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Fluorine in Animal Nutrition

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FLUORINE IN ANIMAL NUTRITION

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INTRODUCTION

It has been repeatedly demonstrated by numerous investigators that rations composed of cereal grains and their by-products are low in calcium and, to some extent, in phosphorus. Therefore, such rations should be supplemented with materials which contain large amounts of these two elements, since they are essential to bone formation and other life processes. Bone meal, a by-product of the meat-packing industry and the manufacture of gelatin, was recommended as a mineral supplement to cereal grain rations for swine. Such a rapid increase in the demand for this material resulted that the available supply became inadequate, and, consequently, the price was increased. In seeking another and possibly cheaper source of calcium and phosphorus, rock phosphate was considered. This mineral contains approximately the same amounts of calcium and phosphorus as bone meal and, therefore, might be expected to replace bone meal efficiently as a mineral supplement for livestock and poultry at a considerably lower cost. However, in the attempts to use rock phosphate for this purpose uniformly good results have not always been obtained.

Rock phosphates are known to contain appreciable amounts of fluorine. Jacob and Reynolds (25) made exhaustive studies of the fluorine content of various rock phosphates from the principal deposits of the world and found this element present in amounts ranging from 2.62 to 4.08 per cent in the different grades of the commercial materials. The fluorine in these products appears to be associated in some manner with the phosphorus of the mineral, since Reynolds, Jacob, and Hill (39) found a fairly constant ratio between these two elements in products from various sources. Phosphatic limestones, which contain approximately one-third as much phosphorus as rock phosphates, also contain approximately one-third as much fluorine.

It has been suggested that the fluorine content of rock phosphate was the cause of the lack of uniformity in the results obtained in feeding trials where this mineral has been used to supplement the rations of farm animals. Such an hypothesis appears to be well grounded, in view of the fact that good results have been obtained with phosphatic limestone and some questionable results with rock phosphates.

REVIEW OF THE LITERATURE

As early as 1909, Hart, McCollum, and Fuller (21) improved the efficiency of low-phosphorus rations for the growth of pigs by the addition of rock phosphate. Later, Hart, Steenbock, and Fuller (22) reported that "floats" increased the calcium and phosphorus retention of growing pigs and tended to form heavier skeletons. These trials were all of relatively short duration.

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Forbes and co-workers (17) were the first to report poor results with swine when rock phosphate was used as the mineral supplement. They found it to be less efficient than limestone, steamed bone meal, whiting, marl, precipitated bone, or calcium carbonate in improving bone formation and calcium and phosphorus retention of growing pigs. The femurs of the animals which received rock phosphate were less dense, contained less ash per unit volume, and were actually weaker than those of the animals on the control ration which received no mineral supplement. Chemical analyses of these tibiae, softened as a result of feeding rock phosphate, were characterized by maximum magnesium and phosphorus contents and minimum calcium and carbon dioxide contents.

Considering the results of a comparative test of rock phosphate and other minerals, Konantz (28) stated that "the best bones were produced by rock phosphate". However, no tests were made of the comparative strengths of the bones of the animals, his criteria being other physical measurements. It does not follow that the largest bones were necessarily the strongest or best. In this test, precipitated phosphate proved decidedly toxic. This latter observation agrees with the observations of Bohstedt and co-workers (10) who fed precipitated bone flour. Whether or not this toxicity was due to fluorine was not definitely determined. It is quite possible that a concentration of the fluorine from the original material may take place in the process of the manufacture of these products.

A decidedly favorable effect on the gains made by pigs on a ration of corn and soybeans was demonstrated by Vestal (50) when acid phosphate alone or in combination with wood ashes or limestone and salt was added to the ration. Later, the Indiana Agricultural Experiment Station (3) reported poor results with a combination of equal parts of rock phosphate and limestone. The Kentucky Agricultural Experiment Station (5) obtained good results by supplementing the rations of swine which were hogging down corn with a mineral mixture composed of 3 parts rock phosphate, 1 part limestone, and 1 part salt. Soft bones occurred in both pigs and rats which had been fed on rations containing rock phosphate at the Arkansas Agricultural Experiment Station (2). Bohstedt and co-workers (11) found no ill effects on growth and fattening when pigs were fed rock phosphate at a level of 0.4 per cent. In some cases 0.8 per cent of rock phosphate retarded growth, while 1.6 per cent had a deleterious effect on both rate and economy of gains. However, no injurious effects were noted on the livers, kidneys, intestines, or teeth of these animals, and there were no appreciable alterations in the percentage of bone ash.

McClure and Mitchell (33) compared rock phosphate with synthetic mineral mixtures of tricalcium phosphate and varying amounts of calcium fluoride, fed in amounts to insure 5 grams of calcium per pig per day. They found that rock phosphate was more harmful than the mixture of the two salts and stated that a mineral containing 2 per cent of fluorine, when fed at this level, had a detrimental effect on the growth of pigs and reduced feed consumption. A mineral containing 3 per cent of fluorine was decidedly deleterious. Metabolism trials showed a decreased calcium retention when the ration contained 0.017 to 0.026 per cent of fluorine.

In a comparison of phosphatic limestone with a mixture of bone meal and limestone supplying approximately the same amounts of calcium and phosphorus, Tolle and Maynard (48) found both supplements equally efficient for growth and bone formation in pigs.

The inclusion of rock phosphate in the rations of mature dairy cows was studied by Reed and Huffman (38). They reported that the animals were thrown off feed when 1.5 per cent of rock phosphate was included in the grain ration. Taylor (46) obtained poor results with dairy heifers when rock phosphate was fed at the same level used by Reed and Huffman. The animals became unthrifty, and their teeth showed an abnormal amount of wear and a characteristic "step" formation. When calcium fluosilicate was fed at the same fluorine levels similar results were obtained. In a comparative test with dairy cattle, Huffman and Reed (24) fed bone flour, rock phosphate, limestone, and a complex mineral mixture at a level of 1.5 per cent of the grain ration. They found that the rock phosphate – fed animals made slower gains, were in a poorer state of health, showed a lowered milk production, and had more badly worn teeth than did the animals receiving the other supplements. These investigators also reported that after the first parturition, when the cows were approximately 2.5 years old, the teeth became sensitive to cold water and the jaw bones and metatarsal bones showed a thickening and an exostosis. Reproduction was not affected.

The Wisconsin Agricultural Experiment Station (11) reported no injurious effects when rock phosphate was fed at a level of 2.5 per cent of the grain ration to dairy heifers for 2 years. This was attributed to the fact that only 4 pounds of grain were fed daily per head. After parturition, as the grain ration was increased, these animals lost their appetites, lost in weight, and their milk flow declined. Changes in the teeth similar to those reported by Huffman and Reed (24) were noted in these animals.

Kennard and White (26) found that steamed bone meal and rock phosphate were equally efficient in mineral mixtures fed to pullets or cockerels. Mixtures consisting of equal parts of limestone, salt, and either rock phosphate or steamed bone meal were equally effective for growth and egg production. The same investigators (27) reported good results with laying hens from the use of mineral mixtures composed of 60 parts rock phosphate or steamed bone meal, 20 parts ground limestone, and 20 parts common salt.

Studies by Buckner, Martin, and Peter (13) on the availability of calcium in different compounds for laying hens showed that the calcium of rock phosphate could be utilized for the growth of bone but not for the production of egg shells. The mature birds which received rock phosphate were troubled with diarrhea and showed a decreased egg production. Later, Buckner, Martin, and Insko (12) reported that rock phosphate interfered with the growth of chicks during the first few weeks but later permitted a continuance of normal growth.

From a study of various levels of rock phosphate feeding to chicks, Halpin and Lamb (20) concluded that no harmful effects were indicated at a 1-per cent level; some depression of growth resulted at a 2-per cent level; and seriously harmful effects were observed at a 3-per cent level. The 3-per cent level also caused a decrease in egg production, but there was no observable effect on the ash content of the bones of the birds at 17 to 20 weeks of age.

In studies with rats, Tolle and Maynard (47) reported that the addition of bone meal to a low-calcium ration resulted in a higher bone ash than did the inclusion of rock phosphate on an equivalent calcium percentage. They also noted poor growth on the rock phosphate ration. These differences in growth and bone ash were also accompanied by a noticeable change in the incisors of the animals which received the rock phosphate. These teeth became soft, white in color, and greatly elongated. The same investigators (48) observed similar changes in the teeth of rats which were fed rations containing 2 per cent or more of phosphatic limestone. No ill effects on reproduction were observed even when the latter supplement was fed at levels as high as 3 per cent.

Investigators at the Wisconsin Agricultural Experiment Station (11) found that 0.6 per cent of rock phosphate was the maximum amount which could safely be fed to rats. No deleterious effect on growth was observed when a ration containing 1 per cent was fed, although a decreased efficiency in reproduction and retarded growth of the young resulted. A 2-per cent level proved detrimental to growth.

The physiological effects of fluorine have recently been reviewed in an excellent article by McClure (31). No attempt will be made to review the literature in this field and those interested are referred to the above-mentioned paper.

From the review of the work done on the feeding of rock phosphate and phosphatic limestone, it is obvious that great variance exists in the results obtained. In most of the work no effort was made to control the fluorine intake, and no systematic attempt was made to feed graded amounts of comparative fluorine-containing minerals or salts such as rock phosphate or sodium fluoride. With these observations in mind, the authors concerned themselves chiefly with a study of the specific effects of fluorine in the form present in rock phosphate and chemically pure fluorides on growth, reproduction, bones, and various other tissues of the pig, rat, and chick. An endeavor was also made to determine if the excessive fluorine content of rock phosphate is directly responsible for the poor results encountered when this mineral is used at a relatively high percentage in practical livestock rations.

EXPERIMENTS WITH PIGS

The pig was selected as one experimental animal because, under ordinary farm conditions, rock phosphate is more likely to be fed to this animal than to other classes of livestock. Also, its shorter life cycle and more rapid reproductive period allow results to be procured in a shorter time than with other species. In addition, the larger size of pigs makes them better adapted for more detailed chemical analyses of the bones than are smaller laboratory animals.

Five separate trials were conducted for the purpose of comparing the effects produced by rock phosphate and other mineral supplements and combinations and of studying the effects of fluorides fed at different levels. While the fluorine present in rock phosphate is undoubtedly not in the forms of the fluorides used, the latter salts were arbitrarily chosen since they were readily available in chemically pure form.

The basal mixtures used throughout the experimental work with pigs were as follows:

	Pigs weighing under 125 lb.	Pigs weighing over 125 lb.
Ground yellow corn	55.0	75.0
Flour wheat middlings	25.0	14.0
Linseed oil meal	19.5	10.5
Salt (NaCl)	0.5	0.5
Cod-liver oil	0.5	0.5
Mineral supplement	as indicated	

In those experiments which were conducted out-of-doors, the cod-liver oil was omitted since the antirachitic factor was supplied by sunlight. These rations when properly supplemented with minerals are satisfactory for pigs, as shown by previous trials. While not the best rations to feed under ordinary farm conditions, they have the advantage, from the experimental viewpoint, of being low in mineral content, thus throwing the added burden upon the mineral supplements. The proportions of the ingredients were altered at the time the animals attained approximately 125 pounds in weight in order to widen the nutritive ratio of the mixture. At the beginning of each experiment all the feeds were individually analyzed for calcium, phosphorus, magnesium, and fluorine. The corn was ground in a hammer mill, using a medium fine screen. All the various ingredients, including the minerals, were thoroughly mixed together.

Yellow corn was used to supply vitamin A. The flour wheat middlings, linseed oil meal, salt, ground limestone, steamed bone meal, and phosphatic limestone were the ordinary commercial products used in livestock feeding operations. The treble superphosphate was of the grade commonly used as a fertilizer. The precipitated bone flour, according to the manufacturer, was an hydrochloric acid – precipitated by-product from the manufacture of gelatin. During the course of the study four different rock phosphates were used. Rock phosphate A was supplied by the Ruhm Phosphate and Chemical Company. It was a brown phosphate rock, mined near Mount Pleasant, Maury County, Tennessee. Rock phosphate B, used only in one experiment, was a gray land-pebble phosphate, mined in Florida. Both products contained approximately 3.5 per cent fluorine. The rock phosphates designated as "treated" and "untreated" were obtained through the courtesy of the Mellon Institute of Pittsburgh, Pennsylvania. The "untreated" material was comparable to rock phosphate A in mineral composition; the "treated" product had been processed to remove the fluorine and contained approximately the same amounts of calcium and phosphorus as the "untreated" but considerably less fluorine.

Previous to being placed on experiment, all pigs were immunized against cholera and treated for worms. They were then divided into their respective lots, according to litter, sex, and weight. In the experiments which were conducted indoors the animals were kept on a concrete floor; whereas those experiments performed out-of-doors were conducted on brick-paved lots which were supplied with adjoining houses. The animals were weighed individually every 2 weeks, and complete records of growth, feed consumption, and general physical behavior were maintained.

At the close of the experiments the animals were slaughtered for postmortem examinations. The femurs, kidneys, and mandibles were removed from each animal. Pieces of the kidneys were preserved in a 4 per cent solution of formaldehyde for subsequent histological study. The femurs were kept in a refrigerator at approximately 28° F. They were cleaned of adhering tissue, and their maximum lengths were determined by means of a micrometer calibrated to read to 1 millimeter. The smallest diameter of the femurs was measured with a vernier micrometer calibrated to read to 0.1 millimeter. The volume of the bone was determined by the difference between the weights of the bone in air and in water. These measurements were made with a Toledo balance calibrated to read to 1 gram. When weighing the bone in water, several sources of error were encountered—namely, air bubbles collected around the bone, especially in the crevices, and some water was absorbed.

However, with a little care and rapid reading of the weight, both these sources of error were minimized and a fairly accurate measurement was obtained. The breaking strength was determined with an Olsen dynamometer calibrated to read to 1 pound.

After the physical measurements had been completed, the entire femur was crushed in a bone cutter and dried in a drying oven for 24 hours at approximately 100° C. The dried material was then transferred to flasks and extracted with three applications of warm alcohol and two applications of ether. This treatment removed sufficient fat and lipoid material to allow the bone to be finely ground in a burr mill. To insure the complete removal of the fats and lipoids, the finely-ground samples were further extracted for 18 to 24 hours with ether in Soxhlet extractors. They were then dried and analyzed for ash, carbon dioxide (49), and fluorine (40). Calcium (36), phosphorus (6), and magnesium (35) were determined on the ash of each bone.

The mandibles were cleaned of adhering tissue and kept in the refrigerator at approximately 28° F. They were examined for gross changes in the bones and teeth. In one experiment, various physical measurements were obtained.

In the first two experiments, four incisors and six molars were extracted from the mandibles of each animal and a composite sample made for each lot. These were dried, ground, extracted with alcohol and ether, and dried again as in the procedure followed with the femurs. The moisture- and fat-free teeth were analyzed for ash, carbon dioxide, and fluorine. Calcium, phosphorus, and magnesium determinations were made on the ash. In the case of the sows (Experiment 5) histological studies were made on representative teeth and mandibles and on the femurs.

EXPERIMENT 1

The first experiment was planned to study the effects of the addition of various amounts of sodium fluoride to a control ration and to compare the

TABLE 1.—Mineral Composition of the Rations—Calculated

Experiment 1

Lot No.	Mineral additions to 100 lb. basal ration	Calcium	Phosphorus	Magnesium	Fluorine	Ca:P ratio
		Pct.	*Pct.*	*Pct.*	*Pct.*	
1	908 gm. ground limestone	0.802* 0.766	0.527 0.422	0.269 0.212	Trace Trace	1.5 1.8
2	908 gm. ground limestone 30 gm. sodium fluoride	0.802 0.766	0.527 0.422	0.269 0.212	0.029 0.029	1.5 1.8
3	908 gm. ground limestone 60 gm. sodium fluoride	0.802 0.766	0.527 0.422	0.269 0.212	0.058 0.058	1.5 1.8
4	908 gm. ground limestone 100 gm. sodium fluoride	0.801 0.766	0.526 0.421	0.268 0.211	0.097 0.097	1.5 1.8
5	454 gm. ground limestone 454 gm. rock phosphate A	0.786 0.751	0.676 0.570	0.254 0.197	0.033 0.033	1.2 1.3
6	908 gm. rock phosphate B	0.772 0.736	0.827 0.721	0.236 0.180	0.070 0.070	0.9 1.0
7	908 gm. rock phosphate A	0.770 0.734	0.824 0.719	0.240 0.183	0.065 0.065	0.9 1.0
8	908 gm. steamed bone meal	0.735 0.700	0.822 0.717	0.240 0.183	Trace Trace	0.9 1.0

*Upper figures represent starting ration. Lower figures represent finishing ration.

results with those produced by rations in which rock phosphate was fed at different levels. This was done in order to determine, if possible, whether or not the fluorine content of the rock phosphate was responsible for the results obtained when this mineral supplement was used.

For this purpose, 64 weanling pigs were divided equally into eight lots. All lots were kept indoors and hand-fed their respective rations twice daily. The rations for each lot, together with the mineral analyses and the Ca:P ratios, are recorded in Table 1. The mineral analyses of the rations were calculated from the following analyses of the various ingredients:

Feed	Calcium	Phosphorus	Magnesium	Fluorine
	Pct.	*Pct.*	*Pct.*	*Pct.*
Ground yellow corn*	0.014	0.301	0.109	....
Wheat middlings*	0.057	0.921	0.326	....
Linseed oil meal*	0.365	0.743	0.489	....
Ground limestone	36.49	....	1.96	....
Rock phosphate A	34.79	15.20	0.48	3.34
Rock phosphate B	34.90	15.34	0.30	3.61
Steamed bone meal	33.01	15.09	0.48	....
Sodium fluoride	....	....	....	44.75

*Analyses taken from Henry & Morrison's "Feeds and Feeding".

Lots 1 and 8, which served as the control lots, received the basal mixture supplemented with 2 parts of ground limestone and 2 parts of steamed bone meal, respectively. The rations of Lots 2, 3, and 4 consisted of the basal mixture supplemented with 2 parts of ground limestone and increasing amounts (30, 60, and 100 gm. per 100 pounds of mixture) of sodium fluoride. In the ration of Lot 5, one-half of the ground limestone of the control ration was replaced by Tennessee rock phosphate A, while in the rations of Lots 6 and 7 the ground limestone was replaced entirely by the Florida rock phosphate B and the Tennessee rock phosphate A, respectively.

The calcium, phosphorus, and magnesium contents of the rations of Lots 1, 2, 3, and 4 were the same, while the percentage of fluorine was increased by the additions of sodium fluoride (Table 1). The substitutions of the rock phosphates in the rations of Lots 5, 6, and 7 slightly altered the calcium and phosphorus contents and the Ca:P ratios. The magnesium and fluorine percentages of these rations were increased. The fluorine contents of the rations of Lots 6 and 7 (2 parts of rock phosphates) were approximately equivalent to the addition of 70 grams of sodium fluoride to 100 pounds of the basal mixture. The Ca:P ratios of all the rations fell within favorable limits, according to the work of Bethke, Edgington, and Kick (8). The amount of calcium consumed daily by the pigs was in excess of that fed by either Forbes and co-workers (17) or McClure and Mitchell (33), so that there was sufficient of this element present for maximum growth.

The pigs were continued on experiment for 144 days, at which time the animals were slaughtered and the tissues removed for analyses. In order to study the effects of these rations over a long-time feeding period and on reproduction, one gilt from Lot 1 and two gilts from Lot 5 were saved and continued on their respective rations.

It is evident, from the data presented in Table 2, that, when more than 30 grams of sodium fluoride were added to 100 pounds of the ration, the average daily gain was decreased and the feed required to produce a unit of gain was increased. The addition of 30 grams of sodium fluoride to 100 pounds of the basal ration slightly decreased the average daily gain and the feed requirements per unit of gain. However, these decreases were so slight that they might well be within the limits of experimental error. The decrease in the average daily gain when one-half of the limestone was replaced by rock phosphate A was also questionably significant, but the feed required per unit of gain was definitely increased. The lots in which rock phosphates comprised the sole mineral supplements (Lots 6 and 7) showed a marked decrease in daily gain and a large increase in the feed requirement. None of the lots compared favorably with Lot 8 (steamed bone meal) in either gains or feed requirements. The average daily feed for the animals in the different lots closely followed the average daily gains. The presence of sodium fluoride or rock phosphate in the rations caused a decrease in feed consumption in proportion to the amounts of these materials present.

TABLE 2.—Gains and Feed Requirements

Experiment 1

Lot No.	Mineral additions to 100 lb. basal ration	Fluorine content of ration	Average initial weight	Average final weight	Average daily gain	Average daily feed	Feed required per 100 lb. gain
		Pct.	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*
1	908 gm. ground limestone	Trace	54.6	204.6	1.04	4.92	472.4
2	908 gm. ground limestone ..... 30 gm. sodium fluoride	0.029	52.0	192.0	0.97	4.48	461.3
3	908 gm. ground limestone ..... 60 gm. sodium fluoride	0.058	54.0	182.6	0.89	4.31	483.1
4	908 gm. ground limestone ..... 100 gm. sodium fluoride	0.097	52.5	138.0	0.59	3.62	610.5
5	454 gm. ground limestone ..... 454 gm. rock phosphate A	0.033	54.0	196.0	0.99	4.84	491.7
6	908 gm. rock phosphate B.......	0.070	51.1	139.4	0.59	3.89	635.8
7	908 gm. rock phosphate A	0.065	53.1	130.3	0.54	3.48	650.1
8	908 gm. steamed bone meal......	Trace	52.1	217.0	1.14	5.02	438.7

From daily observations it was evident that the animals in Lots 6 and 7 (2 parts of rock phosphates) were consuming considerably more water than the animals in the other lots. The paved pens in which these animals were kept were always wetter than those of the other animals, showing that their excretion of water was also considerably greater. Unfortunately, no record of the water consumption of any of the lots was kept during this experiment. It was also noted that several of the animals in the rock phosphate and sodium fluoride – fed lots manifested a reluctance to move and showed definite signs of stiffness. This was especially true in the higher fluorine lots. One pig in Lot 6 (2 parts rock phosphate B) died at 103 days. Post-mortem examination revealed contracted, roughened, firm, pale kidneys.

It was apparent, on gross examination, that certain differences existed between the femurs of the pigs in the various lots. Those of the pigs in Lots 1 and 8 (2 parts ground limestone and 2 parts steamed bone meal, respectively) were smooth and dense in appearance, with a normal yellowish color and a definite luster; whereas those from Lots 5, 6, and 7 (rock phosphates) were rough in appearance, with some exostoses, whiter in color, and lacking in luster. This contrast became more exaggerated as the amounts of sodium fluoride or rock phosphates in the rations were increased. The weight, volume, length, and smallest diameter of the femurs are obviously dependent upon the type and size of the animal at the time of slaughter. Taking this fact into consideration, there is some evidence, from the data presented in Table 3, that a slight increase in the diameters of the femurs accompanied the feeding of high levels of rock phosphate or sodium fluoride. The evidence is rather difficult to interpret and is, therefore, somewhat inconclusive.

