THE RELATIONSHIP OF CERTAIN NUTRITIONAL STRESS FACTORS TO PARAKERATOSIS OF SWINE

by

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INTRODUCTION

Parakeratosis is a skin condition of swine which has taken on a greater degree of incidence in the past seven years. The condition was recognized as a disease entity in the early 1940's but was not named and described until 1953 by Kernkamp and Ferrin (10). The naming of the disease was based upon the histological alteration of the strateum corneum. In 1955 Tucker and Salmon (23) showed that in a high calcium ration the incidence of parakeratosis was greater and that zinc supplementation would alleviate the condition. As a result of this study, these workers thought parakeratosis was due to a zinc deficiency being precipitated by a high level of calcium in the diet. It does not appear logical that the zinc requirements for swine would be increased from a previous level of about 4 mgm. per day to that of over 25 mgm. of zinc per day in a few years. Therefore, it seems that other factors are involved besides a zinc deficiency induced by a high calcium diet in the etiology of parakeratosis.

These experiments herein described were designed to show whether parakeratosis could be produced by feeding a balanced diet with a low zinc level and higher than recommended calcium level. The latter experiments were designed to establish more accurately the various nutritional and other stress factors contributing to the etiology of parakeratosis. The purpose for which all experiments were directed was to produce zinc

depleted animals or to render zinc physiologically unavailable on practical rations. The approaches used were high calcium feeding, calcium borogluconate injections, bleeding, use of chelating agents, and omitting various essential nutrients from the diet. Theoretically if the addition of zinc to the diet of parakeratotic pigs cures the condition the substraction of zinc from healthy animals by any of the above methods should cause the disease. Careful observation is the only test as to whether or not parakeratosis is present. Weight studies, blood value determinations, and study of histological sections gives an indication of the health status of the animals and were used for such in these experiments.

REVIEW OF LITERATURE

Definition

Parakeratosis is an abnormality of the strateum corneum of the skin in which there is hypertrophy and nucleation of this layer. The disease is characterized by the presence of keratinous crust separated by crevices containing a brown exudate.

Pathogenesis and Signs

The disease process begins as a reddening of the skin and subsequent development of small (1-5 mm.) papules or pustules (Plates III and IV). According to Lewis et al. (13) many cases do not progress further and may go unnoticed.

There may be a decrease in feed intake and a brown exudation from the eye with no other symptoms present. These pustules form small crater lesions and progress to form keratinous crust (Plates V and VI). These crust are first seen over the buttocks, on the tail, behind the ears and on the feet. As the disease progresses, crust will appear over the entire body. Crevices filled with a brown exudate separate the areas of incrustation. Tucker and Salmon (23) have noticed scouring as a clinical symptom of parakeratosis.

Differential Diagnosis

Parakeratosis is similar to seborrhea oleosa (so-called greasy pig disease), seborrhea crustosa, sarcoptic mange, and allergic dermatitis. The seborrheic diseases affect pigs during the suckling period. The crusty form has a greater similarity to parakeratosis than does the oily form. Age differentiates the seborrheic disorders from parakeratosis. In animals weaned at six to eight weeks of age the highest incidence of parakeratosis is from ten to twelve weeks of age. If pigs are weaned at an earlier age, parakeratosis may also appear earlier (21).

Sarcoptic mange clinically appears similar to parakeratosis. Microscopical examination of skin scrapings differentiates the conditions. With sarcoptic mange there is no consistent age occurrence. When mange is observed in pigs usually the parent stock will be affected.

Various undefined allergic conditions occur in swine producing a dermatitis which could easily be confused with parakeratosis. The etiology of parakeratosis is not known; therefore, one can not dismiss the fact that an allergin may be involved although the condition does not appear to be an allergic reaction. In the summer of 1957, a herd problem of dermatitis in suckling pigs was referred to Kansas State College, School of Veterinary Medicine. The history revealed that the pens were washed daily with a cresol disinfectant. The pigs were affected at about two weeks of age with a dry skin and rough hair coat. The skin showed an increased thickness over the body except on the ventral surface and on the inside of the posterior legs. Mange mites were not demonstrated in skin scrapings. Erythrocyte counts, leukocyte counts, hematocrit, and hemoglobin determinations were within the usual physiological range. Leukocyte differentiation counts revealed 10 percent esinophiles which are higher than normal. No abnormalities could be demonstrated from skin biopsies. It was thought that this dermatitis was an allergic condition; however, it could not be differentiated from seborrhea crustosa. The above case history is presented as an example of a condition which requires differentiation from parakeratosis.

Parakeratosis in Other Animals

A condition similar to parakeratosis in swine has occurred in cattle. The outbreak and the condition was observed in a herd of Holstein cattle near Rockford, Illinois by Link (16).

Since the animals responded dramatically to zinc therapy the condition was thought to be parakeratosis. The skin of these animals became thickened with a rough hair coat, but returned to normal shortly after zinc supplementation. Other reports of similar situations have not come to the author's attention.

Studies with rats and mice on purified zinc deficient diets indicate some similarity to parakeratosis of swine (3,5,22). The histological changes on the experimental animals included: extreme parakeratosis of the esophagus with a layer of partially keratinized cells and with the buccal cavity affected to a lesser extent; the skin underwent hyperkeratinization with thickening of the epidermis, loss of hair follicles and with persistance of the sebaceous glands; and corneal changes were present in a few animals. These changes were increased vascularization and leukocytic infiltration of the cornea. However, similar corneal changes occur in riboflavin deficiency which may indicate a relationship between these two essential nutrients (6,12).

Etiology

Parakeratosis is referred to by some workers as a zinc deficiency induced by a higher than normal calcium level intake (12). On the other hand, the exact etiology of parakeratosis is not known. As stated previously, this condition apparently has been on the increase during the past seven years. Prior to this period zinc supplementation was not considered necessary and zinc requirements were listed as approximately 4 mgm. per day.

Presently prevention of parakeratosis is advocated by the addition of 25 mgm. of zinc per day to each animal but this amount does not always prevent occurrence of the condition. Apparently parakeratosis is not a primary zinc deficiency but a deficiency induced by other factors.

Workers in Wisconsin and Michigan (9,13,14,15,17,18) have published numerous reports showing that a ration high in calcium (1 to 2 percent) and containing 40 to 50 p.p.m. of zinc resulted in 80 to 100 percent incidence of parakeratosis. After the pigs were showing severe symptoms the condition could easily be alleviated by the addition to the ration of 50 to 100 p.p.m. of zinc in the form of carbonate or sulfate salt. Tucker and Salmon (23) at Alabama were the first to demonstrate this and since that time, numerous reports of the same nature have appeared in the literature (1,4,8,9,11,13,14,15,17,18,24). Many of these rations have been analyzed using the National Research Council publication as a guide (2) and many have been found to be deficient in several vitamins. The vitamins consistently deficient were pantothenic acid and riboflavin. In only a few cases was a balanced basal ration fed (13,14,15). The basal ration used in these experiments (13,14,15) contained an analyzed calcium level of 1.5 percent and 31 p.p.m. of zinc. This ration when fed to weanling pigs produced parakeratosis in all of the animals.

