CCLXXXI. CEREALS AND RICKETS.

IV. THE EFFECT OF IMMATURITY OF THE MAIZE KERNEL UPON ITS RACHITOGENIC PROPERTIES[1].

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(*Received October 7th, 1933.*)

INTRODUCTION.

THE present report on the effect of the ripening process on the anticalcifying properties of maize had its origin in two lines of interest. In the first place, it was expected that if differences between immature and ripe grains were found to exist, the foundation might thereby be laid for profitable studies on the nature of an anticalcifying factor or factors [Mellanby, 1921; 1922; 1924; 1925; 1926; 1930; Green and Mellanby, 1928]. In the second place, the irregularity in the production of rickets on Ration 2965 [Steenbock and Black, 1925] as reported by Harris and Bunker [1930; 1931] might be explained. In seven or more years of use of Ration 2965, even though we have obtained our supply of maize from numerous sources on the open market, we have not experienced the irregularities in the production of rickets reported by Harris and Bunker. They claim to have overcome the difficulty by ageing the ground maize for 6 months or more, but storage of the whole kernels as distinguished from the ground maize was not found to result in improvement. Goldblatt [1931] has suggested that the difficulty was not caused by a variation in the quality of the maize but in a variable settling out of the calcium carbonate from the other ingredients of the ration. He has proposed the incorporation of gelatin in the diet to prevent this.

EXPERIMENTAL.

Series I and II—Field maize.

On August 25, 1931, yellow dent maize still in the milk stage was brought to the laboratory from the university farm. Only good sized ears which had the husks fully closed and which carried a browning silk were selected. These were husked in the laboratory, and the kernels were cut from the cob. Scraping successfully removed the germ from the cob also. A moisture determination at this time showed the presence of 66·0 % of water.

The maize, spread in granite-ware pans in a layer not over one kernel in depth, was dried in the laboratory before an electric fan at a temperature not exceeding 29°. If any fermentation occurred, it was kept at a minimum by the low humidity of the atmosphere during the days of drying. The product had a sweet odour and in 24 hours was sufficiently brittle to be ground in a burr mill.

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station, Madison.

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The temperature during grinding never rose above 42°. Immediate cooling was accomplished in a refrigerator. After a day of further desiccation at reduced pressure in the presence of dry $CaCl_2$, it was stored in a cloth bag.

A second portion of the maize prepared in the same manner was desiccated in a drying room with the aid of a fan. After 15 hours' drying at a temperature slightly below 50° it was sufficiently dry to grind. A third portion, which was too mature to be classified in the milk stage, was dried on the cob in an air current at room temperature preparatory to shelling and grinding.

For control purposes normally matured ears of maize were removed from the shocks of the same field on September 29. These were shelled and dried by exposure to an air current at room temperature for 5 days. The kernels were ground at once and feeding was started a week later. For additional control purposes there was fed maize from the previous year's harvest.

In Series I each sample of maize was fed as the cereal component of our high calcium-low phosphorus rickets-producing Ration 2965 [Steenbock and Black, 1925] which has the following composition: yellow maize 76 parts, wheat gluten 20; $CaCO_3$ 3; and NaCl 1. The rations were fed to four litters of rats ranging in age from 23 to 25 days and in weight from 54 to 67 g. The rats were confined individually in cages provided with screen bottoms. They were so distributed that each ration was fed to one representative of a litter. Distilled water and food were fed *ad libitum*, but a daily record was kept of the food consumed. Body weights were recorded weekly.

In Series II there was used the same maize as in Series I, but the ration and technique of feeding were changed. Four parts of the wheat gluten were replaced by dry yeast[1] to improve food consumption, and the food intake was equalised by limiting it for each rat in the series to the level of that rat which had the lowest intake, disregarding distinctly abnormal animals. Each ration was fed to 6 rats taken from representative litters at the age of 25 or 26 days and at a range in weight of 52 to 63 g.

At the close of a 5-week feeding period the rats of both Series I and II were killed and the femurs removed and dissected free from soft tissues. The femurs were extracted in a Soxhlet extractor for five days with 95 % alcohol and then ashed in a muffle furnace at red heat. The distal ends of radii and ulnae were preserved in 10 % formalin and later split longitudinally and placed in dilute $AgNO_3$ solution for exposure to light to reveal the width of the uncalcified metaphyses as carried out in the line test described by McCollum *et al.* [1922]. Inspections of the costochondral junctions were made at the time of termination of the experiment.