The outstanding difference noted was in the breaking strengths of the femurs (Table 3). The addition of 100 grams of sodium fluoride (Lot 4), or the substitution of rock phosphates (Lots 6 and 7) for the 2 per cent of limestone in the control ration (Lot 1), caused a marked decrease in the breaking strength of the femurs. Smaller amounts of sodium fluoride or rock phosphate had no significant effect on the breaking strength of the bones. In the breaking strength of the bones none of the lots approached the one that received 2 parts of steamed bone meal as the mineral supplement (Lot 8). The most obvious factor affecting the breaking strength is the size of the bone. In order to take this factor into consideration it was thought advisable to put the breaking strength on the basis of a unit of weight of bone. This was done by dividing the breaking strength by the weight of the femur, thus obtaining an arbitrary value which should approach the relative strengths of the bones in the various lots. On this basis, Lot 2 (30 gm. sodium fluoride) and Lot 5 (1 part each of limestone and rock phosphate A) showed an increased strength over Lot 1 (2 parts ground limestone). On the same basis the femurs of Lot 3 (60 gm. sodium fluoride) are equal in strength to those of Lot 1, while the addition of 100 grams of sodium fluoride (Lot 4) decreased the strength of the bone, as did the substitution of the rock phosphates (Lots 6 and 7) for all the limestone (Lot 1). Likewise, the femurs of the animals in Lot 5 (1 part each of ground limestone and rock phosphate A) were as strong as those from the animals receiving steamed bone meal as their mineral supplement (Lot 8).

The ash contents of the moisture- and fat-free bones (Table 3) show no significant variations from those of the control lots (Lots 1 and 8), with the exception of Lot 4 (100 gm. sodium fluoride). The femurs of the pigs from the latter group show a small decrease in the percentage of ash. When the ash contents are put on a unit value basis (ash per cc. volume), the resulting values approximate the densities of the bones; i. e., weight per unit volume. There is a noticeable correlation between the ash per unit volume and the breaking strength.

The detailed chemical analyses of the femurs of the different lots (Table 4) show that the amounts of carbonates in the moisture- and fat-free bones decreased with increasing fluorine intakes. The fluorine compound present in rock phosphate seemed to exert a greater effect in this respect than did sodium fluoride, since Lots 6 and 7 had the lowest carbonate content, while the fluorine contents of these rations were lower than that of Lot 4 (100 gm. sodium fluoride). The percentages of fluorine in the dry, fat-free bones increased in direct proportion to the amounts of this element present in the rations of the various lots. The calcium and phosphorus contents of the bone ash were the same in all the lots regardless of the mineral supplement fed; whereas the percentage of magnesium increased with increasing amounts of sodium fluoride or rock phosphate in the rations, but independently of the magnesium intake.

TABLE 3.—Physical Measurements and Ash Contents of the Femurs

Experiment 1

Lot No.	Mineral additions to 100 lb. basal ration	Fluorine content of ration	Average weight	Average volume	Average length	Average smallest diameter	Average breaking strength	Average of breaking strength divided by weight of bone	Average ash	Average ash per cc. volume
		Pct.	*Gm.*	*Cc.*	*Cm.*	*Cm.*	*Lb.*		*Pct.*	*Gm.*
1	908 gm. ground limestone	Trace	177	135	18.4	2.00	690 ± 62	3.94	61.3 ± 0.39	0.805
2	908 gm. ground limestone 30 gm. sodium fluoride	0.029	164	124	17.5	2.06	733 ± 34	4.51	62.3 ± 0.27	0.825
3	908 gm. ground limestone 60 gm. sodium fluoride	0.058	167	131	16.8	2.21	647 ± 35	3.92	60.5 ± 0.31	0.770
4	908 gm. ground limestone 100 gm. sodium fluoride	0.097	135	111	16.3	2.03	393 ± 19	2.93	58.0 ± 0.29	0.708
5	454 gm. rock phosphate A 454 gm. ground limestone	0.033	160	126	17.1	2.12	766 ± 51	4.81	61.9 ± 0.51	0.760
6	908 gm. rock phosphate B	0.070	133	111	16.3	2.05	407 ± 67	3.21	59.4 ± 0.68	0.707
7	908 gm. rock phosphate A	0.065	134	109	16.1	2.01	456 ± 40	3.34	60.8 ± 0.54	0.743
8	908 gm. steamed bone meal	Trace	177	134	18.3	1.95	841 ± 55	4.80	61.6 ± 0.32	0.816

TABLE 4.—Chemical Analyses of the Femurs

Experiment 1

Lot No.	Mineral additions to 100 lb. basal ration	Fluorine content of ration	Bone ash			Moisture- and fat-free bone	
			Calcium	Phosphorus	Magnesium	Carbon dioxide	Fluorine
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	908 gm. ground limestone	Trace	38.4 ± 0.11	17.5 ± 0.21	0.79 ± 0.013	2.76 ± 0.07	0.04 ± 0.003
2	908 gm. ground limestone 30 gm. sodium fluoride	0.029	38.5 ± 0.13	18.0 ± 0.09	0.96 ± 0.009	2.72 ± 0.05	0.53 ± 0.016
3	908 gm. ground limestone 60 gm. sodium fluoride	0.058	38.3 ± 0.12	17.7 ± 0.18	0.97 ± 0.011	2.37 ± 0.05	0.78 ± 0.014
4	908 gm. ground limestone 100 gm. sodium fluoride	0.097	38.4 ± 0.09	17.6 ± 0.04	1.14 ± 0.011	2.34 ± 0.04	1.11 ± 0.028
5	454 gm. ground limestone 454 gm. rock phosphate A	0.033	38.1 ± 0.12	17.9 ± 0.15	1.04 ± 0.019	2.57 ± 0.05	0.59 ± 0.016
6	908 gm. rock phosphate B	0.070	37.8 ± 0.20	17.9 ± 0.23	1.17 ± 0.031	1.92 ± 0.07	1.04 ± 0.034
7	908 gm. rock phosphate A	0.065	37.9 ± 0.20	18.1 ± 0.17	1.14 ± 0.025	2.06 ± 0.07	1.09 ± 0.032
8	908 gm. steamed bone meal	Trace	38.3 ± 0.10	17.8 ± 0.14	0.82 ± 0.004	2.81 ± 0.03	0.04 ± 0.001

The mandibles of the rock phosphate and sodium fluoride – fed animals, on gross examination, showed conditions similar to those noted in the femurs. They were rough in appearance, with exostoses, white in color, and lusterless; and the bodies of the bones were thicker than in the control animals. This condition became more evident as the amounts of sodium fluoride or rock phosphates in the rations increased. The teeth showed no definite differences in any of the lots and the amount of wear on the molars was quite comparable. However, there was a tendency for the teeth from the animals in the higher fluorine-fed lots to chip more easily than those from the controls.

The chemical analyses of the teeth (Table 5) revealed no significant differences in the ash contents or in the calcium, phosphorus, or magnesium percentages of the ash in any of the lots. The carbon dioxide contents of the dry, fat-free teeth were not affected, except possibly in the cases of Lots 6 and 7 (2 parts rock phosphates A and B) where evidence of a slight decrease existed. The percentage of fluorine present in the teeth was directly proportional to the amounts of this element present in the ration in all instances.

TABLE 5.—Chemical Analyses of the Teeth

Experiment 1

Lot No.	Mineral additions per 100 lb. basal ration	Fluorine content of ration	Ash	Tooth ash			Dry fat-free tooth	
				Calcium	Phosphorus	Magnesium	Carbon dioxide	Fluorine
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	908 gm. ground limestone........	Trace	74.0	35.8	17.5	1.45	2.03	0.083
2	908 gm. ground limestone...... 30 gm. sodium fluoride........	0.029	75.0	36.3	17.8	1.54	2.09	0.181
3	908 gm. ground limestone...... 60 gm. sodium fluoride........	0.058	74.2	36.8	17.9	1.63	2.07	0.281
4	908 gm. ground limestone...... 100 gm. sodium fluoride........	0.097	74.7	37.0	17.9	1.53	2.15	0.361
5	454 gm. ground limestone...... 454 gm. rock phosphate A	0.033	74.8	36.7	17.7	1.51	2.11	0.228
6	908 gm. rock phosphate B.......	0.070	74.4	36.7	17.6	1.50	1.83	0.331
7	908 gm. rock phosphate A	0.065	75.2	37.0	17.4	1.56	1.95	0.375
8	908 gm. steamed bone meal......	Trace	75.8	36.9	17.3	1.79	2.12	0.092

Gross examination of the various organs at the time of slaughter revealed no abnormalities, except that the kidneys from the animals which had been fed rock phosphate exhibited a chronic, parenchymatous nephritis. They were pale in color, contracted and firm in texture, with numerous nodules and depressions covering the surface. The capsules were slightly thickened and, in some instances, firmly adherent to the surface. Occasionally, small cysts containing a clear or amber colored fluid protruded above the surface or were deeply situated in the kidney. On section, the cortex appeared reduced in width, and frequently the medulla contained a considerable amount of fat.

Microscopically, the kidneys showed a nephritis with a varying degree of degeneration of the tubular epithelium and, as a terminal result, the replacement of many tubules and glomeruli with fibrous tissue. None of the animals in either the control lots or the sodium fluoride – fed lots exhibited this condition.

From the data presented, it is evident that, with the exception of the detrimental effect upon the kidneys, the sodium fluoride in the ration produced practically the same results as did the rock phosphate. It would appear that the poor results obtained with rock phosphate in pig feeding are due, in part at least, to the fluorine content of this material.

EXPERIMENT 2

The second test was designed to check the results of the previous trial and also to study the effects of including smaller amounts of sodium fluoride and rock phosphate in the rations of growing, fattening pigs.

A group of 48 weanling pigs was divided into six lots of eight pigs each. All lots were kept indoors on a concrete floor and were handled as in the previous experiment. The animals were continued on experiment for 160 days, at which time they were slaughtered, post-mortem examinations made, and tissues removed for analyses.

TABLE 6.—Mineral Composition of the Rations—Calculated

Experiment 2

Lot No.	Mineral additions per 100 lb. basal ration	Calcium	Phosphorus	Magnesium	Fluorine	Ca:P ratio
		Pct.	*Pct.*	*Pct.*	*Pct.*	
1	454 gm. ground limestone...... 454 gm. steamed bone meal....	0.714* 0.672	0.654 0.556	0.348 0.260	Trace Trace	1.1 1.2
2	454 gm. ground limestone..... 454 gm. steamed bone meal.... 10 gm. sodium fluoride........	0.714 0.672	0.654 0.556	0.348 0.260	0.010 0.010	1.1 1.2
3	454 gm. ground limestone...... 454 gm. steamed bone meal.... 30 gm. sodium fluoride........	0.714 0.672	0.654 0.556	0.348 0.260	0.029 0.029	1.1 1.2
4	454 gm. ground limestone ..... 454 gm. steamed bone meal.... 60 gm. sodium fluoride........	0.714 0.672	0.654 0.556	0.348 0.260	0.058 0.058	1.1 1.2
5	429 gm. ground limestone...... 227 gm. steamed bone meal.... 222 gm. rock phosphate A	0.714 0.671	0.654 0.557	0.346 0.258	0.016 0.016	1.1 1.2
6	404 gm. ground limestone...... 444 gm. rock phosphate A	0.714 0.672	0.654 0.557	0.343 0.255	0.032 0.032	1.1 1.2

*Upper figures represent starting ration. Lower figures represent finishing ration.

The rations were so planned that the percentages of calcium and phosphorus were maintained at a constant level throughout (Table 6). The rations of Lots 1, 2, 3, and 4 contained the same amounts of steamed bone meal and limestone. Sodium fluoride was added to the rations of Lots 2, 3, and 4 in amounts of 10, 30, and 60 grams per 100 pounds of feed, respectively. Rock phosphate A was substituted, on an equivalent phosphorus basis, for one-half and all of the steamed bone meal of the control ration in Lots 5 and 6, respectively. Sufficient ground limestone was added to the rations of the latter lots to make the calcium content of these rations comparable to that of the other lots. The amounts of magnesium in the rations of Lots 5 and 6 varied slightly from those of the other lots. The fluorine percentages varied from a trace to 0.0578 per cent. The mineral compositions of the various rations were calculated from the analyses of the various ingredients as given below:

Feed	Calcium	Phosphorus	Magnesium	Fluorine
	Pct.	*Pct.*	*Pct.*	*Pct.*
Ground yellow corn*	0.014	0.301	0.109	....
Wheat middlings	0.077	0.814	0.465	....
Linseed oil meal	0.417	0.779	0.645	....
Ground limestone	32.39	....	4.63	....
Rock phosphate A	34.13	15.32	0.48	3.34
Steamed bone meal	29.84	14.99	0.49	....
Sodium fluoride	....	....	....	44.75

*Analyses taken from Henry & Morrison's "Feeds and Feeding".

The gain and feed-requirement data are presented in Table 7. It is evident that neither the addition of 10 grams of sodium fluoride to 100 pounds of the ration (Lot 2) nor the substitution of rock phosphate for one-half of the steamed bone meal of the control ration (Lot 5) had any effect on the average daily gains. When 60 grams of sodium fluoride were added to 100 pounds of the ration (Lot 4) and also when all the steamed bone meal was replaced by rock phosphate A (Lot 6), the average daily gain was decreased. The feed requirements of Lots 2 and 3 (10 and 30 grams sodium fluoride) were somewhat less than for the control lot (Lot 1). However, it is questionable whether the differences are significant. The substitution of rock phosphate A for one-half of the steamed bone meal of the control ration (Lot 5) made practically no difference in the feed requirements, while the addition of 60 grams of sodium fluoride (Lot 4) and the substitution of rock phosphate A for all the steamed bone meal of the control ration (Lot 6) increased the feed requirements. The average daily rations of the pigs in the various lots decreased with increasing amounts of sodium fluoride or rock phosphate A in the rations.

TABLE 7.—Gains and Feed Requirements

Experiment 2

Lot No.	Mineral additions per 100 lb. basal ration	Fluorine content of ration	Average initial weight	Average final weight	Average daily gain	Average daily feed	Feed required per 100 lb. gain
		Pct.	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*
1	454 gm. ground limestone.. 454 gm. steamed bone meal	Trace	39.3	243.4	1.28	5.08	398.4
2	454 gm. ground limestone.. 454 gm. steamed bone meal 10 gm. sodium fluoride....	0.010	37.7	244.6	1.29	4.92	380.2
3	454 gm. ground limestone.. 454 gm. steamed bone meal 30 gm. sodium fluoride....	0.029	38.8	213.6	1.09	4.26	389.9
4	454 gm. ground limestone.. 454 gm. steamed bone meal 60 gm. sodium fluoride....	0.058	39.4	161.3	0.76	3.41	450.2
5	429 gm. ground limestone.. 227 gm. steamed bone meal 222 gm. rock phosphate A	0.016	40.4	246.0	1.29	5.06	394.7
6	404 gm. ground limestone.. 444 gm. rock phosphate A	0.032	40.4	219.4	1.12	4.85	434.0

Toward the end of the feeding period, a careful record of the water consumption of the different lots was kept for a period of 2 weeks. The results are recorded in Table 8. It is evident that, regardless of the differences in the sizes of the animals, all the lots consumed approximately the same amounts of water. When the water consumption is put on the basis of the weight of the animals, there is a direct correlation between water consumption and the amount of fluorine in the ration—that is, the greater the amount of fluorine in the ration, the larger the water consumption.

TABLE 8.—The Effect of Fluorine on Water Consumption

Experiment 2

Lot No.	Fluorine content of ration	Feed consumed in 2 weeks	Water consumed in 2 weeks	Average weight of pigs	Water consumed per 100 lb. live weight
	Pct.	*Lb.*	*Gal.*	*Lb.*	*Gal.*
1	Trace	945	165	212	77.83
2	0.010	964	156	208	75.00
3	0.029	767	153	188	81.38
4	0.058	471	159	144	110.41
5	0.016	927	165	212	77.83
6	0.032	817	168	190	88.42

As in the previous experiment, the femurs of the animals in the various lots exhibited differences upon gross examination. The bones from Lot 1 (control) were smooth, with a normal yellow color and lustrous appearance. The femurs of the animals which received 10 grams of sodium fluoride and approximately 0.5 per cent of rock phosphate A also appeared normal, while those of the other lots showed the characteristic changes which accompany fluorine feeding, as described in the previous experiment. From the data presented in Table 9, it is again evident that there was a slight increase in the diameters of the femurs as the percentages of fluorine in the rations were increased. The breaking strengths showed a decrease with increasing amounts of fluorine in the rations. The differences in the breaking strengths of the bones in Lots 1, 2, and 5 were not significant; however, the decreased breaking strengths of the bones of the lots which had received the rations containing greater amounts of fluorine, either in the form of sodium fluoride or rock phosphate A, were decidedly significant. When the breaking strength was put on the basis of the size of the bone (breaking strength divided by the weight of the bone), the relative positions of the lots were unchanged, and the breaking strength on this basis was directly correlated with the fluorine content of the ration.

There was no significant difference in the ash percentages of the femurs of the different lots (Table 9). However, when the ash content was put on a volume basis (ash per unit volume), it was noted that the addition of 10 grams of sodium fluoride to the ration (Lot 2) increased the density of the bone. Lot 4 (60 gm. sodium fluoride) had the least dense bone, while the lots which received 30 grams of sodium fluoride (Lot 3) or rock phosphate A (Lots 5 and 6) showed only a slight decrease in bone density over the control lot (Lot 1).

The detailed chemical analyses of the femurs (Table 10) substantiated the results of the previous experiment. The percentage of carbon dioxide in the dry, fat-free bones of Lots 2 and 5 showed no significant differences from those

TABLE 9.—Physical Measurements and Ash Contents of the Femurs

Experiment 2

Lot No.	Mineral additions to 100 lb. basal ration	Fluorine content of ration	Average weight	Average volume	Average length	Average smallest diameter	Average breaking strength	Average of breaking strength divided by weight of bone	Average ash	Average ash per cc. volume
		Pct.	*Gm.*	*Cc.*	*Cm.*	*Cm.*	*Lb.*		*Pct.*	*Gm.*
1	454 gm. ground limestone.......... 454 gm. steamed bone meal........	Trace	178	139	18.4	1.96	1013 ± 38	6.06	61.8 ± 0.34	0.801
2	454 gm. ground limestone.......... 454 gm. steamed bone meal....... 10 gm. sodium fluoride............	0.010	182	140	18.3	2.04	976 ± 55	5.33	62.9 ± 0.42	0.826
3	454 gm. ground limestone.......... 454 gm. steamed bone meal........ 30 gm. sodium fluoride............	0.029	168	132	17.3	2.12	871 ± 42	5.25	62.5 ± 0.41	0.792
4	454 gm. ground limestone.......... 454 gm. steamed bone meal........ 60 gm. sodium fluoride............	0.058	157	131	17.1	2.22	550 ± 23	3.49	60.7 ± 0.51	0.732
5	429 gm. ground limestone.......... 227 gm. steamed bone meal........ 222 gm. rock phosphate A	0.016	176	138	18.1	2.03	1050 ± 58	6.17	62.0 ± 0.25	0.796
6	404 gm. ground limestone.......... 444 gm. rock phosphate A	0.032	179	142	18.3	2.10	827 ± 37	4.76	62.6 ± 0.30	0.792

of the control lot, while the bones of the animals which received larger amounts of sodium fluoride or rock phosphate A (Lots 3, 4, and 6) showed a decrease in the carbon dioxide contents with increasing amounts of fluorine in the ration. The percentages of fluorine in the dry, fat-free bones varied directly with the amounts of this element present in the rations. The percentages of calcium and phosphorus in the bone ash of the various lots showed no significant differences, regardless of the fluorine intakes. The percentages of magnesium in the bone ash, on the other hand, were increased by fluorine feeding. This increase is directly correlated with the amounts of fluorine ingested.

TABLE 10.—Chemical Analyses of the Femurs

Experiment 2

Lot No.	Mineral additions to 100 lb. basal ration	Fluorine content of ration	Bone ash			Moisture- and fat-free bone	
			Calcium	Phosphorus	Magnesium	Carbon dioxide	Fluorine*
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	454 gm. ground limestone.. 454 gm. steamed bone meal	Trace	37.6±0.10	18.5±0.11	0.92±0.011	2.93±0.02	0.06
2	454 gm. ground limestone.. 454 gm. steamed bone meal 10 gm. sodium fluoride....	0.010	37.6±0.09	18.3±0.08	0.97±0.011	3.01±0.03	0.32
3	454 gm. ground limestone.. 454 gm. steamed bone meal 30 gm. sodium fluoride....	0.029	37.6±0.09	18.3±0.04	1.00±0.028	2.71±0.03	0.67
4	454 gm. ground limestone.. 454 gm. steamed bone meal 60 gm. sodium fluoride....	0.058	37.7±0.05	18.2±0.05	1.21±0.107	2.31±0.02	1.08
5	429 gm. ground limestone.. 227 gm. steamed bone meal 222 gm. rock phosphate A	0.016	37.7±0.05	18.2±0.05	1.04±0.013	2.88±0.03	0.41
6	404 gm. ground limestone.. 444 gm. rock phosphate A	0.032	38.2±0.13	19.0±0.18	1.10±0.014	2.78±0.04	0.62

*Fluorine run on composite samples.

On gross examination the mandibles of the animals which had been fed sodium fluoride or rock phosphate A presented a rough, white, lusterless appearance, with many exostoses. In the lots which were fed smaller amounts of sodium fluoride or rock phosphate A (Lots 2 and 5), these changes were very slight but they became more exaggerated as the percentages of fluorine in the rations increased. The total lengths and heights of the mandibles in the different lots showed considerable variation which could not be correlated with the fluorine ingested (Table 11). These differences were probably due to such factors as size, type, etc., rather than to any effects of the rations. The buccolingual dimensions, taken at the positions of the three molars, were increased with the increase of fluorine in the rations. The vertical dimensions showed no significant differences in any of the lots. The width of the dental arch, at the position of the second molar, was increased with the thickening of the body of the mandibles (Figs. 1a and 1b).

TABLE 11.—Physical Analyses of Mandibles

Experiment 2

Lot No.	Fluorine content of ration	Average length of mandible	Average height of mandible	Average buccolingual dimensions			Average vertical dimensions			Average width of dental arch at second molar
				First molar	Second molar	Third molar	First molar	Second molar	Third molar	
	Pct.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
1	Trace	199	102	21.2	21.7	23.1	42.1	40.5	41.1	42.4
2	0.010	205	103	21.3	22.3	23.4	42.3	41.8	42.2	45.6
3	0.029	194	102	22.7	24.6	25.3	40.7	41.0	41.2	46.8
4	0.058	192	102	24.1	25.0	25.1	41.3	40.5	41.1	45.6
5	0.016	203	105	23.0	22.9	24.2	42.3	41.2	41.6	45.5
6	0.032	200	104	24.7	25.8	25.9	43.5	42.0	42.3	44.0

Fig. 1a Fig. 1b

Fig. 1.—Mandibles from pigs in Lot 1 (control, 1a) and Lot 6 (0.032% of fluorine in diet, 1b)

The teeth of all the lots showed no significant differences in the amount of wear; however, those of the animals which received the highest percentages of fluorine in the form of sodium fluoride or rock phosphate A had a greater tendency to chip than those of the controls (limestone and steamed bone meal). The chemical analyses of the teeth (Table 12) showed no significant differences in the ash contents or in the percentages of calcium, phosphorus, and magnesium present in the ash. The carbon dioxide contents of the moisture- and fat-free teeth were the same for all lots, while the amount of fluorine varied in direct proportion to that present in the rations.

Post-mortem examinations revealed no abnormalities in the various organs, with the exception of the kidneys of Lot 6 (approximately 1 per cent of rock phosphate A). These kidneys were rather pale in color and slightly mottled in appearance. Histological examinations showed the presence of a chronic, parenchymatous nephritis. The epithelium of the convoluted tubules was degenerated, with an infiltration of fibrous tissue throughout the organ. This condition was the same as that noted in the previous experiment when rock phosphates were fed at the same or higher levels.

