CCLXXXII. CEREALS AND RICKETS.

V. THE EFFECT OF GERMINATION AND AUTOLYSIS ON THE RACHITOGENIC PROPERTIES OF THE MAIZE KERNEL[1].

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INTEREST has been centred not only on the effect of maturity on the calcifying properties of grains [Templin and Steenbock, 1933] but also on changes effected by the process of germination. Stepp [1925] observed that the substitution of sprouted grains for the cereal component of McCollum's rachitogenic ration 3143 [McCollum *et al.*, 1921] did not alter its rickets-producing effect.

Mellanby [1926] reported that the rachitogenic property of oats was not materially altered by exposure to a temperature of 100° for 18 hours or by germination for as long as a week, but the combined application of these treatments distinctly reduced interference with bone calcification. He further stated that the same relations held for barley, making the kilned grain a comparatively good cereal product from the point of view of bone formation and much better than the original barley.

György and Schall [1929] extended the study of the rickets-producing property of seeds germinated in the dark to include wheat, barley, oats and beans (Puffbohnen). He used extracts of these germinated seeds prepared with a mixture of alcohol, ether and chloroform of such concentration that 0·1 g. of extract represented 1 g. of seed. No evidence of healing was obtained when daily doses of 0·1 g. of extract were administered to rachitic rats over a period of 8 to 14 days. These workers were also unsuccessful in their attempt to activate ergosterol by exposing it to mitogenetic rays from the roots of hyacinth bulbs.

The observations of Schittenhelm and his co-workers stand in opposition to the results just reviewed. Rubner and Schittenhelm [1926] expressed interest in the promotion of the therapeutic use of preparations made from dried sprouts obtained as a by-product of barley malted in the dark. Schittenhelm and Eisler [1928, 1] claim to have demonstrated the presence of vitamin D in these sprouts by feeding rats with an extract obtained with alcohol, light petroleum and chloroform. They found that 1 or 2 mg. of extract daily were sufficient to induce healing in 8 days. They further state that the active principle follows the phytosterol fraction and is sensitive to oxygen and to light. However, the incompleteness of the data reported as well as the inadequate use of controls make it difficult properly to evaluate these results.

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At a later date these same investigators [1931, 2, 3, 4] reported more carefully controlled experiments strengthening their claim that antirachitic activity is present in dried barley sprouts. In prophylactic tests, when dried sprouts comprised 6 % of the otherwise rachitogenic ration, rickets was prevented, and bone with a normal ash content was formed. Their failure to do more than arrest further development of rickets by feeding the same dried sprouts at even higher levels to rachitic rats they attribute to deranged functions of the digestive tract occurring in rats suffering with rickets. Analysis revealed that twice as much crude fibre was eliminated by the rachitic animals as by normal rats of a similar age. Because of this complication, they found it more satisfactory to work with an extract prepared with a mixture of acetone and light petroleum. Daily doses of 1 mg. of the extract were sufficient to prevent or cure rickets consistently in rats and further fractionation resulted in even more potent products.

Rubner [1930] has reported improvement in the retention of nitrogen, calcium and phosphorus in two patients who were given 50 g. of dried barley sprouts daily; but these results were complicated by the feeding of a bone-marrow supplement.

On the basis of such findings together with other clinical data, Schittenhelm [1928] and in collaboration with Eisler [1928, 2; 1931, 1] has advanced the thesis that the formation of vitamin D is not dependent upon the presence of ergosterol or activation by light. They favour the point of view that it is formed during the process of germination, even in the dark, since they have failed to detect provitamin in the seed from which their preparations were made.

Our objective in the present series of studies was to determine if any alteration in the rickets-producing property of maize occurred during germination in the absence of light. We relied primarily upon prophylactic technique in which the products tested were substituted for the cereal component of a high calcium-low phosphorus rachitogenic ration. A few curative tests were conducted with alcoholic extracts, but the data accumulated are as yet too limited to warrant presentation.