The symptoms of pantothenic acid and riboflavin deficiency as listed by Hammond (6) are poor appetite, retarded rate of growth, rough hair coat, reddening and scaliness of skin often caked with sebaceous exudate, colitis and normolcytic anemia.

These symptoms are listed to point out the similarity to parakeratosis. Yagi (26) reports that chlorotetracycline will form a complex with flavin adenine dinucleotide. In forming this complex a riboflavin deficiency will develop. The role antibiotics may have in the etiology of parakeratosis is not known. However, parakeratosis occurred before the discovery of antibiotics; therefore, other factors may be involved and are needed to explain many of the outbreaks of the disease. In the majority of the outbreaks which have been brought to the attention of the author, the rations fed to the animals contained chlorotetracycline. Apparently parakeratosis has taken on a higher incidence since the employment of antibiotics. The use of antibiotics in swine feeds has brought about an increased rate of growth which entails increased requirements of nutrients. Therefore some relationship may exist between antibiotics and parakeratosis.

Wong (21) reports in the Pfizer symposium that he has made attempts to grow a parakeratotic virus on kidney tissue. Presently certain commercial laboratories are working on the possibility of the disease being associated with a virus. It is suspected by some that a viral agent is a contributing factor in the etiology of parakeratosis. It is feasible that this is true, because the disease appears infectious in nature. It is possible the animal may have to be conditioned before the infectious agent will multiply in its tissues. Parakeratosis occurs on well managed farms where feeding and management is optimum and the disease occurs during a period of very rapid growth.

With the feeding of better rations which results in more rapid weight gain it may be possible that more animals are conditioned today for an infectious agent than was true prior to the introduction of antibiotics, thus, explaining the increase in incidence in the past few years. The best therapeutic agent for the treatment of parakeratosis is zinc. This does not appear compatible with the etiology being infectious in nature.

Various high protein feeds have been incriminated in being associated with parakeratosis including linseed meal, soybean meal, and dehydrated alfalfa. Little data are available concerning these products and their relationship to parakeratosis.

Treatment

At the present time zinc is superior in the treatment and prevention of parakeratosis. It is recommended that zinc sulfate be incorporated at the rate of 1/4 lb. per ton of feed for prophylaxis and 1/2 lb. per ton of feed for therapy. Zinc carbonate can be substituted for zinc sulfate and requires 10 percent less of the compound. Manganese can be used also but is needed at a higher level and is less effective (23).

Research work to date has shown that there are two factors consistently involved in parakeratosis. A high calcium diet brings about a higher incidence of parakeratosis and zinc supplementation (50 to 150 p.p.m.) will rapidly cure or satisfactorily prevent the condition. With these facts established, research work was designed to attempt to reproduce the disease.

EXPERIMENTAL RESEARCH

Introduction to Research - General Materials and Methods

There are eight approaches to the study of parakeratosis included in this thesis. Each of these has been labeled a separate experiment for the purpose of organization and reference. The results, materials, and methods of each experiment are discussed as a unit. Certain materials and methods are common to all of the experiments and will be outlined prior to the individual experiments. A combined discussion and summary on the work as a whole is found at the conclusion of the thesis.

All experiments with the exception of two groups of animals in Experiment VI were conducted on concrete floors with no access to direct sunlight. The feeding and watering area was cleaned daily. The bedded area was kept supplied with clean bright wheat straw. In the first five experiments all animals were hand fed from wooden troughs and were watered from automatic watering troughs attached to 55 gallon metal drums. In Experiment VI the animals were full fed and watered from wooden troughs. The rations fed all animals are indicated in Table 1. The most susceptible period for parakeratosis is shortly after weaning; therefore, weanling pigs were used in all cases with the exception of the studies in Experiment I (b) and II. The breed of swine used in these studies was Durocs (Experiment I through IV) and Hampshires (Experiment V and VI).

Experiment I: (a) Excess Calcium and Low Zinc Intake in Relation to Parakeratosis of Swine with Bleeding as a Stress Factor.

Materials and Methods. Ten pigs were divided at random according to weight into four groups. Each group received the respective ration as indicated in Table 1. Two animals were placed in groups I and IV and three animals in groups II and III. One animal from each group was bled twice a week at the rate of 5 percent of the blood volume per week for the first seven weeks of the experiment. Experiment I lasted 65 days with the pigs being treated as indicated in Table 3. Weights of the pigs were taken at the beginning and end of the experiment. Hematocrit values were determined on the 1st, 15th and 50th day using Winthrop's hematocrit tubes and centrifuged in an angle centrifuge at 3,000 r.p.m. for 30 minutes. Hemoglobin values were determined on the 1st, 15th, and 50th day using standard laboratory technic. The rations were analyzed for zinc content by the Chemistry Department, Kansas State College. At the end of the experiment, skin biopsies were taken from each animal on the dorsal midline of the withers. The animals were observed closely for evidence of developing parakeratosis.

Results. During the time of the experiment the animals did not show any signs of developing parakeratosis. Histological appearance of the biopsies appeared normal. The animals in groups I and II receiving the diets containing normal levels of calcium gained an average of .12 pound per day more than those animals in groups III and IV receiving high calcium diets.

The addition of zinc to the high calcium diet in group IV did not appear to overcome the growth inhibition of the added calcium (Table 4). From Table 5 it can be seen that the hematocrit and hemoglobin values were little affected from bleeding. The highest zinc content of the rations analyzed was 31.6 p.p.m. which is considered to be lower than the average reported from rations which have produced parakeratosis (Table 2).

Experiment I: (b) Stress Factor Injections of Calcium Borogluconate

Materials and Methods. Six nursing pigs, two weeks of age, were injected intraperitoneally twice a week for 22 weeks at the rate of 1 cc of 20 percent calcium borogluconate per pound of body weight. The male pigs were castrated and all pigs were vaccinated at seven weeks of age and at eight weeks of age all pigs were weaned. After weaning the pigs were placed on ration II. The animals were observed closely for parakeratosis. At the conclusion of the 22 week period skin biopsies were taken from the midline dorsal to the withers.

Results. During the time of the experiment the animals did not show clinical signs of developing parakeratosis. Histological examination of the skin biopsies revealed 50 percent of the tissues contained areas of nucleation in the strateum corneum with the strateum corneum being of normal thickness. Plates VII, VIII, and IX are photomicrographs of these nucleated areas.

Table 1. Composition of various rations fed experimental animals.