TABLE 12.—Chemical Analyses of the Teeth

Experiment 2

Lot No.	Mineral additions to 100 lb. basal ration	Fluorine content of ration	Ash	Tooth ash			Dry fat-free teeth	
				Calcium	Phosphorus	Magnesium	Carbon dioxide	Fluorine
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	454 gm. ground limestone.. 454 gm. steamed bone meal	Trace	73.5	36.7	17.9	1.65	1.76	0.043
2	454 gm. ground limestone.. 454 gm. steamed bone meal 10 gm. sodium fluoride....	0.010	74.4	37.0	17.8	1.67	1.66	0.127
3	454 gm. ground limestone.. 454 gm. steamed bone meal 30 gm. sodium fluoride....	0.029	74.0	37.1	17.7	1.60	1.76	0.262
4	454 gm. ground limestone.. 454 gm. steamed bone meal 60 gm. sodium fluoride....	0.058	73.9	36.7	17.7	1.66	1.76	0.373
5	429 gm. ground limestone.. 227 gm. steamed bone meal 222 gm. rock phosphate A	0.016	74.6	36.8	17.7	1.70	1.64	0.124
6	404 gm. ground limestone.. 444 gm. rock phosphate A	0.032	74.2	36.8	17.1	1.74	1.65	0.228

The data, in general, substantiated those of the previous experiment. It was evident that sodium fluoride had precisely the same effects as rock phosphate on the bones. These changes became more exaggerated as the fluorine contents of the rations were increased. Rock phosphate produced changes in the kidneys which were not produced when fluorine in the form of sodium fluoride was included in the ration, even at much higher levels than in the form of rock phosphate.

EXPERIMENT 3

The third experiment was planned to study the comparative efficiency of precipitated bone flour, "treated" and "untreated" rock phosphates, and a combination of treble superphosphate and limestone, as compared with steamed bone meal, for growing, fattening pigs.

Six lots of eight pigs each were housed indoors on a concrete floor and hand-fed their respective rations twice daily. The experiment was continued for 140 days, at which time the animals were slaughtered and subjected to post-mortem examinations, tissues being removed for analyses.

The mineral compositions of the various rations were calculated from the analyses of the ingredients which were as follows:

Feed	Calcium	Phosphorus	Magnesium	Fluorine
	Pct.	*Pct.*	*Pct.*	*Pct.*
Ground yellow corn*........	0.014	0.301	0.109	....
Wheat middlings........	0.089	0.488	0.309	....
Linseed oil meal........	0.361	0.872	0.519	....
Ground limestone........	34.10	....	3.900	....
Steamed bone meal........	31.55	14.45	0.389	....
Precipitated bone flour........	25.45	16.59	0.062	....
Treble superphosphate........	14.34	20.45	0.215	1.08
"Treated" rock phosphate........	33.15	14.25	0.598	0.50
"Untreated" rock phosphate........	33.30	14.50	0.452	3.56

*Analyses taken from Henry & Morrison's "Feeds and Feeding".

The rations of the various lots, together with their mineral compositions, are presented in Table 13. The rations of Lots 1 and 2 contained 2 per cent of "untreated" and "treated" rock phosphate, respectively. Those of Lots 3 and 4 were the same, each containing 2 per cent of steamed bone meal, except that 90 grams of calcium fluoride were added to each 100 pounds of the basal mixture in the former group. Treble superphosphate and limestone were added to the ration of Lot 5 in such amounts that the calcium and phosphorus contents of this ration were comparable to those of Lot 4 (2 per cent steamed bone meal). In the ration of Lot 6, the steamed bone meal of the control ration was replaced, part for part, by precipitated bone flour.

TABLE 13.—Mineral Composition of the Rations—Calculated
Experiment 3

Lot No.	Mineral additions to 100 lb. basal ration	Calcium	Phosphorus	Magnesium	Fluorine	Ca:P ratio
		Pct.	*Pct.*	*Pct.*	*Pct.*	
1	908 gm. "untreated" rock phosphate	0.750* 0.711	0.729 0.658	0.239 0.182	0.071 0.071	1.1 1.1
2	908 gm. "treated" rock phosphate	0.747 0.708	0.724 0.653	0.242 0.184	0.010 0.010	1.1 1.1
3	908 gm. steamed bone meal 90 gm. calcium fluoride	0.811 0.771	0.726 0.656	0.238 0.180	0.091 0.091	1.2 1.2
4	908 gm. steamed bone meal	0.715 0.677	0.728 0.657	0.238 0.180	Trace Trace	1.0 1.0
5	642 gm. treble superphosphate 570 gm. ground limestone	0.710 0.671	0.723 0.653	0.279 0.222	0.015 0.015	1.0 1.0
6	908 gm. precipitated bone flour	0.596 0.559	0.770 0.699	0.232 0.175	Trace Trace	0.8 0.8

*Upper figures represent starting ration. Lower figures represent finishing ration.

It is apparent, from the data presented in Table 14, that neither the substitution of "treated" rock phosphate (Lot 2) nor the addition of calcium fluoride (Lot 3) to the steamed bone meal of the control ration (Lot 4) had any effect on the average daily gains. However, when treble superphosphate and limestone in combination or when precipitated bone flour was substituted for the steamed bone meal, the average daily gain was slightly reduced; whereas the substitution of "untreated" rock phosphate caused the average daily gain to be reduced by one-half, at the same time materially reducing the average daily feed intake. The feed requirements of Lot 4 (steamed bone meal) were slightly less than those of Lots 2, 3, and 5 ("treated" rock phosphate, 90 gm. calcium fluoride, and the combination of limestone and treble superphosphate, respectively), decidedly less than those of Lot 6 (precipitated bone flour), and very materially less than those of Lot 1 ("untreated" rock phosphate).

With the exception of Lot 1, the physical analyses of the femurs of the various lots (Table 15) showed no significant differences in weight, volume, length, and smallest diameter. The femurs of the animals in the latter lot were lighter in weight, smaller in volume, shorter in length, but larger in diameter than those of the other lots. Despite the fact that these bones were larger in diameter, they had a much lower breaking strength than those of the other animals.

TABLE 14.—Gains and Feed Requirements

Experiment 3

Lot No.	Mineral additions to 100 lb. basal ration	Fluorine content of ration	Av. initial weight	Av. final weight	Av. daily gain	Av. daily feed	Feed required per 100 lb. gain
		Pct.	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*
1	908 gm. "untreated" rock phosphate....	0.071	39.7	123.6	0.60	3.54	592.7
2	908 gm. "treated" rock phosphate.......	0.010	39.9	213.2	1.24	4.89	395.2
3	908 gm. steamed bone meal............ 90 gm. calcium fluoride...............	0.091	39.9	207.5	1.20	4.74	396.2
4	908 gm. steamed bone meal..............	Trace	39.4	224.1	1.23	4.95	374.9
5	642 gm. treble superphosphate........ 570 gm. ground limestone..............	0.015	38.6	201.4	1.16	4.62	397.0
6	908 gm. precipitated bone flour	Trace	39.0	190.7	1.08	4.71	435.6

The percentage of ash in the moisture- and fat-free bones exhibited some variations among the lots. The femurs from Lot 2 ("treated" rock phosphate) had a significantly higher ash content than those of Lot 1 ("untreated" rock phosphate); whereas the femurs from those which had received the addition of fluorine as calcium fluoride to the steamed bone meal ration (Lot 3) caused no significant difference in the percentage of ash in the bones. The ash contents of the bones from Lots 5 and 6 (treble superphosphate and bone flour, respectively) showed no significant differences when compared to those of Lot 4 (steamed bone meal).

The chemical analyses (Table 16) revealed, in contrast to the results of the previous trials, significant differences in the calcium and phosphorus contents of the bone ash from the different lots. The femurs of Lot 4 (steamed bone meal) had a significantly higher percentage of calcium in the ash than did any of the other lots, with the exception of Lot 6 (precipitated bone flour). There was no significant difference between Lots 1 and 2 in the percentage of calcium or in the percentage of phosphorus in the bone ash. The percentages of phosphorus in the bone ash of Lots 3, 4, and 5 (calcium fluoride, steamed bone meal, and treble superphosphate, respectively) showed no significant variations but the phosphorus contents of the bone ash of the control group (Lot 4) were significantly lower than those of Lots 1, 2, and 6 ("untreated" and "treated" rock phosphate and precipitated bone flour, respectively). The magnesium contents of the bone ash of Lots 2 and 1 ("treated" and "untreated" rock phosphates) varied directly with the fluorine contents of the rations, while the addition of calcium fluoride (Lot 3) to the control ration (Lot 4) had no significant effect in this respect. The percentage of magnesium in the bone ash of Lot 4 was significantly higher than that of Lots 5 and 6 (treble superphosphate and precipitated bone flour, respectively) regardless of the differences in the fluorine or magnesium contents of the three rations. As in the previous experiments, the substitution of rock phosphate ("untreated") for the steamed bone meal of the control ration caused an increase in the fluorine content of the ration and a decrease in the carbon dioxide content of the dry, fat-free bone. This decrease did not occur when the steamed bone meal was replaced by "treated" rock phosphate, which contained a low fluorine content, nor when calcium fluoride was added to the ration. The outstanding difference

TABLE 15.—Physical Measurements and Ash Contents of the Femurs
Experiment 3

Lot No.	Mineral additions to 100 lb. basal ration	Fluorine content of ration	Average weight	Average volume	Average length	Average smallest diameter	Average breaking strength	Average of breaking strength divided by weight of bone	Average ash	Average ash per cc. volume
		Pct.	*Gm.*	*Cc.*	*Cm.*	*Cm.*	*Lb.*		*Pct.*	*Gm.*
1	908 gm. "untreated" rock phosphate	0.071	110	108	15.9	2.03	570 ± 67	5.18	62.8 ± 0.25	0.640
2	908 gm. "treated" rock phosphate	0.010	139	122	17.2	1.92	1046 ± 29	7.53	64.1 ± 0.18	0.737
3	908 gm. steamed bone meal; 90 gm. calcium fluoride	0.091	133	119	17.0	1.91	866 ± 11	6.51	63.9 ± 0.28	0.714
4	908 gm. steamed bone meal	Trace	137	120	17.4	1.87	939 ± 22	6.85	63.1 ± 0.27	0.720
5	642 gm. treble superphosphate; 570 gm. ground limestone	0.015	139	120	17.5	1.88	938 ± 34	6.75	63.1 ± 0.16	0.731
6	908 gm. precipitated bone flour	Trace	135	117	17.2	1.83	861 ± 42	6.38	63.8 ± 0.14	0.736

TABLE 16.—Chemical Analyses of the Femurs
Experiment 3

Lot No.	Mineral additions to 100 lb. basal ration	Fluorine content of ration	Bone ash			Moisture- and fat-free bone	
			Calcium	Phosphorus	Magnesium	Carbon dioxide	Fluorine
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	908 gm. "untreated" rock phosphate	0.071	38.7 ± 0.09	18.9 ± 0.13	1.51 ± 0.036	2.05 ± 0.07	1.07 ± 0.031
2	908 gm. "treated" rock phosphate	0.010	38.8 ± 0.09	18.6 ± 0.17	0.96 ± 0.014	2.85 ± 0.03	0.13 ± 0.017
3	908 gm. steamed bone meal; 90 gm. calcium fluoride	0.091	38.6 ± 0.10	17.0 ± 0.09	1.02 ± 0.014	2.80 ± 0.05	0.07 ± 0.003
4	908 gm. steamed bone meal	Trace	39.6 ± 0.09	17.3 ± 0.19	1.08 ± 0.024	2.83 ± 0.03	0.03 ± 0.001
5	642 gm. treble superphosphate; 570 gm. ground limestone	0.015	38.8 ± 0.09	17.9 ± 0.19	0.92 ± 0.015	2.86 ± 0.02	0.03 ± 0.004
6	908 gm. precipitated bone flour	Trace	39.1 ± 0.18	18.9 ± 0.21	0.92 ± 0.009	2.83 ± 0.02	0.03 ± 0.001

in the chemical analyses lay in the fluorine contents of the bones of the various lots. It was clear that the fluorine of the calcium fluoride and the treble superphosphate was not transferred to the bones as efficiently as the fluorine from the two samples of rock phosphate. Comparing the results obtained with calcium fluoride in this experiment with those obtained with sodium fluoride in the previous experiments, it is obvious that the calcium salt is not so deleterious to pigs with respect to growth, feed consumption, or bone composition as is the sodium salt.

The mandibles of the pigs in Lot 1 ("untreated" rock phosphate) showed the characteristic symptoms of excessive fluorine feeding. They were white in color and rough in appearance, while the bodies of the bones were considerably thickened in contrast to the yellowish, lustrous color and smooth appearance of those in the other lots. This same roughened, white appearance was also apparent in the femurs of the animals in Lot 1.

The kidneys of the animals in Lot 1 ("untreated" rock phosphate), on gross examination, were firm to the touch and exhibited various degrees of fibrosis. Those of Lot 2 ("treated" rock phosphate) generally were slightly congested, while those of the animals in the other lots appeared normal. Microscopic examination showed varying degrees of fibrosis in the kidneys of Lot 1, congestion of the blood vessels and dilation of the capsular space in those of Lot 2, and normal conditions in the kidneys from Lots 3, 4, 5, and 6.

It is apparent, from the results of this trial, that calcium fluoride does not exert the same effects upon swine as does sodium fluoride or fluorine in the form present in rock phosphate. The various effects on the bones and kidneys noted in the previous trials when rock phosphate was fed were again demonstrated. No such kidney changes as those reported by Bohstedt and co-workers (10), as a result of feeding precipitated bone flour, were observed. Since the kidney changes in Lots 1 and 2 ("untreated" and "treated" rock phosphate) were not of the same magnitude and no pathological changes were noted in any of the previous experiments when sodium fluoride was fed, it would appear that these kidney changes must be caused either by the form of fluorine present or by some other substance present in the rock phosphate which was removed in part when the mineral was processed for the removal of the fluorine.

EXPERIMENT 4

The fourth experiment involved a study of the maximum amount of rock phosphate which could economically be fed to growing pigs and a comparison of the effects of phosphatic limestone (Fos-for-us) and a combination of rock phosphate and limestone when fed at the same calcium and phosphorus levels.

Forty weanling pigs were divided into five equal lots. All groups were confined in outdoor, brick-paved pens and self-fed the respective rations presented in Table 17. The mineral compositions of the rations were calculated from the following analyses of the various constituents:

Feed	Calcium	Phosphorus	Magnesium	Fluorine
	Pct.	*Pct.*	*Pct.*	*Pct.*
Ground yellow corn*	0.014	0.301	0.109	....
Wheat middlings	0.084	0.562	0.192	....
Linseed oil meal	0.364	0.714	0.420	....
Ground limestone	34.10	....	3.900	....
Rock phosphate A	33.83	12.90	0.216	3.62
Steamed bone meal	31.55	14.45	0.389	....
Phosphatic limestone	34.87	4.55	0.664	1.20

*Analyses taken from Henry & Morrison's "Feeds and Feeding".

The ration of Lot 1 was supplemented with 2 parts of rock phosphate A; Lot 2 had half of the rock phosphate A replaced by limestone; while Lot 3 received a combination of steamed bone meal and limestone in such amounts that the ration contained the same quantities of calcium and phosphorus as did the ration of Lot 2. The ration of Lot 4 contained 2 parts of phosphatic limestone; this was replaced in the ration of Lot 5 by a combination of rock phosphate A and limestone having the same calcium, phosphorus, and fluorine contents. The experiment was continued for 148 days, at which time the animals were slaughtered. Post-mortem examinations were made and tissues were removed for analyses.

TABLE 17.—Mineral Composition of the Rations—Calculated

Experiment 4

Lot No.	Mineral additions to 100 lb. basal ration	Calcium	Phosphorus	Magnesium	Fluorine	Ca:P ratio
		Pct.	*Pct.*	*Pct.*	*Pct.*	
1	908 gm. rock phosphate A	0.761* 0.723	0.695 0.628	0.190 0.150	0.071 0.071	1.1 1.2
2	454 gm. rock phosphate A .... 454 gm. ground limestone.........	0.764 0.725	0.568 0.501	0.226 0.189	0.036 0.036	1.3 1.4
3	405 gm. steamed bonemeal .... 530 gm. ground limestone..........	0.763 0.724	0.568 0.501	0.233 0.197	Trace Trace	1.3 1.4
4	908 gm. phosphatic limestone	0.781 0.743	0.531 0.464	0.199 0.162	0.024 0.024	1.5 1.6
5	320 gm. rock phosphate A .... 611 gm. ground limestone..........	0.780 0.742	0.531 0.464	0.238 0.202	0.025 0.025	1.5 1.6

*Upper figures represent starting ration. Lower figures represent finishing ration.

The data presented in Table 18 show that 2 parts of rock phosphate A in the ration were not as economical from the standpoints of average daily gains and feed required to produce 100 pounds of gain as when only one-half as much rock phosphate was fed (Lot 2). On the other hand, the ration supplemented with one part of rock phosphate A (Lot 2) caused a slightly greater average daily gain and a slightly smaller feed requirement than did the ration of Lot 3, which contained its supplementary phosphorus in the form of steamed bone meal. The average daily gains and the feed requirements of the pigs in Lots 4 and 5 (phosphatic limestone and rock phosphate A, respectively) were comparable to those of Lots 2 and 3.

TABLE 18.—Gains and Feed Requirements

Experiment 4

Lot No.	Mineral additions to 100 lb. basal ration	Fluorine content of ration	Av. initial weight	Av. final weight	Av. daily gain	Av. daily feed	Feed required per 100 lb. gain
		Pct.	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*
1	908 gm. rock phosphate A ..	0.071	42.3	170.0	0.86	4.21	488.4
2	454 gm. rock phosphate A 454 gm. ground limestone..	0.036	42.0	224.4	1.23	5.13	415.9
3	405 gm. steamed bone meal 530 gm. ground limestone..	Trace	42.1	194.9	1.02	4.45	434.6
4	908 gm. phosphatic limestone	0.024	41.7	209.6	1.13	4.81	424.6
5	320 gm. rock phosphate A 611 gm. ground limestone..	0.025	42.1	228.3	1.25	5.22	415.5

TABLE 19.—Physical Measurements and Ash Contents of the Femurs
Experiment 4

Lot No.	Mineral additions to 100 lb. basal ration	Fluorine content of ration	Average weight	Average volume	Average length	Average smallest diameter	Average breaking strength	Average of breaking strength divided by weight of bone	Average ash	Average ash per cc. volume
		Pct.	*Gm.*	*Cc.*	*Cm.*	*Cm.*	*Lb.*		*Pct.*	*Gm.*
1	908 gm. rock phosphate A	0.071	145	129	16.8	2.22	827 ± 43	5.70	59.6 ± 0.78	0.726
2	454 gm. rock phosphate A 454 gm. ground limestone	0.036	153	134	17.4	1.93	869 ± 30	5.68	63.2 ± 0.49	0.767
3	405 gm. steamed bone meal 530 gm. ground limestone	Trace	130	112	16.7	1.80	861 ± 33	6.62	61.3 ± 0.51	0.783
4	908 gm. phosphatic limestone	0.024	155	134	17.6	1.95	960 ± 27	6.19	61.6 ± 0.58	0.786
5	320 gm. rock phosphate A 611 gm. ground limestone	0.025	169	147	18.4	2.03	1011 ± 28	5.98	60.8 ± 0.59	0.777

TABLE 20.—Chemical Analyses of the Femurs
Experiment 4

Lot No.	Mineral additions to 100 lb. basal ration	Fluorine content of ration	Bone ash			Moisture- and fat-free bone	
			Calcium	Phosphorus	Magnesium	Carbon dioxide	Fluorine
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	908 gm. rock phosphate A	0.071	37.8 ± 0.20	16.9 ± 0.16	1.19 ± 0.018	2.53 ± 0.04	0.70 ± 0.013
2	454 gm. rock phosphate A 454 gm. ground limestone	0.036	38.6 ± 0.07	17.6 ± 0.13	1.03 ± 0.033	3.04 ± 0.03	0.33 ± 0.011
3	405 gm. steamed bone meal 530 gm. ground limestone	Trace	38.4 ± 0.06	16.6 ± 0.16	0.99 ± 0.022	3.04 ± 0.02	0.04 ± 0.002
4	908 gm. phosphatic limestone	0.024	37.8 ± 0.32	17.1 ± 0.20	1.09 ± 0.012	2.81 ± 0.07	0.26 ± 0.011
5	320 gm. rock phosphate A 611 gm. ground limestone	0.025	37.9 ± 0.20	16.6 ± 0.22	1.02 ± 0.018	2.90 ± 0.04	0.26 ± 0.007

The femurs of the animals in Lot 1, on gross examination, showed the characteristic rough, white, lusterless appearance found in the previous experiments when the rations contained 2 per cent of rock phosphate. In contrast to the previous experiments, those of Lot 2 (1 per cent of rock phosphate A) appeared normal in color and shape. The femurs of the animals in the other lots all had the smooth, yellow, lustrous, normal appearance. The smallest diameters of the bones of the various lots increased as the amounts of fluorine in the rations increased. The data on the breaking strengths of the bones (Table 19) did not show the extreme changes found in the other trials, especially when the ration contained 2 per cent of rock phosphate A. The percentage ash in the bones showed no significant differences in any of the lots when compared with Lot 3 (control). However, the ash content of the femurs of Lot 2 (1 per cent rock phosphate A) was significantly higher than that of Lot 1 (2 per cent rock phosphate A). No differences were noted in the results obtained on phosphatic limestone and a combination of rock phosphate A and limestone containing equivalent quantities of calcium, phosphorus, and fluorine (Lots 4 and 5).

Table 20 gives the data on the chemical analyses of the femurs. The calcium contents of the bone ash of the various lots do not vary significantly from that of the control lot (Lot 3); however, that of Lot 2 (1 per cent rock phosphate A) is significantly higher than that of Lot 1 (2 per cent rock phosphate A). The phosphorus content of the bone ash of Lot 2 is significantly higher than that of the other lots. These results are not in accord with those of the previous experiments. However, as in the other trials, the magnesium contents of the bones increased as the fluorine contents of the rations increased. The carbon dioxide content of the dry, fat-free bones showed a decrease in the case of Lot 1 (2 per cent rock phosphate A), and in all cases the percentage of fluorine in the bones increased in direct proportion to the fluorine contents of the rations. The increase was, however, not of the same magnitude as in the previous trials, when fluorine was fed at the same levels. There is no obvious explanation for the apparent discrepancies in this experiment when compared with the previous trials.

EXPERIMENT 5

LONG-TIME FEEDING TRIALS WITH SWINE

Inasmuch as the effects of feeding rock phosphate and sodium fluoride over short periods of time had been established, it was desired to determine the extent to which these effects would proceed if such feeding was continued over long periods of time. Furthermore, it was desired to determine if such feeding had a detrimental effect upon reproduction and lactation.

To this end, gilts from the previous experiments had been saved and continued on their respective rations. Three animals were fed the limestone control ration; two each were fed rations containing 2 parts of limestone and 30 and 60 grams of sodium fluoride per 100 pounds of ration, and two more received as a mineral supplement 1 part each of ground limestone and rock phosphate A. Five animals were maintained on the basal ration supplemented with 2 parts of rock phosphate A.

The same basal ration was used as that fed during the latter part of the fattening period in the previous experiments. The ingredients of the rations were analyzed separately and the mineral compositions of the rations calculated from the following results:

Feed	Calcium	Phosphorus	Magnesium	Fluorine
	Pct.	*Pct.*	*Pct.*	*Pct.*
Ground yellow corn*..................	0.014	0.301	0.109	....
Wheat middlings*....................	0.057	0.921	0.326	....
Linseed oil meal*....................	0.365	0.743	0.489	....
Ground limestone....................	36.49	....	1.96	....
Rock phosphate A....................	34.79	15.20	0.48	3.34
Sodium fluoride......................	....	....	....	44.75

*Analyses taken from Henry & Morrison's ''Feeds and Feeding''.

