
Percent niacin in water-extract	73.2	66.4	68.4	63.2
Lowland corn				
Total (mg/100 g)	2.11	2.05	1.93	2.09
Water extract (mg/100 ml)	1.46	1.36	1.31	1.31
Residue (mg/100 g)	0.65	0.69	0.62	0.78
Percent niacin in water extract	69.2	66.4	68.0	62.7

The enzymatic release *in vitro* of niacin from corn and corn preparations, using 72-hour enzymatic hydrolysis, is shown in Table 4 for the lowland corn, and in Fig. 1 for the highland corn. Fig. 2 shows 24-hour hydrolysis results for the highland corn and its tortilla.

Pepsin hydrolysis yielded better than 69% of the original niacin, as shown in Table 4 and Fig. 1. This amount was probably not due to the action of the enzyme, however, since similar amounts of niacin were extractable with water. Enzymatic hydrolysis with trypsin, following the pepsin digestion, increased niacin availability to about 78% of the original value, whereas final digestion with pancreatin raised the amount of niacin to values approaching 100% of the niacin present in corn as determined by 1*N* sulfuric acid hydrolysis.

In all cases, lime-treated corn, raw corn, and the other corn preparations yielded niacin by enzymatic hydrolysis at the same rate. In the experiment shown in Fig. 2, the enzymatic hydrolysis lasted 24 hr. Tortillas had a lower initial niacin content but released more niacin than raw corn on a percent basis. Amounts of niacin expressed on an absolute basis were essentially the same for raw corn and tortillas after pancreatin

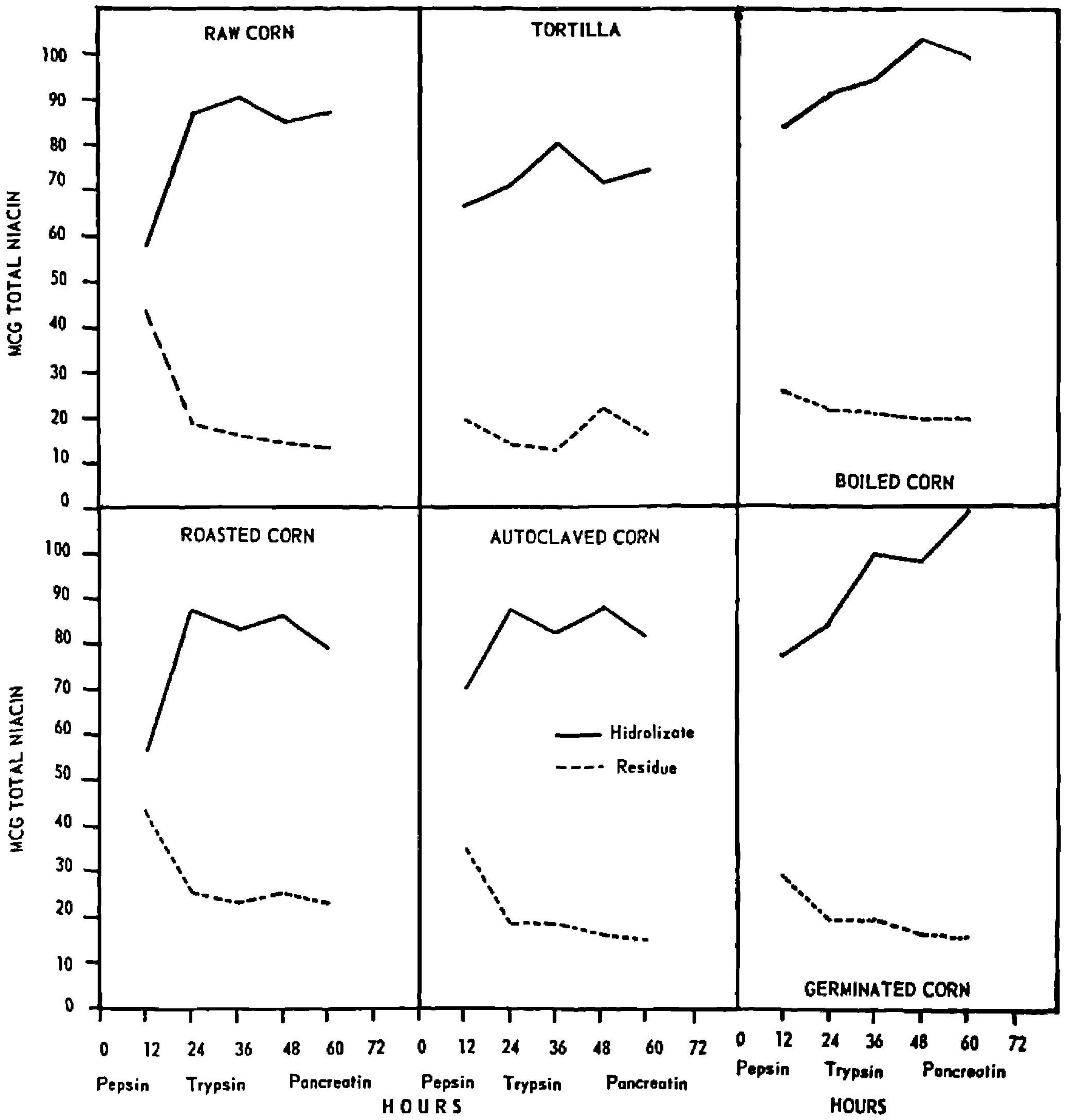

Fig. 1. Release of niacin from a 5-g sample of corn and various corn preparations by enzymatic hydrolysis (72-hour trial).

digestion. Residue analysis showed only small amounts of niacin, and the recoveries using the niacin values of the hydrolysate and the residue approximated 100%.

