Reprinted from THE JOURNAL OF NUTRITION
Vol. 67, No. 3, March 1959

A COMPARISON OF THE EFFECT OF RAW CORN AND TORTILLAS (LIME-TREATED CORN) WITH NIACIN, TRYPTOPHAN OR BEANS ON THE GROWTH AND MUSCLE NIACIN OF RATS [1,2]

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(Received for publication August 15, 1958)

Pellagra, although traditionally associated with a high dietary intake of corn (*Zea mays*), is rare in Central America and Mexico where this cereal may contribute as much as 80% of the Calories of the rural diet (Anderson et al., '48; Sogandares et al., '53; Flores and Reh, '55). Evidently in these areas the combined niacin and tryptophan activity of the diets is sufficient to prevent the appearance of clinical pellagra. This may be due in part to the fact that beans (*Phaseolus vulgaris*) make up the second most common ingredient in the diet and are a good source of niacin (Bressani et al., '54). Moreover, it has been shown that rats fed lime-treated corn grow better than those fed raw corn (Cravioto et al., '52; Laguna and Carpenter, '51). Pearson et al. ('57) conclude that the conversion of "bound" niacin to free niacin by this process would appear to explain the superiority of tortillas over raw corn in the diet of the rat.

[1] A portion of the information in this publication has been presented in abstract form in Federation Proc., *14:* 451, 1955.

[2] Supported in part by the Williams Waterman Fund of the Research Corporation. INCAP Publication 1–116.

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The present rat growth experiments were designed to study the nutritive value of corn and beans in Central American diets. Lime-treated corn in the form of ground tortillas with and without beans was substituted for ground raw corn in a niacin-free, low-tryptophan diet. The effects on growth and skin condition, as well as on the protein and niacin content of blood serum, liver and muscle were measured. Niacin-deficient animals were also irradiated in an effort to induce pellagroid skin lesions of the type which occur in niacin-deficient humans upon exposure to sunlight.

MATERIALS AND METHODS

Weanling rats of the Wistar strain were housed in individual all-wire cages with raised screen bottoms in a room at approximately 24°C. Food and water were provided ad libitum. For each experiment, all rats were depleted for a 21-day period on the following niacin-deficient, low-tryptophan basal diet: vitamin test casein, 7; zein, 14; sucrose, 74.15; L-lysine, 0.55; U.S.P. XII mineral mixture, 4; and cod liver oil, 0.30. The following nutrients were added to supply the specified amounts per 100 gm of diet: choline chloride, 345 mg; thiamine chloride, 0.52 mg; pyridoxine, 0.52 mg; riboflavin, 0.52 mg; folacin, 0.52 mg; calcium pantothenate, 3.45 mg; and biotin, 0.017 mg. This basal ration contained approximately 18.5% of crude protein and 0.16% of tryptophan. Under the conditions of this laboratory, rats fed this basal diet did not gain more than an average of 5 gm during the 21-day depletion period and developed an appreciable alopecia. White corn (*Zea mays*) of known moisture and nitrogen content was ground finely to facilitate its incorporation into the diet. Tortillas were prepared from the same corn by cooking the whole grain in water containing about 0.43% calcium oxide at 90°C. for one hour and then letting it stand in this solution at room temperature overnight. The water was discarded with most of the episperm, but without removing the germ. The material so treated was then ground, prepared and cooked as flat circular cakes (tortillas) for one to two

minutes on a hot plate. The procedure commonly used for the preparation of tortillas and the corresponding nutrient changes have been described in detail (Bressani et al., '58; Bressani and Scrimshaw, '58). The resulting tortillas were dried to about 10% moisture and finally ground. Corn or tortillas, when fed, replaced 48% of the zein and 92% of the sucrose of the basal ration. This rate of substitution gave the various test diets a uniform crude protein content. Black beans (*Phaseolus vulgaris*) were cooked in water, dried, ground, and included in the diet at a 15% level, equivalent in relative quantity to that consumed by some rural populations of Guatemala. In order to equalize the crude protein content of the diets, the beans replaced 27% of the zein and 20% of tortillas of the basal diet. When niacin was added, one mg was given orally every day to each rat. Tryptophan, when required, was added at the rate of 0.24%, which increased the total content of the diet to 0.40%. At the end of a depletion period the rats were stratified by clinical appearance, weight and sex, and then distributed equally among the experimental groups. At the end of the experimental period the rats were sacrificed and the blood sera, livers and leg muscles analyzed for proteins and niacin. Protein in the tissue was determined by the Kjeldahl method and niacin by the micro-biological method described in the U.S. Pharmacopoeia, XIV edition ('50). For these analyses the animals in each group were autopsied and the livers as well as the muscles of the right hind leg pooled to give single samples.

