No other selection or restriction of type localities seems to us called for, and no other such restrictions seem to us legally binding on other workers. We therefore regard the restrictions of Smith and Taylor as without legal status ("incompetent, irrelevant, immaterial") and do not consider them as binding on us or other workers.

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The Effect of Distillers' Solubles Containing Fluorine on the Development of Dental Enamel in Swine's Teeth [1, 2]

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This report is the result of a surprise finding of hypoplastic lesions in the enamel of the developing teeth of some swine that were being studied as "normal" animals. The experimental material upon which this investigation was based was obtained[1] in order to make supportive studies of normal development of the teeth and jaws for comparison with a large group of experimental swine. The discovery of developmental defects in well-bred swine that had been raised under ideal conditions pertinent to nutritional experimentation gave emphasis to the desire to find the cause for these lesions. Inasmuch as the animals were raised on a series of high-quality rations for the purpose of observing rate of growth and weight gains, it seemed doubtful that nutritional insufficiency (*1, 2*) would be a likely cause for the lesions. An examination of the rations fed (Table 1) will support this view. Also, since no sickness had been reported in the swine, it was not reasonable to believe that the lesions were the result of infectious processes. The most likely field for investigation seemed to be in relation to effects of some toxic substances that may inadvertently have got into the diet (*3, 4*). An examination of the diets, for the purpose of finding possible toxic substances, led to a consideration of distillers' solubles, which made up 10% of the diet of 5 animals. A discussion of these substances with our associate bacteriologist, J. L. Nemes, who has had personal experience in large distillery operations, led us to consider the possible presence of fluorine in the diets. It is the practice of many large distilleries to incorporate fluorine ($NH_4F \cdot HF$) into the mash during the brewing process in order to inhibit bacterial development (*5, 6*). Since the yeasts have been acclimatized to bifluoride during preparation, this does not interfere with fermentation. In subsequent distillation processes, the fluorine remains in the residue or "slops." These by-products of distillation frequently are used to supplement basal rations fed to livestock (*7, 8*).

The swine obtained for this study were from 26 to 29½ weeks old, 206–225 lbs in live weight, and were equally divided between the sexes. They were well-bred, as indicated in Fig. 1. Mandibles were disarticulated and split at the symphysis. Roentgenograms were made of both sides of each mandible. Primary sections were made for low-power microscopic study by cutting through the undecalcified mandibular teeth and bones by means of high-speed cutting disks, as previously described (*9*). These sections, 0.5–1.5 mm in thickness, were studied under reflected light through a research binocular microscope. There were 9 permanent first molar teeth included in the sections and 10 developing second molar teeth, all from the right half of the mandibles. Other histologic sections were made from decalcified, celloidin-embedded tissue, for high-power microscopic study. The celloidin sections included 5 developing second molar teeth.

Roentgenograms of the mandibles showed no changes in periosteal bone formation similar to those reported as resulting from fluorine intoxication (*10*). There were some irregularities in bone density, but this followed no characteristic pattern. The development of the teeth and jaws was as nearly equal among these swine as one would expect considering their variation in age.

The primary sections revealed developmental defects in enamel formation that ranged from a complete break in the contiguity of enamel in 2 second molar teeth (Nos. 278 and 7,675) to a mere thinning or gnarling of the enamel in a number of the others. A diagrammatic illustration of these changes is shown in Fig. 1. It was observed that only 1 first molar tooth (No. 7,823) contained a developmental fault. The characteristic region which seemed susceptible to developmental interference was an area about one third the distance from the occlusal surface, on the buccal aspect of the second molar teeth. Seven of the swine showed some irregularities in the architectural pattern of the enamel in this region, but animals 271, 278, and 7,675 were outstanding in this respect. Other irregularities were noted on the occlusal surfaces of 5 swine. A low-power picture of a developing second molar tooth with typical hypoplasia of the buccal and occlusal surfaces is shown in Fig. 2. A high-power photomicrograph of a hypoplastic lesion observed in the enamel of swine No. 271 is shown in Fig. 3. In this animal there was some enamel covering the dentin over all the tooth shown, but there was a definite fault over the buccal surface. Similar lesions were observed in

[1] Eleven swine heads were obtained from the Agricultural Research Center, Bureau of Animal Industries, USDA, Beltsville, Md. Ten of these were sectioned for study. We are indebted to John H. Zeller and N. R. Ellis, of the Bureau, for assistance in obtaining the swine tissue, feed samples, and the data on nutrition and swine characteristics of Table 1.

[2] Acknowledgment is made of the fluorine analysis of feeds done by the Food Division of the Food and Drug Administration.

[3] CDR, DC, USN. The opinions or assertions contained herein are those of the writer and are not to be construed as being official or as reflecting the views of the Department of the Navy or the naval service at large.