Experimental.

Series I. In order to correlate results with those already published [Templin and Steenbock, 1933], the maize selected for these experiments was of the same origin and was raised in the same field and harvested and stored according to the usual agricultural practice.

The maize was germinated in the following manner. A weighed quantity was thoroughly scrubbed with three changes of distilled water and then treated in a vacuum with a 1·0 % solution of formalin for 10 minutes. After an additional 10 minutes at atmospheric pressure the formalin was drained off, the maize was washed once or twice with sterile water and finally covered and allowed to soak in sterile water from one and a half to six hours. Tinned or galvanised iron pans $12 \times 20 \times 25$ inches were used as germinators. In them was placed a thin layer of clean pine shavings which was covered with two layers of paper towelling. A litre of water was poured in, the pan was wrapped with heavy manila paper and sterilised for 15 minutes in an autoclave at 15 pounds pressure. Approximately 250 to 300 g. of prepared maize were then spread in a single layer between the towelling. Care was exercised to reduce contamination to a minimum. The germinator was incubated at 26°. In the longer germination periods it was found necessary to add more sterile water later. In 48 hours 95 % of the kernels had grown roots not exceeding 1 inch in length and 5 % had roots between 1 and 2 inches in length. In 95 hours only 23 % of the roots were less than 1 inch in length, 65 % between 1 and 3 inches, and 12 % between 3 and 4 inches.

At the termination of the germination period, the maize was ground in a food chopper. A portion was spread in granite-ware pans in a layer not over ½ inch deep and dried before a fan at a temperature of 35 to 37° for 24 to 48 hours. The dry product was ball-milled for incorporation in the test ration.

For autolysis a second portion of the sprouted maize was ground, and approximately 200 g. quantities were placed immediately in 2 quart glass jars. One litre of distilled water, with chloroform and toluene, was added; the cover was lightly closed, and the whole was placed in an incubator for 10 days at 31°. The product was dried in the same manner as the germinated preparation. Difficulty was encountered in desiccating the autolysed corn which had been germinated 95 hours because enough sugars were present to form sticky lumps which failed to release all moisture and toluene even when placed in a $CaCl_2$ desiccator provided with a circulating air current. Final desiccation was attained in an evacuated desiccator kept at 45°. In all it required about two weeks to dry this product sufficiently to allow grinding in a burr mill preparatory to incorporation in the ration. A 90 % recovery of the initial weight of the maize was obtained in the products of maize germinated 48 hours and an 83 % recovery in the case of maize germinated 95 hours.

The dry, ball-milled maize products were substituted for the yellow maize in Ration 2965 [Steenbock and Black, 1925] with the further addition of 4 % yeast known to be rich in the vitamin B complex. This was added at the expense of the wheat gluten fraction.

Seven litters of rats between 24 and 27 days of age were used to provide 6 rats for each test ration. The daily food intake of each rat was restricted to that amount voluntarily taken by the rats with the lowest food consumption. The limiting individuals of the entire series were rats receiving the maize which had been sprouted 95 hours and autolysed 10 days. During the first three weeks approximately three times the requirement of vitamin A was administered to each rat weekly in the form of a crude carotene extract dissolved in corn oil. During the last two weeks a similar amount of vitamin A was fed as red palm oil. Data on the composition of the bones and blood were obtained as described in the preceding publication [Templin and Steenbock, 1933].

Data. The results of this series of experiments are to be found in Table I. It will be noted that weight gains and food consumption were both satisfactory and uniform throughout the various groups. Distinctly the best calcification was obtained with the rations which contained autolysed sprouted maize. Whereas untreated maize and maize sprouted for 48 and 95 hours respectively gave 27·9, 28·4 and 29·6 % of ash, maize sprouted for 48 and 95 hours and then autolysed raised these figures to 46·6 and 56·7 respectively. Thus the sprouting process altered the calcifying activity only to a very slight degree if at all, but when followed by autolysis practically normal bone was produced. Soaking the maize for 16 hours increased the ash content to 35·5 %, but in a later test this result could not be duplicated.

