THE PANTOTHENIC ACID REQUIREMENT OF THE BABY PIG

By

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INTRODUCTION

A great many of the discoveries in animal nutrition can be traced to the experimental use of purified diets. However, with larger animals such as the weanling pig the use of this type of diet is extremely expensive and only a very limited number of animals can be used. Many of the disadvantages encountered in the use of purified diets with older pigs can be eliminated through the use of suckling pigs. The diet used in the latter case is actually a liquid purified diet commonly called a synthetic milk, and merely consists of the addition of water to the purified constituents of the diet followed by homogenization to a liquid with many of the characteristics of milk.

Work with baby pigs and the use of synthetic milk diets has been reported as early as 1939 (Wintrobe). Since that time other research workers have carried out various nutrition studies with the greater part of the work being done in the past three or four years. The introduction and commercial production of sow's milk replacement diets for baby pigs within the past year has stimulated an increased interest in this subject.

The use of synthetic milk diets in raising baby pigs permits a detailed study of the physiology and nutritional requirements of pre-weanling pigs. The fact that the commercial use of sow's milk replacers will probably become more extensive within the next few years necessitates more research into the quantitative requirements of the baby pig for various nutrients.

The importance of correct vitamin supplementation in pig rations has been shown during the last ten to fifteen years by various research workers. The work done in the last four to five years has shown the need of adequate amounts of B vitamins in the ration, particularly niacin and pantothenic acid. Luecke et al. (1949) produced pantothenic acid deficiencies experimentally on diets of natural feedstuffs. Additional evidence by these workers has shown the importance of pantothenic acid supplementation to corn-soybean oil meal diets fed to weanling pigs for optimum growth. Since pantothenic acid has been shown to be so essential for weanling pigs, its importance for baby pigs fed synthetic milk diets should not be overlooked.

Work by Wiese et al. (1951) has demonstrated pantothenic acid deficiency in baby pigs fed a synthetic milk diet. No attempt however, was made to estimate the quantitative requirement of pantothenic acid for the baby pig.

It is a well known fact that a large proportion of the number of cases veterinarians are called upon to treat are due to nutritional deficiencies or imbalances. However, few veterinarians actually know and recognize all of the disorders of a nutritional nature, especially in baby pigs.

The importance of pantothenic acid has already been mentioned and if deficiency symptoms for baby pigs can be established, it will be of importance not only to the swine industry, but to the veterinary profession.

With these two ideas in mind, i.e.

1. To establish the requirement of baby pigs for pantothenic acid, and
2. To elucidate the little information available on pantothenic acid deficiency in baby pigs,

the work presented in this thesis was done during the winter and spring of 1952. All of the work was done at Michigan State College through the cooperation of the Animal Husbandry, Agricultural Chemistry, and Animal Pathology Departments.

REVIEW OF LITERATURE

R. J. Williams and his associates (1933) first extracted 'pantothenic acid' from very diverse tissues. The extracts stimulated in a very striking way the growth of Gebrüde Meyer yeast. The name 'pantothenic acid' is derived from the Greek words meaning 'from everywhere'. Other research workers gave different names to compounds which eventually were declared similar to each other and identical with pantothenic acid -- the name used in all literature today.

Norris et al. (1930) reported the occurrence of a pellagra like syndrome in chicks. This stimulated further research and workers using purified casein or heated diets of natural foodstuffs produced a pellagra-like condition or dermatitis and through the name developed "chick anti-dermatitic factor" and "chick anti-pellagra factor".

Work by Rohrman et al. (1934) in determining the pantothenic acid content of animal tissues based on yeast growth, found the liver to be the richest tissue in pantothenic acid. Elvehjem et al. (1935) stated that flavins proved inactive in the prevention of chick pellagra and, liver extract from which the flavins had been removed was active in the prevention of pellagra. Later work, using

liver extracts, eventually gave rise to the name 'filtrate factor' for pantothenic acid. However, considerable discussion arose as to whether the filtrate contained only pantothenic acid, and work by Oleson et al. (1939) and Woolley (1940) postulated the multiple nature of the 'filtrate factor'.