Ingredient (In lbs. unless otherwise stated)	Rations						
	Used in Exp. I,(abc) II, III				Used in Exp. IV, V, VI		
	I	II	III	IV	V	VI	VII
Yellow corn	66.0	66.0	64.5	64.5	87.0	79.0	15.0
Soybean oil meal	28.0	28.0	28.0	28.0	4.4	17.0	17.6
50% meat scraps					4.4		2.5
Linseed oil meal					1.1		
Rolled oats							50.0
Dehydrated alfalfa					1.1		
Brown sugar							7.5
Dried whey							2.5
Antibiotic vitamin premix	1.0[1]	1.0	1.0	1.0		1.0[1]	1.0[4]
Sodium chloride	1.0	1.0	1.0	1.0		0.5	0.5
Ground limestone	1.0	1.0	2.5	2.5	1.8	2.3	1.3
Tr. Min. (in gm/100#)	10.0[2]			10.0			
Tr. Min. W/o zinc (in gm/100#)		9.67[3]	9.67				5.72[5]
Dicalcium phosphate	1.2	1.2	1.2	1.2		.3	1.0

1. Antibiotic vitamin premix: Thiamin, 25 mgm.; Riboflavin, 106 mgm.; Pantothenic Acid, 416 mgm.; Cobalamin Concentrate (3 mgm. vit. B_{12} activity/gm.) 333 mgm.; Irradiated Yeast, (9,000 U.S.P. units vitamin D/gm) 1333 mgm.; Oxytetracycline (25 gm. activity/lb.) 20 gm. soybean oil meal q.s. 1 lb.

Footnotes to Table 1 Continued.

2. Trace mineral mixture.

	% of element contained in final premix	Amount of compound for mixture
$FeSO_4 \cdot 7H_2O$	77.0	383.1 gms.
$CuSO_4 \cdot 5H_2O$	8.0	31.4 gms.
$MnSO_4 \cdot H_2O$	8.0	24.6 gms.
$CoCl_2 \cdot 6H_2O$	3.5	14.1 gms.
$ZnSO_4 \cdot 7H_2O$	3.5	15.4 gms.

3. Trace mineral mixture W/o zinc is the same as above except omit $ZnSO_4 \cdot 7H_2O$.

4. Antibiotic vitamin premix for creep ration.

Ingredient	Amount/100 lbs.
Vitamin A (1 million I.U./gm.)	0.3 cc vitamin A acetate
Vitamin D (9000 U.S.P. units/gm.)	5.5 gm. Irridated yeast
Vitamin B_{12} concentrate (3 mgm./gm.)	0.7 gm.
Nicotinic acid	3.0 gm.
Choline chloride	7.5 gm.
Pantothenic acid	0.3 gm.
Riboflavin	0.3 gm.
Aeuromycin (25 gm. activity/lb.)	20.0 gm.
Soybean oil meal q.s.	1 lb.

5. Trace mineral mix for creep ration.

Trace mineral	Amount of element /lb.	Amount of compound /100 lbs.
$MnSO_4 \cdot H_2O$	2.5 mgm.	720 mgm.
$CuSO_4 \cdot 5H_2O$	2.0 mgm.	500 mgm.
$FeSO_4 \cdot 7H_2O$	15.0 mgm.	4100 mgm.
$CoCl_2 \cdot 6H_2O$	1.0 mgm.	400 mgm.

Table 2. Zinc analysis in p.p.m. of rations I, II, III and IV[1]

Sample	Rations			
	I	II	III	IV
1 Antibiotic not present	0	15.4	25.0	23.2
2 Antibiotic present	31.6	23.6	4.5	6.0
3 Analytical grade dicalcium phosphate used in ration instead of commercial grade	27.2	13.4	0	.2

1. Zinc analyses were carried out by the chemistry department of Kansas State College under the direction of W. S. Ruliffson, Asst. Professor.

Table 3. Treatment of pigs in Experiment I

Pig Number	Group
52	I
61*	I
58	II
51	II
55*	II
56	III
57	III
64*	III
50*	IV
59	IV

*Indicates animal which was bled.

Table 4. Weights of pigs in Experiment I

Pig Number	Group	Weight 1st day	Weight 65 days	Rate of Gain
52	I	36.2	137	1.55
61*	I	43.2	141	1.50
58	II	39.0	133	1.45
51	II	39.8	152	1.72
55*	II	37.0	[1]	
56	III	38.0	116	1.20
57	III	35.8	120	1.29
64*	III	40.3	122	1.26
50*	IV	43.0	127	1.29
59	IV	35.3	123	1.35

1. Animal 55 died the 50th day while attempting to remove blood. Cause of death was suffocation from blood entering the thorasic cavity from the ruptured anterior vena cava.

* Indicates animal which was bled.

Table 5. Hematocrit and hemoglobin values of animals in Experiment I.

Pig No.	Group	Hematocrit			Hemoglobin		
		1st day	15th day	50th day	1st day	15th day	50th day
52	I	40	40	43	14.0	13.0	14.2
61*	I	36	36	41	15.0	11.7	14.2
58	II	41	41	45	14.5	13.6	15.1
51	II	32	32	43	12.8	10.3	14.3
55*	II	36	36		15.0	12.1	
56	III	43	43	43	15.0	14.5	14.2
57	III	42	42	42	15.0	14.0	14.0
64*	III	40	40	37	14.5	13.2	12.0
50*	IV	40	40	35	14.0	13.2	11.5
59	IV	41	41	43	14.5	13.2	13.6

* Indicates animals bled.

Experiment I: (c) Stress Factor Daily Injection of Calcium Borogluconate.

Materials and Methods. Sixteen nine-week-old pigs were divided at random according to weight into four groups and fed the rations I, II, III and IV as indicated in Table 1. These pigs were wormed with 1 percent sodium fluoride; vaccinated with Rovac(R) plus 10 cc of antihogcholera serum; and castrated shortly before being placed on experiment. Two animals from each group were injected intraperitoneally with 20 percent calcium borogluconate at the rate of 2 cc per pound of body weight at each injection. The animals were injected daily for the first eight weeks of the experiment. The animals were observed 12 weeks for evidence of developing parakeratosis. Weights of the animals were taken at 1, 28 and 56 days. The dosage of the calcium borogluconate was changed at weekly intervals. Between weighings, the rate of growth was considered to be one pound per day and the dosage rate for each animal was computed from this figure.

Results. Alopecia developed in all of the animals but it did not develop greater in any one group over the other groups (Plates X, XI). The animals receiving calcium borogluconate injections had a greater degree of alopecia. Parakeratosis was not observed in any of the groups. The animals receiving high calcium diets did not gain as well as those on normal calcium level diets (Table 6). Calcium borogluconate injection caused greater growth depression irregardless of diet.

Rovac(R) Trade name for American Cyanamid Company's modified live hog cholera vaccine.

Table 6. Weights and Rate of Gain of Animals in Experiment I: (c).

Animal No.	Group	Weight (1 day)	Weight (28 days)	Weight (56 days)	Av. Rate of Gain
81	I	22.4	71.5	108.3	1.45
88	I	29.4	79.5	105.5	
84*	I	15.6	59.5	62.5	.82
82*	I	34.6	67.5	89.3	
92	II	25.0	77.0	105.8	1.37
79	II	26.6	80.0	109.3	
89*	II	17.2	55.0	72.8	.89
87*	II	35.2	80.0	89.8	
78	III	22.0	48.5	78.5	1.12
90	III	26.4	60.0	95.3	
80*	III	31.5	61.0	70.5	.88
86*	III	23.8	53.0	83.8	
83	IV	20.0	43.5	63.3	1.10
85	IV	28.8	72.5	108.8	
91*	IV	25.4	60.0	87.8	.61
99*1	IV	25.4	36.0	41.5	

* Indicates animals injected with 20% calcium borogluconate.