Experiment 1

Supplementation of niacin-deficient, low-tryptophan diets with corn or tortillas with and without niacin

Trial 1. Corn and tortillas were compared as sources of niacin. Forty-eight rats were matched for weight and distributed among 6 experimental groups, each containing 4 males and 4 females. The 6 groups received the basal ration with one of the following supplements: group 1, none; group

2, niacin; group 3, corn; group 4, tortillas; group 5, corn + niacin; and group 6, tortillas + niacin. At the end of 20 days on the basal diet alone it was apparent that the rats would soon die. To test their recuperative ability, the diets of groups 1 and 2 were reversed. The other 4 groups remained on the same diets for 30 additional days, making a total of 50 days on trial for all 6 groups.

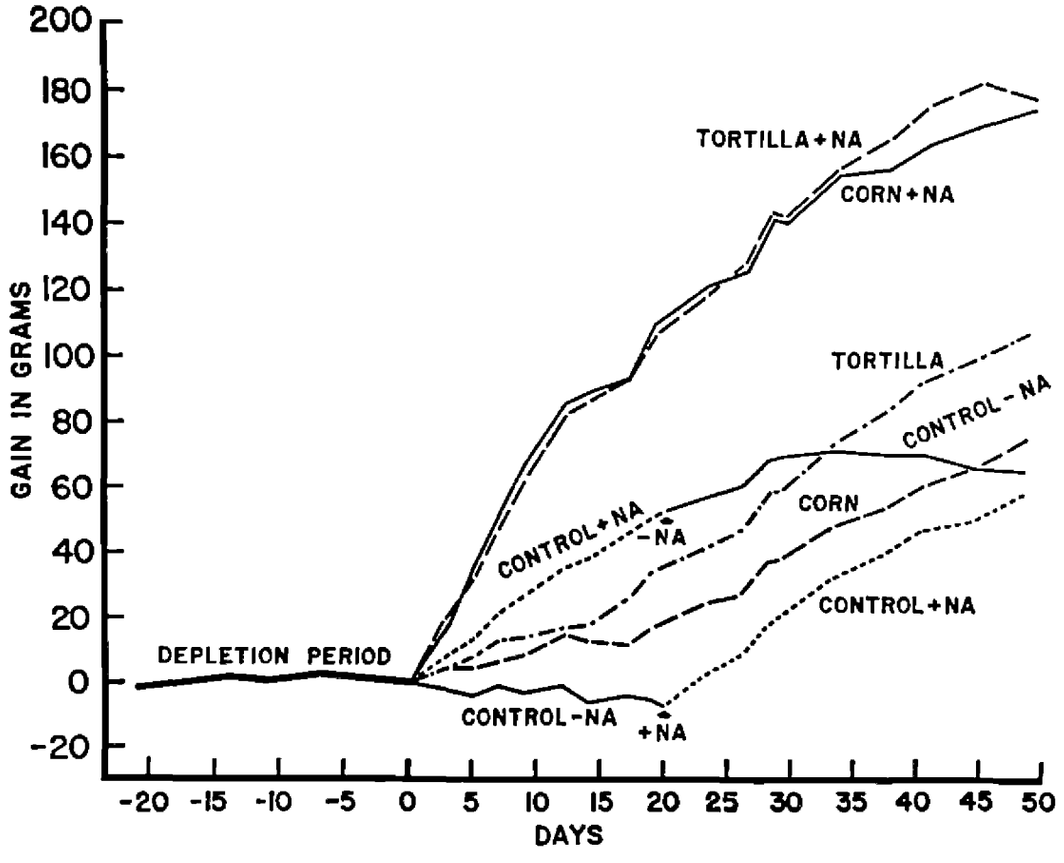

Fig. 1 Weight gain of rats fed diets based on corn or tortilla with and without the addition of niacin. The diets of the groups initially receiving the basal diet alone and the basal + niacin were reversed after 20 days.