The average results for the groups studied in Series I and II are assembled in Table I. In the series which received the rations *ad libitum* but without yeast, the range of average individual daily food intake was 6·2 to 9·1 g. with group averages ranging from 7·0 to 8·2 g. In the duplicate series of rations containing yeast, the individual range was 6·5 to 7·3 g. with group averages between 6·9 and 7·2 g.

The weight gains as well as food consumption of the rats were somewhat disturbed during the fifth week by infections of their respiratory tracts. Although in most cases the infections were of a very mild nature, in 10 animals they were of a moderate or severe grade as indicated by a definite wheeze and the presence of a serous discharge from the nostrils. Although these symptoms were suggestive of a vitamin A deficiency, only two rats showed evidence of the initial stages of ophthalmia.

[1] Obtained from The Northwestern Yeast Company, Chicago.

Table I. *Effect of immaturity of field maize on calcification.*

Ration	Body weight		Average daily food consumption	Femur data				
	Initial g.	Gain g.	g.	Weight of extracted bone g.	Weight of ash g.	% of ash	Metaphyses	Costochondral junctions
Ad libitum food consumption—(4 animals)								
R 37. Unripe maize air-dried below 29°	58	42	7·9	0·1005	0·0331	33·0	Wide	Much enlarged; double beading; angulation
R 38. Unripe maize dried at 50°	59	46	7·9	0·1069	0·0346	32·3	,,	,,
R 39. Nearly ripe maize air-dried on cob	60	18*	8·2	0·0833	0·0203	24·3	,,	,,
R 40. Ripe maize, from shock	58	17*	7·2	0·0893	0·0250	27·8	,,	,,
R 41. Ripe maize, from usual stock supply	61	24	7·0	0·0929	0·0243	26·2	,,	,,
Equalised food consumption—(6 animals)								
R 42. Unripe maize air-dried below 29°	57	44	7·2	0·1050	0·0330	31·5	,,	,,
R 43. Unripe maize dried at 50°	57	44	7·2	0·1069	0·0357	33·2	,,	,,
R 44. Nearly ripe maize air-dried on cob	(55)	(25)	(7·0)	(0·0840)	(0·0213)	(25·5)	,,	,,
	57	24*†	7·0	0·0902†	0·0243†	26·8†	,,	,,
R 45. Ripe maize, from shock	55	33*	6·9	0·0924	0·0220	24·0	,,	,,
R 46. Ripe maize, from usual stock supply	56	30*	7·0	0·0903	0·0210	23·2	,,	,,

* 2 or 3 animals lost weight during the last week.
† 3 animals died before 31st day.
( ) Average for the 3 animals which lived to the close of the experiment.

The values for weights of extracted femurs, weights of ash and percentages of ash ran remarkably parallel in the two series. Although all animals were in a very rachitic condition, the ash analyses indicated that the immature maize was less rickets-producing than the ripe maize. Group averages for percentage of ash in the femurs of rats fed immature maize, whether dried below 29° or at approximately 50°, fell between the values 31·5 and 33·2 %. On the other hand the somewhat immature maize dried on the cob, ripe maize taken from the shock and maize from the previous year's stock supply produced femurs with an ash content falling between 23·2 and 27·8 %. Thus immature maize rations consistently produced a bone with approximately 7 % higher ash content than that formed from ripe maize rations. The average weight of extracted femurs from the immature maize rations fell between 0·1005 and 0·1069 g. as compared with the ripe maize range of 0·0833 to 0·0929 g. A similar relationship held for average ash weights, the immature maize values falling between 0·0330 and 0·0357 g. in comparison with a range of 0·0203 to 0·0250 g. for the ripe maize products.

The silver nitrate staining test as applied to the wrist bones showed the presence of wide rachitic metaphyses in all animals of both series. Likewise no differentiation was possible by means of the costochondral junctions, all of which were much enlarged with double beading or angulation in most animals. These observations were in harmony with the results obtained by ash analysis of the femurs.

It is evident that the immature maize contained nutrients or a combination thereof, which promoted better bone calcification than the corresponding mature maize. Analysis of two of the rations of Series I ruled out the possibility that this was due to a variation in total phosphorus content. The ration compounded from immature maize dried below 29° contained 378 mg. of phosphorus per 100 g., and that compounded from ripe maize from the shock contained 391 mg. per 100 g.

Series III—Sweet maize.