1. Animal 99 developed numerous superficial abcesses.

Experiment II. Excess Calcium and Low Zinc Rations Fed for Two Generations and Its Relation to Parakeratosis of Swine.

Materials and Methods. Four gilts (animals 57, 64, 50 and 59) from Experiment I: (a) were selected because of marked alopecia and were all placed on ration III (Table 1) (Plates XII, XIII). These gilts were bred at eight months of age with one gilt failing to conceive. After the normal gestation period, the remaining three gilts farrowed 19 normal pigs. Seven weeks of age all pigs were vaccinated with Rovac(R) and 10 cc of antihogcholera serum and the males were castrated.

At eight weeks of age the pigs were weaned and were placed on ration III (Table 1). The experiment was continued until the pigs were 16 weeks of age.

Results. It was felt studies other than visual observation would tend to cause a disturbance among the animals; thus, bringing about abnormal field conditions and possibly some growth inhibition. After vaccination, castration, and weaning the animals were observed only for evidence of developing parakeratosis. As in the experiments thus far the only abnormality observed was slight alopecia. There was no evidence of developing parakeratosis.

Experiment III. The Use of Chelating Agents to Induce Zinc Depletion of Tissues in an Attempt to Produce Parakeratosis of Swine.

Materials and Methods. The chelating agents employed were Diphenylthiocarbazone (dithiazon) and Ethylene-Diamine-Tetra-Acetic Acid (EDTA). Dithiazon was prepared according to the method of Wolff et al. (25) into a solution containing 750 mgm. per 100 cc and having a pH of 8.6. The EDTA solution was prepared by using six and one-half grams of EDTA (disodium salt) in 100 cc. of an 8.7 percent calcium gluconate solution resulting in a calcium salt of EDTA. Using stoichiometric calculations each gram of EDTA will unite with approximately 120 mgm. of calcium; thus, the reason for using 8.7 percent calcium gluconate.

Wolff *et al.* reports that in dogs dithiazon is a specific *in vivo* chelator of zinc, i.e., other metals were not found in the urine in increased amounts following the injection of the chelator. EDTA is a non specific chelator which readily chelates calcium but preferrentially chelates the trace minerals. The toxic side effects of EDTA are less than those of dithiazon thus the purpose of using this non specific chelator.

The first approach to the administration of dithiazon was the intravenous route. Much difficulty was encountered in this procedure as the tissue reaction to this material was severe and occurred almost instantly resulting in apparent collapse or perforation of the vein. The tissue adjacent to the vein became white and very hard which also added to the difficulty in this approach. Because of the long injection period (45 minutes) all animals were anesthesized with sodium pentobarbital. The femoral and jugular veins were exposed and cannulated for administration of the dithiazon. The first rate of dosage attempted was 50 mgm./lb. of body weight. All injections were preceeded 24 hours with 1 gm. of vitamin E which, according to Wolff *et al.* (25), prevented the hemolysis of the erythrocytes. At this level and rate of dithiazon administration the animals would die before the injection was completed. Autopsies on these animals revealed tissues throughout the entire body which were dyed the amber color of the dithiazon solution. The lungs were emphesematous and the pleural cavity filled with amber colored serous fluid.

One animal on autopsy possessed a large hematocyst dorsal to the heart. The usual symptoms prior to death was labored breathing. Blood samples taken at this point proved to have adequate circulating erythrocytes to maintain life, i.e., death was not due to erythrocyte hemolysis. Of seven trials using the above described procedure one animal (No. 17) lived and subsequent tests, post-injection urine and serum zinc levels, were performed and are reported in Table 8.

After preliminary trials on rabbits it was found that intraperitoneal injection was less toxic. The dithiazon was absorbed because it left the peritoneal cavity and appeared in the sera. The above animal (No. 17) was placed with three other animals in which all injections were made intraperitoneally. Two animals were given dithiazon and two animals were given EDTA (Table 7). All animals were pre-injected with one gram of vitamin E. The animals were fed ration III as described in Table 1. The animals were observed for four weeks. At the end of this period low level injections were given intraperitoneally three times a week for six weeks. The same chelating agent was used on each animal as was used previously. The dosage rate was 150 mgm. of dithiazon and 132 mgm. of EDTA which was given at each injection. Twenty-four hours after the last injection each animal was operated on for a biopsy of the pancreas for further histochemic studies not reported in this thesis. The animals were observed for eight weeks after which euthanasia was performed. Tissues were taken and gross pathology observed.

Table 7. Chelating agents used and the injection rates.

Pig No.	Chelating Agent	Weight	Dose (mgm./lb.)
17*	Dithiazon	46	25
36	"	24	25
37	EDTA	34	34
38	"	30	34

* Pig No. 17 was previously injected intravenously.

Results. During the eighteen week duration of this study no cases of parakeratosis occurred. The method to show that zinc was being transported out of the body was to determine pre-injection and post-injection serum and urine zinc levels (Table 8).

Table 8. Zinc levels of blood sera and urine from animal No. 17. Zinc expressed in μgm %[1]

	Pre-injection	Post-injection			
		6 hrs.	12 hrs.	28 hrs.	96 hrs.
Urine	215	290	17	214	40
Serum	45	124	194	72	--

1. Analysis, courtesy W. S. Ruliffson, Department of Chemistry, Kansas State College.

The skin sections taken from these animals appeared normal. Gross pathology was normal disregarding the adhesions from the operation and numerous intraperitoneal injections. The results of the zinc content of urine and serum from Table 8 can not be

used to show a significant chelation of zinc and resulting zinc depletion because an insufficient number of animals were used. Wolff et al. (25) reported an increase of 101.2 μ percent urinary zinc level in the dog 10 hours after injection. The injection rate was 68 mgm./lb. of body weight. It was shown an injection of this level produced subsequent diabetes and pancreatic tissue zinc could not be demonstrated using a histochemic technic.

Animals No. 17 and 36 received an average total of 120 mgm. dithiazon/lb. of body weight over a 10 week period. Animals No. 37 and 38 received an average total of 225 mgm. EDTA/lb. of body weight over a 10 week period.

Experiment IV. Observations and Studies Carried Out on Swine Obtained from the Animal Husbandry Department, Kansas State College.

History of Pigs. This study was carried out under the supervision of Aubel (1). It is referred to in this thesis because it is the basis for Experiment V. Histological sections were prepared from these animals and the appearance of the syndrome described.

Eight weanling pigs were placed in dry lot on December 1st and fed ration V (Table 1) which was pelleted. The first lesions of parakeratosis appeared in 28 days. By 40 days seven of the eight pigs developed parakeratosis. Five of these animals showing the most severe symptoms were obtained for closer observations and skin biopsies. The animals were maintained on the same feed and placed in clean concrete pens. Within 30 days after the animals were moved they were normal.

Description of the Condition. Skin biopsies obtained when the condition was well developed revealed a thickened and nucleated strateum corneum. The corium was hyperemic and infiltrated with leukocytes (Plates XIV and XV). The gross appearance of the skin showed abnormal thickness with a dry crusty appearance. The brown exudate as observed by others was not present. The most severely affected areas were on the inside of the posterior limbs, tail and on the edges of the ears. The affected areas varied from one-fourth to five inches in diameter. Photographs were not obtained from these animals as it was thought the condition would progress further in the lesser affected animals instead the condition rapidly cleared. Appearances of the animals were similar to that of the typical field cases of parakeratosis (Plates V and VI).