DISCUSSION

The niacin concentrations were similar in the germ and endosperm of the grain, and much higher in the seed coat. Nevertheless, the endosperm contributes the most niacin to the whole grain because it is the largest part of the kernel. This fact is important because, during tortilla preparation in Central America, the seed coat is discarded and its niacin lost (Bressani and Scrimshaw, 1958; Bressani *et al.*, 1958), whereas elsewhere in Latin America, the germ may also be removed (Jaffe *et al.*, 1950) in preparing corn for human consumption.

Part of the niacin in each of the three fractions of the uncooked but ground corn kernel is not extractable with water under the conditions used. The results show that on the basis of 10% moisture, only about 42% of the niacin in the seed coat and 54% of that in the endosperm is soluble in water, whereas the quantity extractable from the germ in this manner is approximately 73%. Sweeney (1951) and Sweeney and Parrish (1954) reported that most of the niacin in the seed coat of the corn grain is in the "bound" form, and that its full activity could be obtained by treating the sulfuric acid hydrolysate with sodium hydroxide at pH 12. The seed coat is of further interest since Borrow *et al.* (1948) suggested that a pellagragenic factor is present for the rat.

Table 4. In vitro release of niacin by four hydrolytic agents from the lowland corn preparations (averages of duplicate samples: values adjusted to 10% moisture).

	1*N* H_2SO_4 (reference)		Pepsin (12 hr)				Trypsin (36 hr)				Pancreatin (72 hr)			
	Hydrolysate	Residue	Hydrolysate		Residue		Hydrolysate		Residue		Hydrolysate		Residue	
Sample	μg/100 g	μg/100 g	μg/100 g	% ref.	μg/100 g	% rec.	μg/100 g	% ref.	μg/100 g	% rec.	μg/100 g	% ref.	μg/100 g	% rec.
Raw corn	103.5	0	62.0	69.5	24.0	93.2	79.0	76.4	19.5	95.2	85.3	82.4	17.5	99.3
Lime-treated corn	85.5	0	56.0	65.5	28.0	98.3	68.5	80.2	27.5	112.2	74.0	86.5	24.0	114.5
Roasted corn	105.5	0	73.0	69.2	28.5	96.2	85.0	80.5	27.0	106.0	95.0	90.0	24.0	112.7
Steamed corn	99.5	0	71.0	71.5	20.5	92.0	82.0	82.5	15.0	97.5	103.0	103.5	10.0	113.5

Bressani *et al.* (1958) and Bressani and Scrimshaw (1958) showed that, during tortilla preparation in Central America, about 17% of the niacin is lost in the cooking water when the raw corn is cooked and soaked in lime water before grinding. The largest part of this loss comes from the physical loss of the seed coat. The seed coat contributes about 9% of the niacin of the whole grain, or about 0.19 mg per 100 g, compared with a total loss of niacin of about 0.43 mg per 100 g. Since the water extractability of the niacin is not significantly greater in nixtamal (masa) and tortillas than in raw corn, the more favorable effects on growth attributed to lime-treated corn are apparently not due to a change in niacin solubility.

Furthermore, the processing treatments given the other corn preparations did not hydrolyze the niacin, since the quantity of the vitamin extractable with water from these materials was also comparable to that obtained from raw corn. The quantity of niacin extracted from these preparations was about two-thirds of the vitamin present in the grain, leaving one-third of the total niacin in the residue, a figure only slightly lower than the corresponding values for the nixtamal (masa) and the tortilla. Nearly 20% more water-extractable niacin was found in this study than reported by Pearson *et al.* (1957), probably because of minor differences in cooking procedure.

The *in vitro* enzymatic studies, carried out for both 60 and 72 hours, indicate that niacin is available *more rapidly* from tortillas and roasted, germinated, steamed, or autoclaved corn than from raw corn. Similar *total quantities* of niacin were available from the treated and raw corn samples. The amount of niacin found after the pepsin hydrolysis appears to represent no more than the amount of free niacin in the grain. The addition of the other proteolytic enzymes liberated the niacin, which was not extractable with water. More than 80% of the niacin in corn was obtained after 24 hours of enzymatic hydrolysis in all cases studied, which indicates that the vitamin in corn can be freed almost totally by prolonged proteolytic action.