Other names given to pantothenic acid at one time or another and eventually discarded were Factor II, Vitamin B_2 or G, and the anti-chromotrichia factor. The name anti-chromotrichia factor resulted from work with rats. However, various research workers disagreed about pantothenic acid being the sole causative factor. Ansbacher (1941) established para-aminobenzoic acid as one of the factors, while other research workers since have concluded other factors and interrelationships to be involved.

It was not until 1938 that R. J. Williams and his associates determined the chemical nature of pantothenic acid. The chemical name given to pantothenic acid is (+)-2, 4-dihydroxy-3, 3-dimethylbutyryl-beta-alanine. It is predominantly of acid character but shows also some basic properties. The vitamin is readily soluble in water and is sensitive toward acids, bases, and heat.

The part that pantothenic acid plays in the metabolism of animals has only recently been established. Work by

Lipmann et al. (1947) showed pantothenic acid to be a constituent of coenzyme A. As such it is involved in acetylation processes. Decreased acetylation of aromatic amines in the pantothenic acid deficient rat have been reported in 1948 by Riggs et al. and in 1949 by Shils et al.

Today pantothenic acid is commercially available in the form of its crystalline synthetic calcium or sodium salt. In the work presented in this thesis, the crystalline synthetic calcium salt was used.

Good natural sources of pantothenic acid are brewer's dried yeast, dried buttermilk, dry whey, dry skim milk, peanut meal, cane molasses, alfalfa, liver meal, and fermentation residues.

As mentioned, the early work with pantothenic acid and its physiological activity was done with rats and chicks. However within the past ten years more work and emphasis has been placed on the role of pantothenic acid in the nutrition of the pig.

No attempt will be made to give a complete summary of all the research in species other than swine. Instead, it will be treated in a more general way in an attempt to make comparisons where similarities or differences are quite obvious.

In 1916, Wehrbein reported on an undiagnosed paralysis in pigs which lasted 2 - 4 months. The pigs had good appetites but anemia developed and emaciation became more and more apparent. He concluded that it was not an infectious disease, and that certain strains of pigs were more susceptible than others. Doyle (1937) reported on a paralysis he believed due to an infectious form of disease. The "disease" however was sporadic and had been observed in suckling pigs. He noted inflammatory changes of the large nerve trunks from the hind legs of cases characterized by spastic or "springhalt" symptoms.

The fact that both of these research workers were probably reporting on much the same condition, "goose-stepping" as a result of pantothenic acid deficiency in pigs should not be overlooked. However at that time not as much was known about nutrition and deficiency symptoms as today. Chick (1938) reported on pantothenic acid deficiencies in pigs and stated that the symptom corresponded closely to the description given by Wehrbein (1916). The pigs were fed a purified basal diet deficient in pantothenic acid and developed a flaccid palsy in the hind quarters.

Hogan (1940) reported on observations made on baby pigs, which exhibited leg weaknesses such as goose-stepping,

weaving gait, and sickle hocks, as well as other abnormalities probably attributable to vitamin deficiencies. He concluded that the rations commonly used for brood sows were deficient in some factor or factors.

Wintrobe (1940) published work on the relation of diet to the occurrence of ataxia and degeneration in the nervous system of pigs. Forty-four pigs averaging three weeks of age were raised on a basal diet containing casein, sugar, lard, a mineral mixture, cod liver oil, ascorbic acid and varying amounts of yeast. If the yeast content was reduced to a low level or omitted entirely and thiamine, riboflavin, and nicotinic acid added to the diet, a disturbed gait and extensive lesions of the nervous system developed. Wintrobe made an intensive study of the nerve damage that was attributed to pantothenic acid deficiency.