Experiment V. The Relationship of Certain B-Complex Vitamins and Lysine to the Etiology of Parakeratosis.

Materials and Methods. Thirty-two nine-week-old Duroc pigs were used in this experiment. They were previously vaccinated with Rovac(R) and the males were castrated. The animals were divided by weight into eight groups (Table 9). The basal ration used for this experiment was ration V (Table 1) with the exception of 2.3 percent ground limestone employed instead of 1.8 percent which gives a resulting calculated calcium content of 1.35 percent.

Table 9. Groups and rations for Experiment V

Group	Ration
I	Basal, not pelleted
II	Basal, pelleted
III	Basal + Lysine[1]
IV	Basal + Riboflavin
V	Basal + Pantothenic Acid
VI	Basal + Folic Acid
VII	Basal + Riboflavin + Pantothenic Acid
VIII	Basal + Riboflavin + Pantothenic Acid + Folic Acid

1. Lysine, courtesy E. I. DuPont D. Nemours and Company, Inc. Wilmington, Delaware.

The various nutrients which were added to the basal ration as listed in Table 9 were added after the pelleting process. This was done to prevent destruction of these nutrients by heat as they are labile or partially labile to heat. These vitamins were placed in a sugar carrier and dissolved in water which was sprinkled over the feed as it was mixed in a 50 pound Homart mixer.

The mixing procedure used to prevent contamination among the added nutrients were groups IV, VII, and VIII which were mixed in sequence. The vat was then washed and re-washed between mixing of each subsequent ration.

The added nutrients as listed in Table 9 were added at the following rates: Lysine .75 percent, pantothenic acid 500 mgm./lb. of feed, riboflavin 100 mgm./pound of feed, and folic acid 5 mgm./ pound of feed. The addition of these nutrients provided for some of the intended deficiencies, alterations, and deterioration

due to pelleting present in the basal ration. These animals were housed inside on concrete floors. They were watered from automatic watering troughs attached to 55 gallon metal drums and fed daily from wood troughs. An oat straw bedded area was provided with the feeding and watering area free of straw and cleaned as indicated.

This experiment lasted 118 days during which time the animals were observed for evidence of developing parakeratosis. Weights were obtained and blood studies were made at periodic intervals. A skin biopsy was obtained at the termination of the experiment from the posterior aspect of the buttocks about three inches lateral and ventral to the tail.

Results. During the period of this experiment there was no indication of developing parakeratosis. The zinc content of the ration was similar to reports of other workers (Table 10.) Since the zinc content of the water and bedding were lower than the consumed feed it is felt this would not be a significant source of zinc for preventing development of parakeratosis.

The rate of gain for the 31 pigs (Table 11) of this experiment was below average but can be accounted for by the low protein content (12.86%) and the high calcium content (1.38%) of the ration.

Blood studies (Tables 12 through 18) show that the animals were normal at the beginning of the experiment and there was little deviation from the beginning values.

The histological analysis of the biopsies revealed 50 percent nucleation of the strateum corneum without clinical symptoms.

Table 10. Zinc analyses[1]

	Zinc p.p.m.
Water from metal barrels	.05
Water directly from city water supply	.12
Basal ration	30.0
Straw bedding	27.0

1. Zinc analyses, courtesy W. S. Ruliffson, Department of Chemistry, Kansas State College.

Table 11. Average weights of animals

Group	Av. Beginning Wt. in Lbs.	Av. Wt. in Lbs. at end of Experiment	Av. Daily Rate of Gain in Lbs.
I	33.2	157.5	1.05
II	36.5*	183.0	1.24
III	33.5	172.5	1.18
IV	33.5	202.0	1.43
V	33.5	182.0	1.26
VI	33.5	182.0	1.26
VII	33.5	150.0	0.99
VIII	33.5	177.5	1.22

* One animal from this group developed an infection with Ballantidium Coli and was removed from the experiment. Average beginning weight 33.8 lbs.; Average weight at the end of the experiment 175.8 lbs.; Average rate of gain 1.20 lbs.

Table 12. Average erythrocytes 10^6 (Million/cu. mm.)

Group	Av. RBC 10^6 at beginning of exp.	Av. RBC 10^6 at end of exp.
I	7460	8210
II	7610	7730
III	8720	8370
IV	7530	8300
V	8120	7810
VI	8390	8520
VII	7160	9060
VIII	8210	8540

Table 13. Average hematocrit (%)

Group	Av. Ht. at beginning of exp.	Av. Ht. at end of exp.
I	39.3	43.3
II	41.0	44.0
III	42.8	44.5
IV	39.5	44.3
V	40.5	44.8
VI	42.8	44.5
VII	38.5	43.0
VIII	44.3	44.3

Table 14. Average hemoglobin (gm./100 cc. blood)

Group	Av. Hb. at beginning of exp.	Av. Hb. at end of exp.
I	12.3	14.5
II	13.0	14.5
III	13.5	14.5
IV	12.5	14.3
V	13.0	14.0
VI	12.8	14.5
VII	12.0	14.0
VIII	13.5	14.5

Table 15. Average mean corpuscular volume (cu.μ gram)

Group	Av. MCV at beginning of exp.	Av. MCV at end of exp.
I	52.7	52.7
II	53.9	56.9
III	49.1	53.2
IV	52.5	53.4
V	49.9	57.4
VI	51.0	52.2
VII	53.8	47.5
VIII	54.0	51.9

Table 16. Mean corpuscular hemoglobin (Expressed in μ gm.)

Group	Av. MCH at beginning of exp.	Av. MCH at end of exp.
I	16.5	17.7
II	17.1	18.8
III	15.5	17.3
IV	16.6	17.9
V	16.0	17.9
VI	15.3	17.0
VII	16.8	15.5
VIII	16.4	17.0

Table 17. Mean corpuscular hemoglobin concentration (Expressed in %)

Group	Av. MCHC at beginning of exp.	Av. MCHC at end of exp.
I	31.3	33.5
II	31.7	33.0
III	31.5	32.6
IV	31.6	32.1
V	32.0	31.3
VI	29.9	32.6
VII	31.2	32.6
VIII	30.5	32.7

Table 18. Average leukocyte counts (per cu. mm.)

Group	Av. WBC at beginning of exp.	Av. WBC at end of exp.
I	17,837	21,375
II	22,225	22,863
III	20,150	19,762
IV	21,225	25,875
V	21,725	18,025
VI	23,225	25,312
VII	22,400	23,612
VIII	21,162	22,462

Experiment VI. The Effect of Sunlight on the Incidence of Parakeratosis.