The mineral compositions and the Ca:P ratios of these rations are presented in Table 21. Subsequent to the removal of the gilts at the close of the fattening period, all the animals were hand-fed in order to prevent excessive fattening and to avoid breeding troubles from this source. After parturition, the amounts of the rations were again increased so that the animals were receiving all the feed they would consume in order to cover the requirements of lactation. No record was kept of the water consumption, but from daily observation it was obvious that the animals on the high levels of rock phosphate and sodium fluoride consumed much larger quantities of water than did the animals on the other rations. Judging by the condition of their lots and houses, water elimination was also much greater in those lots on the high-fluorine levels.

TABLE 21.—Mineral Composition of the Rations—Calculated
Experiment 5

Lot No.	Mineral additions to 100 lb. basal ration	Calcium	Phosphorus	Magnesium	Fluorine	Ca:P ratio
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	908 gm. ground limestone............	0.766	0.422	0.212	Trace	1.8
2	908 gm. ground limestone.......... 30 gm. sodium fluoride............	0.766	0.422	0.212	0.029	1.8
3	908 gm. ground limestone.......... 60 gm. sodium fluoride............	0.766	0.422	0.212	0.058	1.8
4	454 gm. ground limestone.......... 454 gm. rock phosphate A.......	0.751	0.570	0.197	0.033	1.3
5	908 gm. rock phosphate A..........	0.734	0.719	0.183	0.065	1.0

The gilts were kept out-of-doors on brick-paved lots and shelter was provided in well-ventilated houses adjoining the lots. They were first bred when approximately 7 to 8 months of age and subsequently to have pigs twice a year. At each breeding period all the animals were mated to the same boar so that in a comparison of the litters the male factor remained constant. The sows were allowed to farrow in the houses adjoining the lots and at no time were they allowed off the paved lots. The young pigs were confined to the pens until they were 2 weeks old, at which time they were given the run of an adjoining bluegrass pasture. The litters were weighed at birth and weekly thereafter until they were 6 weeks old, at which time they were weaned.

The sows were maintained under these conditions for approximately 2 years, with the exception of four animals in the lot receiving 2 parts of rock phosphate. One of these sows died after 10 months on the ration without having produced a litter; two others died after being on the ration 14 and 19 months, respectively, each having produced and suckled one litter; the fourth animal was a replacement but had been on the test 19 months before the termination of the experiment. At the time of slaughter, or after death, postmortem examinations were made and tissues were removed for analyses and histological examinations.

Until the time of their first parturition, all the animals remained in good physical condition, with the exception of Sow 12 (2 parts rock phosphate A). This animal began to lose weight and became unthrifty after she had been on the ration approximately 7 months. She was hesitant to move and usually stood with her head down and her hind feet drawn far up beneath her body. When forced to move she did so with a stiff, stilted gait and walked on the tips of her toes, with her "knees" bent. The sow continued to live in this condition for about 3 months.

Naturally, all the animals lost some weight after parturition, but the greatest losses occurred in the lots which received the largest amounts of fluorine (Lots 3 and 5). An attempt was made to increase the food consumption but it was found that the animals, except those in Lot 1 (control), refused to eat all of the extra amounts provided. The lot which received 2 per cent of rock phosphate was especially outstanding in this respect. After the young were weaned, none of the sows, with the exception of the control lot (Lot 1), consumed enough feed to bring them back to good condition until after a long period of time. None of the sows on the 2 per cent rock phosphate ration ever returned to good condition after having produced one litter, and in only one case (Sow 34) did a sow produce more than one litter. When feed was offered to the sows in Lots 2, 3, 4, and 5, particularly to those in Lot 5, they would take a mouthful and, with a backward toss of their heads, attempt to swallow the feed without allowing it to come in contact with their teeth. No effort was made to chew the feed. Cold water was taken very reluctantly, and the greatest part of the water was consumed after the sun had warmed it in the troughs. The reason for this behavior became apparent when the teeth were examined at the time of slaughter.

Data on reproduction (Table 22) are too meager to be conclusive. Sows 20 and 31 (2 per cent rock phosphate) produced only one litter each. The condition of these sows after their pigs were weaned became similar to that described in the case of Sow 12. They became unthrifty and were disinclined to move, standing most of the time in the position previously described. They walked with the same stiff, stilted gait as Sow 12 and lived only a short time after their litters had been weaned. The data indicate that, when the fluorine content of the ration does not exceed 0.058 per cent in the form of sodium fluoride or 0.065 per cent in the form of rock phosphate, no deleterious effect is produced on the number or size of the young at birth. However, the feed intake of the animals on rations containing 0.028 per cent or more of fluorine was curtailed to such an extent that lactation, as judged by the growth of the young, was impaired and the sows became unthrifty (Fig. 2 Upper). These effects became more exaggerated as the amounts of fluorine in the rations were increased. Sow 20 produced only one litter of nine pigs and lactation was so inadequate that had the pigs not been fed skimmilk the entire litter probably

TABLE 22.—Reproduction Data on Sows

Experiment 5

Lot No.	Mineral supplements per 100 lb.	Sow No.	No. of litters	Av. number per litter: Living	Av. number per litter: Dead	Birth weight	Average per cent weaned	Weaning weight
						Lb.		Lb.
1	908 gm. ground limestone	35	3	9.0	0	2.5	100.00	16.7
		42	3	10.0	0	2.0	63.63	17.9
		53	2	6.5	0	2.4	40.00	23.0
	Average	....	....	8.5	....	2.3	71.21	19.2
2	908 gm. ground limestone; 30 gm. sodium fluoride	22	2	11.0	0	2.5	63.63	17.9
		36	3	9.7	0	2.3	77.27	12.7
	Average	....	....	10.4	....	2.4	70.45	15.3
3	908 gm. ground limestone; 60 gm. sodium fluoride	39	2	9.0	0	2.0	88.88	12.0
		44	2	7.5	0	2.6	80.00	15.3
	Average	....	....	8.3	....	2.3	84.44	13.7
4	454 gm. limestone; 454 gm. rock phosphate A.	25	3	10.0	0	2.3	35.29	19.5
		65	3	7.1	0	1.9	45.45	17.5
	Average	....	....	8.6	....	2.1	40.37	18.5
5	908 gm. rock phosphate A.	12	....	....	....	....	....	....
		20	1	9.0	0	2.4	....	....
		23	1	5.0	1	1.9	60.00	21.3
		31	1	8.0	0	2.5	87.50	11.0
		34	2	7.5	0	1.8	46.66	16.2
	Average	....	....	7.4	....	2.2	48.54	16.2

would have succumbed. The other sows on the same ration (2 per cent rock phosphate) produced more milk than Sow 20 but apparently not so much as the control animals (Lot 1).

The femurs of the sows on gross examination showed conditions similar to but more extensive than those observed in the femurs of the pigs from the previous experiments. The femurs of the animals in Lot 1 (control) had a normal yellowish color, with smooth exteriors, a dense appearance, and a decided luster. The bones of the animals in the other lots had a lusterless appearance, were white in color, had many protuberances, and showed a decided thickening (Fig. 3).

The physical measurements of the femurs (Table 23) are not especially conclusive, due to the small number of animals in each lot. Considering the differences in the size of the animals in the various lots, the data seem to indicate that the diameters of the bones were increased with increasing amounts of fluorine in the ration. The data showed no significant differences in the breaking strengths of the femurs of the various lots, even when put on the basis of the weights of the bones, except in the case of Lot 5 (2 per cent rock phosphate) which showed a considerable decrease in tensile strength over the control lot (ground limestone).

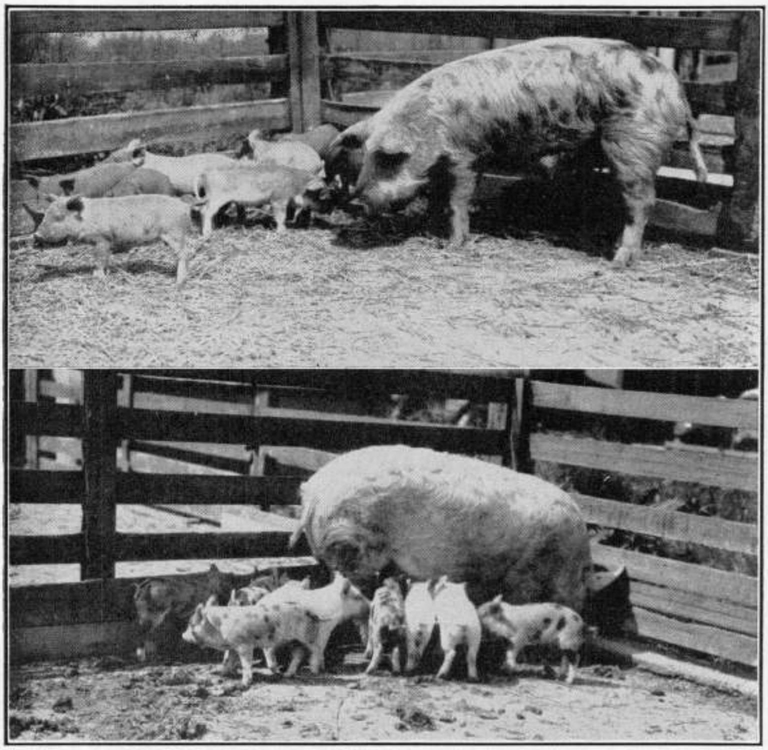


Fig. 2.—*Upper*—Sow 20 (2 parts of rock phosphate A) with her litter, which she was incapable of suckling. The young were fed skimmilk. Note the poor condition of the mother.

Lower—Sow 42 (2 parts of ground limestone) with her first litter of nine pigs, which she suckled successfully.

TABLE 23.—Physical Measurements and Ash Contents of Sow Femurs
Experiment 5

Lot No.	Mineral supplements per 100 lb. basal ration	Sow No.	Time on ration	Weight	Volume	Length	Smallest diameter	Breaking strength	Breaking strength divided by weight of bone	Ash†	Ash per cc. volume
			Mo.	*Gm.*	*Cc.*	*Cm.*	*Cm.*	*Lb.*		*Pct.*	*Gm.*
1	908 gm. ground limestone	35	26	304	236	21.8	2.76	822	2.70	65.7	0.846
		42	25	272	206	21.5	2.79	1041	3.83	67.1	0.885
		53	25	263	189	20.5	2.56	1116	4.24	67.6	0.940
	Average			280	210	21.3	2.70	993	3.59	66.8	0.890
2	908 gm. ground limestone 30 gm. sodium fluoride	22	25	325	274	22.1	3.15	629	1.94	67.0	0.795
		36	25	283	227	20.6	3.18	772	2.72	67.7	0.844
	Average			304	251	21.4	3.17	701	2.33	67.3	0.820
3	908 gm. ground limestone 60 gm. sodium fluoride	39	25	245	176	18.5	2.97	1087	4.44	67.3	0.937
		44	25	221	186	18.9	2.93	584	2.64	66.1	0.780
	Average			233	181	18.7	2.95	836	3.54	66.7	0.862
4	454 gm. ground limestone 454 gm. rock phosphate A	25	26	318	234	22.2	3.00	874	2.75	68.7	0.934
		65	26	455	346	25.6	3.02	2000‡	4.40‡	67.9	0.892
	Average			387	290	23.9	3.01	1437‡	3.58‡	68.3	0.913
5	908 gm. rock phosphate A	12	10*	176	175	19.6	2.97	248	1.41	63.4	0.637
		20	14*	197	187	19.5	2.83	322	1.63	64.4	0.678
		23	19	344	282	21.5	3.57	613	1.78	67.1	0.819
		31	19*	238	222	19.9	2.99	379	1.59	66.6	0.714
		34	25	232	201	18.4	3.37	645	2.78	65.8	0.760
	Average			237	213	19.8	3.15	441	1.84	65.4	0.722

*Sows died. †Ash based on moisture- and fat-free bone. ‡Beyond the limits of th dynamometer.

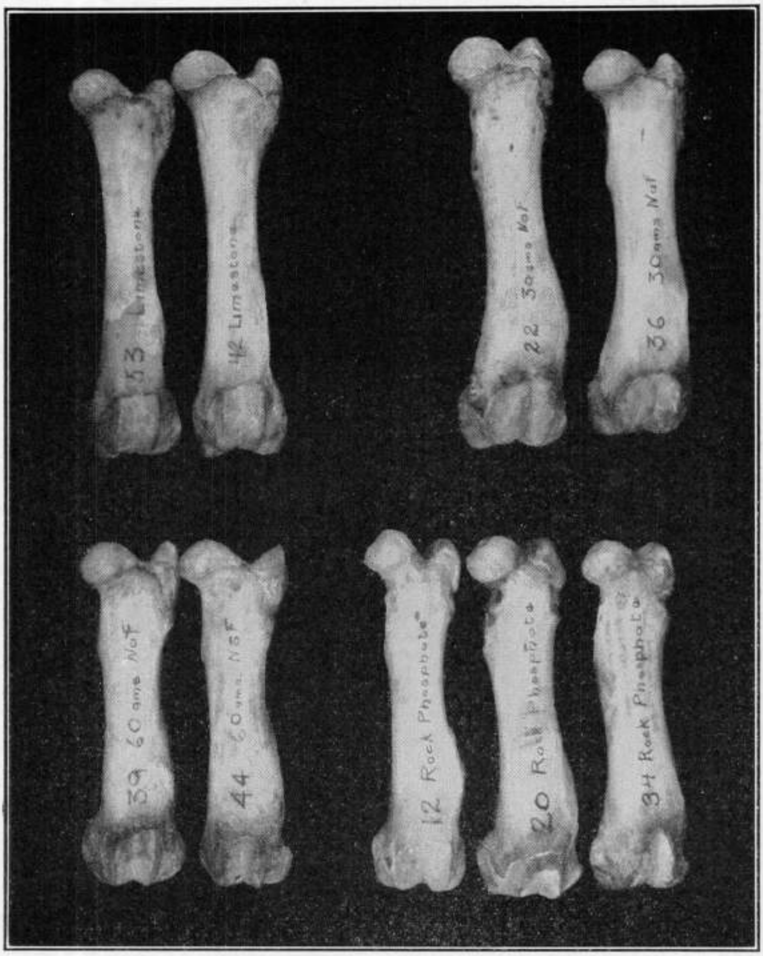


Fig. 3.—Femurs of sows fed various mineral supplements. The increase in the diameters and roughness of the bones with increasing amounts of fluorine in the ration may be observed.

Sows 42 and 53, basal ration plus 2 parts of ground limestone.

Sows 22 and 36, basal ration plus 2 parts of ground limestone and 30 gm. of sodium fluoride per 100 lb. of ration.

Sows 39 and 44, basal ration plus 2 parts of ground limestone and 60 gm. of sodium fluoride per 100 lb. of ration.

Sows 12, 20, and 34, basal ration plus 2 parts of rock phosphate A.

The ash contents of the bones (Table 23) were practically constant in all the lots, if the age and condition of Sows 12 and 20 at the time of their deaths and the fact that the latter had just passed through a lactation period are taken into consideration. There were indications that the bones of all the lots, with the exception of Lot 4 (1 part each of ground limestone and rock phosphate), were less dense than those of the control lot (Lot 1), as shown by the ash per unit volume. These results are in agreement with the findings in the previous experiments with growing, fattening pigs.

TABLE 24.—Chemical Analyses of the Sow Femurs*

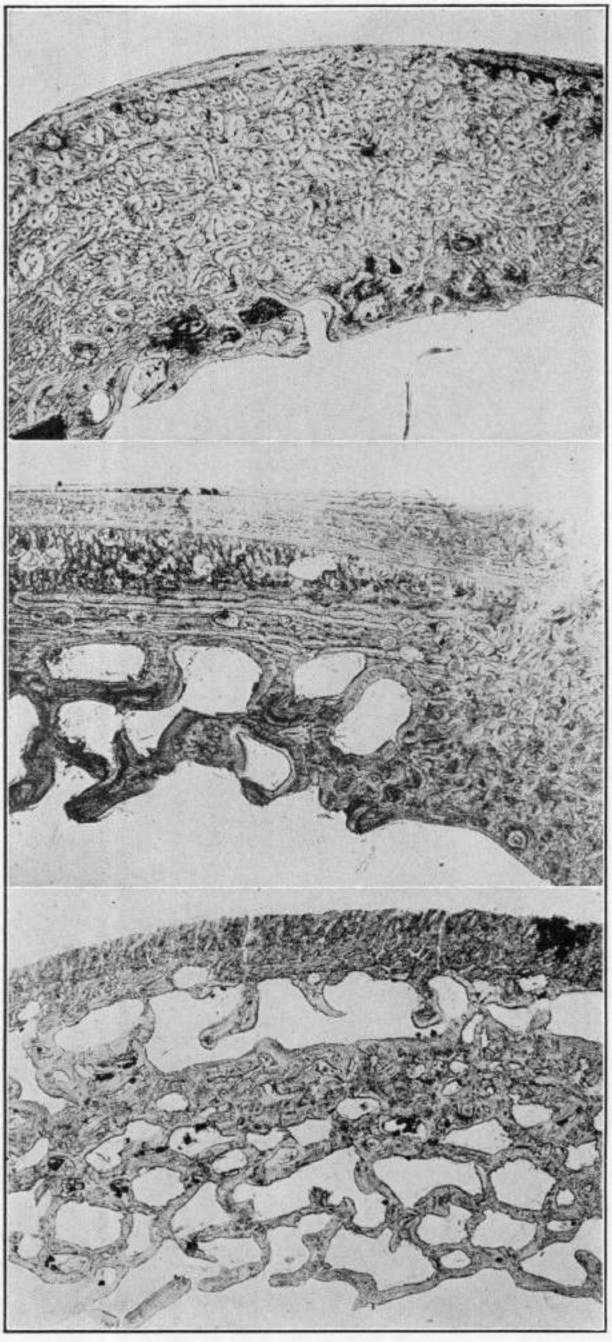
Experiment 5

Lot No.	Mineral supplement to 100 lb. basal ration	Sow No.	Calcium	Phosphorus	Magnesium	Carbon dioxide	Fluorine
			Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	908 gm. ground limestone	35	39.15	18.17	0.754	2.90	0.141
		42	38.61	18.39	0.760	3.27	0.230
		53	39.25	18.46	0.703	3.42	0.101
	Average	....	39.00	18.34	0.739	3.20	0.124
2	908 gm. ground limestone, 30 gm. sodium fluoride	22	39.94	17.74	0.931	2.97	1.090
		36	38.46	18.44	0.896	2.91	0.867
	Average	....	39.20	18.09	0.914	2.94	0.979
3	908 gm. ground limestone, 60 gm. sodium fluoride	39	39.46	18.31	0.964	2.40	1.307
		44	39.30	18.18	0.978	2.64	1.375
	Average	....	39.38	18.25	0.971	2.52	1.341
4	454 gm. ground limestone, 454 gm. rock phosphate A	25	38.95	17.86	0.915	2.24	0.927
		65	39.10	18.30	0.839	2.39	0.829
	Average	....	39.03	18.08	0.877	2.32	0.878
5	908 gm. rock phosphate A	12	38.26	18.66	1.281	1.58	1.473
		20	37.96	18.46	1.248	1.32	1.575
		23	39.00	17.75	1.040	2.51	1.278
		31	38.75	17.74	1.029	2.43	1.367
		34	38.56	17.65	1.121	2.33	1.621
	Average	....	38.51	18.05	1.144	2.03	1.463

*Calcium, phosphorus, and magnesium based on bone ash. Carbon dioxide and fluorine based on dry, fat-free bone.

The detailed chemical analyses of the femurs, presented in Table 24, were also in agreement with the results obtained in the previous experiments. They showed a decrease in the amounts of carbonates in the bones with an increase in the fluorine content of the rations. The percentage of fluorine in the bones varied directly with the fluorine content of the rations. The percentages of calcium and phosphorus in the bone ash did not vary; whereas the amounts of magnesium increased with increasing percentages of fluorine in the rations, the increase being independent of the magnesium intakes.

Ground sections were made from the central portions of the shafts of the femurs from all the sows. A decided difference in the structures of the bones from the various lots (Fig. 4) was observed. The bones of the animals in Lot 1 (control) presented the appearance of normal diaphyseal bones; whereas the bones from the animals in the other lots showed evidence of markedly increased rate of production of subperiosteal bone, lack of uniformity in the process of converting this bone into compact bone, and varying degrees of



Upper—Sow 35, basal ration plus 2 per cent of ground limestone.

Middle—Sow 65, basal ration plus 1 per cent each of ground limestone and rock phosphate A.

Lower—Sow 23, basal ration plus 2 per cent of rock phosphate A.

Fig. 4.—Transverse ground sections of femurs

secondary resorption of the compact bone to form large spaces filled with yellow marrow. These departures from normal were most marked in those animals which had received the higher amounts of fluorine. This phase of the study is receiving further investigation.

The mandibles of the sows which were fed rock phosphate or sodium fluoride appeared rough, uneven, and less translucent than those of the normal controls. The most striking change was a marked increase in the size of the bodies of the mandibles. The mandibles of Sow 35 (control), Sow 65 (1 part each of limestone and rock phosphate), and Sow 23 (2 parts rock phosphate A) were studied in detail. The average buccolingual dimensions in the molar regions of Sows 65 and 23 were respectively 42 and 60 per cent greater than those of the control, Sow 35. The width of the dental arch, taken at the position of the second molars, was definitely increased by increasing percentages of fluorine in the ration. In addition to the general thickening, exostoses were common. In slices cut transversely through the mandibles of Sows 35, 65, and 23, extensive local thickening of the compacta was observed. The medullary space was markedly increased in size as the amount of fluorine in the ration was increased (Fig. 5). Microscopical examination of celloidin sections through the mandible showed that the compact bone in certain thickened regions was imperfectly transformed from its cancellous, precursory state. The concentric lamellar systems were irregular and meandering in their course, and the Haversian canals were often greater in diameter than in normal bone. Aside from the irregularity of the concentric lamellar systems, the appearance was that of compact bone being formed with great rapidity. The resulting effect was hyperplasia of certain portions of the compacta, chiefly restricted to the buccal plate of the mandible. The medullary spaces of the mandibles of all three sows were filled with yellow marrow. Red marrow was lacking, even in the marrow spaces of cancellous bone near cortical bone.

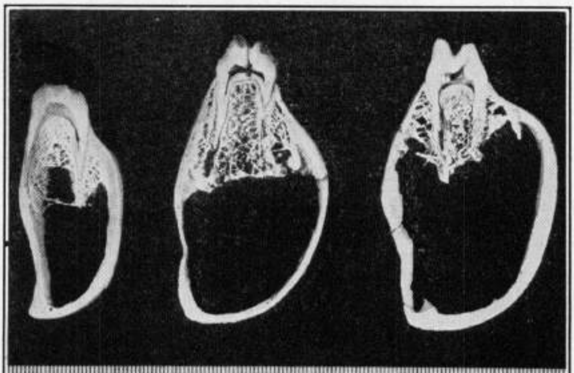


Fig. 5.—Sections of mandibles cut through distal roots of second molars of Sows 35, 65, and 23. (Metric scale)

While some mild gross hypoplasia of the enamel was occasionally observed in sows on the control ration, those which had been on rations containing high levels of fluorine showed severe hypoplasia of the enamel. This condition involved those portions of the enamel which were formed after the time when experimental feeding began. For the purpose of comparison, the three permanent molars were selected for microscopical examination because the calcification of these teeth covers a range of time, including before and during

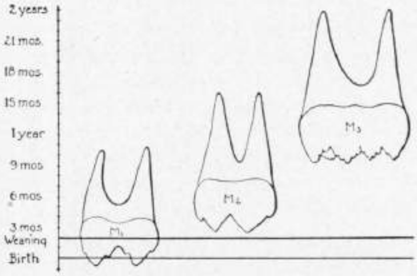


Fig. 6.—Schema showing progress of calcification of permanent molars of swine

experimental feeding. Besides the gross hypoplasia observed in the enamel, many of the teeth exhibited a longitudinal fluting where the surface was formed after experimental feeding had begun. A marked example of this is shown in Figure 7. Abrasion was not notably different in the teeth of the controls and of those on rations of high fluorine contents, but friability was evidenced by many chipped and broken teeth of animals on rations containing additions of fluorine.