Criteria other than percentage variation in bone ash confirmed the indicated relations. Whereas the blood-serum-calcium was normal, the inorganic phosphorus was very low in all groups except the one receiving maize which had been sprouted 95 hours and autolysed 10 days. This group had a normal value of 7·8 mg. per 100 cc. The width of the rachitic metaphyses and to a lesser extent the enlargement of the costochondral junctions also confirmed these relations.

From the results of this series of experiments it appears that maize germinated in the dark for as long as 4 days does not acquire additional antirachitic

Table I. *Effect of germination and autolysis of maize upon calcification.*

Ration	Body weight		Average daily food consumption	Blood-serum analyses*		Femur data			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 47. Untreated	57	33	7·0	12·4	2·7	0·0976	0·0277	27·9	0·17	Much enlarged, angulation, double beading
R 48. Soaked 16 hours	56	34	7·0	13·2	4·6	0·1094	0·0393	35·5	0·17	Moderately to severely enlarged, angulation and double beading in 2 animals
R 49. Soaked and sprouted totalling 48 hours	57	38	7·0	13·2	3·3	0·1024	0·0293	28·4	0·17	Much enlarged, angulation, double beading
R 50. Soaked and sprouted totalling 48 hours, autolysed 10 days	56	36	7·0	14·0	4·5	0·1307	0·0615	46·6	0·08	Slightly to moderately enlarged
R 51. Soaked and sprouted totalling 95 hours	57	24†	6·9†	11·6	2·7	0·0900	0·0270	29·6	0·15	Much enlarged, angulation, double beading
R 52. Soaked and sprouted totalling 95 hours, autolysed 10 days	57	32	7·0	13·2	7·8	0·1751	0·0977	56·7	0·05	Normal

* Blood of animals having advanced respiratory trouble was not used.
† 2 animals had severe respiratory trouble.

potency. On the other hand autolysis of this germinated maize results in changes which materially aid calcification. However, the effect of heat as a complicating factor was not completely ruled out.

Series II. To determine the part which heat and moisture conditions played in the previous series, the following group of maize rations was fed: (1) germinated 96 hours and autolysed 10 days; (2) germinated 96 hours—stored dry at same temperature as (1); (3) germinated 96 hours—boiled—stored wet at same temperature as (1); (4) soaked 16 hours and dried for 24 hours; (5) ground and dried for 24 hours; (6) untreated.

The maize used was a high grade yellow dent seed maize grown in Wisconsin. The process of germination was conducted as previously described except for the elimination of the soaking before germination and the use of a larger amount of maize, *viz.* 400 g. per pan. Germination proceeded vigorously at 31°. In 96 hours 45 % of the kernels had rootlets 3 to 5 inches in length, 45 % 1 to 2 inches and 10 % less than 1 inch. Autolysis was conducted at 31° using $CHCl_3$ as an antiseptic. By an increase in the temperature of drying to a range of 45 to 49°, it was possible to shorten the time of drying of the autolysed product to 6 days when carried out in open pans before an electric fan. Controls for such heat treatment were provided by the following two preparations. Dried germinated maize was stored alongside the autolysed product both during its autolysis and drying. The other control was germinated maize which had been boiled 5 minutes to inactivate the enzymes, then stored and dried by the methods used in the preparation of the autolysed product. Other groups were included in the series to check the result on the effect of soaking the whole kernels and to determine the result of heat treatment on untreated ground maize.

The prepared samples were fed in Ration 2965 according to the technique described for the preceding series. During the first 13 days of feeding the average daily intake of ration per rat was only 4·5 g. owing to the fact that the rats did not like the autolysed and boiled maize rations. Since this amount was not sufficient to maintain good growth, it was decided to replace only half of the

yellow maize of the ration with these products for the remainder of the 5-week period. This necessitated a similar change in the ration of germinated maize which had been stored under dry conditions. Such an alteration in the dietary regimen resulted in a 7 to 8 g. daily intake per rat and a resumption of satisfactory growth. Calculations in terms of the total ration eaten over the entire experimental period indicate that these prepared products comprised 49 % of the ingested food.