Materials and Methods. Four Hampshire sows were obtained a few days prior to their farrowing dates and each was placed in a different group as described in Table 19. The rations received by these sows and weights in groups are listed in Table 20. At weaning time, the pigs were placed on the same diets which their dams received. The creep ration was fed only to litters I and II during the suckling period. Litters III and IV were allowed access to their dam's ration during the suckling period. The male pigs were castrated at three weeks of age. All pigs were vaccinated with Rovac(R) at seven weeks of age and weaned at eight weeks of age. All animals were restricted to concrete flooring, watered from automatic watering troughs connected to 55 gallon drums, and were fed from wood self feeders. At weekly intervals beginning at one week of age until five weeks old, each pig received 1½ cc. of a syrup containing iron sulfate and copper sulfate to prevent anemia.

The animals were observed for evidence of developing parakeratosis. Weights were taken at birth, weaning time, and at the end of the experiment.

Table 19. Animals: Groups in Experiment VI

	Ration V	Ration VI
Sunshine	Litter I (8 pigs)	Litter III (5 pigs)
No sunshine	Litter II (7 pigs)	Litter IV (9 pigs)

Table 20. Rations and group weights of animals in Experiment VI

	Birth Wt. lbs.	Weaning Wt. lbs.	140 Day Wt. lbs.	Av. Gain Weaning to 140 days
Sunshine				
Ration V Litter 1	2.5	31.2	132.9	1.2
Ration VI Litter 4	2.5	48.1	197.9	1.8
No sunshine				
Ration V Litter 2	2.5	26.1	87.9	0.7
Ration VI Litter 3	2.5	18.7	123.4	1.2

Skin biopsies were removed from the buttocks of each animal as described in Experiment V at the end of the experiment. This experiment was 140 days in duration.

Results. During the period of this experiment no cases of parakeratosis developed. The weights of the pigs are indicated in Table 20. The biopsies appeared normal.

DISCUSSION

The data of these experiments fail to agree with reports in the literature (1,9,13,14,15,17,18,23). The typical report from the above references was 80 to 100 percent parakeratosis with a 1.25 percent calcium content of the ration and 0 to 10 percent parakeratosis with .65 percent calcium content of the ration. Using similar rations and calcium contents which varied from .65 to 1.6 percent and in one experiment 1.6 percent calcium plus an injection of 18 gm. calcium/100 lb. body weight/day failed to cause a single case of clinical parakeratosis. The total number of animals used in these experiments including controls was 97.

Several experiment station workers have indicated that parakeratosis could be produced in certain years and in other years could not be produced when the same procedures were repeated. From the many contradictory reports one would assume the true etiology of parakeratosis has not been solved. Tucker and Salmon (23) reported the syndrome of parakeratosis could be induced by a zinc deficiency. They did not elaborate as to whether the condition is a simple or conditioned zinc deficiency. From all evidence available the possibility of parakeratosis being a primary zinc deficiency is very remote. It is questionable whether it is a conditioned deficiency because manganese supplementation will also correct the condition but at a slower rate. The apparent curative effect of zinc may be in its action as an alterant in the body. Various elements are included in this class such as arsenic, iodides, iron, zinc, and manganese. The most commonly used alterant is arsenic. The mechanism of action of an alterant is not known. Possibly, these elements tend to stimulate the enzyme systems of the body thus eliciting their response. As was pointed out above this may be the action of zinc but has not been proven.

By using a chelating agent in Experiment III to substantially lower the available zinc to carry on enzyme action it was not possible to produce parakeratosis. An experiment of this type definitely is limited as to the value one can place on the results because of the many unknown side effects of the toxic chelating agent. However, it is felt the results of this work tend to minimize the importance of zinc in the

etiology of parakeratosis. A study using zinc free purified diets should also be used to study the effects of minimal zinc on the production of parakeratosis. Work of this type has not come to the author's attention.

In Experiments I (b) and V considerable nucleation was observed in the strateum corneum. According to Habel and Biberstein (7) this is abnormal. It is not known whether this is an indication of the first stages of developing parakeratosis. The presence of this histological finding in the absence of clinical symptoms of parakeratosis has not been recorded in the literature to the author's knowledge. This condition should be studied further.

Yagi et al. (26) reports the formation of a complex of chlorotetracycline and flavin adenine dinucleotide which may be one of the factors in the etiology of ariboflavinosis that is caused by this antibiotic. It has been noted by the author that most of the rations used to produce parakeratosis contained chlorotetracycline. This indicates a possible relationship of the etiology of parakeratosis to this antibiotic. This is possibly the explanation as to why parakeratosis has occurred with greater frequency in the past seven years.

SUMMARY AND CONCLUSIONS

Ninety-seven pigs involving six experiments were used in these studies to elucidate the etiology of parakeratosis. The purpose was to produce zinc depleted animals or to make zinc physiologically unavailable on practical rations. The various

approaches used to accomplish this were high calcium feeding, calcium borogluconate injections with and without high calcium feeding, bleeding with and without high calcium intake, injection of chelating agents into animals on a high calcium diet and omitting certain essential nutrients from the ration to complement the known factors reportedly involved in the production of the syndrome of parakeratosis.

The first approach to the study of parakeratosis was to use calcium as a zinc antagonist. The production of parakeratosis by feeding a higher than normal calcium level has been reported numerous times in the literature. Certain stress factors such as bleeding, biweekly calcium borogluconate injection and daily calcium borogluconate injection were employed, also. All attempts failed to produce parakeratosis using these approaches.

Two chelating agents were used to make zinc physiologically unavailable. Diphenylthiocarbazone is reported to be a specific chelator of zinc. In using this compound it was found that its toxic side reactions minimized the value as an *in vivo* chelator. Ethylene-diamine-tetra-acetic acid is a non specific chelator. In general the heavy metals which includes the trace minerals are chelated to varying degrees. However, pathology resulting from the use of this chelator can not be traced to the removal of a specific element. Utilizing these agents it was found that one, diphenylthiocarbazone was effective in eliminating increased output of zinc via the urinary tract. Both failed in inducing the parakeratotic syndrome.

Five parakeratotic pigs were obtained from the Department of Animal Husbandry for histological studies. These animals were moved to sheltered concrete pens and fed the same ration. After this change the condition rapidly cleared up without treatment. This identical ration was used in Experiment V in an effort to reproduce parakeratosis. This ration was also supplemented with various vitamins and lysine in different combinations to determine whether these nutrients were contributing factors in inducing the disease. Further it was thought that removing these parakeratotic animals, obtained from the Department of Animal Husbandry, out of the sunlight might possibly have aided in the recovery of the condition. An experiment was designed (Experiment VI) to study the effect of sunlight on the occurrence of parakeratosis. For this particular experiment two rations were used. One ration was the same pelleted ration fed by the Department of Animal Husbandry. The other ration was a more desirable, better balanced ration.

The nutritional stress factors employed in these studies using a total of ninety-seven pigs failed to cause the parakeratotic syndrome as recorded in the literature. Histological studies made on the experimental animals revealed in about 50 percent of the cases a microscopic nucleation of the strateum corneum. The importance of this finding in relation to the parakeratotic syndrome is not known. The presence of this histological finding in the absence of clinical symptoms of parakeratosis has not been recorded in the literature to the Author's knowledge.

It is suggested that factors other than a high calcium and low zinc diet are involved in the etiology of parakeratosis. The data of this thesis fails to agree with existing reports on this condition.

ACKNOWLEDGMENT

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E. I. DuPont D. Nemours and Company generously supplied the lysine required for Experiment V.