TABLE 1

Hog No.	Sex	Breed	Sire	Dam	Age (weeks)	Live shrunk wt at slaughter	Ration fed*	Presence of lesions	
								Primary section	Celloidin section
271	M	Crossbred	L-D-H	L-LB	27	212 lb	B	++++	+++
276	F	"	"	"	27	213	B	++++	
278	F	"	"	"	27	225	B	++++	++
318	F	"	"	Y-D-L-H	26.5	210	E	–	+
7,975	F	Landrace-Duroc Hampshire			26.5	221	C	–	
7,675	F	Chester White-Landrace			29.5	206	B	–	
7,823	M	Landrace			28.5	212	B	+++	
7,934	M	Yorkshire-Duroc-Landrace-Hampshire			27	216	A	++	+
7,983	M	Landrace			26.5	206	E	–	
8,013	M	Landrace-Duroc			26	216	C	–	

* The composition of the various rations follows. These are considered high-quality rations.

Ration	A	B	C	E
Yellow corn	68.5	66.0	69.5	70.0
Soybean meal	12	10.5	24.	10.5
Meat and bone scrap	7.	7.		
Alfalfa meal	7.	5.	5.	6.
Linseed meal	6.			6.
Corn distillers' soluble		10.		
Mineral	1.5	1.5	1.5	1.5
Vitamin B_{12} concentrate			2.1 mg/100 lb	
Tankage				3.0
Fishmeal				3.0

Protein content of rations A, B, C,—17.5 to pigs from 30 to 125 lbs
" " " " " —14.5 " " " 125 lbs to slaughter wt

animal No. 278. Table 1 includes a listing of lesions observed.

Three samples of distillers' solubles used in preparing the ration fed to hogs that received ration B were obtained from the Agricultural Research Center, Beltsville, Md. These samples were analyzed for fluorine by

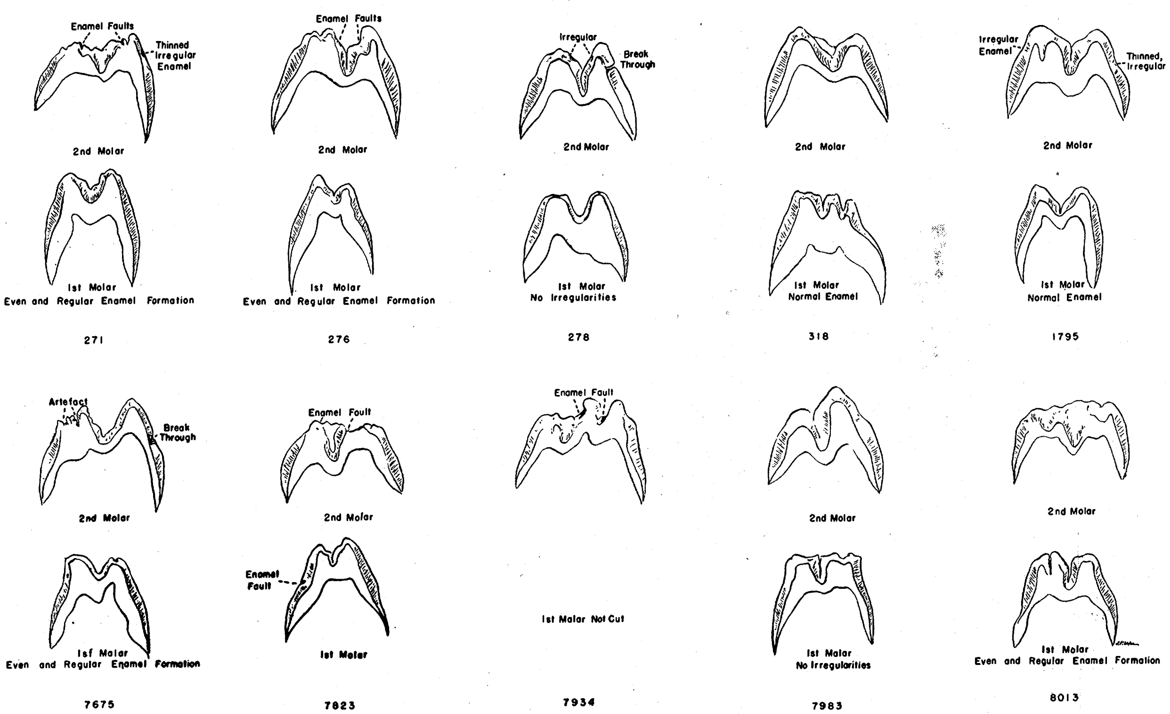

FIG. 1. Diagrammatic illustration of enamel formation in teeth of 10 hogs raised on varied high-quality rations.

the Food Division of Food and Drug Administration. The results are shown in Table 2. The malt process and fungal amylase process solubles were processed by one distillery for a special study conducted by the Beltsville Agricultural Research Center, and the composite sample was made up of equal parts of solubles obtained from four of the leading distilleries of the country.