The study of the relation between the ripening process of maize and a change in its power to promote bone calcification was continued on canned sweet maize. Two canned products of "Golden Bantam" sweet maize and "Golden Bantam" seed were obtained, from a commercial canning company[1]. One product was vacuum-packed and the other brine-packed. According to information furnished by the packers the sweet maize canned by the vacuum process had received no addition other than salt. In the brine process of packing, salt, sugar and water had been added. For further comparison a brine-packed whole kernel white sweet maize, probably of the "Country Gentleman" variety, was bought in the open market.

The desiccation of the above canned products was carried out in granite-ware pans placed before a fan in a room held at a temperature of 47 to 51°. By spreading the maize in a layer not over one kernel in thickness a product suitable for grinding was obtained in 18 to 22 hours. The vacuum-packed maize yielded 31 % of dry matter and the brine-packed maize an average of 20 % dry matter.

The three samples of canned sweet maize were compared with ripened Golden Bantam seed. In order to relate the results to those obtained in the immediately preceding series, immature yellow dent maize dried below 29° was also included. The possible effect of heating during the drying process was evaluated by using a ration which included maize which had been ground coarsely, then covered with water and dried under conditions comparable to those used in desiccating the canned samples.

The 6 samples of maize were fed in Ration 2965 which was modified by the inclusion of 4 % dry yeast as described in Series II. To eliminate a possible vitamin A deficiency indicated in the previous experiments, 2 drops of red palm oil were administered to each rat twice weekly. This was five times the amount required to cure ophthalmia in rats depleted of vitamin A.

Before feeding the red palm oil as a source of vitamin A, it was tested for vitamin D to make certain that it did not add appreciable amounts of this factor. The assay was carried out in the usual manner of conducting the line test. Rachitic rats were fed the oil at levels of 2, 5 and 7 drops, delivered from a calibrated dropper. These amounts, equivalent to 40, 100 and 140 mg. of oil, were administered over a 10 day period. In no case was any evidence of healing obtained in the distal ends of the radii and ulnae.

Each ration was fed to 6 rats taken from litters 24 to 27 days of age. The distribution, caging and feeding of the animals were the same as in Series II, including equalisation of food consumption for all rats.

At the close of the 5 week feeding period pooled blood samples were obtained for estimation of serum-calcium and inorganic phosphorus. The rats were placed under light ether anaesthesia and the blood from the left carotid artery was collected in a chilled centrifuge-tube. After standing overnight in a refrigerator, the blood was centrifuged and the serum was analysed.

The inorganic phosphorus was determined by the method of Fiske and Subbarow [1925]. Calcium was determined by the McCrudden [1911–12] method modified as follows. An aliquot of the protein-free filtrate obtained as in the phosphorus determination and representing 1 cc. of serum was brought to neutrality in the presence of alizarin with dilute NH_4OH and HCl. One drop of 5 % HCl in excess was added and the calcium precipitated by sodium oxalate in

[1] Obtained from The Mid West Canning Corporation, Rochelle, Illinois.

a medium buffered with sodium acetate to a p_H sufficient to give a red colour with alizarin. After standing overnight, the calcium oxalate was centrifuged off, washed with dilute NH_4OH, dissolved in H_2SO_4 and titrated with 0·01 *N* $KMnO_4$ delivered from a micro-burette.

The ash analyses were made on only one femur of each animal except in those cases where a possible discrepancy needed checking. The metaphyses of the wrists were measured after silver nitrate staining, with a steel scale graduated to one-hundredth of a centimetre. Observations on the costochondral junctions were included.

The results of this series of tests are assembled in Table II. The gains in body weight were both satisfactory and uniform throughout all groups. The food intake averaged 6·6 g. per animal per day for the entire period.

Table II. *Effect of immaturity of sweet maize on calcification.*

Ration	Body weight		Average daily food consumption	Blood-serum analysis*		Femurs			Maximum width of metaphyses	Costochondral junctions
	Initial g.	Gain g.	g.	Ca mg./100 cc.	P mg./100 cc.	Weight of extracted bone g.	Weight of ash g.	% of ash	cm.	
R 61. Golden Bantam seed	63	47	6·6	11·4	2·5	0·1138	0·0378	33·0	0·15	Much enlarged, some angulation and double beading
R 62. Golden Bantam ground seed moistened and dried	62	52	6·6	10·8	2·7	0·1174	0·0424	36·2	0·14	Much enlarged
R 63. Golden Bantam, vacuum pack	62	51	6·6	11·0	4·3	0·1450	0·0653	45·0	0·10	Slightly enlarged
R 64. Golden Bantam, brine pack	60	54	6·6	11·2	3·9	0·1419	0·0619	43·6	0·10	Slight to moderately enlarged
R 65. White sweet maize, brine pack	62	57	6·6	12·0	4·4	0·1620	0·0749	46·1	0·10	Slightly enlarged
R 66. Unripe maize, air-dried below 29° (yellow dent)	61	55†	6·6	10·8	3·9	0·1338	0·0534	39·7	0·11	Moderately enlarged

* Blood from rats with severe respiratory trouble was not used.
† One rat had severe respiratory trouble at the close of the experiment.