The average results of the data obtained by the same methods employed in Series I are to be found in Table II. Body weight gains of 30 to 40 g. were procured on an average daily food intake of 6·0 g. Such gains were comparable with those obtained in preceding tests.

Table II. *Effect of germination, autolysis and heat treatment of maize upon calcification.*

Ration	Body weight		Average daily food consumption	Blood-serum analyses		Femur data			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 72. Germinated 96 hours and autolysed 10 days	57	30	6·0	13·0	3·4	0·1149	0·0540	46·8	0·06	Slightly enlarged
R 73. Germinated 96 hours —stored dry at same temperature as R 72	58	32	6·0	11·5	3·0	0·0901	0·0265	29·3	0·15	Much enlarged with double beading and angulation
R 74. Germinated 96 hours —boiled, stored wet at same temperature as R 72	59	29	5·9	13·1	4·0	0·1056	0·0419	39·3	0·12	Moderately to severely enlarged
R 75. Soaked 16 hours, dried 24 hours	58	37	6·1	12·2	3·3	0·0997	0·0333	33·3	0·15	Much enlarged
R 76. Ground, dried 24 hours	59	41	6·1	11·6	3·5	0·1070	0·0343	31·7	0·14	Much enlarged
R 77. Untreated	57	39	6·1	12·6	4·0	0·1033	0·0364	35·2	0·13	Much enlarged

The blood-serum picture was as expected, namely, a normal range of calcium and a greatly depressed inorganic phosphorus content without significant variations among the various groups.

As in the preceding series, the ash content of the femurs was highest for those rats fed on the maize which had been germinated 96 hours and autolysed for 10 days. Although the autolysed product had been fed at a level considerably lower than in the preceding series, the femurs contained 46·8 % ash. The control ration of the cooked maize produced femurs containing 39·3 % ash. These figures are respectively 17·5 % and 10·0 % higher than those obtained with the germinated maize (29·3 %); but only 11·6 and 4·1 % higher than values obtained with the untreated maize (35·2 %). Moreover when consideration is given to the fact that the value obtained with germinated maize is 5·9 % lower than that obtained with untreated maize, the significance of the difference in calcification produced by the cooked maize, if any, becomes questionable. On the other hand, the values obtained with the autolysed product indicate the formation of distinctly better calcified bone even when these relations are taken into account. The percentage of ash (46·8 %) obtained with the autolysed maize also compares favourably with the 56·7 % obtained in the preceding series when allowance is made for the difference in levels of this constituent in the two rations, 49 % in the former and 76 % in the latter. Sprouting of the maize *per se* gave no

evidence of increasing its antirachitic activity; in fact the percentage of femur ash was actually lowered in this series. Such a failure to demonstrate any change in antirachitic activity due to the process of germination is in accord with the results obtained in the earlier test. The slight increase in calcification observed when whole kernel maize soaked for 16 hours was fed in the preceding series of experiments was not substantiated in this series. In fact the 33·3 % ash obtained was nearly 2 % below that found with untreated maize. Neither did heating the ground maize for 24 hours produce any significant alteration from the result obtained with the untreated maize. The percentages of ash were 31·7 and 35·2 % respectively.

These relations, based upon a comparison of the ash content of the femurs, were substantiated not only by the average weights of the extracted femurs and the average weights of ash but also by the ranges of individual values from which these averages were calculated. Confirmation was also obtained from the width of the uncalcified metaphyses observed in the silver nitrate staining test and to a lesser degree by the extent of involvement of the costochondral junctions.