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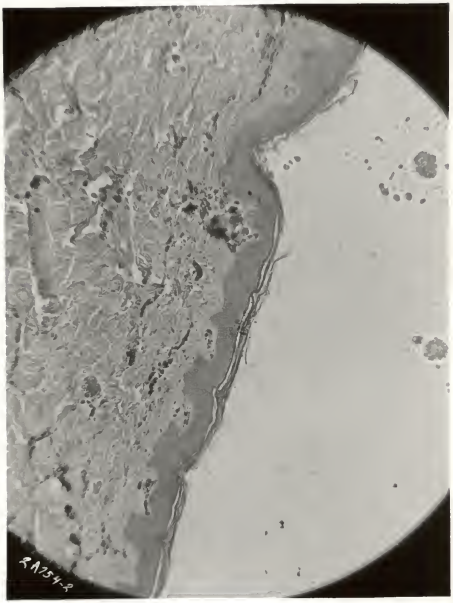
APPENDIX

EXPLANATION OF PLATE I

Section of normal skin. (95x).

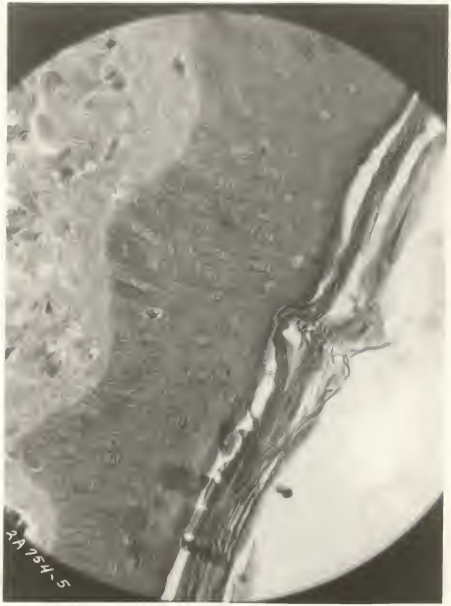
PLATE I



EXPLANATION OF PLATE II

Section of normal skin (450x).

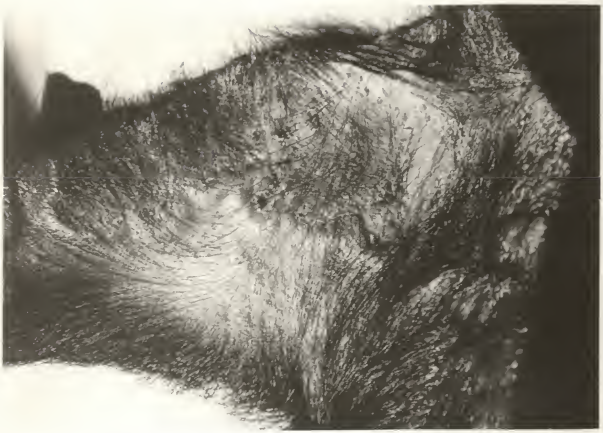
PLATE II



EXPLANATION OF PLATE III

Photograph of a field case of parakeratosis showing raised hyperemic areas on the inside of the foreleg. This demonstrates the first stage of parakeratosis.

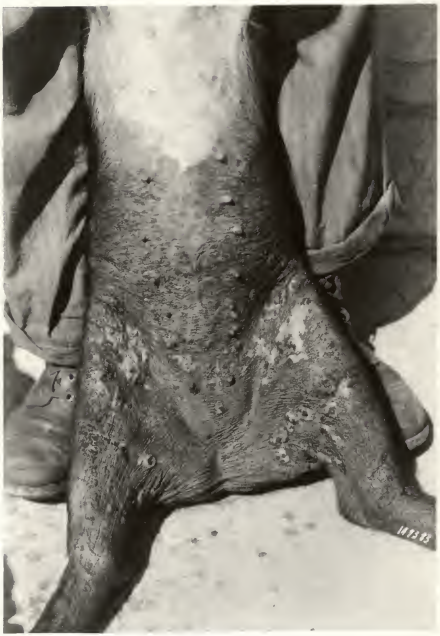
PLATE III



EXPLANATION OF PLATE IV

Photograph of a field case of parakeratosis showing pustule and papule formations. This stage of development immediately follows the hyperemic stage.

PLATE IV



EXPLANATION OF PLATE V

Photograph of a field case showing advanced parakeratosis.

PLATE V

EXPLANATION OF PLATE VI

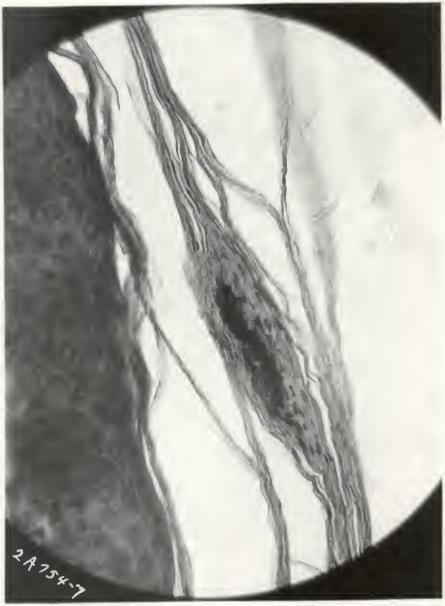
Photograph of a field case of advanced parakeratosis. Note the cracks and crevices in the skin.

PLATE VI

EXPLANATION OF PLATE VII

Section demonstrating a small microscopic area of nucleation in the strateum corneum. This section was a biopsy taken from an animal in Experiment I (b). (450x).

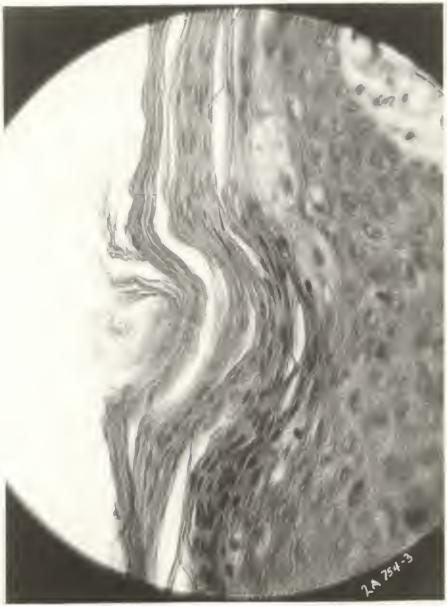
PLATE VII



EXPLANATION OF PLATE VIII

Section demonstrating a diffuse type of nucleation in the strateum corneum. This section was taken from the buttocks of an animal on Ration II, Experiment V. (450x).

PLATE VIII



EXPLANATION OF PLATE IX

Section demonstrating a localized area of nucleation of the strateum corneum. This section was taken from buttock of an animal on Ration VII, Experiment V. (450x).