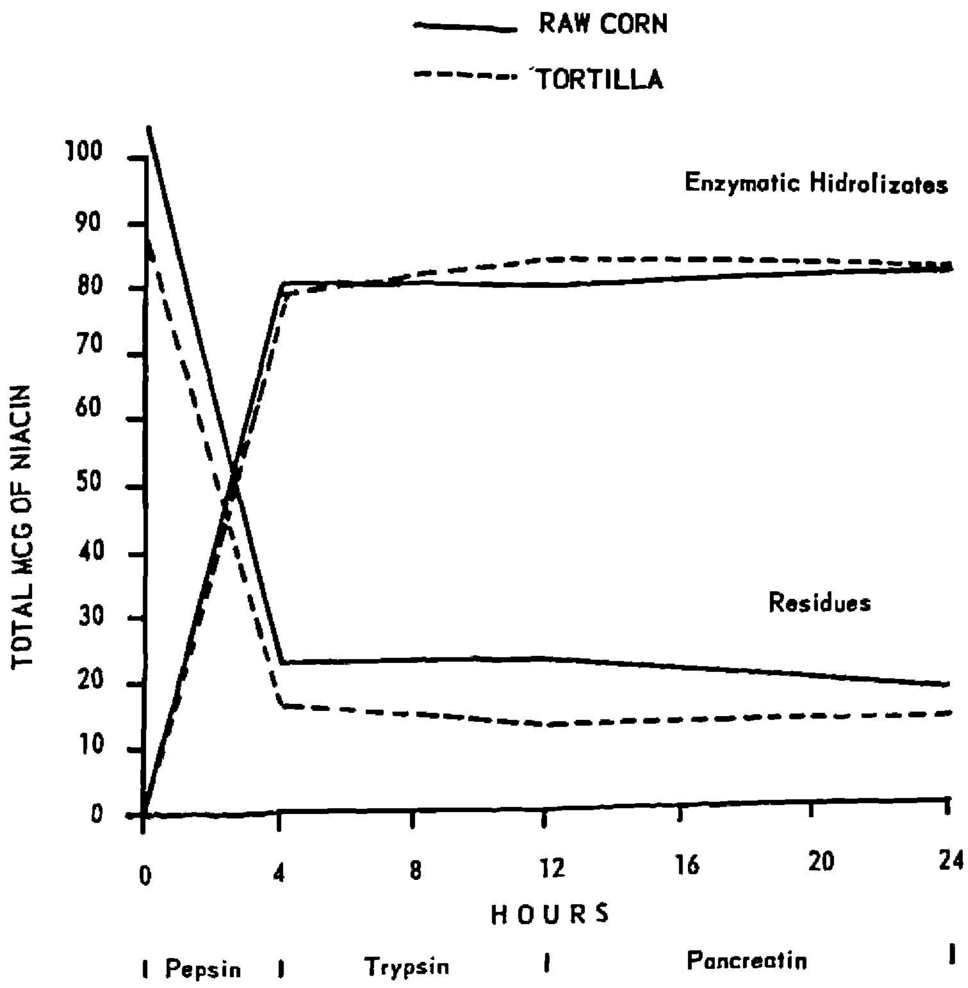

Fig. 2. Release of niacin from a 5-g sample of corn and tortilla by enzymatic hydrolysis (24-hour trial).

Since the digestive process does not last 72 hours, shorter periods of enzymatic hydrolysis were studied, using three proteolytic enzymes on both corn and tortilla. At the end of 4 hr of hydrolysis, similar absolute quantities of niacin were obtained from corn and tortillas. As in the previous cases, the trypsin and pancreatin hydrolysis increased the quantity of niacin in the hydrolysate through their action on the residual niacin of corn and tortilla.

The data thus indicate that, under the conditions employed, niacin is as available in raw corn as in lime-treated corn. As suggested previously (Bressani and Scrimshaw, 1958; Cravioto *et al.*, 1952), other factors, such as a more favorable balance of available amino acids, must be responsible for the better growth observed in experimental animals fed lime-treated corn than in those fed raw corn.

Kodicek (1960), Carpenter *et al.* (1960), and Chaudhuri and Kodicek (1960) reported that the "bound" niacin in corn and other cereal grains can be released only by an alkaline, and not by enzymatic, digestion, either *in vitro* or *in vivo* in rats and pigs. Instead of using corn as the sole source of protein, as in our studies, these workers used a diet supplementing corn with casein or peas to raise the protein level. It is possible that this procedure accentuates the imbalance of tryptophan by supplying relatively more of the other two principal limiting amino acids in corn, lysine and isoleucine. Since tryptophan serves as a precursor of niacin, it would have the effect of lowering the relative niacin activity of the diet. This could be interpreted in *in vivo* experiments as a binding of niacin, whereas it is really an amino acid imbalance of the type described by Elvehjem and Krehl (1955). This proposed explanation does not account, however, for their failure to find a release of "bound" niacin in enzymatic digestion with papain, but this enzyme may be less effective for the purpose than the combination of pepsin, trypsin, and pancreatin used in this study.

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