The percentage of ash in the femurs disclosed the same relations as those observed in the previous experiments; that is, the immature maize promoted better calcification than the corresponding ripe maize. Whereas the Golden Bantam seed produced femurs containing 33·0 % of ash, the same variety of maize at the canning stage raised the ash content to 45·0 and 43·6 %. The white variety of sweet maize gave an ash content of 46·1 %, a figure comparable to that given by the Golden Bantam variety. Moistening and redrying the Golden Bantam seed raised the ash content to 36·2 %—a 3·2 % increase above the untreated seed. This suggested the need for further study of the complicating factors of moistening and heating. The 39·7 % of ash in the bones produced on immature yellow dent maize dried below 29° fell between the percentages obtained for the immature and ripe sweet maize. It was also 7 to 8 % higher than the figure obtained for the same sample used in the previous tests. This latter variation can be accounted for by such factors as difference in initial weight of animals and food consumption.

These differences in calcifying properties were verified by the analyses of the blood-sera. Whereas all calcium values fell within the normal range, the inorganic phosphorus was greatly depressed. Although it was impossible to obtain

particularly accurate results at the low levels encountered, the relative distribution of values was the same as in the data already reviewed.

Another confirmation of these relations was observed in the width of the rachitic metaphyses of the wrist bones and in the degree of involvement of the costochondral junctions.

Since any variations in calcification might be caused by differing phosphorus content of the rations, the dried maize samples were analysed for this element using the method described by The Association of Official Agricultural Chemists [1930]. The three samples of canned sweet maize were found to contain between 264 and 273 mg. phosphorus per 100 g. dry product, the Golden Bantam seed 355 and 367 mg. and the immature yellow dent maize 410 mg. From these differences calculations based on a daily food intake of 7 g. indicated that the maximum variation in total phosphorus intake over a 5 weeks' period was 272 mg. It is obvious that these phosphorus variations were not responsible for the observed differences in calcification since the sweet maize rations with the lower phosphorus content produced better calcified bones.

Sweet maize at the canning stage possessed properties favouring better skeletal calcification than the same maize fed as ripened seed. This is in accord with the previous results obtained with immature yellow dent field maize.

Summary.

Immature yellow dent field maize promoted better calcification than corresponding mature maize of the same variety and grown under identical conditions.

Commercially canned sweet maize promoted better calcification than the seed from which it had been grown.

The differences in calcifying value cannot be explained by more favourable calcium/phosphorus ratios.

The authors wish to express their appreciation to the Quaker Oats Company whose financial support made these experiments possible. The authors are indebted to Mr James T. Lowe and Mr Robert W. Haman for assistance rendered in analytical work.

REFERENCES.

Association of Official Agricultural Chemists (1930). Methods of analysis, p. 14, 3rd edition.
Fiske and Subbarow (1925). *J. Biol. Chem.* **66**, 375.
Goldblatt (1931). *Science*, **73**, 494.
Green and Mellanby (1928). *Biochem. J.* **22**, 102.
Harris and Bunker (1930). *Amer. J. Pub. Health*, **20**, 1287.
—— —— (1931). *Science*, **73**, 95.
McCollum, Simmonds, Shipley and Park (1922). *J. Biol. Chem.* **51**, 41.
McCrudden (1911–12). *J. Biol. Chem.* **10**, 187.
Mellanby (1921). *Med. Res. Coun. Special Rep. Series*, No. 61.
—— (1922). *Brit. Med. J.* ii, 849.
—— (1924). *Brit. Med. J.* i, 895.
—— (1925). *Med. Res. Coun. Special Rep. Series*, No. 93.
—— (1926). *J. Physiol.* **61**, *Proc.* xxiv.
—— (1930). *Brit. Med. J.* i, 677.
Steenbock and Black (1925). *J. Biol. Chem.* **64**, 263.