Series III. Further tests of the effect of heat and moisture upon ungerminated and germinated maize were necessary to interpret properly the results already obtained. To this end the following maize products were prepared: (1) untreated; (2) ground, moistened, dried 27 hours; (3) ground, moistened, dried 10 hours; remoistened, dried 17 hours; (4) germinated 96 hours, dried 27 hours; (5) germinated 96 hours, dried 10 hours; remoistened, dried 17 hours; (6) germinated 96 hours, dried 2 weeks.

It was necessary to determine if heat and the presence of moisture had a different effect upon the germinated maize from that which they had on the untreated seed. Furthermore, it was possible that heat functioning in the presence of moisture might have an effect different from that in the absence of moisture.

The ground products were spread loosely in granite-ware pans in a layer of about a half an inch in depth. The conditions of drying approximated to those used in the process of desiccating former preparations. For the first 10 hours the temperature was held between 47 and 50°, during the following 12 hours the lowest point reached was 33°; throughout the final 5-hour period it was again maintained between 47 and 50°. Moisture additions were made in amounts barely sufficient to cover the ground corn and the pans were immediately placed in front of the fan.

As in the former series these maize preparations were fed as the cereal portion of Ration 2965 in the same manner as described for Series III in a previous publication [Templin and Steenbock, 1933].

Six rats from as many litters and ranging in age from 23 to 30 days were placed on each ration for a five-week feeding period in which the food consumption of all rats was maintained at the same level.

The data are presented collectively in Table III. Regardless of whether or not the maize had been germinated or subjected to varying types of heat and moisture treatment below a temperature of 50°, all rats became very rachitic. The untreated maize produced femurs containing 32·7 % of ash. After moistening and drying, this same maize produced femurs with 31·5 and 33·3 % ash content. The ash contents of femurs produced on the ration containing germinated corn subjected to 27 hours of drying were 27·1 and 30·5 %. An extension of the time of drying to 2 weeks did not alter the rachitogenic property of the germinated maize, for the ash content of the femurs was 30·8 %. These same relations were found for the weights of extracted femurs and the weights of

Table III. *Effect of heat treatment and germination of maize upon calcification.*

Ration	Body weight		Average daily food consumption	Blood-serum analyses		Femur data			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 91. Untreated	61	44	6·9	13·6	3·5	0·1085	0·0355	32·7	0·17	Much enlarged, some angulation and double beading
R 92. Ground, moistened, dried 27 hours	60	43	6·9	12·8	4·6	0·1081	0·0341	31·5	0·18	,,
R 93. Ground, moistened, dried 10 hours; remoistened, dried 17 hours	59	45	6·9	11·0	4·2	0·1095	0·0365	33·3	0·18	,,
R 94. Germinated 96 hours, dried 27 hours	61	34	6·8	11·6	4·0	0·0994	0·0269	27·1	0·18	Much enlarged, severe angulation, triple beading
R 95. Germinated 96 hours, dried 10 hours; remoistened, dried 17 hours	61	33	6·8	—	—	0·1001	0·0307	30·5	0·19	Much enlarged, moderate angulation and some double beading
R 96. Germinated 96 hours, dried 2 weeks	59	36	6·9	11·6	4·1	0·1011	0·0312	30·8	0·17	Much enlarged, moderate angulation, double beading

femur ash. Confirmation of the very rachitic condition of all the groups of animals was obtained in the low level of blood-serum-phosphorus, in the presence of wide rachitic metaphyses in the wrist bones and in the manifestations of severe involvement of the costochondral junctions.

Good growth was obtained on an average food intake of 6·9 g. per rat per day. The fact that the average weight gains made by rats on the ungerminated maize were 9 g. greater than on the germinated maize is not, in our opinion, a complicating factor in the interpretation of the results of this series. Neither is it significant in itself since such a difference was not uniformly obtained in the previous tests.

The results of this series again demonstrated that the germination of maize did not alter its rachitogenic property. Furthermore the heat treatment incident to the process of drying the maize products was not responsible for the variations in calcification observed with immature maize.