The growth of the rats receiving the different treatments is shown in figure 1. Those consuming the basal ration lost weight steadily and showed severe alopecia over their entire bodies; the addition of niacin increased growth without appreciably affecting the alopecia. The alopecia that was present after 20 days on the unsupplemented basal diet disappeared, however, in the groups receiving corn or tortillas. When the diets of groups 1 and 2 were interchanged at the end of 20 days, the growth of the rats showed a cross-over

effect. The increase in weight compared with that of rats on the control diet was highly significant with both the corn and tortilla diets although the latter caused the greater response. Niacin, when added to either the basal diet or the diets containing corn or tortillas, produced a further significant increase in the growth of the rats. In this case niacin supplementation obscured or eliminated the "tortilla effect" on rat growth. The efficiencies of food utilization, calculated at the end of 20 days on trial before the cross-over was initiated, showed that to produce 1 gm gain in weight the rats required 7.77 gm of the diet containing corn, 4.43 gm for tortillas, 3.55 gm for corn + niacin, and 3.21 gm when the diet contained tortillas + niacin. The corresponding absolute gains in weight at the end of 50 days were 77 gm for the group receiving corn, 110 gm for that fed tortillas, and 179 and 182 for the groups receiving corn + niacin and for tortillas with added niacin, respectively.

Trial 2. This was a replicate of trial 1 except that the groups fed the basal ration and the basal ration + niacin were not included. Thirty-two depleted rats were distributed among the 4 experimental groups, each containing 4 males and 4 females. At the end of 50 days on these diets the results were closely similar to those of trial 1.

Experiment 2

Supplementation of raw corn and tortilla diets with niacin, tryptophan and beans

In this trial, 66 depleted rats were distributed among 6 experimental groups, matched for weight, each containing 5 males and 6 females. The substitutions in the basal diet were as follows: group 1, corn; group 2, tortillas; group 3, tortillas + niacin; group 4, tortillas + tryptophan; group 5, tortillas + beans; and group 6, tortillas + beans + tryptophan.

As may be seen in figure 2, tortillas again significantly increased the growth rate of the rats over those fed corn. While the supplementation of the tortilla diet with beans, trypto-

phan and niacin increased the rate of growth of the rats over those fed tortillas, these gains were not significant.

The data of table 1 show no significant differences in serum proteins among rats fed corn or tortillas or when the latter was supplemented with tryptophan or niacin. Nor was there