Lesions were noted in the peripheral nerves and in the spinal cord. In the nerves there was irregularity and swelling of the myelin sheath in isolated areas. Fragments of myelin in the form of small and large clumps were present. Many droplets of free fat could be seen in these areas side by side with clumps of degenerating myelin. It was noted that degeneration was more advanced in the sciatic nerves than in the brachial nerves. In many cases no lesions were

seen in the latter, while early changes appeared in the former. Degeneration of the myelin sheaths in the spinal ganglia and proliferation of sheath cell degeneration in posterior roots and dorsal funiculi of the spinal cord was noted.

Hughes (1942) reported on pantothenic acid deficiency in swine, on pigs started at 35 to 43 pounds and carried to final weights of 65 to 95 pounds. A purified ration containing fifteen per cent casein was used in the trial. Symptoms of a pantothenic acid deficiency were an early decrease in appetite and slow growth as well as an inability to move about in a normal manner within about one month after being placed on a deficient diet. The deficient pigs apparently lost their sense of equilibrium and coordination for they goose-stepped and often fell. After about 70 days, two of the deficient pigs lost most of their hair and had diarrhea which was somewhat bloody. Autopsies of the deficient pigs showed gastritis which included a reddened area on the floor of the stomach about the size of a normal hand. Scattered throughout this area were haemorrhagic spots ranging in size from one to two millimeters. Some inflammation of the large intestine occurred and in one pig many abscesses were present. It took the pigs on experiment 6 to 10 weeks to gain thirty to fifty pounds respectively.

Hughes (1942) also published a paper establishing the minimum requirement of pantothenic acid for the growing pig to be between 7.8 and 11.8 milligrams per hundred pounds live weight. The same basal diet was used as before - 81% sugar, 15% purified casein, salt mix 4% plus the required vitamins. The pantothenic acid deficient pigs exhibited the same symptoms as mentioned in his previous work. Extreme goose-stepping and rhythmic kicking with first one hind leg and then the other were symptoms observed.

Following up earlier work (1941), research by Ellis et al. (1943) showed that the addition of calcium pantothenate to a heated diet greatly reduced the incidence and severity of locomotion incoordination, but the further addition of pyridoxine appeared necessary for the full prevention.

Wintrobe (1943) made another intensive study of pantothenic acid deficiency in swine with more reference to symptoms other than the neurological ones that he reported in 1940. His report was more detailed than Hughes (1942) but the gross symptoms were much the same in each case. The pigs used were started at 3 to 5 weeks of age. Deficiency symptoms developed within eleven days. Diarrhea, a loss of appetite, rough hair coats, abnormal gait, and a failure to gain weight, were observed. The

pantothenic acid deficient pigs gained 20 to 92 grams per day and in one case a loss of weight occurred. The controls gained 433 grams per day. The impairment of growth was far more severe than was obtained in deficiencies of B_1, B_2, nicotinic acid or B_6. The onset of the gait abnormality took 32 to 52 days in pigs started at 3 weeks of age and 93 to 107 days for pigs started at 5 weeks of age. Diarrhea began about 32 to 52 days after the start of the experiment. A patchy alopecia developed over the rump within three weeks after the start of the experiment. The bowel was congested and edematous. In the colon, injury to cells lining the glands was observed. The mucus vacuoles had disappeared and the cells became atrophic. Wintrobe stated that the absence of mucous secretion is due to the lack of some enzyme or enzymes normally secreted by the colonic epithelium; thus the mucous vacuoles disappeared. He attributed the poor growth to diarrhea and loss of appetite, although a specific effect as well was possibly involved. The diarrhea which developed soon became constant and sometimes considerable mucous was present. In many instances a bloody discharge appeared.

Wintrobe noted that in the very pronounced pantothenic acid deficiency, a cough developed in many pigs and a watery discharge from the nose. Patchy areas of reddening appeared

at the margin of the tongue surrounded by a serrated border of white. Wintrobe observed as in his earlier work the degeneration of sensory neurons. A moderate normocytic anemia was observed in thirteen out of eighteen pigs. A fall in serum chlorides was observed and sometimes hypoglycemia developed. Treatment with pantothenic acid alleviated the anemia. Administration of five hundred or more milligrams of pantothenic acid per killogram by body weight was accompanied by cessation of diarrhea. Gradual improvement in the condition of the bowel, growth of hair and gain in weight occurred. The abnormal gait improved but complete restoration of function did not occur.

