possible that the continuous feeding of *A. aerogenes* and *E. coli* materially aided the antibiotic in reducing the numbers of less desirable organisms in the intestinal tract, thereby permitting more rapid chick growth.

Summary. Pure cultures of *E. coli* and *A. aerogenes* were grown, lyophilized, and fed to chicks as dietary supplements both in the presence and in the absence of procaine penicillin G. Little or no chick growth response was obtained when either of these organisms were added to the ration in the absence of the antibiotic. Greater gains were obtained when 10 ppm procaine penicillin G were fed. When viable cultures of *A. aerogenes* and *E. coli* were fed in combination with penicillin, growth was further increased significantly. The effectiveness of the antibiotic in promoting chick growth was increased 64 and 80% when these organisms were added to the feed. The results obtained illustrate the influence of bacterial environment on the antibiotic growth effect and in nutritional studies.

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Skim Milk Powders and Experimental Rat Caries. (20252)

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In a previous publication(1) it was reported that a diet containing 4 heat-processed cereal foods and 18% cerelose produced dental caries in white rats. The results were important because of the type of caries which developed on the smooth surfaces of the teeth, and because neither an excessive quantity of sugar nor coarse food particles were implicated as cariogenic factors. Moreover, the results suggest that serious attention should be given to the possibility of an effect of nutritive deterioration which may accompany the heat-processing of foods, on the etiology of experimental dental caries in white rats.

Perhaps no constituent of the diet has been more widely studied for the effects of heat-processing, than have the skim milk powders. Such studies are of obvious importance when it is realzed that in 1951 approximately 700,000,000 lb of skim milk powder, and 130,000,000

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lb of whole milk powder were produced in the United States for human consumption, particularly in bakery products, confectioneries and ice creams(2). In general, there is adequate evidence in numerous previous studies that skim milk powders during preparation and storage undergo nutritive losses in the protein fraction, as shown by effects on growth, digestibility and biological value(3-7). However, none of the published results of studies on nutritive properties of skim milk powders *per se* have reported observations on dental caries. In one prior abstract report on dental caries in white rats, placed on "low fat, high sugar diets consisting chiefly of skimmed milk (powder) and cane sugar, supplemented with cod liver oil," surface caries were observed "on the buccal and lingual surfaces of both the upper and lower molar teeth of albino rats in addition to proximal surface and occlusal fissure caries"(8). The Syrian hamster also may develop a cervical type of dental lesion on buccal and lingual surfaces when fed a diet composed essentially of cornstarch and whole milk powder(9). In these and other prior caries studies the cariogenic role of milk powders in the diets may have an added significance in the light of the results of this experiment. The incrimination of heat-processing or storage deterioration of foods such as cereals and milk powders, in the production of dental caries is a relatively new approach in experimental caries research.

Materials and plan. The primary object of these experiments was to study the cariogenic properties of several dry skim milk powders as obtained from commercial sources, and to determine the effect of an additional heat-treatment on their cariogenic action. One spray-process and 2 roller-process powders were studied before and after being given an additional heat-treatment. The milk powders were present at a 35% level in all the diets. The other components of the diets were: 18% glucose (commercial cerelose), 45.0% cornstarch and 2.0% dehydrated liver (excepting Diet 598 which contained 63.0% cerelose and no cornstarch). A concentrated vit. A, D and E supplement was given by mouth weekly. The diets were finely powdered before they were fed.

The analysis of the diets was as follows:

Diet No.		598	614	615	616	633	634	635	636
Protein (Nx 6.25),	%	13.56	14.31	13.68	13.56	13.25	13.50	13.56	13.75
Ash	%	3.04	3.04	3.02	3.04	2.83	2.92	3.01	2.92
Calcium	%	.48	.46	.44	.44	.44	.43	.47	.46
Phosphorus	%	.38	.39	.38	.39	.36	.36	.35	.36
Fluorine (ppm)		.4	.5	.5	.5	.9	.9	1.0	1.0