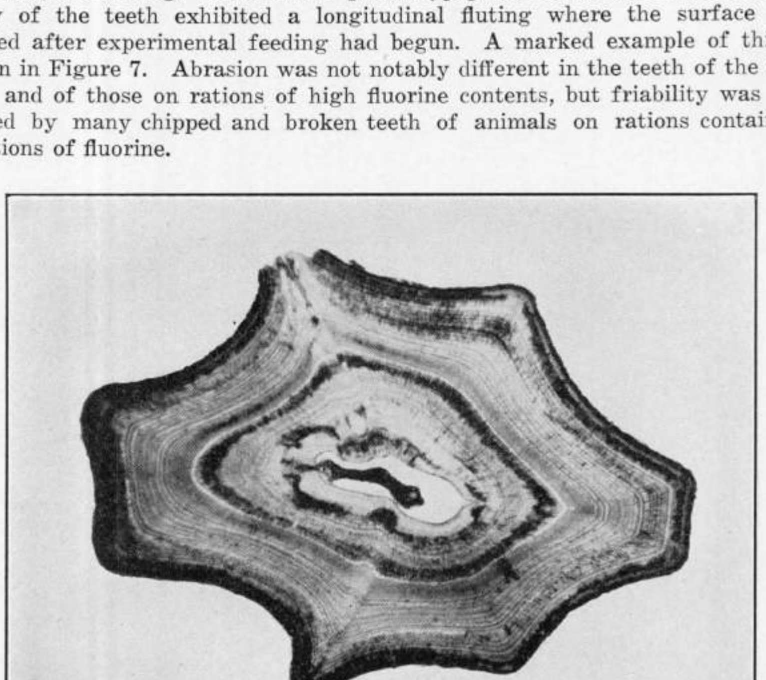


Fig. 7.—Ground cross-section of fluted central incisor from Sow 23. (x 7)

Microscopical examination of the teeth of Sow 35 (limestone), Sow 65 (1 part each limestone and rock phosphate), and Sow 23 (2 parts rock phosphate) showed a progressive increase in the hypoplasia of both enamel and dentin with increasing amounts of fluorine in the rations. A synopsis of the microscopical findings is made in Table 25. Figure 8, showing a buccolingual

TABLE 25.—Microscopical Observations of Molars of Sows

Age of sow and number	Diet	Per cent fluorine	First molar	Second molar	Third molar
Control 29 mo. 35	Basal	0.000 Trace (?)	Enamel good	Enamel good	Partially erupted. Enamel good except for two thin zones of mild hypoplasia
			Dentin good. Two zones of mildly hypoplastic dentin	Dentin good with two zones of mild hypoplasia	Dentin good except for two zones of mild hypoplasia
29 mo. 65	Basal + 1 part rock phosphate A and 1 part ground limestone	0.033	Enamel good	Enamel hypoplastic and reduced in amount	Unerupted. Very little enamel. Extreme hypoplasia except near cervix, where it is mild
			Dentin fair in superficial half; hypoplastic in pulpal half	Dentin mildly hypoplastic in superficial half and severely hypoplastic in pulpal half	Dentin has severe hypoplasia
14 mo. 23	Basal plus 2 parts rock phosphate	0.065	Enamel good.	Crown nearly devoid of enamel. When present, less than 1/10 normal thickness and extremely hypoplastic.	Unerupted. Slightly more enamel than 2nd molar but all extremely hypoplastic.
			Dentin in superficial third good; in pulpal two-thirds, severe hypoplasia	Dentin hypoplastic in superficial third and extremely hypoplastic in pulpal two-thirds	Dentin has severe hypoplasia throughout

section of a first molar of Sow 23, illustrates well-formed dentin deposited before the experimental feeding was begun and marked hypoplastic dentin formed after the inclusion of rock phosphate at a 2 per cent level in the ration. In a section of the second molar of this sow (Fig. 9), calcification of which was almost entirely subsequent to the start of fluorine feeding, the dentin exhibits severe hypoplasia throughout. Enamel, extremely meager in amount, is found chiefly in sulci and on cervical portions of the crown. Figure 10, showing higher magnification of one cusp of an unerupted third molar from the same sow, illustrates extreme hypoplasia of the enamel.

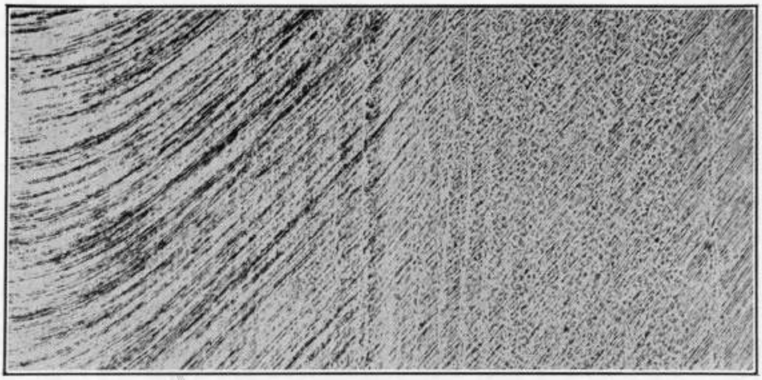


Fig. 8.—Ground section of dentin of first molar from Sow 23, illustrating dentin formed before and after experimental feeding. (x 100)

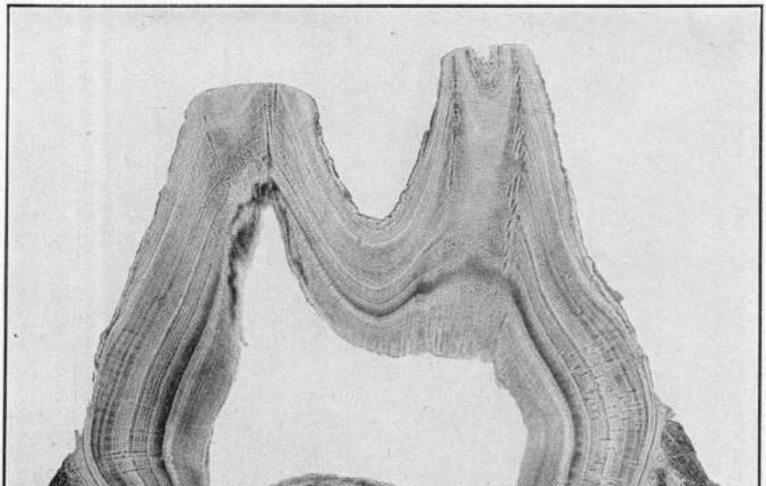


Fig. 9.—Buccolingual ground section of second molar from Sow 23, showing hypoplastic dentin and scarcity of enamel. (x 6)

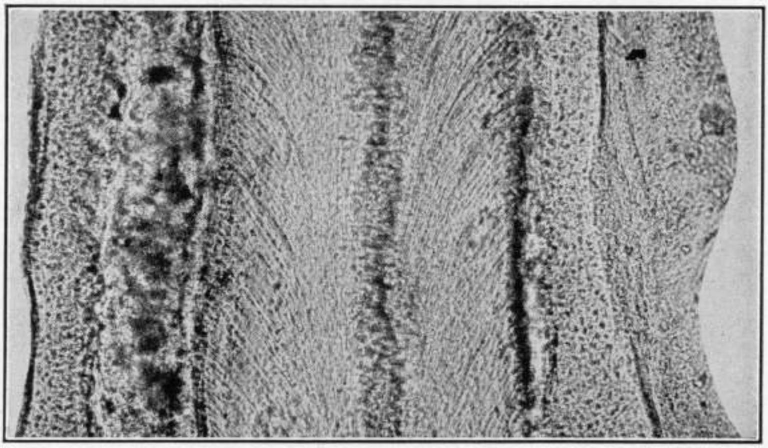


Fig. 10.—Buccolingual ground section of buccal cusp of unerupted third molar from Sow 23, illustrating extreme hypoplasia of enamel. (x 150)

Post-mortem examination revealed no abnormalities in any of the other organs, with the exception of the kidneys of Lots 4 and 5 (1 and 2 per cent rock phosphate A, respectively). These organs were firm, pale in color, and presented a "hobnailed" appearance (Fig. 11). The kidneys from the animals

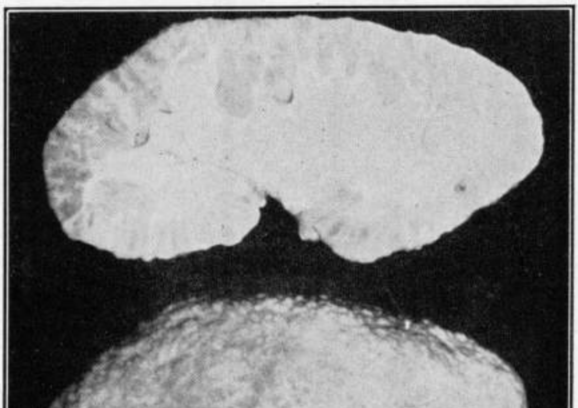


Fig. 11.—Kidney of Sow 12 fed the basal ration supplemented with 2 parts of rock phosphate A for 10 months. The lower picture shows the characteristic "hobnailed" condition of the exterior of kidneys of sows fed rock phosphate. Note the small amount of active tissue in the upper picture.

fed 2 per cent of rock phosphate showed more extensive lesions than those fed 1 per cent. Histological examination showed a destruction of the epithelium of the convoluted tubules and a marked infiltration of fibrous tissue throughout the organ (Fig. 12). In the cases of Sows 12, 20, and 31 (2 per cent rock phosphate A), the degeneration had progressed to such an extent that it apparently was the cause of the deaths of these animals. The kidneys from the sows of Lots 1, 2, and 3 (ground limestone, 30 and 60 gm. sodium fluoride, respectively) showed no abnormalities upon microscopical examination. The livers, in all cases, showed no histological changes.

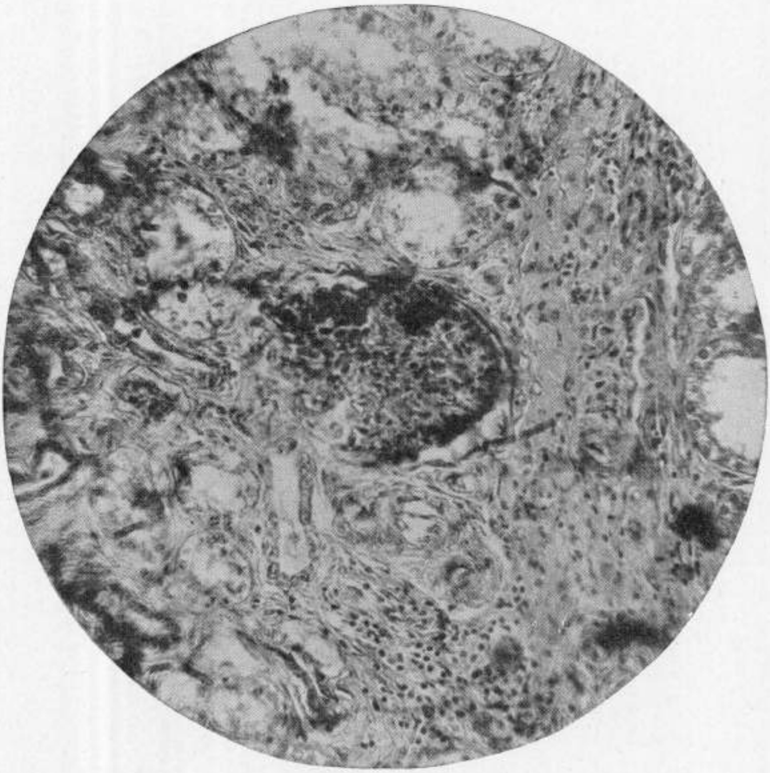


Fig. 12.—Kidney section from Sow 12, fed the basal ration supplemented with 2 parts of rock phosphate A for 10 months, showing degeneration of the convoluted tubules and presence of fibrous tissue. H. and E. (x 225).

From these data it is evident that the feeding of rock phosphate and sodium fluoride over long periods of time produced the same effects as noted in the previous short-time experiments, although in a more exaggerated form. No direct effect upon reproduction was noted, but, due to the serious curtailment in the consumption of feed, poor lactation occurred when the ration contained 0.029 per cent or more of fluorine.

EXPERIMENTS WITH CHICKS

The investigations cited in the review of the literature suggest that fluorine, as found in rock phosphate, may be a detrimental factor in the nutrition of the chick. The data do not give any definite information as to the tolerance of the bird for this element or the specific effects of a surplus. The present investigations were undertaken to study the effects of rock phosphate and various fluorides on the chick and to obtain information regarding the tolerance and physiological reactions of this species to fluorine.

Four separate trials were conducted. The basal rations used in all the experiments consisted of: ground wheat, 20; soybean oilmeal, 20; wheat bran, 5; dried skimmilk, 5; cod-liver oil, 2 in Experiments 1 and 2 and 1 in Experiments 3 and 4; sodium chloride, 1; and ground yellow corn to make 100 parts after the addition of the minerals. This ration, when properly supplemented with minerals, had given good results under similar experimental conditions. The mineral supplements were from the same sources as those used in the experiments with swine.

White Leghorn chicks were used throughout the several trials. They were started on their respective rations when one day old. The birds were confined in battery brooders provided with false screen bottoms, and feed was kept before them at all times in hoppers attached to the outside of the brooders. Tap water was supplied from ordinary drinking fountains. The chicks were weighed individually each week and accurate records kept of their feed consumption. Twenty chicks were placed in each lot at the start of the experiment.

At the end of 8 weeks the birds were killed. In three of the trials the tibiae were removed for ash determinations from 10 representative birds of each surviving lot. The ash values are expressed on a moisture- and fat-free basis.

In the calculations of the coefficients of variability, the male weights were put on a female basis, using the factor of Ackerson and Mussehl (1). The biweekly weights given in the tables are the mean weights of the birds without regard to sex distribution.

EXPERIMENT 1

The initial trial consisted of eight lots which received the basal ration supplemented as indicated in Table 26. Rock phosphate A, containing 3.17 per cent fluorine, was fed at levels of 3 and 5 per cent (Lots 2 and 4, respectively). As control rations, steamed bone meal was fed at levels which furnished the same amounts of calcium as did the rock phosphate (Lots 1 and 3). Sodium fluoride was added in amounts of 1, 2, 5, and 10 grams per kilo of ration to that containing the lower amount of steamed bone meal (Lot 1), and these rations were fed to Lots 5, 6, 7, and 8, respectively.

From the results presented in Table 26, it is obvious that rock phosphate exerted a detrimental effect on growth and feed consumption when fed at levels of 3 per cent or more of the ration (0.095 per cent fluorine). The addition of sodium fluoride in amounts that supplied more than 0.045 per cent fluorine also decreased feed consumption and growth. The birds which received a ration containing 0.22 per cent of fluorine as sodium fluoride (Lot 7) were all dead by the end of the fifth week; whereas those that received the ration containing

TABLE 26.—Effect of Additions of Rock Phosphate and Sodium Fluoride to the Rations of Chicks

Experiment 1

Lot No.	Mineral additions per kilo basal ration	Fluorine content of ration	Feed consumed per chick	Fluorine consumption per chick	Average biweekly weight, in grams					Coefficient of variability	Average ash in tibiae
					0	2	4	6	8		
		Pct.	*Gm.*	*Gm.*							*Pct.*
1	32.8 gm. steamed bone meal	Trace	1547	....	34	86	189	313	435	13.64	50.6 ± 0.31
2	30.0 gm. rock phosphate A	0.095	1171	1.11	34	78	156	270	387	23.08	51.1 ± 0.28
3	54.7 gm. steamed bone meal	Trace	1500	....	34	88	192	311	450	10.25	49.2 ± 0.20
4	50.0 gm. rock phosphate A	0.158	886	1.40	34	72	125	163	226	44.50	50.7 ± 0.45
5	32.8 gm. steamed bone meal 1.0 gm. sodium fluoride	0.045	1450	0.65	34	92	182	299	444	11.31	49.9 ± 0.22
6	32.8 gm. steamed bone meal 2.0 gm. sodium fluoride	0.089	1350	1.20	34	68	154	270	393	15.09	50.0 ± 0.26
7	32.8 gm. steamed bone meal 5.0 gm. sodium fluoride	0.221	322	0.71	34	43	55	....	....	....	....
8	32.8 gm. steamed bone meal 10.0 gm. sodium fluoride	0.442	123	0.54	34	40	....	....	....	....	....

0.44 per cent fluorine (Lot 8) survived only 3 weeks. The data clearly show that feed consumption and growth of chicks are adversely affected when the ration contains approximately 0.09 per cent of fluorine in the form of either rock phosphate or sodium fluoride.

The lots which received the largest amounts of fluorine in their ration also showed the greatest variability in growth. This is probably due to the individual ability of the birds to withstand the toxic effects of fluorine.

A comparison of the ash contents of the bones from the birds of Lots 1, 2, 5, and 6 showed that the addition of fluorine, either in the form of sodium fluoride or rock phosphate, had no significant effect on calcification. However, the ash contents of the tibiae of Lot 3 (steamed bone meal) were significantly lower than those of Lot 4 (5 per cent rock phosphate A), but it is doubtful whether this difference can be attributed to the fluorine content of the rock phosphate.

EXPERIMENT 2

The second test was in part a repetition of the first trial, except that calcium fluoride was used in place of sodium fluoride and high- and low-fluorine rock phosphates were also fed. These latter minerals represented the "treated" and "untreated" rock phosphates used in Pig Experiment 3. Both products were of the same origin. The "treated" product, which had been processed to remove the greater portion of the fluorine, contained only 0.50 per cent fluorine, in contrast to 3.56 per cent for the "untreated". The calcium and phosphorus in the rations were adjusted so that they were comparable to either 3 or 5 per cent rock phosphate A. The calcium fluoride was added in amounts to make the fluorine content of the rations equivalent to either 3, 5, or 7.5 per cent of rock phosphate A.

Table 27 gives the amounts of minerals included in the rations, the fluorine contents of the rations, and the feed consumption and growth data of the different lots. Again the data show that 3 per cent or more of rock phosphate in the ration decreased feed intake and retarded growth. The addition of calcium fluoride, even at a fluorine level equivalent to 7.5 per cent of rock phosphate (0.27 per cent fluorine), had no effect on either feed consumption or growth. These results are in direct opposition to those obtained with chicks with sodium fluoride (Experiment 1) but are in accord with the results obtained with pigs. The growth of the birds in Lot 8 (5 per cent "untreated" rock phosphate) was significantly less than that of the birds in Lot 9 (5 per cent "treated" rock phosphate), due to the higher fluorine content of the former ration (0.178 per cent fluorine as compared to 0.025 per cent). No bone ash determinations were made in this trial.

EXPERIMENTS 3 AND 4

Since the first two trials had definitely shown that a ration containing approximately 0.09 per cent or more of fluorine, either in the form present in rock phosphate or sodium fluoride, had a deleterious effect on growth and feed consumption, it was desired to determine the effects of smaller additions of rock phosphate and also to ascertain the comparative effect of fluorine in the form of the phosphorite mineral and sodium fluoride.

Two separate experiments were conducted to answer these questions. In Experiment 3, two lots of 20 chicks each were fed the same ration; in Experiment 4, each lot of 20 chicks received one of the seven different rations.

TABLE 27.—Effect of Additions of Rock Phosphate and Calcium Fluoride to the Rations of Chicks

Experiment 2

Lot No.	Mineral additions per kilo basal ration	Fluorine content of ration	Feed consumed per chick	Fluorine consumption per chick	Average biweekly weights, in grams					Coefficient of variability
					0	2	4	6	8	
		Pct.	*Gm.*	*Gm.*						
1	27.0 gm. steamed bone meal; 5.0 gm. ground limestone	Trace	1889	....	34	104	230	407	539	14.02
2	45.0 gm. steamed bone meal; 8.0 gm. ground limestone	Trace	1709	....	34	107	224	378	531	11.27
3	30.0 gm. rock phosphate A	0.108	1710	1.89	34	93	211	360	493	15.22
4	50.0 gm. rock phosphate A	0.181	1527	2.76	34	72	138	233	326	24.82
5	27.0 gm. steamed bone meal; 1.6 gm. ground limestone; 2.3 gm. calcium fluoride	0.108	1813	1.96	34	112	246	425	574	15.88
6	27.0 gm. steamed bone meal; 3.9 gm. calcium fluoride	0.182	1916	3.49	34	109	242	426	570	12.83
7	27.0 gm. steamed bone meal; 5.8 gm. calcium fluoride	0.271	1868	5.06	34	107	236	393	559	18.84
8	50.0 gm. rock phosphate (untreated)	0.178	1531	2.73	34	99	180	291	401	28.02
9	50.0 gm. rock phosphate (treated)	0.025	1756	0.44	34	86	201	332	470	21.87

The calcium and phosphorus contents of the rations were kept comparable throughout both trials. Steamed bone meal and ground limestone were added to the control ration (Lot 1) in such amounts that the percentages of calcium and phosphorus were equivalent to those supplied by 3 per cent of rock phosphate A. The latter mineral was fed at 1, 2, and 3 per cent levels and replaced such amounts of bone meal and limestone in the control ration as to maintain the same calcium and phosphorus contents. Sodium fluoride was added to the control ration in such amounts that the fluorine contents of these rations were comparable to those containing 1, 2, and 3 per cent of rock phosphate A. Bone ash determinations were made on the tibiae of 20 chicks on each ration in Experiment 3 and of 10 individuals of each lot in Experiment 4.

The results of Experiment 3 are shown in Table 28; Table 29 presents those of Experiment 4. The results of both trials showed that when the ration contained approximately 0.07 per cent or more of fluorine, either in the form of sodium fluoride or rock phosphate, a decrease in feed consumption and a retardation in growth resulted. These effects became more pronounced as the percentages of fluorine in the rations were increased. With smaller amounts of fluorine no deleterious effects were noted.

As in the previous trials with chicks, there were no significant differences in bone ash, in either experiment, between the control lot (steamed bone meal and limestone) and 3 per cent of rock phosphate (Lot 4). However, the substitution of 1 and 2 per cents of rock phosphate for part of the steamed bone meal and limestone (Lots 2 and 3, respectively) resulted in a significantly higher ash content of the tibiae. In Experiment 3 the ash contents of the bones of the birds in Lots 5, 6, and 7 (sodium fluoride additions) all had a significantly higher bone ash than the control lot (Lot 1); whereas in Experiment 4 none of the corresponding lots (Lots 5, 6, and 7) showed any significant differences in bone ash when compared to Lot 1. It would appear that a small amount of fluorine tended to increase the percentage of bone ash but more work will be required before any definite conclusion can be drawn in this respect.