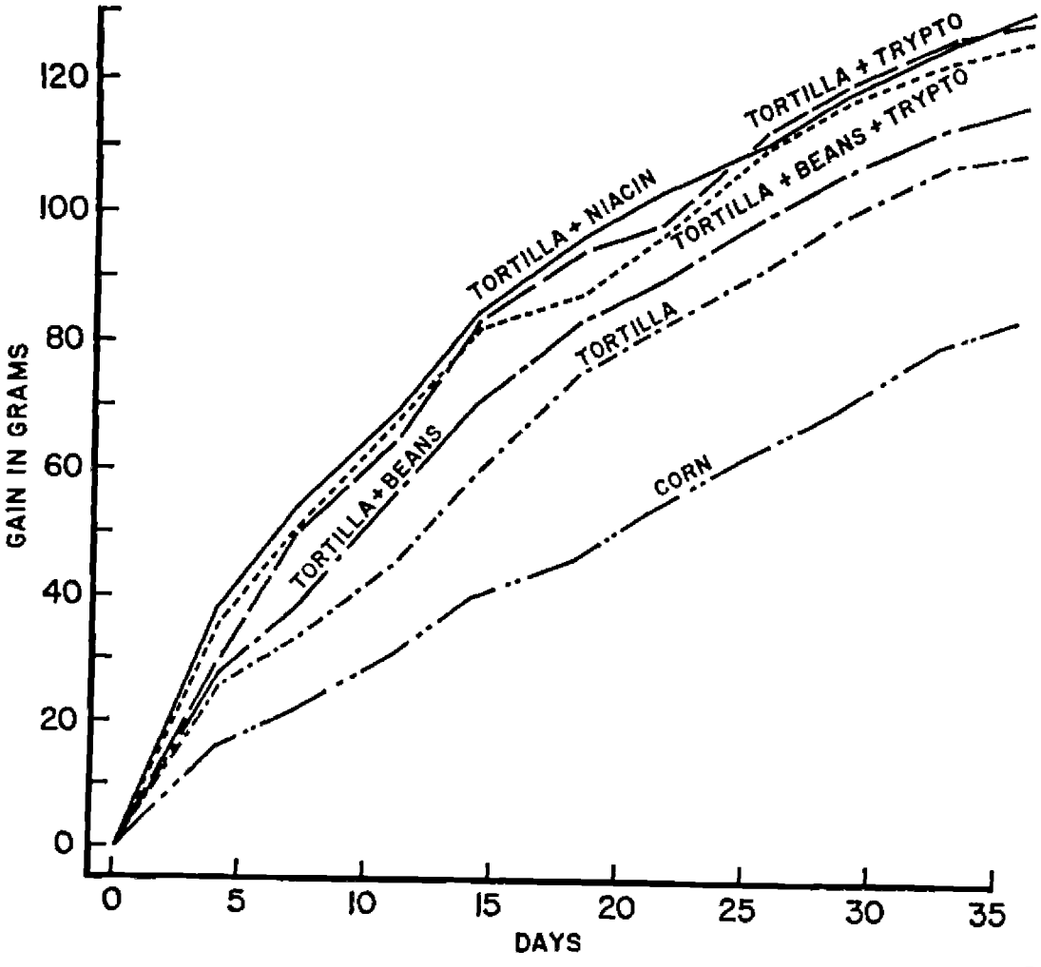

Fig. 2 Weight gains of rats fed corn, tortilla or tortillas supplemented with combinations of beans, niacin and tryptophan.

TABLE 1

Protein and niacin content of rat sera, livers and muscle

GROUP	SERUM PROTEINS	LIVER PROTEINS	MUSCLE PROTEINS	MUSCLE NIACIN
	gm %	*mg/100 gm*	*mg/100 gm*	*mg/100 gm*
Corn	5.77	19.5	22.3	3.52
Tortilla	5.87	20.6	23.3	4.30
Tortilla + niacin	6.31	19.8	22.4	7.74
Tortilla + tryptophan	6.43	21.1	24.2	8.22
Tortilla + beans	5.91	21.3	23.0	6.30
Tortilla + beans + tryptophan	6.11	22.3	23.9	8.51

any appreciable influence of the various diets on liver or muscle protein. The substitution of tortillas for corn resulted in a slight increase in muscle niacin which became more marked with the addition of beans, niacin or tryptophan to the tortilla diet.