Discussion.

It is probably premature to attempt to give an explanation of the results obtained by Schittenhelm and his co-workers in the light of our results. Whereas our studies dealt with maize, the German workers fed only dried barley sprouts or extracts thereof. Mellanby *et al.* [1928; 1929] postulated that vitamin D may be formed from ergosterol by the growing plant independently of ultra-violet radiations. De Ruyter de Wildt and Brouwer [1932] reported that barley meal prepared from the entire grain contains vitamin D, whereas that prepared from maize does not. Proof of the above statements is not at present forthcoming and other possibilities can be entertained. For instance, since calculations based on data reported by Täufel and Rusch [1929] indicate that the weight of dry barley sprouts equals less than 4 % of the weight of the barley from which they are formed, it is possible that the Schittenhelm group of workers fed more concentrated products than investigators who used the entire grain and sprouts or even extracts prepared from them. Perhaps the specific rachitogenic factor

referred to by Mellanby [1925] was retained in the grain, thus allowing the nutrients in the sprouts to function without the presence of such antagonistic effects; or the rachitogenic factor may have been destroyed during the progress of germination. Again, the conversion of phosphorus compounds into forms more available to the growing animal would not be revealed by ordinary analysis but would have the same effect as an increase in the phosphorus of the ration. Some of these considerations apply equally well to the interpretation of the results reported here in connection with germinated and autolysed maize.

SUMMARY.

Yellow maize germinated for 95 hours, with roots not exceeding 4 inches in length, was found as rachitogenic as ungerminated maize.

Germinated maize dried below 50° had practically the same rachitogenic properties as the maize from which it had been prepared.

Autolysed germinated maize was definitely less rachitogenic than germinated or untreated maize.

The antirachitic effect of autolysed germinated maize was most pronounced in maize which had been germinated for a long period of time.

Soaked whole kernels and moistened ground maize dried by the same methods as those employed for the sprouted products did not differ from untreated maize in rachitogenic properties.

Heat treatment of ground maize at a temperature less than 50° did not alter its rickets-producing tendency, neither did germinated maize subjected to this temperature for 2 weeks undergo a change in this property.

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REFERENCES.

György and Schall (1929). *Jahrb. Kinderheilk.* **124**, 260.
McCollum, Simmonds, Shipley and Park (1921). *J. Biol. Chem.* **47**, 507.
Mellanby (1925). *Med. Res. Coun. Special Rep. Series*, No. 93.
—— (1926). *J. Physiol.* **61**, *Proc.* xxiv.
—— Surie and Harrison (1928). *J. Physiol.* **65**, *Proc.* xxix.
—— —— —— (1929). *Biochem. J.* **23**, 710.
De Ruyter de Wildt and Brouwer (1932). *Biedermanns Zentr.* Abt. B, *Tierernährung*, **4**, 573.
Rubner (1930). *Deutsch. med. Woch.* **56**, 295.
—— and Schittenhelm (1926). *Deutsch. med. Woch.* **52**, 2065.
Schittenhelm (1928). *Klin. Woch.* **7**, 1214.
—— and Eisler (1928, 1). *Z. ges. exp. Med.* **58**, 645.
—— —— (1928, 2). *Klin. Woch.* **7**, 118.
—— —— (1931, 1). *Klin. Woch.* **10**, 1014.
—— —— (1931, 2). *Z. ges. exp. Med.* **75**, 737.
—— —— (1931, 3). *Z. ges. exp. Med.* **75**, 745.
—— —— (1931, 4). *Z. ges. exp. Med.* **75**, 758.
Steenbock and Black (1925). *J. Biol. Chem.* **64**, 263.
Stepp (1925). *Z. Biol.* **83**, 99.
Täufel and Rusch (1929). *Biochem. Z.* **209**, 55.
Templin and Steenbock (1933). *Biochem. J.* **27**, 2055.