At the time the birds in these two experiments were killed for bone analysis it was thought advisable to determine the coagulation time of the blood, since some decidedly contradictory results on the effect of fluorine on blood coagulability had been reported in the literature. The drop plate method was used. The average of the individual determinations is presented in Table 30. It is apparent that fluorine, fed either in the form of sodium fluoride or rock phosphate, decreased the clotting time of the blood.

Considerable mortality was experienced in Experiment 3 as a result of pullorum infection. The mortality, however, was not associated with the fluorine content of the ration. The results, in general, were the same as those in Experiment 4 or in the other trials in which mortality was not a factor.

TABLE 28.—Effect of Additions of Rock Phosphate and Sodium Fluoride to the Rations of Chicks

Experiment 3

Lot No.	Mineral additions to 100 lb. basal ration	Fluorine content of ration	Feed consumed per chick	Fluorine consumption per chick	Average biweekly weights, in grams					Coefficient of variability	Average ash in tibiae
					0	2	4	6	8		
		Pct.	*Gm.*	*Gm.*							*Pct.*
1	1380 gm. steamed bone meal 199 gm. ground limestone	Trace	1237	....	34	84	177	328	486	21.18	47.9 ± 0.26
2	920 gm. steamed bone meal 133 gm. ground limestone 454 gm. rock phosphate A	0.036	1269	0.46	34	84	171	314	492	18.43	51.1 ± 0.32
3	460 gm. steamed bone meal 66 gm. ground limestone 908 gm. rock phosphate A	0.071	1074	0.76	34	81	160	280	424	24.40	50.5 ± 0.26
4	1362 gm. rock phosphate A	0.107	663	0.71	34	67	101	153	203	34.76	47.8 ± 0.57
5	1380 gm. steamed bone meal 199 gm. ground limestone 37 gm. sodium fluoride	0.036	1268	0.46	34	88	182	325	491	22.48	50.3 ± 0.26
6	1380 gm. steamed bone meal 199 gm. ground limestone 74 gm. sodium fluoride	0.072	1059	0.76	34	77	151	274	421	20.22	49.6 ± 0.30
7	1380 gm. steamed bone meal 199 gm. ground limestone 111 gm. sodium fluoride	0.108	827	0.89	34	66	122	202	297	33.87	50.1 ± 0.30

TABLE 29.—Effect of Additions of Rock Phosphate and Sodium Fluoride to the Rations of Chicks

Experiment 4

Lot No.	Mineral additions to 100 lb. basal ration	Fluorine content of ration	Feed consumed per chick	Fluorine consumption per chick	Average biweekly weight, in grams					Coefficient of variability	Average ash in tibiae
					0	2	4	6	8		
		Pct.	*Gm.*	*Gm.*							*Pct.*
1	1380 gm. steamed bone meal 199 gm. ground limestone	Trace	1606	....	34	113	246	426	621	11.17	49.9 ± 0.33
2	920 gm. steamed bone meal 133 gm. ground limestone 454 gm. rock phosphate A	0.036	1466	0.53	34	103	223	393	575	20.31	52.6 ± 0.29
3	460 gm. steamed bone meal 66 gm. ground limestone 908 gm. rock phosphate A	0.071	1450	1.03	34	104	218	375	546	19.92	51.5 ± 0.43
4	1362 gm. rock phosphate A	0.107	1073	1.15	34	79	146	250	386	26.36	49.2 ± 0.34
5	1380 gm. steamed bone meal 199 gm. ground limestone 37 gm. sodium fluoride	0.036	1572	0.57	34	110	247	430	616	13.47	50.9 ± 0.34
6	1380 gm. steamed bone meal 199 gm. ground limestone 74 gm. sodium fluoride	0.072	1252	0.90	34	96	195	312	456	24.81	51.0 ± 0.31
7	1380 gm. steamed bone meal 199 gm. ground limestone 111 gm. sodium fluoride	0.108	959	1.04	34	69	127	202	288	26.45	47.8 ± 0.36

TABLE 30.—Effect of Fluorine Feeding on the Coagulability of Chicken Blood

Lot No.	Fluorine content of ration	Exp. 3	Exp. 4
	Pct.	*Seconds*	*Seconds*
1	Trace	159	196
2	0.036	128	140
3	0.071	66	70
4	0.107	32	27
5	0.036	68	211
6	0.072	34	38
7	0.108	17	17

EXPERIMENTS WITH RATS

At the time the previously reported experiments were in progress, it was considered advisable to check and extend this work, using the rat as the experimental animal. This species affords several advantages over the larger animals. The relatively short life cycle of the rat greatly reduces the length of time required to obtain data on growth and reproduction. Also, the ease with which the animals are handled and the rations controlled are distinct advantages from the experimental point of view. Another advantage is the fact that the permanently growing incisors furnish opportunity to test the effects of different rations on tooth structure.

Several separate trials were conducted for the purpose of studying the comparative availability of fluorine in different compounds and its effect on the formation and composition of bones and teeth and on reproduction.

EXPERIMENT 1

This experiment was planned to study the effects of the addition of varying amounts of sodium and calcium fluorides to a synthetic ration on the development of the bones and teeth of rats. It involved seven lots of five rats each—three females and two males per lot. The animals were confined in wire cages provided with false screen bottoms and were started on their experimental rations when 24 days of age.

The basal synthetic ration was composed of: casein, 18; dextrin, 67; yeast, 7; agar, 2; cod-liver oil, 2; and Osborne-Mendel salt mixture, from which the sodium fluoride had been omitted, 4. To this ration were added 0.01, 0.02, or 0.05 per cent of either sodium or calcium fluorides of C. P. grade. One lot which received the unsupplemented basal ration served as a control. All rations were fed *ad libitum*, and distilled water was used for drinking.

After the animals had been on experiment for 19 weeks, they were chloroformed and the heads, femurs, humeri, and tibiae were removed. The heads were preserved in a 4 per cent solution of formaldehyde for examination of the teeth. One of each of the three sets of cleaned, dried, extracted bones was used for ash determinations. Composite samples of the ash of the bones of each lot were analyzed for calcium, phosphorus, and magnesium. The remaining femurs, humeri, and tibiae from each lot were composited and used for carbon dioxide and fluorine determinations.

The data presented in Table 31 show that the incorporation of calcium fluoride in the rations had no effect upon feed consumption, growth, or percentage of bone ash. Sodium fluoride did not influence the ash content of the bones but had a depressing effect upon feed consumption and growth when fed at a level of 0.05 per cent of the ration. The slower growth was directly correlated with the decreased feed consumption of the animals in this group (Lot 6).

The detailed chemical analyses of the moisture- and fat-free bones, recorded in Table 32, show that with increasing amounts of fluorides in the rations there was an increase in the percentage of fluorine in the bones. This increase was greater when sodium fluoride was fed than when the ration contained corresponding amounts of calcium fluoride. The carbon dioxide content of the bones of Lots 6 and 7 (0.05 per cent sodium and calcium fluoride, respectively), when compared with the bones of Lot 1 (control), showed a tendency to decrease. Analyses of the bone ash showed no significant differences in the calcium and phosphorus contents of the various lots. The magnesium contents of the bone ash varied considerably, but there appeared to be a tendency for this to increase in the lot receiving the highest amount of sodium fluoride (Lot 6).

The teeth of the rats in the control lot (Lot 1) and those of the lots which received 0.01 and 0.02 per cent of calcium fluoride (Lots 3 and 5) were normal in size, shape, and color. The lots which were fed 0.05 per cent calcium fluoride or 0.01 per cent sodium fluoride (Lots 7 and 2, respectively) had teeth which were normal in shape but which were characterized by lack of the normal orange-yellow color of the enamel. The presence of 0.02 per cent or more of sodium fluoride in the ration caused the enamel to lose all color and become soft and white. The mandibular incisors showed considerable wear, while the maxillary incisors became greatly elongated and curved backwards, forming the greater part of a circle. Figure 13 shows the skulls and teeth of representative animals from the various lots. The changes in the teeth were more severe when sodium fluoride was fed than when calcium fluoride was included in the ration at the same levels. This is in agreement with the effects of these two salts upon the chemical composition of the bones and upon growth as previously reported with pigs and chickens.

EXPERIMENT 2

Experiment 2 was designed to obtain data on the effects of feeding rock phosphate, phosphatic limestone, and calcium fluoride on reproduction and lactation in the rat and also to study the effects of these supplements on growth and bone and tooth development in this species. It was also expected that this experiment would serve as a check on the swine investigations.

Weanling albino and hooded rats, 24 days old, were divided into 11 lots of five rats each, according to litter, sex, and weight. Two males and three females were placed in each group and were given access to feed and distilled water. All rats were weighed individually each week. The males and females were kept in the same cage and were allowed to breed at will. When the females became pregnant, they were removed to individual cages for parturition and lactation. All litters were weighed at birth and reduced to six in number. The young were allowed to suckle until they were 24 days old, at which time they were weighed, weaned, and destroyed. The female was returned to her original cage until she again became pregnant. Two or three

TABLE 31.—Effects of Adding Varying Amounts of Sodium and Calcium Fluorides to the Ration of Rats on Growth, Feed Consumption, and Bone Ash

Experiment 1

Lot No.	Fluorine additions	Fluorine in ration	Initial weight	Final weight	Feed consumption per rat	Average ash in bones		
						Femurs	Humeri	Tibiae
	Pct.		*Gm.*	*Gm.*	*Gm.*	*Pct.*	*Pct.*	*Pct.*
1	None	0.0 ?	56	279	1637	65.6 ± 0.47	66.2 ± 0.43	65.8 ± 0.30
2	0.01 sodium fluoride	0.0044	55	279	1645	66.4 ± 0.23	66.9 ± 0.15	66.8 ± 0.12
3	0.01 calcium fluoride	0.0046	56	289	1754	64.3 ± 0.18	66.2 ± 0.27	65.7 ± 0.21
4	0.02 sodium fluoride	0.0088	55	282	1687	65.3 ± 0.57	66.0 ± 0.28	66.3 ± 0.27
5	0.02 calcium fluoride	0.0092	54	261	1634	65.0 ± 0.28	65.9 ± 0.29	65.2 ± 0.30
6	0.05 sodium fluoride	0.0220	51	201	1269	64.3 ± 0.27	65.5 ± 0.24	65.5 ± 0.11
7	0.05 calcium fluoride	0.0230	61	285	1593	65.6 ± 0.13	65.8 ± 0.07	64.6 ± 0.54

TABLE 32.—Effects of Adding Varying Amounts of Sodium and Calcium Fluorides to the Ration of Rats on the Chemical Composition of the Bones*

Experiment 1

Lot No.	Fluorine additions	Calcium			Phosphorus			Magnesium			Carbon dioxide	Fluorine
		Femurs	Humeri	Tibiae	Femurs	Humeri	Tibiae	Femurs	Humeri	Tibiae		
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	None	37.2	37.4	37.4	18.0	18.1	18.7	0.70	0.75	0.75	3.35	0.05
2	0.01 sodium fluoride	37.5	38.1	37.9	18.5	18.4	18.6	0.76	0.89	0.84	3.42	0.43
3	0.01 calcium fluoride	37.5	37.9	37.8	18.3	18.2	18.5	0.67	0.88	0.68	3.42	0.06
4	0.02 sodium fluoride	37.9	38.2	37.9	18.3	18.4	18.5	0.68	0.70	0.69	3.20	0.58
5	0.02 calcium fluoride	37.9	38.0	38.0	18.1	18.5	18.9	0.67	0.63	0.63	3.20	0.11
6	0.05 sodium fluoride	37.4	38.0	37.6	18.9	17.9	18.3	0.81	0.82	0.79	2.81	1.10
7	0.05 calcium fluoride	37.7	38.0	37.8	18.4	18.5	18.4	0.65	0.68	0.62	3.02	0.17

*Calcium, phosphorus, and magnesium based on bone ash. Carbon dioxide and fluorine based on dry, fat-free bone.

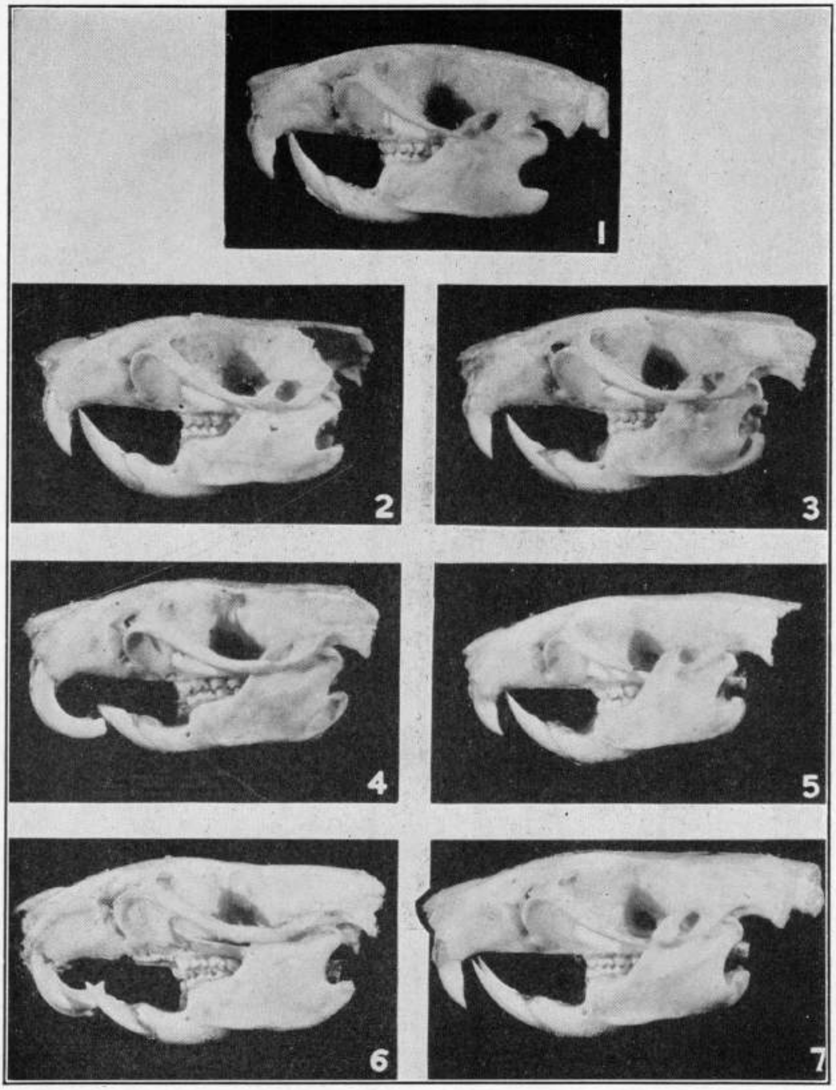


Fig. 13.—Skulls and teeth of rats showing the effects of varying amounts of calcium and sodium fluorides in the ration

1. Control ration
2. 0.01 per cent sodium fluoride
3. 0.01 per cent calcium fluoride
4. 0.02 per cent sodium fluoride
5. 0.02 per cent calcium fluoride
6. 0.05 per cent sodium fluoride
7. 0.05 per cent calcium fluoride

representative young were selected from the third litter of the females in each lot and continued on the same ration. After the majority of the females in a lot had produced three litters, the entire lot was chloroformed and the heads, femurs, humeri, and tibiae were removed. The heads were preserved in a 4 per cent solution of formaldehyde for gross examination and histological study. The bones were cleaned of adhering tissue, dried, and extracted with alcohol and ether for ash determinations.

The basal ration used in this study was composed of: 10 per cent casein, 10 per cent soybean oilmeal, 3 per cent yeast, 2 per cent cod-liver oil, 1 per cent sodium chloride, minerals as indicated in Table 33, and ground yellow corn to make 100 per cent after the additions of the minerals. Table 33 records the amounts of calcium, phosphorus, and fluorine in the mineral supplements included in the rations of the different lots.

TABLE 33.—Amounts of Calcium, Phosphorus, and Fluorine in the Mineral Supplements Added to the Ration

Experiment 2

Lot No.	Mineral supplements in 100 gm. of the ration	Calcium	Phosphorus	Fluorine
		Gm.	*Gm.*	*Gm.*
1	2.00 gm. rock phosphate A	0.677	0.258	0.072
2	2.00 gm. rock phosphate (untreated)	0.666	0.290	0.071
3	2.00 gm. rock phosphate (treated)	0.663	0.285	0.010
4	1.78 gm. steamed bone meal 0.34 gm. ground limestone	0.678	0.289	Trace
5	2.00 gm. phosphatic limestone	0.697	0.091	0.024
6	4.00 gm. phosphatic limestone	1.395	0.182	0.048
7	0.70 gm. rock phosphate A 1.35 gm. ground limestone	0.697	0.090	0.025
8	1.41 gm. rock phosphate A 2.69 gm. ground limestone	1.394	0.182	0.051
9	1.78 gm. steamed bone meal 0.11 gm. ground limestone 0.16 gm. calcium fluoride	0.678	0.257	0.075
10	0.63 gm. steamed bone meal 1.39 gm. ground limestone 0.05 gm. calcium fluoride	0.697	0.091	0.024
11	1.26 gm. steamed bone meal 2.78 gm. ground limestone 0.10 gm. calcium fluoride	1.394	0.182	0.048

The experiment was continued throughout four successive generations, with no change in rations nor any introduction of other animals. The rats in Lot 1, which received 2 per cent of rock phosphate A, failed to reproduce in the third generation and were necessarily discontinued. Those which received rock phosphate A and limestone in such amounts as to make the calcium, phosphorus, and fluorine contents of their ration equivalent to 4 per cent of phosphatic limestone (Lot 8) failed to reproduce in the second generation. Stock males of known fertility were placed in both cages in order to check the fertility of the females, but no pregnancies occurred in either lot. These results cannot be attributed to the fluorine contents of the rations entirely, since the animals in Lot 2, which received a ration equivalent to or higher in fluorine than Lots 1 and 8, respectively, reproduced normally.

The rats in Lots 1, 2, and 5 (Table 33) grew more slowly than those in the other lots and never attained the size of the others. They were in poor condition, as evidenced by the degree of fatness and the condition of their hair (which was longer and lacked the glossiness of well-fed animals). This condition in the cases of Lots 1 and 2 (2 per cent rock phosphate A and "untreated" rock phosphate) may be attributed to the high fluorine contents of their rations (0.072 and 0.071, respectively), but the poor growth and condition in Lot 5 (2 per cent phosphatic limestone) cannot be accounted for on this basis since the ration of Lot 6 (4 per cent phosphatic limestone) contained twice as much fluorine and the animals grew well and appeared to be in excellent condition. Other than Lots 1, 2, and 5, growth was comparable in all the lots and all the animals were in good condition at the end of the experiment.

The ash content of the bones from rats of some of the generations are presented in Table 34. These specific generations were chosen because of the similarity in age of the animals at the time they were killed. No significant differences were observed in the percentage of bone ash between any of the lots with the exception of the lot which received 2 per cent of "treated" rock phosphate (Lot 3). The bone ash of these animals was significantly higher than that of the other lots. All the rats were approximately 29 weeks of age at the time they were killed.

TABLE 34.—Effect of the Additions of Mineral Supplements Containing Varying Amounts of Fluorine to the Rations of Rats on Bone Formation

Experiment 2

Lot No.	Mineral supplements per 100 gm. basal ration	No. of rats	Av. age of rats	Av. final weight	Generations	Per cent ash	
						Femurs	Humeri
			Wk.	*Gm.*			
1	2.00 gm. rock phosphate A	7	28	267	P and F_1	65.5±0.40	65.7±0.35
2	2.00 gm. rock phosphate (untreated)	9	29	298	P and F_3	65.2±0.27	66.5±0.17
3	2.00 gm. rock phosphate (treated)	11	31	347	P and F_3	67.0±0.23	67.7±0.22
4	1.78 gm. steamed bone meal 0.34 gm. ground limestone	10	29	309	P and F_3	65.0±0.33	66.1±0.30
5	2.00 gm. phosphatic limestone	9	29	280	P and F_3	65.0±0.34	65.8±0.36
6	4.00 gm. phosphatic limestone	10	29	325	P and F_3	65.4±0.41	66.4±0.24
7	0.70 gm. rock phosphate A 1.35 gm. ground limestone	11	29	305	P and F_3	65.8±0.19	66.2±0.22
8	1.41 gm. rock phosphate A 2.69 gm. ground limestone	5	28	324	P	65.7±0.38	65.4±0.57
9	1.78 gm. steamed bone meal 0.11 gm. ground limestone 0.16 gm. calcium fluoride	11	29	321	P and F_3	64.7±0.31	65.9±0.19
10	0.63 gm. steamed bone meal 1.39 gm. ground limestone 0.05 gm. calcium fluoride	11	29	304	P and F_3	65.4±0.28	66.5±0.19
11	1.26 gm. steamed bone meal 2.78 gm. ground limestone 0.10 gm. calcium fluoride	10	29	324	P and F_3	65.3±0.38	66.0±0.33

The reproduction data are presented in Table 35. The number of infertile females in Lots 1 and 2 (2 per cent rock phosphate A and "untreated" rock phosphate, respectively) was greater than in any of the other lots. However,

TABLE 35.—Reproduction Data on Rats Fed Fluorine in Varying Forms and Amounts

Experiment 2

Lot No.	Mineral supplements per 100 gm. basal ration	Fluorine content of rations	Number of fertile females	Number of infertile females	Total number of litters	Number of litters per fertile female	Average number per litter	Average birth weight	Average weight at weaning	Per cent of young left with female weaned
		Pct.						*Gm.*	*Gm.*	
1	2.00 gm. rock phosphate A	0.072	5	3	9	1.8	5.6	5.0	32.4	48.84
2	2.00 gm. rock phosphate (untreated)	0.071	7	4	25	3.6	7.3	5.4	36.2	87.02
3	2.00 gm. rock phosphate (treated)	0.010	14	0	54	3.8	8.4	5.7	45.6	82.08
4	1.78 gm. steamed bone meal 0.34 gm. ground limestone	Trace	14	1	54	3.9	7.8	5.5	42.8	86.51
5	2.00 gm. phosphatic limestone	0.024	11	1	28	2.5	7.4	5.8	48.6	87.67
6	4.00 gm. phosphatic limestone	0.048	13	1	59	4.5	8.5	5.9	49.4	80.42
7	0.70 gm. rock phosphate A 1.35 gm. ground limestone	0.025	13	2	47	3.6	8.4	5.7	43.7	87.64
8	1.41 gm. rock phosphate A 2.69 gm. ground limestone	0.051	6	1	12	2.0	7.2	5.8	44.9	70.15
9	1.78 gm. rock phosphate A 0.11 gm. ground limestone 0.16 gm. calcium fluoride	0.075	14	1	55	4.0	8.3	6.1	48.2	95.37
10	0.63 gm. steamed bone meal 1.39 gm. ground limestone 0.05 gm. calcium fluoride	0.024	15	0	53	3.5	7.5	5.8	47.3	88.49
11	1.26 gm. steamed bone meal 2.78 gm. ground limestone 0.10 gm. calcium fluoride	0.048	12	2	65	5.4	8.8	5.8	42.9	90.21

since infertile females occurred in all the lots, with the exception of Lots 3 and 10, the presence of the larger number in Lots 1 and 2 is probably not significant, especially in view of the small total number of females involved. More data must be accumulated before it can be definitely stated that rock phosphate causes infertility in females. The average number of young per litter and the average birth weight of the young were not affected by the feeding of either rock phosphate, phosphatic limestone, or calcium fluoride, except in the case of Lot 1 (2 per cent rock phosphate A) which performed poorly in all respects throughout the entire test. The average weaning weights of the young were lower in Lots 1 and 2, which received 2 per cent of rock phosphate A and 2 per cent "untreated" rock phosphate, respectively, than in the other lots. This indicated poor lactation on the part of the females receiving these rations. The feeding of calcium fluoride and phosphatic limestone appeared to have no effect on reproduction or lactation. The percentage of selected young weaned was not significantly different in any of the lots, with the exception of Lots 1 and 8. The animals of these two lots died out in the third and second generations, respectively. However, these discrepancies cannot be correlated with the fluorine contents of the rations, since Lot 2, with the same percentage of fluorine as Lot 1 and more than Lot 5, reproduced normally.