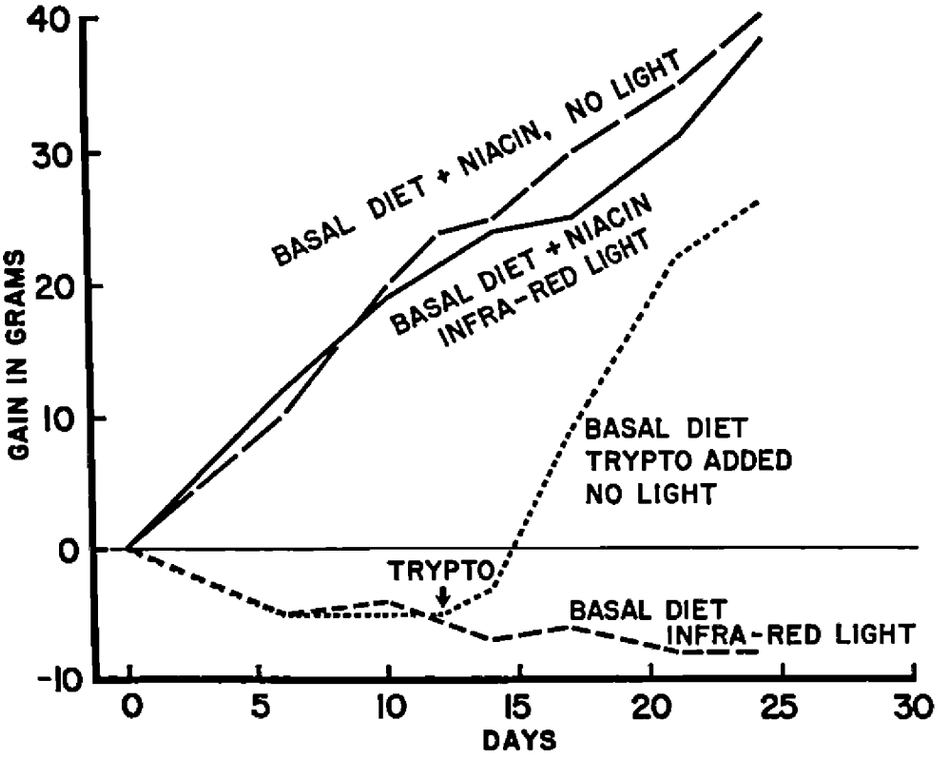

Fig. 3 Effect of infra-red light on the growth of niacin-depleted rats receiving the basal diet with and without added tryptophan or niacin.

Experiment 3

Effect of infra-red light and of supplementation with niacin and tryptophan on niacin-depleted rats

Forty depleted rats with medium-to-acute alopecia were shaved clean over an area 1-inch square on their backs and then distributed among 4 experimental groups of 5 males and 5 females each matched for weight and severity of alopecia. The 4 groups were treated during a 24-day period as follows: group 1, housed under normal rat colony conditions (24°C) and fed the basal diet; group 2, same treatment as group 1 but with supplementary niacin; group 3, same diet as group 1 but subjected to continuous infra-red light which raised the temperature within the cages to 31°C; and group 4

received the same treatment as group 3 but was given supplementary niacin similar to group 2.

There was no effect of light on the rate of growth of rats (fig. 3), although niacin supplementation again significantly increased the growth rate over the corresponding control groups. Tryptophan supplementation of the depleted rats in group 1, at the end of 12 days, significantly increased their growth rate, but neither niacin nor tryptophan supplementation had an apparent effect on the alopecia. Furthermore, no morphological changes were observed in the depleted rats as a result of exposure to light. The rats deficient in niacin, however, showed a tendency to huddle as if they had become light-sensitive. Huddling was not observed in the irradiated group which was supplemented with niacin. Similar results were observed with ultra-violet light.

DISCUSSION

A niacin-deficient, low-tryptophan basal ration containing 18.5% of crude protein has proved useful for studying niacin deficiency in rats. Weanling rats placed on this diet failed to grow and developed a mild-to-acute alopecia within 21 days. The alopecia observed in rats fed the basal ration, while not affected by the addition of niacin, was improved by the addition of raw or lime-treated corn. The alopecia observed may have been related to the content and availability of the amino acids of the diet.

It was apparent from the data of these trials that supplementation of a niacin-deficient, low-tryptophan basal diet with raw corn, tortillas or niacin increased the growth rate and muscle niacin of the rats. The greater growth of the rats fed tortillas over those fed raw corn was apparently due to increase in the availability of niacin, a result of the lime treatment of the corn used in their preparation. This is substantiated by the lack of significant differences in the growth of rats fed raw as compared with lime-treated corn (tortilla) when both diets were supplemented with niacin. Kodicek ('56), Pearson et al. ('57) and more recently McDaniel and

Hundley ('58) have presented evidence that alkaline hydrolysis of corn increases the availability of a "bound niacin." While niacin supplementation of raw corn and tortilla-containing diets apparently eliminated the "tortilla growth effect" observed by Cravioto et al. ('52) and Laguna and Carpenter ('51) and shown also in the present study, the rats fed the basal control diet supplemented with 1 mg of niacin per rat per day did not grow as well as when either raw corn or tortillas were present in the diet. This may have been due to insufficient niacin supplementation but was more likely the result of an imbalance of the amino acids of the basal ration which contained in addition to 7% casein only the zein fraction of the protein of corn.

Under the conditions of these experiments it was not possible to induce skin lesions in the niacin-deficient rats. A continuous source of infra-red or ultra-violet light had no apparent effect on bare skin areas of niacin-depleted or supplemented rats. The tendency of the depleted rats to huddle under light treatment, however, was lessened by niacin supplementation. This indicates that even though no morphological changes in the skin areas were apparent, a niacin deficiency does increase light sensitivity in rats.

The lime treatment of corn for the preparation of tortillas apparently increases the availability of niacin. However, the relatively large effect of niacin supplementation on the growth of the rats fed either raw corn or tortillas, would indicate that the quantity of niacin released by lime treatment is small. While lime treatment of corn may still be a minor contributing factor to the absence of pellagra among Central American and Mexican rural populations consuming high corn diets, the rat studies reported here suggest that the relatively high consumption of beans is a factor of greater significance. The higher levels of niacin observed in the muscle tissue of the bean-supplemented group support this conclusion. It has also become evident that coffee may play a role as a source of dietary niacin and its contribution in Central America has recently been demonstrated by Bressani and Navarrete ('59).