Considering the lactose present in skim milk powder, the total lactose plus cerelose content of Diet 598 was 81.2%; the other diets 36.2%. The milk powders present in the diets and the heat treatments were as follows: *Powder A* was an improved low-heat spray-process, non-fat dry milk powder. *Powder B* was prepared by placing powder A about one-half inch deep in a pan sealed with adhesive tape(5) and heating in an autoclave for 15 minutes at approximately 121°C. *Powder C* was a roller process non-fat dry milk powder from the same commercial source as powder A. *Powder D* was prepared from powder C by the same heat treatment described for powder B. *Powder E* was a roller process powder obtained from a commercial source different from powder C. *Powder F* was prepared by placing powder E one to 1½ inches deep in a sealed pan and heating in an autoclave 15 minutes at approximately 121°C. *Powder G* was prepared by placing powder E one to 1½ inches deep in an open pan and heating in an autoclave 15 minutes at 15 lb. Milk powders B & D, heated in closed pans, were subjected to essentially a dry heating and were a pale cream color only slightly darker than the unheated powders from which they were prepared. Milk powder G, which was heated in an open pan received the full effect of the moist heat of autoclaving and was a dark brown color. This additional heat-treatment may be compared in some respects to heating processes required for the preparation of certain commercial foods which contain skim milk powders. The *moisture and nitrogen* contents of the raw milk powders were respectively 3.83% and 5.61%, powder

TABLE I. Results of Feeding Diets Containing Heated and Unheated Skim Milk Powders on the Caries Experience of Young White Rats.

Exp. No.	1	2	3	4	5	6	7	8	9
Diet No.	633	634	635	636	598	614	615	616	616
Skim milk powder	A	B	C	D	E	E	F	G	G
" " " heated	—	+	—	+	—	—	+	+	+
No. of rats	40	40	39	38	35	40	39	39	30
" " litters	34	33	30	29	7	17	17	8	22
Days on exp.	90	90	90	90	91	91	91	35	42
Initial wt	26.7	26.9	26.3	26.2	31.6	28.2	28.2	27.5	27.8
Final "	216.7	83.6	199.1	46.7	163.2	193.2	178.8	23.5	23.6
Avg daily gain	2.11	.63	1.92	.23	1.45	1.81	1.65	−.10	−.10
				Caries incidence—%					
Carious rats	12.5	40.0	51.3	84.2	48.6	57.5	74.4	56.4	23.3
Occlusal carious rats	.0	.0	7.7	5.3	8.5	.0	5.1	.0	.0
Surface " "	12.5	40.0	43.6	84.2	42.9	57.5	71.8	56.4	23.3
				Caries distribution and severity—No./rat					
Upper carious teeth	.00	.08	.03	.13	.09	.00	.10	.00	.00
Lower " "	.20	1.03	1.00	2.82	1.40	1.35	2.05	1.69	.57
Total " "	.20	1.10	1.03	2.95	1.49	1.35	2.15	1.69	.57
Occlusal carious teeth	.00	.00	.15	.11	.29	.00	.15	.00	.00
Surface " "	.20	.10	.87	2.89	1.23	1.35	1.97	1.69	.57
Occlusal carious areas	.00	.00	.21	.13	.31	.00	.15	.00	.00
Lingual " "	.00	.00	.00	.05	.09	.00	.00	.00	.00
Buccal " "	.28	1.78	1.15	4.08	1.89	1.93	3.03	2.64	.60
Score/rat	.30	2.63	2.72	7.11	2.57	2.15	3.95	2.85	.67
Score/carious rat	2.40	6.56	5.30	8.44	5.29	3.74	5.31	5.05	2.86

A; 3.31% and 5.63%, powder C; and 4.13% and 5.24%, powder E. Powders A, B, C, and D were kept at approximately 5°C as were all the diets; powders E, F, and G, were stored in a constant temperature room at 20°C. *Two strains of rats* were used in these studies, Sprague-Dawley in Exp. 5, 6, 7, and 8, Holtzman in Exp. 9, and both strains in Exp. 1, 2, 3, and 4. In the latter 4 experiments the results on both strains were essentially the same and the data were pooled to obtain the final results. Litter mates were used to compare heated *vs* unheated milk powders and for the comparison of powder A *vs* powder C. The rats were started at weaning age, were fed *ad libitum* in screen-bottom cages (2 rats per cage) and were given distilled water. At the end of 90 or 91 days, or when the rats died, as in Exp. 8 and 9, the heads were autoclaved, cleaned of adhering soft tissues, and the teeth, *in situ*, kept under water in the cold. The dental examinations were made with a low-power microscope, starting with the teeth in the moist state and continuing while the tooth surfaces dried in the heat of the microscope lamp. The dental lesions were recorded and scored as follows: occlusal, lingual and buccal surfaces were divided into two or three areas (interproximal surfaces were divided between lingual and buccal surfaces) and each carious defect was scored one, 2, or 3, according to the extent of the carious destruction. As has been mentioned previously(1) the initial surface-caries process was observed as a white, opaque, usually elongated area, following most generally the line of the gingival attachment. Frequently these opaque areas were small and did not seem to penetrate the dentin; in other instances a very considerable destruction of both enamel and dentin occurred. The final diagnosis of the dental defects included 3 essential items as shown in Table I, *i.e.*, a) the per cent of affected rats in the group, b) the number and location of the affected teeth, and c) the severity of the caries as indicated by the number of surface areas involved and the caries score. It should be emphasized that our evaluation of the data on the experimental caries diagnosis includes all 3 of the above essential items.