The teeth of the animals in Lots 1, 2, 5, 6, 7, and 8 showed the characteristic effects of fluorine feeding. All these lots received rock phosphate or phosphatic limestone (0.024 to 0.074 per cent fluorine) in varying amounts. The mandibular incisors showed extensive wear, while the maxillary incisors were greatly elongated. All the teeth were chalk white in appearance. It became necessary to clip the maxillary incisors of these animals at frequent intervals in order to keep them from growing against the roof of the mouth. The teeth of the calcium fluoride-fed rats lost most of their normal pigmentation, but in no case was any elongation evident.

Post-mortem examination showed no gross abnormalities in any of the tissues. However, the results obtained with pigs made it advisable to retain certain of the tissues for histological examination. Accordingly, the livers, kidneys, spleens, thyroids, and parathyroids were removed and preserved in formalin.

The thyroid glands were weighed at the time of dissection to determine whether the feeding of rock phosphate, phosphatic limestone, or calcium fluoride had exerted any effect upon the size of these glands. The weights of the glands from the various lots are presented in Table 36. Thyroids were not obtained from the animals in Lots 1 and 8, which had previously died. It can be seen from the data presented that the weights of the thyroids from the different lots varied, even when placed on the basis of the body weights of the animals (mg. thyroid per 100 gm. body weight). No correlation existed, however, between the fluorine content of the rations and the weights of the glands. Histological examination of the glands, made by Dr. H. Goldblatt of the Pathological Institute of Western Reserve University, Cleveland, Ohio, revealed no changes which could be attributed to the fluorine in the rations.

Histological examinations of the livers, spleens, kidneys, and parathyroids of the animals from the different lots of the third generation revealed no abnormalities.

TABLE 36.—Effect of Fluorine in Different Forms on the Size of the Thyroid of the Rat

Experiment 2

Lot No.	Mineral supplement per 100 gm. basal ration	Fluorine content of rations	Number of rats	Average weight of thyroids	Average weight of rats	Mg. of thyroid per 100 gm. rat
		Pct.		*Mg.*	*Gm.*	
2	2.00 gm. rock phosphate (untreated)	0.071	7	16.3	308	5.3
3	2.00 gm. rock phosphate (treated)..	0.010	12	21.4	339	6.3
4	1.78 gm. steamed bone meal....... 0.34 gm. ground limestone	Trace	11	17.1	292	5.9
5	2.00 gm. phosphatic limestone	0.024	7	24.0	294	8.2
6	4.00 gm. phosphatic limestone	0.048	10	25.4	360	7.1
7	0.70 gm. rock phosphate A........ 1.35 gm. ground limestone	0.025	13	21.5	282	7.6
9	1.78 gm. steamed bone meal....... 0.11 gm. ground limestone .... 0.16 gm. calcium fluoride..........	0.075	12	22.1	324	6.8
10	0.63 gm. steamed bone meal....... 1.39 gm. ground limestone......... 0.05 gm. calcium fluoride..........	0.024	11	20.8	289	7.2
11	1.26 gm. steamed bone meal....... 2.78 gm. ground limestone .... 0.10 gm. calcium fluoride..........	0.048	10	22.7	326	7.0

At the time the rats of the fourth generation were killed for bone analysis, blood coagulability tests were made in order to check the results obtained in the chick experiments and also to attempt to clarify some of the conflicting data in the literature on this point. Table 37 presents the results of these determinations. It is apparent that in the case of the rat the coagulation time was definitely lengthened by increasing amounts of fluorine in the ration. This is contrary to the results obtained with chicks.

TABLE 37.—Effect of Fluorine Feeding on the Coagulability of Rat Blood

Lot No.	Fluorine content of ration	Time
	Pct.	*Seconds*
1	0.071	230
2	0.010	95
3	Trace	98
4	0.036	133
5	0.077	168
6	0.071	156

EXPERIMENT 3

The third experiment was planned to study the effect of fluorine in different forms on the rate of calcification. According to the results of Tolle and Maynard (47) and investigators at the Arkansas Agricultural Experiment Station (2), the feeding of rock phosphate resulted in a decreased ash content of the bones of rats 5 weeks old. The results of the previous experiments reported in this paper showed no such differences in the rats killed when from 19 to 31 weeks of age. Accordingly, it was thought advisable to study the effect of fluorine in various forms on the ash content of the bones from rats of different ages.

Six lots of weanling rats, consisting of 12 animals each, were placed on the same corn-casein-soybean oilmeal ration as that used in the previous experiment. The rations of Lots 1 and 2 were supplemented with 2 per cent of "untreated" and "treated" rock phosphate, respectively. Lot 3 received the control ration which was supplemented with 1.78 per cent of steamed bone meal and 0.34 per cent of ground limestone. The rations of Lots 5 and 6 were the same as that of Lot 3, except that 0.16 per cent of calcium and sodium fluorides was added, respectively. A proportionate amount of ground limestone was omitted from the ration of Lot 5 in order to maintain the same calcium content as in the control ration. Lot 4 received a ration containing 1 per cent each of steamed bone meal and "untreated" rock phosphate. These rations were fed *ad libitum* and distilled water was used for drinking. At the end of 5 weeks six animals in each lot were killed and the femurs and humeri were removed for ash determinations. The remaining six animals in each lot were maintained on their respective rations for 5 additional weeks, when they were killed and similar material collected for ash determinations.

The data are presented in Table 38. It was noted that the ash contents of the bones of the animals which received 0.0712 per cent of fluorine as "untreated" rock phosphate and 0.0708 per cent of fluorine as sodium fluoride (Lots 1 and 6) were definitely lower than those of the other lots at the end of 5 weeks. At the end of 10 weeks, the animals which had received "untreated" rock phosphate (0.0712 per cent fluorine) had overcome the difference to some extent, although the ash contents of their bones were still not so great as those of the other lots (Lots 2, 3, 4, and 5). The existing difference, however, was less than it had been at 5 weeks. On the contrary, the bone ash of the animals in Lot 6 (0.16 per cent sodium fluoride) when compared with the ash values of Lots 2, 3, 4, and 5 still showed a difference of approximately the same magnitude at 10 weeks as it had at 5 weeks. These reduced ash contents of the bones were not correlated with the amounts of fluorine consumed but rather with the percentages of fluorine in the rations. Considering the results of the two previous experiments, in which the rats had been killed at 19 and 28 to 31 weeks, it is apparent that the bone ash reaches a normal value at the time the animals become mature. It is clear, therefore, that high fluorine feeding retards calcification of bones of the rat during the early weeks of their life, but with the progress of age the bones become completely calcified. Calcium fluoride feeding exerted no effect on the rate of calcification, which is in agreement with the other observations previously reported.

EXPERIMENT 4

The evidence presented in the preceding experiments showed that fluorine in the form of calcium fluoride was not so detrimental to either pigs, chicks, or rats as fluorine in the form of rock phosphate or sodium fluoride. This is in agreement with the reports of many other investigators. Workers at the Iowa Agricultural Experiment Station (4) suggested that the difference in action between sodium and calcium fluorides was due to their difference in solubility. In order to obtain information relative to the rate of retention and paths of excretion of different fluorine-containing materials, metabolism trials, involving young male stock rats weighing approximately 125 grams, were conducted. Three rats were placed on each ration and were housed in a metal cage designed for the purpose of making quantitative collections of feces and urine.

TABLE 38.—Effect of Fluorine on Growth and Calcification in the Rat

Lot No.	Mineral additions to 100 gm. of the rations	Fluorine content of ration	Rats killed at 5 weeks				Rats killed at 10 weeks			
			Average fluorine consump-tion	Average gain	Bone ash		Average fluorine consump-tion	Average gain	Bone ash	
					Femurs	Humeri			Femurs	Humeri
		Pct.	*Mg.*	*Gm.*	*Pct.*	*Pct.*	*Mg.*	*Gm.*	*Pct.*	*Pct.*
1	2.00 gm. rock phosphate (untreated)....	0.0712	281	124	54.7 ± 0.57	56.6 ± 0.65	660	220	62.9 ± 0.20	62.9 ± 0.30
2	2.00 gm. rock phosphate (treated)	0.0100	41	147	60.9 ± 0.19	60.3 ± 0.01	96	243	64.9 ± 0.16	64.6 ± 0.14
3	1.78 gm. steamed bone meal........... 0.34 gm. ground limestone.............	0.0004	2	151	60.7 ± 0.19	60.0 ± 0.28	4	231	64.4 ± 0.18	63.7 ± 0.20
4	1.00 gm. steamed bone meal........... 1.00 gm. rock phosphate (untreated)..	0.0358	187	138	60.6 ± 0.27	60.1 ± 0.27	378	238	64.9 ± 0.15	64.4 ± 0.11
5	1.78 gm. steamed bone meal........... 0.11 gm. ground limestone............. 0.16 gm. calcium fluoride..............	0.0768	335	146	61.1 ± 0.28	60.3 ± 0.16	736	238	64.8 ± 0.18	64.1 ± 0.20
6	1.78 gm. steamed bone meal........... 0.34 gm. ground limestone............. 0.16 gm. sodium fluoride	0.0708	153	29	54.6 ± 0.61	53.9 ± 0.67	302	74	57.2 ± 0.75	58.0 ± 0.35

The same corn-soybean oilmeal-casein ration as used in the previous trials was fed. For two groups this was supplemented with "untreated" rock phosphate at a 2 per cent level. This material was from the same source as that used in Experiment 2. Two other groups were fed a ration containing 1.78 per cent steamed bone meal, 0.34 per cent ground limestone, and 0.16 per cent sodium fluosilicate; another group received the same ration, except that the sodium fluosilicate was replaced by sodium fluoride. In the ration of the sixth group, calcium fluoride replaced the sodium fluoride of the previous group and the limestone was reduced proportionately in order to maintain the same calcium content as that of the other rations. Three hundred grams of each ration were mixed and fed *ad libitum* until consumed. When the 300 grams of the experimental ration had been consumed, the animals were given ground yellow corn for a period of 3 days in order to clear the digestive tract of any residues of the experimental rations. The feces and urine were composited and analyzed for fluorine.

The data acquired are presented in Table 39. Approximately 30 per cent of the fluorine ingested as "untreated" rock phosphate, sodium fluosilicate, and sodium fluoride was retained in the body. In the case of calcium fluoride less than 2 per cent of the ingested fluorine was absorbed and this was excreted quantitatively in the urine. Apparently, the absorbed fluorine which is not retained by the body is excreted through the kidneys. Since the amounts of ingested fluorine retained in the body in the cases of rock phosphate, sodium fluosilicate, and sodium fluoride were of much greater magnitude than in the case of calcium fluoride and since the majority of the fluorine of the latter supplement was excreted in the feces, the reason for the difference in the effects of these supplements is apparent. The extremely small amount of fluorine in the basal ration was not taken into consideration in these calculations.

TABLE 39.—Availability of Fluorine in Various Forms

Fluorine supplement	Time on ration	Fluorine ingested	Fluorine in feces	Fluorine absorbed	Fluorine in urine	Fluorine balance	Fluorine retained
	Days	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Pct.*
Rock phosphate (untreated)	11	217.2	128.7	88.5	31.5	+ 57.0	26.2
Rock phosphate (untreated)	10	213.6	131.5	82.1	20.5	+ 61.6	28.8
Sodium fluosilicate	23	269.9	94.3	175.6	93.6	+ 82.0	30.4
Sodium fluosilicate	22	269.9	94.4	175.5	90.2	+ 85.3	31.6
Sodium fluoride	18	211.2	116.5	94.7	25.8	+ 68.9	32.6
Calcium fluoride	11	229.6	225.5	4.1	4.2	— 00.1	0.0

SKULL AND TEETH STUDIES

In order to study the alterations in the skulls and teeth due to the ingestion of fluorine, the heads of the rats from Experiments 1 and 2 were preserved in formalin. Jaws from five lots were examined histologically. The teeth and jaws from all 18 lots were used for gross examination and comparison.

Sixteen measurements were made on each skull, as follows: length of entire skull, fronto-occipital length, length of nasal bone, zygomatic width, squamosal width, length and width of both dental arches, distance from dental arch to foramen magnum, height and length of mandibles, distance from anterior alveolar crest to angle of jaw, distance from last molar to condyle, distance from last molar to angle of jaw, and thickness of left mandible in plane passing between first and second molars. A comparison of the average measurements of the skulls from control groups with groups on rations containing added fluorine showed no significant differences for either sex. Contrary to the findings with swine, the mandibular thickness was not altered. No decrease in the width of the dental arch, such as was reported by Bunting (34), was found. In spite of the inability to demonstrate differences in the proportions of the mandibles by actual measurements, those from rats on high fluorine rations had blunter contours and were less graceful in appearance than those of normal rats. As has been observed by others, the bone was whiter in appearance and had a rougher surface, lacking the normal luster. Microscopically, the bone from fluorized rats exhibited no significant differences from normal bone. The marrow spaces in the mandibles from all groups contained red marrow showing no deviations from normal.

The addition of fluorine to the ration resulted in gross changes in the incisors, similar to those described by McCollum, Simmonds, Becker, and Bunting (34), Schultz and Lamb (41), Bergara (7), Chaneles (14), and others. These changes appeared as a graded series, their severity depending upon the amount and compound of fluorine fed. The nature and severity of the ensuing changes are briefly indicated for individual rats of all lots in Table 40. At low levels of fluorine intake, the pigment normally present in incisor enamel was reduced in amount; at higher levels, the pigment was further reduced—the enamel lost its luster and gross hyperplasia began to appear. At still higher levels, the chisel edge of the incisors became worn and dulled and the angle at which the plane of abraded surface met the enamel surface was increased. Continuation of this condition resulted in either excessive wear or the breaking of one or more incisors and consequent loss of occlusion. Incisors without an antagonist continued to grow and became markedly elongated. Any or all incisors might have become elongated, depending upon deficiency or loss of occlusal relations through wear or divergence. We were unable to confirm the reports of investigators who have claimed or inferred that elongation is peculiar to upper incisors, that it results from more rapid growth of the incisors, or that it involves a change in their radius of curvature. Bunting's explanation of the elongation of incisors was confirmed. With fluorine toxicity sufficient to produce conditions of elongation, incisors usually exhibited longitudinal corrugations of the dental surfaces—referred to in Table 40 as fluting. In most rats which had incisors that were fluted and elongated, periodontal tissues immediately overlying the enamel organ near the alveolar crest appeared blood-red after the gum had been excised. This condition was not found in rats in which fluting and elongation were absent. Microscopical examination and microchemical tests showed that this condition was due to an iron-containing blood pigment, which was chiefly intracellular, and was confined to tissues immediately adjacent to or in the remains of the enamel organ.

TABLE 40.—Microscopical Observations of the Incisors of Rats

Lot No.	Fluorine addition to diet	Form in which fluorine was added	Identification number and sex of rats	Shape and occlusion of incisors	Pigment in enamel of incisors	Luster and character of enamel of incisors	Gross hypoplasia of enamel of incisors	Blood pigment in and around gingival enamel organ
	Pct.							
1 (Controls)	0.0000	....	8190 ♂	Normal	Normal	Normal	None	None
			8192 ♀	Normal	Normal	Normal	None	None
2	0.0044	Sodium fluoride	8194 ♀	Normal	Trace	Normal	None	None
			8198 ♂	Normal	Uppers pale yellow; trace in lowers	Normal	None	None
3	0.0046	Calcium fluoride	8200 ♀	Normal	Normal	Normal	None	None
			8202 ♂	Normal	Slight decrease	Normal	None	None
4	0.0088	Sodium fluoride	8205 ♂	Increased angle of wear	Lacking	Luster lacking; chalky	Marked	Slight
			8207 ♀	Elongated uppers	Traces in spots	Traces of luster; chipped enamel	Mild	Marked
5	0.0092	Calcium fluoride	8211 ♂	Normal	Slight decrease	Normal	None	None
			8213 ♀	Normal	Decreased, blotchy	Dulled; chalky spots	Mild in uppers	None
6	0.0220	Sodium fluoride	8214 ♀	Elongated uppers; marked fluting	Lacking	Luster lacking; chalky	Marked	Marked
			8216 ♂	Elongated lowers; marked fluting	Lacking	Luster lacking; chalky	Marked	Marked
7	0.0230	Calcium fluoride	8222 ♀	Normal	Marked decrease	Normal	None	None
			8223 ♀	Normal	Marked decrease; blotchy in uppers	Fair luster	None	None
8 and 8 A	0.0720	Rock phosphate A	552 ♀	Elongated uppers and lowers; uppers divergent; fluting	Trace	Dulled luster	Mild in uppers; none in lowers	....
			553 ♀	Normal	Lacking to pale yellow	Fair luster; chalky spots	None	....
			8835 ♀	Elongated uppers; slight fluting	Lacking in lowers; trace in uppers	Luster fair in uppers; lacking in lowers	Marked in uppers; none in lowers	Marked
			8837 ♂	Elongated lowers; marked fluting	Lacking	Luster lacking; chalky	Marked	Marked

TABLE 40.—Microscopical Observations of the Incisors of Rats—Continued

Lot No.	Fluorine addition to diet	Form in which fluorine was added	Identification number and sex of rats	Shape and occlusion of incisors	Pigment in enamel of incisors	Luster and character of enamel of incisors	Gross hypoplasia of enamel of incisors	Blood pigment in and around gingival enamel organ
	Pct.							
9	0.0710	Rock phosphate (Untreated)	1410 ♂	Elongated uppers; slight fluting	Lacking	Luster lacking in uppers; fair in lowers	Marked in uppers; mild in lowers	....
			1411 ♀	Elongated uppers; fluting	Trace in lowers	Luster lacking in uppers; fair in lowers	Marked in uppers; mild in lowers	Marked
10	0.0100	Rock phosphate (Treated)	1413 ♂	Normal	Slight decrease	Normal	None	None
			1415 ♂	Decreased angle of wear; slight fluting	Trace	Fair luster	None	None
11 (Controls)	Trace	None added	200 ♀	Normal	Normal	Normal	None	None
			1418 ♂	Normal	Normal	Normal	None	None
			1419 ♀	Normal	Normal	Normal	None	None
12	0.0240	Phosphatic limestone	1427 ♀	Elongated uppers; slight fluting	Trace	Luster almost lost; chalky	Mild	Marked
			1429 ♂	Elongated uppers; slight fluting	Pale yellow in uppers; lacking in lowers	Fair luster	Mild in uppers	....
13	0.0480	Phosphatic limestone	450 ♀	Increased angle of wear; fluting	Trace in spots	Luster lacking; chalky	Marked in uppers; mild in lowers	Marked
			449 ♀	Increased angle of wear; fluting	Trace in spots	Luster lacking; chalky	Marked in uppers; mild in lowers	Marked
14	0.0250	Rock phosphate A	452 ♀	Elongated uppers	Traces	Fair luster; chalky spots in uppers	Marked in uppers; mild in lowers	Marked
			454 ♂	Increased angle of wear	Pale yellow in uppers; trace in lowers	Fair luster	Marked in uppers; mild in lowers	....
15	0.0510	Rock phosphate A	457 ♀	Elongated uppers; mild fluting	Traces	Luster almost lost	Marked in uppers; mild in lowers	Marked in uppers; mild in lowers
			458 ♂	Elongated uppers; mild fluting	Traces	Luster almost lost	Marked in uppers; mild in lowers	Marked in uppers; mild in lowers

TABLE 40.—Microscopical Observations of the Incisors of Rats—Concluded

Lot No.	Fluorine addition to diet	Form in which fluorine was added	Identification number and sex of rats	Shape and occlusion of incisors	Pigment in enamel of incisors	Luster and character of enamel of incisors	Gross hypoplasia of enamel of incisors	Blood pigment in and around gingival enamel organ
	Pct.							
16	0.0750	Calcium fluoride	464 ♀	Elongated uppers; mild fluting	Pale yellow in spots in uppers; darker in lowers	Luster lacking	Marked in uppers; mild in lowers	Slight in uppers; none in lowers
			468 ♀	Elongated uppers; mild fluting	Pale yellow in spots in uppers; darker in lowers	Luster lacking in uppers; fair in lowers	Marked in uppers; mild in lowers	....
17	0.0240	Calcium fluoride	470 ♀	Increased angle of wear	Pale yellow	Fair luster	None	None
			473 ♀	Increased angle of wear	Dark yellow spots on pale yellow	Fair luster	Mild	None
18	0.0480	Calcium fluoride	475 ♂	Normal	Slight decrease	Normal	None	None
			479 ♀	Increased angle of wear	Slight decrease	Normal	None	None

When the changes in the incisors, listed in Table 40 and described above, are arranged in order of severity and plotted against the amounts of fluorine in the rations (Fig. 14), a graphic approximation is made of relative effects of different fluorine compounds on the formation of enamel and dentin. Since divisions on the ordinate are somewhat arbitrary, a numerical relationship cannot be expressed between the dosage and the effects on the teeth, and yet it is obvious that sodium fluoride produced most and calcium fluoride least damage. Rock phosphate and phosphatic limestone had analogous effects intermediate between sodium and calcium fluorides. This is in agreement with the results reported by Sollman, Schettler, and Wetzel (43) and contrary to the report of McClure and Mitchell (32). The teeth of the control rats had normal dental tissues (Fig. 15). The teeth of fluorized rats showed graded hypoplastic defects, the severity of which was directly proportional to the amount of added fluorine when the form of fluorine was considered. Both upper and lower incisors of Rat 8219 (Experiment 1, Lot 7), which received 0.0230 per cent of fluorine as calcium fluoride in its ration, were histologically normal. The effects of fluorine rations were consistently more marked in maxillary incisors than in mandibular. The lower incisors of Rat 550 (2 per cent rock phosphate A), which received 0.07 per cent of fluorine, showed only mild hypoplasia of the enamel (Fig. 16); whereas the upper incisors exhibited severe hypoplasia

(Fig. 17). Both upper and lower incisors of Rat 8215 (0.05 per cent sodium fluoride) showed the most severe hypoplasia of any microscopically examined (Fig. 18).