PLATE IX

EXPLANATION OF PLATE X

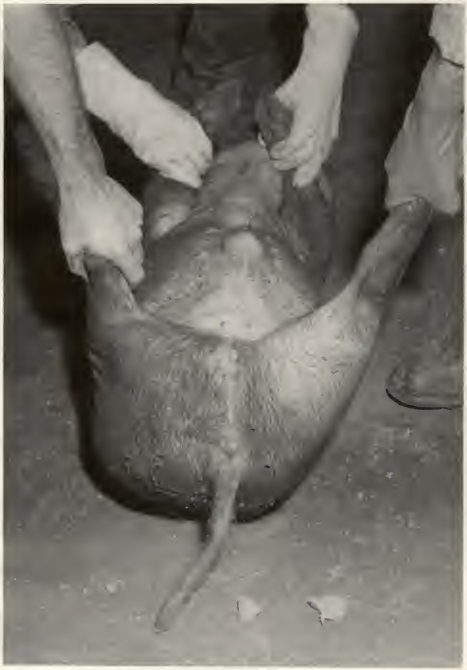
Photograph of a group of animals from Experiment I (c). The two animals in the foreground show slight alopecia but generally the hair coat and skin is normal.

PLATE X

EXPLANATION OF PLATE XI

Photograph of one of the animals pictured in Plate X, demonstrating a normal condition of the ventrum.

PLATE XI



EXPLANATION OF PLATE XII

Photograph showing normal appearance of a group of animals from Experiment I (c).

PLATE XII

EXPLANATION OF PLATE XIII

Photograph of animal showing considerable alopecia. This animal was from Experiment II.

PLATE XIII

EXPLANATION OF PLATE XIV

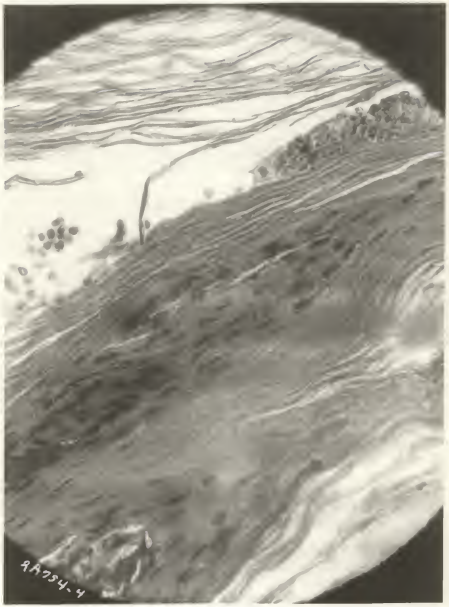
Section from one of the animals from the Animal Husbandry Department with a fully developed case of parakeratosis. The area seen in the photomicrograph is almost completely strateum corneum with keratinous inclusions. At the center and right side of the photomicrograph there is a small area of the remaining layers of the epidermis which appear normal. (95x).

PLATE XIV

EXPLANATION OF PLATE XV

Section from one of the animals from the Department of Animal Husbandry with a fully developed case of parakeratosis. This area shows the nucleation of the strateum corneum. (450x).

PLATE XV



THE RELATIONSHIP OF CERTAIN NUTRITIONAL STRESS FACTORS TO PARAKERATOSIS OF SWINE

by

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AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

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1959

ABSTRACT

Ninety-seven pigs involving six experiments were used in these studies to elucidate the etiology of parakeratosis. The purpose was to produce zinc depleted animals or to make zinc physiologically unavailable on practical rations.

The various approaches used to accomplish this were high calcium feeding, calcium borogluconate injections with and without high calcium feeding, bleeding with and without high calcium intake, injection of chelating agents into animals on a high calcium diet and omitting certain essential nutrients from the ration to complement the known factors reportedly involved in the production of the syndrome of parakeratosis.

Parakeratosis is defined as an abnormality of the strateum corneum of the skin in which there is hypertrophy and nucleation of this layer. This disease is characterized clinically by formation of large keratinous crusts appearing over the buttocks, on the tail, behind the ears, on the feet, and progressing over the entire body as the disease develops.

The etiology of parakeratosis is not completely understood. Research work at this time has shown that there are two factors consistently involved in parakeratosis. A high calcium diet will cause a higher incidence of the disease and zinc supplementation (50 to 150 p.p.m.) will apparently alleviate or satisfactorily prevent the condition. It should be borne in mind that animals will recover spontaneously without treatment.

With these facts established attempts were made to reproduce the disease.

The most susceptible period for development of parakeratosis in swine is shortly after weaning. Therefore, weanling pigs were used in all experiments except I (b) and II. In Experiment I (b) suckling pigs were used and in Experiment II the animals were on experiment for two generations. All experiments were managed in a similar pattern with the individual differences described for each experiment. In the first five experiments, all animals were fed once a day from wood troughs and were watered from automatic watering troughs attached to 55 gallon metal drums. In Experiment IV, the animals were fed from automatic feeders and watered from wood troughs. Seven different rations were used. Some were well balanced and others were deficient in nutrients to approach similarity of conditions which were found in rations reported to induce parakeratosis.

The first approach to the study of parakeratosis was to use calcium as a zinc antagonist. The production of parakeratosis by feeding a higher than normal calcium level has been reported numerous times in the literature. Certain stress factors such as bleeding, biweekly calcium borogluconate injection and daily calcium borogluconate injection were employed, also. All attempts failed to produce parakeratosis using these approaches.

Two chelating agents were used to make zinc physiologically unavailable. Diphenylthiocarbazone is reported to be a specific chelator of zinc. In using this compound it was found that its toxic side reactions minimized the value as an <u>in vivo</u> chelator.

Ethylene-diamine-tetra-acetic acid is a non specific chelator. In general the heavy metals which includes the trace minerals are chelated to varying degrees. However, pathology resulting from the use of this chelator can not be traced to the removal of a specific element. Utilizing these agents it was found that one, diphenylthiocarbazone was effective in eliminating increased output of zinc via the urinary tract. Both failed in inducing the parakeratotic syndrome.

Five parakeratotic pigs were obtained from the Department of Animal Husbandry for histological studies. These animals were moved to sheltered concrete pens and fed the same ration. After this change the condition rapidly cleared up without treatment. This identical ration was used in Experiment V in an effort to reproduce parakeratosis. This ration was also supplemented with various vitamins and lysine in different combinations to determine whether these nutrients were contributing factors in inducing the disease. Further it was thought that removing these parakeratotic animals, obtained from the Department of Animal Husbandry, out of the sunlight might possibly have aided in the recovery of the condition. An experiment was designed (Experiment VI) to study the effect of sunlight on the occurrence of parakeratosis. For this particular experiment two rations were used. One ration was the same pelleted ration fed by the Department of Animal Husbandry. The other ration was a more desirable, better balanced ration.

The nutritional stress factors employed in these studies using a total of ninety-seven pigs failed to cause the parakeratotic syndrome as recorded in the literature. Histological studies made on the experimental animals revealed in about 50 percent of the cases a microscopic nucleation of the strateum corneum. The importance of this finding in relation to the parakeratotic syndrome is not known. The presence of this histological finding in the absence of clinical symptoms of parakeratosis has not been recorded in the literature to the author's knowledge.

It is suggested that factors other than a high calcium and low zinc diet are involved in the etiology of parakeratosis. The data of this thesis fail to agree with existing reports on this condition.