SUMMARY

A niacin-deficient, low-tryptophan basal ration containing 18.5% of crude protein has proved useful for studying niacin deficiencies in rats. Weanling rats of this laboratory placed on such a diet failed to grow and developed mild-to-acute alopecia within 21 days. Daily oral administration of 1 mg of niacin per rat or increasing the tryptophan content of the basal ration to 0.40% restored the growth of the rats but had no influence on the alopecia. When either raw or lime-treated corn was substituted for the zein and sucrose of the basal ration the rate of growth was significantly improved and the growth of hair returned.

The results confirm the increased growth rate of rats fed lime-treated corn (tortillas) compared with those fed raw whole ground corn. Beans fed at a level calculated to be equivalent to that consumed by part of the rural population of Guatemala produced a slight further improvement in growth and significantly increased the muscle niacin of depleted rats. Continuous infra-red or ultra-violet light had no apparent effect on the skin of shaved areas of niacin-depleted or supplemented rats. The addition of niacin, however, lessened the sensitivity of the depleted rats to the light as manifested by a tendency to huddle.

LITERATURE CITED

ANDERSON, R. K., J. CALVO, W. D. ROBINSON, G. SERRANO AND G. C. PAYNE 1948 Nutrition appraisals in Mexico. Am. J. Public Health, *38:* 1126.

BRESSANI, R., E. MARCUCCI, C. E. ROBLES AND N. S. SCRIMSHAW 1954 Nutritive value of Central American beans. I. Variation in the nitrogen, tryptophan, and niacin content of ten Guatemalan black beans (*Phaseolus vulgaris,* L.) and the retention of the niacin after cooking. Food Research, *19:* 263.

BRESSANI, R., AND D. A. NAVARRETE 1959 Niacin content of coffee in Central America. Ibid., in press.

BRESSANI, R., AND N. S. SCRIMSHAW 1958 Effect of lime treatment on the *in vitro* availability of essential amino acids and solubility of protein fractions in corn. J. Agr. Food Chem., *6:* 774.

BRESSANI, R., R. PAZ Y PAZ AND N. S. SCRIMSHAW 1958 Chemical changes in corn during preparation of tortillas. Ibid., *6:* 770.

CRAVIOTO, R. O., G. H. MASSIEU, O. Y. CRAVIOTO AND F. DE M. FIGUEROA 1952 Effect of untreated corn and Mexican tortilla upon the growth of rats on a niacin-tryptophan deficient diet. J. Nutrition, *48:* 453.

FLORES, M., AND E. REH 1955 Estudios de hábitos dietéticos en poblaciones de Guatemala. I. Magdalena Milpas Altas. Suplemento No. 2 del Boletín de la Oficina Sanitaria Panamericana "Publicaciones Científicas del Instituto de Nutrición de Centro América y Panamá," p. 90.

KODICEK, E. 1956 The effect of alkaline hydrolysis of maize on the availability of its nicotinic acid to the pig. British J. Nutrition, *10:* 51.

LAGUNA, J., AND K. J. CARPENTER 1951 Raw versus processed corn in niacin-deficient diets. J. Nutrition, *45:* 21.

MCDANIEL, E. G., AND J. M. HUNDLEY 1958 Alkali-treated corn and niacin deficiency. Federation Proc., *17:* 484.

PEARSON, W. N., S. J. STEMPFEL, J. S. VALENZUELA, M. H. UTLEY AND W. J. DARBY 1957 The influence of cooked vs. raw maize on the growth of rats receiving a 9% casein ration. J. Nutrition, *62:* 445.

PHARMACOPOEIA OF THE UNITED STATES 1950 XIV Edition.

SOGANDARES, L., A. P. DE GALINDO AND H. P. MEJÍA 1953 Estudios Dietéticos de grupos urbanos y rurales de la República de El Salvador. Suplemento No. 1 del Boletín de la Oficina Sanitaria Panamericana "Publicaciones Científicas del Instituto de Nutricíon de Centro América y Panamá," p. 27.