Results. The combined data on the caries

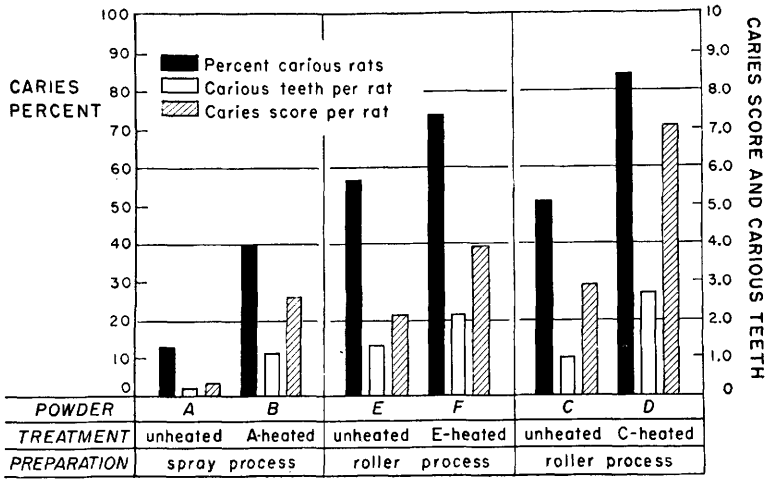

FIG. 1. Incidence and severity of dental caries in white rats fed diets containing heated and unheated dry skim milk powders.

diagnosis and growth effects of the various diets are shown in Table I. The most serious growth failures occurred in Exp. 8 and 9, in which the milk powders were most drastically heat-treated. All the rats in these 2 experiments died by the end of 45 days. Diets 634 and 636 were both inadequate for growth although Diet 634 promoted slightly better growth than Diet 636. Of the unheated milk powders, powder A proved most satisfactory for growth, powder E unheated in Diet 614 and heated in Diet 615, promoted better growth than in Exp. 5, Diet 598. The explanation of differences in growth resulting from heat-treated milk powders seems to relate to the severity of the heat-treatment. The depth of the milk powder in the pan or other unrecognized variations during heating, may have influenced the heat-effects and explain the much better growth results obtained with powder F (Diet 615) *vs* powder D (Diet 636).

The cariogenic effects of the 3 unheated milk powders were most pronounced for powder C (Diet 635) and powder E (Diet 614 and Diet 598), all of which were prepared commercially by the roller-process, whereas powder A, the low-heat, spray-process powder used in Diet 633, was only slightly cariogenic (Table I). There is considerable uniformity in the results obtained with the two roller-process powders C and E, as fed in Diets 598, 614 and 635. It is of interest that these similar effects occurred when as much as 67% cerelose was present in Diet 598 as compared with only 18% cerelose in Diets 614 and 635. A direct comparative study of the effects of heat-treatment on the milk powders was made in Exp. 6 (powder E) *vs* Exp. 7 (powder F); Exp. 1 (powder A) *vs* Exp. 2 (powder B); and Exp. 3 (powder C) *vs* Exp. 4 (powder D). In all these three trials of milk powders, A, C, and E, heated and unheated, a greater incidence of caries and more severe caries resulted from the heated powders (Table I and Fig. 1). This enhanced cariogenic effect is particularly apparent in the comparison of unheated powders A and C *vs* heated powders B and D.

The results with Diet 616 (heated powder G) are somewhat contrary to the results with other heated powders although it is possible that the early death of these rats which permitted only a 35-42-day feeding period caused this limited development of caries.

A survey of the caries data shown in Table I brings out the pertinent fact that the majority of the carious areas occurred in the lower teeth, and on buccal surfaces and that there were practically no carious areas on the occlusal or lingual surfaces. This result is a notable contrast to our previous observations that coarse particle corn meal diets(10) and purified high sugar diets(11) produce

only a fissure type caries on occlusal surfaces. In some respects it would appear that the surface type caries rather than the occlusal fissure caries in experimental animals bears the closest resemblance to human dental caries (1,9).