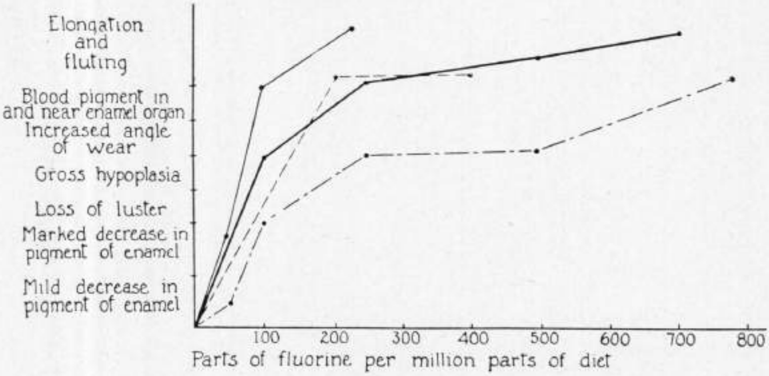


Fig. 14.—Effects of different dosages of F on incisors of rats. (See data in Table 40)
.———. NaF. .———. Rock phosphate. .- - - - - -. Phosphatic limestone. .— - — - — -. CaF_2

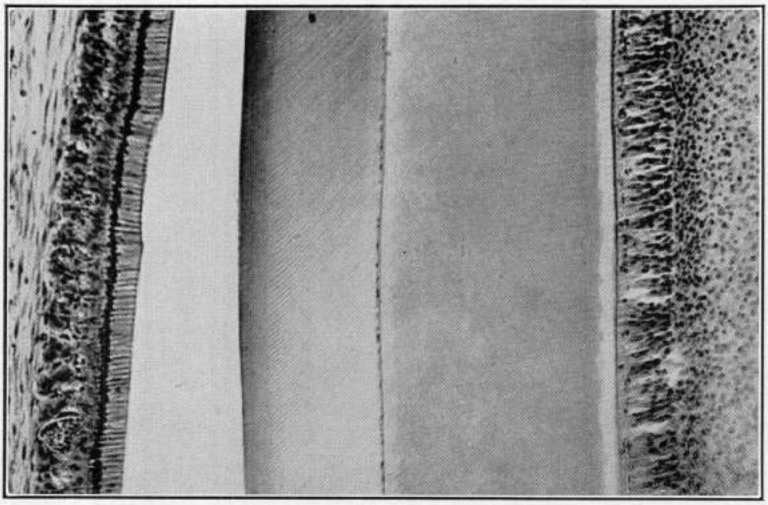


Fig. 15.—Longitudinal section through lower incisor of control rat (Lot 1). H. and E. (x 160)

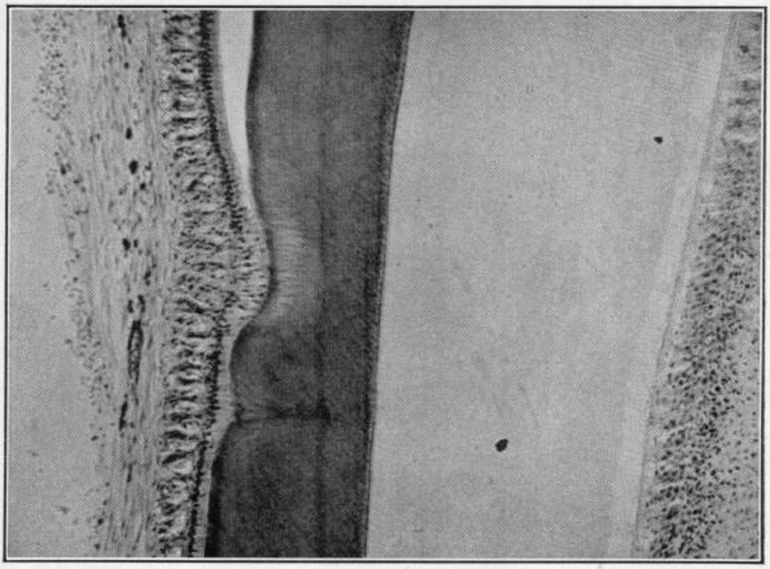


Fig. 16.—Longitudinal section through lower incisor of Rat 550 (Lot 8, 2% of rock phosphate A added to diet). H. and E. (x 160)

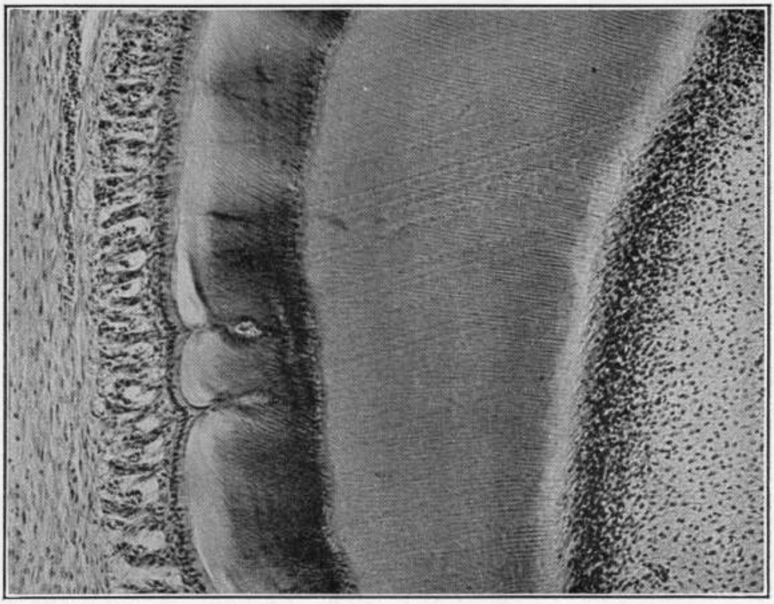


Fig. 17.—Longitudinal section through upper incisor of Rat 550 (Lot 8). H. and E. (x 160). (See Fig. 19)

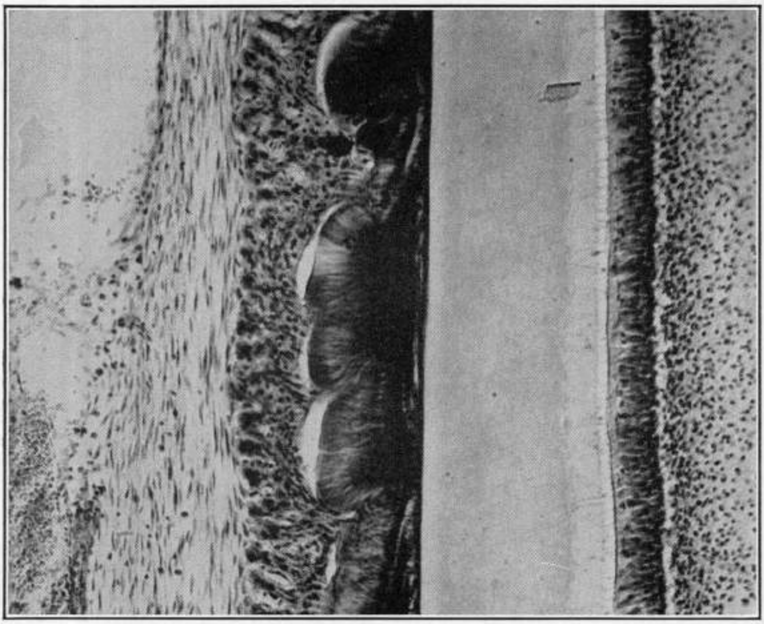


Fig. 18.—Longitudinal section through lower incisor of Rat 8215 (Lot 6, 0.05 per cent of sodium fluoride added to diet). H. and E. (x 160)

The hypoplasia of the enamel involved either general or local decrease in thickness, or both. Concomitant with this decrease in thickness, there were disturbances in the uniformity of the arrangement of the prisms and some difference in the composition of the enamel which rendered it more easily retained following decalcification; its stainability with hematoxylin also was increased. Associated with the changes in the enamel were alterations in the appearance of the enamel organ, involving a decrease in the height of the ameloblasts, an unevenness of the inner ameloblastic surface, and an increase in the length of the interpappillary projections of the enamel organ (Figs. 16-18). In some cases the height of the ameloblasts became so small that they were reduced to a squamous type of cell (Fig. 19). A similar decrease in the heights of the ameloblasts normally occurs toward the end of amelogenesis, in which condition it signifies diminution and ultimate cessation of amelogenic function. In the hypoplasia of the enamel, decrease in height and decrease in function of the ameloblasts occurred before the normal amount of enamel had been formed, a condition that may have been more severe in some locations than in others, resulting in the characteristic pits or grooves of hypoplasia. In Rat 8215 (0.05 per cent sodium fluoride) the lack of function of the ameloblasts was so extreme that enamel was lacking in several regions. Microscopical findings, with respect to the severity of the effects of fluorine in the rations, are confirmatory of the microscopical findings in Table 40. The dentin showed hypoplastic changes which paralleled in severity those of the enamel and were manifested as an increase in the thickness of the predentin, an accentuation of the incremental lines, and the presence of interglobular dentin.

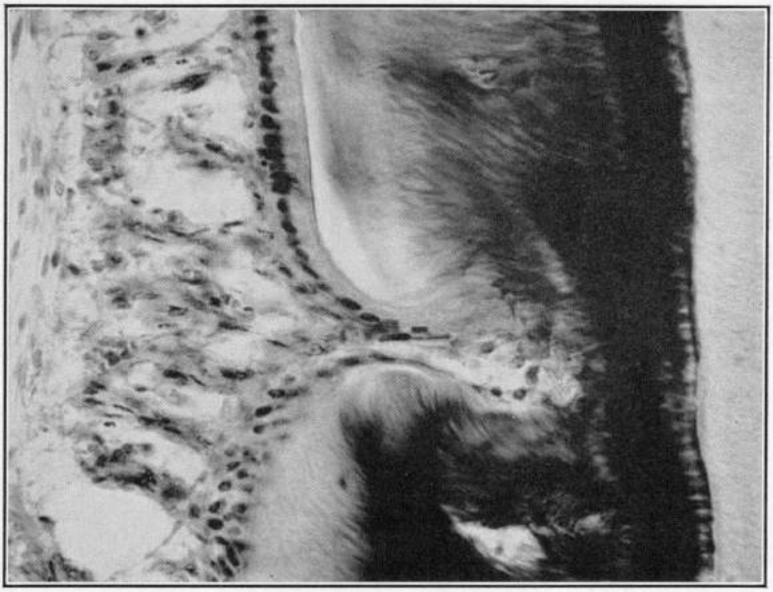


Fig. 19.—Longitudinal section through hypoplastic defect in enamel of upper incisor of Rat 550 (Lot 8). H. and E. (x 600)

DISCUSSION

The results of the foregoing experiments show definitely that fluorine feeding exerts definite physiological effects upon animals. In general, these effects are evident whether the fluorine is fed as a fluoride salt or as present in the naturally occurring minerals, such as rock phosphate and phosphatic limestone, although the severity of the effects are dependent upon the percentage and form of fluorine.

It is evident, from the results of the experiments on pigs, chicks, and rats, that fluorine in the form of calcium fluoride was not nearly so toxic as in the form of the sodium salt, when fed at corresponding levels. This is in agreement with the report of the Iowa Agricultural Experiment Station (4) that rats can tolerate more fluorine as calcium fluoride than as sodium fluoride. We were unable to substantiate the results of McClure and Mitchell (32) that calcium fluoride and sodium fluoride were equally effective in producing changes in the teeth of rats. In our experiments, 0.05 per cent of calcium fluoride in the ration produced approximately the same effects as 0.01 per cent of sodium fluoride in causing changes in rat teeth. On comparable fluorine bases, rock phosphate, phosphatic limestone, and treble superphosphate occupied positions intermediate to sodium and calcium fluorides with respect to the characteristic effects produced by fluorosis.

Metabolism trials with rats showed that very little of the fluorine of the calcium fluoride was absorbed or retained in the body; whereas in the cases of sodium fluoride, sodium fluosilicate, and rock phosphate much greater quantities of the fluorine were absorbed, with approximately 30 per cent being retained in the body. These findings would tend to explain the differences in toxicity between the different forms of fluorine as reported by Marcovitch (29), Cristiani and Chausse (16), and Sollman, Schettler, and Wetzel (43).

The feeding of excessive amounts of fluorine resulted in a decrease in feed consumption and in an inefficient utilization of the feed in the animals studied. Pigs fed rations high in fluorine (0.07 per cent) made significantly smaller average daily gains and required again as much feed to produce a unit of gain as similar animals fed low fluorine rations (trace of fluorine). The curtailment in feed consumption due to fluorine (0.029 per cent) was especially noted in the case of sows suckling pigs. These animals did not consume sufficient feed to prevent them from losing abnormal amounts of weight, and, in most instances, it was not possible to return the animal to good condition even after the young were weaned. Similar observations were made by Huffman and Reed (24) when rock phosphate was included in the rations of dairy cows at levels of 1.5 per cent of the grain ration. It was noted that chicks were able to tolerate higher levels of fluorine in their rations (0.071 per cent) than pigs (0.025 per cent) before showing any deleterious effects. This is in agreement with the findings of Halpin and Lamb (20).

The presence of fluorine in the rations of pigs and rats, either in the form of sodium or calcium fluorides or rock phosphate, exerted no effect on reproduction, as determined by the number of young born and the weight of the young at birth. It was observed, however, that high-fluorine rations adversely affected lactation, since the average weight of the young at weaning was generally less than on similar rations of low-fluorine content. We are of the opinion that this apparent decrease in lactation was not a specific effect of fluorine *per se* but rather due to the curtailment in feed consumption. Similar observations were reported by Huffman and Reed (24) when high-fluorine rock phosphates were fed to dairy cattle.

The data also show that excessive amounts of fluorine produce certain definite changes in the bones of animals. The long bones, as represented by the femurs, become larger in diameter, due to an increased thickness of the walls. This is accompanied by a loss of the normal yellow color and luster and the occurrence of many exostoses. Similar conditions were noted in the flat bones, as typified by the mandibles. However, in the case of the mandibles the increase in the body of the bone was not due to an increase in the thickness of the bone wall, as in the femurs, but to an increase in the size of the medullary space. These medullary spaces were filled with a yellow marrow instead of the normal red marrow. This condition was not observed in the rat mandibles that were examined. In the latter case, normal red marrow was present. High-fluorine rations also caused a marked increase in the width of the dental arch in pigs; whereas no such increases were noted in the case of the rats.

When fluorine was fed to pigs in the form of sodium fluoride or rock phosphate, the outstanding physical effect on the femurs was the decrease in the breaking strength of the bones. Neither the physical changes nor measurements could account for this decrease in tensile strength. Likewise, the decreased breaking strengths could not be explained on the basis of percentage ash in the bones. Although the ash contents of the femurs were somewhat lower in the short-time pig experiments when large amounts of available fluorine were included in the ration, in the case of the sows no differences in ash percentages were noted and yet a great difference in tensile strength occurred. Obviously, the decrease in breaking strength was not directly correlated with the percentage of ash, but, as the data showed, with the ash per unit volume, or what probably approaches the density of the bone itself. Accordingly, it would seem that some change in the structure of the bone must have occurred as a result of excessive fluorine ingestion. Microscopic examination of ground sections of the femurs showed definite differences in bone structure. The data were insufficient to warrant any definite conclusions as to the nature of the change. Work along this line is being continued.

The data pertaining to the effect of fluorine on the ash contents of the bones are of interest. Although high fluorine ingestion did not appear to affect appreciably the percentage of bone ash in the case of pigs, chicks, or mature rats, evidence was obtained which showed that high available fluorine intakes retard or slow up calcification. However, if the fluorized animals are continued on the ration for a sufficient length of time, the bones will acquire a normal percentage of ash. This would tend to account for the occasional decreased ash percentages due to excessive fluorine intakes noted in some of the short-time pig experiments; whereas, in the case of the sows, no differences in ash content due to fluorine were noted.

That a highly available fluorine intake affects the composition of osseous tissue was shown by the analyses of the pig and rat bones. The fluorine content of the moisture- and fat-free bones was directly proportional to the percentages of available fluorine in the rations; whereas the carbonate content was decreased with increased fluorine intakes. The calcium and phosphorus percentages in the bone ash were unaffected, while the magnesium percentages were increased with increased amounts of fluorine in the rations. These results confirm the work of Forbes and co-workers (17) as to the changes in calcium, magnesium, and carbon dioxide contents of bones due to the feeding of rock phosphate, but we were unable to demonstrate any such increase in phosphorus content as was reported by these investigators. It is worthy of note that the high-magnesium and low-carbonate contents of the bones were independent of the percentages of magnesium and carbonates in the ration, the source of calcium and phosphorus in the ration, and the form in which available fluorine was ingested. To determine whether this change in composition in the case of large fluorine intakes is due to a partial replacement of the carbonate by the fluoride or to an actual shift in the percentages of the different bone salts normally present to compensate for the increased fluorine content requires more detailed study.

The effect on the teeth of feeding fluorine was, in general, the formation of imperfect enamel, either in normal or deficient amounts, resulting in varying degrees of hypoplasia. The teeth of sows kept for two or more years on a ration high in fluorine were frequently worn to the gums and the pulp cavities exposed. This was due to the lack of a normally resistant enamel which permitted rapid abrasion of the teeth. The dentin was, by comparison with the enamel, very little affected. Similar changes were observed by Huffman and Reed (24) and Taylor (46) in the case of dairy cattle which had been fed rock phosphate over long periods of time. In rats the condition was evidenced by an overgrowth of the incisors, as has been reported by numerous investigators (7), (14), (32), (34), (41), (43), (47), and (48). Both in case of rats and pigs, hypoplasia of the enamel and, to a much less degree, hypoplasia of the dentin occurred, the severity of which was proportional to the amount and form of fluorine in the ration. Fluorine in the form of sodium fluoride was most effective in causing tooth changes; whereas in the calcium form it was least effective. Rock phosphate and phosphatic limestone occupied intermediate positions in this respect. The chemical analyses of the teeth did not reveal any striking differences in their inorganic composition, aside from the increase in the percentage of fluorine—the increase being directly correlated with the availability of the fluorine and its content in the ration, which is in confirmation of the work of Sonntag (44) with dogs.

The only difference, other than in the severity of the changes noted, between the fluorine derived from rock phosphate and sodium fluoride was the effect that liberal amounts of the natural mineral (1 per cent or more of the ration) exerted on the kidneys. Equivalent, or even higher, levels of fluorine in the form of sodium fluoride had no such effect. Two explanations present themselves as to the cause of this discrepancy. Either the naturally occurring substance must have contained some substance or material, other than fluorine, which caused pathological changes in the kidney, or the fluorine compound in rock phosphate was more toxic to this organ than sodium fluoride.

The increased water consumption of the pigs on the high-fluorine rations may in part be explained on the basis of the results of Gottlieb and Grant (19). These investigators reported that the intravenous injection of sodium fluoride into dogs caused a marked diuresis. Although no urinary volume determinations were made in the experiments reported, it was noted that the litter in the pens of the high fluorine-fed pigs was much more soiled with urine than that in the other lots. A marked increase in urine volume must therefore be accompanied by an increased water intake to avoid a serious dehydration of the body tissues.

The data relative to the effect of fluorine on the coagulability of the blood appear to be contradictory. The coagulation time of rat blood was definitely retarded and that of chicken blood accelerated by rations containing large amounts of fluorine. Much contradictory evidence has been presented in the literature on this point. Schwyzer (42) found that chronic doses of sodium fluoride produced an increased coagulability of the blood of rats, dogs, and pigeons, with which our results with chicks agree. On the contrary, Stuber and Lang (45) suggested that the presence of large amounts of fluorine in the blood decreased its coagulability. Their opinion is based on the observations of a number of hemophilic patients whose blood contained large amounts of fluorine. These investigators also state that goose and rabbit bloods clot slowly and contain relatively large amounts of fluorine, while the blood of cats and dogs, containing no fluorine, clots rapidly. Since no analyses for fluorine were made on the blood of either the rats or chicks in these experiments, it is not possible to state whether the results which we obtained were due to the percentage of fluorine in the blood or to some other factor.

The weight of the thyroid glands of rats was not increased by fluorine feeding. This corroborates the results of Tolle and Maynard (48) and is in disagreement with the findings of Maumene (30) and Goldemberg (18). The thyroids and parathyroids showed no histological changes which could be correlated with the fluorine contents of the rations. Similar results have been reported by Hauck, Steenbock, Lowe, and Halpin (23) with chicks.

The experiments with pigs and chicks bring out certain practical aspects in the use of minerals, such as rock phosphate, which contain high percentages of fluorine. These materials may be used as efficient carriers of calcium and phosphorus providing they are fed at sufficiently low levels to avoid any of the detrimental effects due to excessive fluorine. The pig experiments indicate that rock phosphate containing approximately 3.5 per cent of fluorine may be fed at 0.5 per cent of the total ration to pigs for 20 to 25 weeks without any injurious effects. The results with chicks show that a maximum of 2 per cent of a similar rock phosphate can be included in the total ration without deleterious effects. Since the toxic effects of fluorine are cumulative, it is advisable to feed smaller amounts than the above mentioned if the animals are to be maintained on the ration for extended periods of time.

SUMMARY

1. The toxicity of fluorine varied with the form of fluorine fed. Sodium fluoride was much more toxic to pigs, rats, and chicks than calcium fluoride when these two salts were fed at comparable fluorine levels. Rock phosphate, phosphatic limestone, and treble superphosphate occupied intermediate positions in this respect.

2. Rations containing excessive amounts of available fluorine reduced the growth and the feed consumption of pigs and chicks and definitely increased the feed requirement per unit of gain for the pig.

3. When the rations of pigs contained more than 0.029 per cent of fluorine as sodium fluoride or more than 0.033 per cent as rock phosphate, the bones were characterized by increased thickness, loss of normal color and luster, presence of exostoses, and a decreased breaking strength.

4. The weakened, thickened bones resulting from fluorine feeding contained normal percentages of ash, calcium, and phosphorus, increased amounts of magnesium and fluorine, and decreased percentages of carbonates. These changes were directly correlated with increased amounts of fluorine in the ration.

5. The inclusion of fluorine in the ration of the pig increased the thickness of the walls of the femurs but not of the mandibles. The increased thickness of the mandibles was due to an increase in the size of the medullary spaces. A change also occurred in the type of marrow present.

6. High-fluorine rations increased the width of the dental arch in pigs but not in rats.

7. The inclusion of excessive amounts of fluorine in the rations of pigs and rats caused hypoplasia of the enamel of the teeth. In pigs, the feeding of such rations over long periods of time caused the teeth to become so soft that they were worn down until in some cases the pulp cavities were exposed. The incisors of the rat became white in color and some were elongated with the occluding incisor worn down or broken off. These changes were accompanied by hypoplasia of the enamel. The dentin was similarly affected to a less degree.

8. The percentages of ash, calcium, phosphorus, magnesium, and carbon dioxide in the teeth were unaffected by the fluorine content of the ration but the percentage of fluorine was increased in direct proportion to the amount of this element present in the ration.

9. High levels of fluorine in the ration exerted no direct effect upon reproduction in rats or pigs but adversely affected lactation through decreased feed consumption.

10. Rations containing large amounts of fluorine caused an increased water consumption and a diuresis in pigs.

11. Sodium fluoride, calcium fluoride, and phosphatic limestone had no evident effect on the livers, kidneys, spleens, thyroids, or parathyroids of rats or on the livers or kidneys of pigs.

12. The addition of 1 per cent or more of rock phosphate to the ration of pigs caused a degeneration of the epithelium of the convoluted tubules and a fibrosis of the kidney. This did not occur in the case of the rat.

13. The feeding of sodium fluoride at levels of 0.05 per cent of the ration caused a retardation in the rate of growth of the rat but had no effect on the percentage of bone ash at maturity.

14. When fluorine as rock phosphate or sodium fluoride was fed at levels of 0.071 per cent, a retardation occurred in the calcification of the bones of rats at 5 and 10 weeks of age.

15. The availability of fluorine varied with the form in which the fluorine was fed. Approximately 30 per cent of the fluorine ingested in the form of rock phosphate, sodium fluosilicate, and sodium fluoride was retained in the body by the rat while none of the fluorine in the form of calcium fluoride was retained.

16. The effect of fluorine feeding on blood coagulability varied with the species. High-fluorine rations increased the rate of coagulation in the case of the chick and decreased it in the case of the rat.

17. For practical feeding purposes, rock phosphate may be fed to pigs at 0.5 per cent of the ration and to chicks at 2 per cent of the ration for short periods without danger of fluorine toxicity. If the animals are to be maintained on the ration for long periods of time (12 months or longer), the use of smaller amounts is recommended.

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