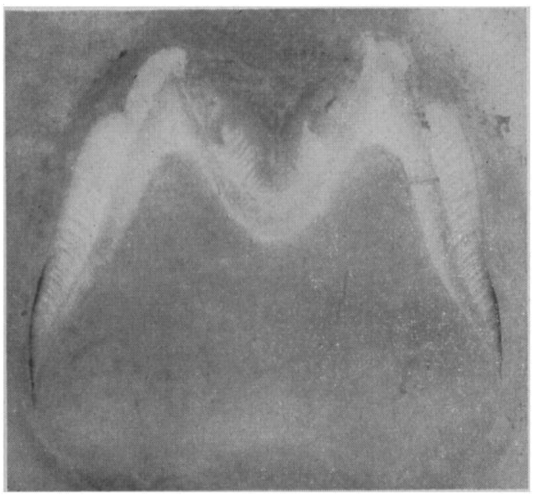

FIG. 2. Buccolingual section through developing second molar tooth, hog No. 271. Break in contiguity of the enamel on the buccal and occlusal surface of this tooth leaves an area of dentin entirely denuded. Inferior to the developmental defect the enamel appears to be normal. No abnormality in the dentin or pulp is evident. (×21.)

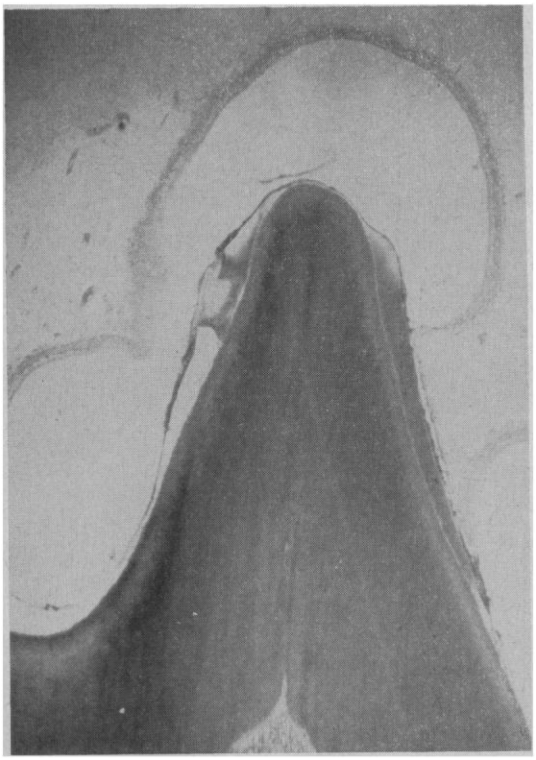

FIG. 3. Celloidin section, hematoxylin and eosin, of decalcified developing second molar tooth, swine No. 271. Decalcification has removed most of the highly inorganic enamel. A thin band of enamel remains over most of the tip of the buccal cusp. There is a hypoplastic lesion on both the buccal and lingual aspects of this cusp. A band of ameloblasts outlines areas where normal enamel has been present, but few ameloblasts are seen in the hypoplastic region. Cells of the stellate reticulum fill the lesion, which is devoid of enamel. The dentin has prominent imbrication lines approximately paralleling the amelodentinal junction. (×5.)

The observations reported herein were made on tissues from 10 swine on varied diets, and a chemical analysis was made of one component which formed 10% of the diets of 5 animals. The distillers' soluble

TABLE 2

Sample No.	Origin of sample	Animals receiving this ration (No.)	F as fluoride (ppm)
I	A malt process corn distillers' soluble	271 276 7,675	115
II	Fungal amylase process soluble	7,823	224
III	A composite sample of corn distillers' solubles	278	24

component of the diets was analyzed for fluorine because the lesions observed were suggestive of a toxic response and because of evidence that bifluoride is sometimes used as a bacterial inhibitor in brewing processes. Inasmuch as this investigation was not planned or specifically controlled for the purpose of relating these substances to toxic effects, the actual finding of fluorine in the rations of 5 of the swine in which serious lesions were observed is not conclusive evidence of a cause-and-effect relationship. In view of the fact that many studies reported in the literature have shown that teeth and bones are subject to developmental changes by the addition of relatively small quantities of fluorine (*3, 7, 10–13*), it is believed that these observations should be reported as specific cases wherein fluorine in the diet may be a factor. Nutritionists and stock raisers should be informed of the possibility of fluorine being present in feed supplements in quantities approaching a toxic level.

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