Discussion. The results of these experiments agree with our previous evidence(1) that a nutritive deterioration of certain processed cereal foods, as brought about in their commercial preparation or by heat-treatment may be associated with the production of carious defects in white rats' molar teeth. In these continuing studies with skim milk powders, the same specific type of surface carious defects have been produced, and there appears to be every reason to incriminate an effect of heating skim milk powders, as one causative factor. The significant difference in the dental results obtained with milk powder A (Diet 633), a low heat spray-process powder *vs* the 2 roller-process powders C (Diet 635) and E (Diets 614 and 598) seem to relate a causative factor with the method of preparation of the 2 types of powders.‡ The spray-process powder was definitely less cariogenic, than the 2 roller-process powders. Substantial evidence that an additional heat-treatment of the 3 milk powders aggravated their cariogenic properties is present in the 3 comparable studies of these powders before and after heating (Table I and Fig. 1). In each of these 3 comparisons the diets containing the heat-treated powders were more cariogenic than the comparable unheated powders.

The nutritive losses which occur in skim milk powders are generally attributed to highly complex chemical changes in the milk proteins. There is conclusive evidence of a major loss of lysine and also evidence of some partial destruction or inactivation of several other essential amino acids(3,6,7,12). Our observed failures in growth are more than likely due to these well-established changes in milk protein values. However, there is no evidence which permits at this time any identification of such changes in protein values with the production of the carious lesions observed in these experimental rats. The role of cerelose as a causative factor is certainly not suggested and mineral and vitamin deficiencies seem unlikely causative agents at this time. Variations in dietary fluorine do not explain the caries differences. It should not be overlooked nevertheless, that any attempt to explain the relation of a dietary factor to dental caries etiology ordinarily cannot avoid considering saliva chemistry, the oral flora, the oral tooth surfaces and both the organic and inorganic chemistry of the teeth.

A comparison of the caries data with the growth data for powders A and C suggest that the cariogenic activity of these powders may not be related to their ability to promote normal growth. Thus the rats given powder C gained only slightly less than their litter mates on powder A, but the rats on powder C had more than 4 times as much caries, and according to average scores, their caries was almost 10 times as severe as that of rats given powder A. Similarly, the growth differences in rats given powders E and F are not accompanied by comparable differences between the two groups in caries incidence or severity.

These results on growth as compared with cariogenicity seem to suggest therefore that the cariogenic factor is possibly not the same as the growth inhibiting factors. Perhaps some additional support of this idea is contained in the observation that heat-processing of powder A (*i.e.* powder B) caused a drastic failure in growth but not a correspondingly high incidence of caries, nor was caries as severe as occurred from powder C. A question regarding the nature of the changes which occurred in preparing powders A and C and the subsequent heat-treatments is raised by the fact that powder B was not as cariogenic as powder D although both were heat-treated

‡ According to the Bureau of Dairy Industry, U.S.D.A. (BDIM-Inf. 25, 1949) dried milks made by the spray-process are finely divided, very dispersible in water, and hydroscopic, while in contrast, because of the high temperatures necessary for drying in the roller-process, there may be a slight discoloration of the product and a partial coagulation of the proteins which makes them "insoluble." The roller powder is practically nonhydroscopic. The roller process is used mainly in the drying of skim milk and buttermilk, while the majority of whole milk powder is prepared by the spray-process.

powders. Likewise powder B although heat-treated was no more cariogenic than the unheated roller-process powder C.

Finally it may be pointed out that additional studies are under way not only to further evaluate the relation of varying heat-treatments to cariogenicity, but to study also the effects of storage of milk powders under controlled conditions of temperature and humidity. As in previous observations on experimental rat caries, caution is indicated in drawing conclusions in the light of the unresolved variations in dental caries susceptibilities which occur among different strains of rats and in different research laboratories. Litter susceptibilities to caries varied in these studies as in previous studies(1,10,11). These results, it should be emphasized, were obtained with two strains of rats, the Holtzman and Sprague-Dawley, as produced in the stock colony maintained at the National Institutes of Health.

The effects of heat-treatment on the development of caries gains added emphasis however when it is realized that in all these 3 experiments the rats compared with litter mates, and the diets were identical in all respects except the source and method of preparation of the milk powders and their subsequent heat-treatment.

Summary. Previous studies on experimental rat caries suggested the possibility of a relation between heat-processed cereal foods and dental caries etiology. Continuing this type of study, the cariogenic role of heat-processed skim milk powders, has been investigated. Diets containing 35% of commercial skim milk powders prepared by the usual spray and roller processing and also given a subsequent heat-treatment prior to feeding, have been identified with the production of a specific type of surface caries in white rats. The extent and severity of the caries was significantly and consistently increased by heat-treatment of these commercial skim milk powders. The results suggest that a cariogenic effect may be produced by heat-treatment of dry skim milk powders. According to present evidence this cariogenic effect has not been identified with the nutritive growth failures usually associated with heat or storage deterioration of milk proteins.

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