Wintrobe noted no changes in the adrenal glands in his report. Other research with swine has not shown this either. Work with pantothenic acid deficient rats by Daft et al. (1940) showed adrenal haemorrhage and necrosis in almost one hundred per cent of the animals. Later work by Mills et al. (1940) and Salmon et al. (1940) confirmed this report. Suppler et al. (1942) suggested an alteration in secretion of adrenal cortical hormone in the case of a pantothenic acid deficient rat. Work by Winters et al. (1952) tends to confirm their hypothesis. They observed a marked depression of adrenal cholesterol in the deficient rats. The reduction of cholesterol content in the adrenal

glands may represent a decreased synthesis rather than an increased utilization of this steroid for conversion to hormone. It may be that coenzyme A, the functional form of pantothenic acid in metabolism, is a necessary part of the enzymatic complement of the adrenal cortex which synthesizes cholesterol.

The work by Chick (1938), Hughes (1942), and Wintrobe (1940, 1943), with pantothenic acid deficient pigs was with purified diets rather than natural feedstuffs, and this work has been stressed in some detail to give an accurate picture of the pantothenic acid deficiency symptoms and its implications. With pigs fed natural feedstuffs, deficiencies appearing may not be as severe or extensive, but where nutritional abnormalities occur one must know all of the possibilities so that accurate diagnosis can be made. Some work has been mentioned however where natural diets were used and symptoms of pantothenic acid deficiency appeared. Hanson (1943) mentioned various degrees of unthriftiness and locomotor incoordination of the rear limbs in pigs fed rations including corn, tankage, and soybean oil meal. The addition of various dried brewer's yeasts to the ration resulted in marked improvement of appetite, rate of gain, and some improvement of coordination. Ellis (1943), previously mentioned, stated that the rather

frequent occurrence of locomotor incoordination in growing swine fed on normal diets of corn with supplements appears to be due to the borderline level of pantothenic acid present in relation to the requirements. Various nutrition reviews since 1943 have reiterated the same idea and stressed the importance of correct B-vitamin supplementation of pig rations.

Work by McMillen et al. (1949) showed that the addition of pantothenic acid, nicotinic acid and riboflavin to a ration of corn, oats, soybean oil meal, meat scraps, alfalfa meal, and complex mineral mixture, gave significant increases in daily gains and reduced feed consumption per unit of gain by 22 to 25 per cent. Work by Luecke et al. (1949, 1950) with pantothenic acid and diets of natural feedstuffs is the latest work reported. A basal ration of corn, casein, soybean oil meal, and minerals did not contain enough pantothenic acid to prevent symptoms of locomotor incoordination and myelin degeneration from appearing. Symptoms of incoordination did not appear until the seventh week of the experiment. In the second trial a pantothenic acid deficiency occurred on low protein corn-soybean meal ration. The deficiency symptoms noted were most severe when thiamine, riboflavin, nicotinic acid, and pyridoxine were added to the basal ration. No incoordination was observed when

the unsupplemented corn-soybean ration was fed, but growth was very poor. It was concluded that the addition of nicotinic acid and riboflavin stimulated growth to such an extent that the levels of pantothenic acid in the ration were insufficient to prevent deficiency. It was noted that two pigs in the pantothenic acid deficient lot were completely paralyzed in the hind quarters, which is quite similar to the symptoms described in the early work by Wehrbein (1916) and Doyle (1937).

Sharma (1952) reported on the pathology of the intestine and other organs of weanling pigs when fed a ration of natural feedstuffs low in pantothenic acid. The large intestine first showed degenerative changes and a few ecchymotic haemorrhages and, in the later stages small superficial discrete ulcers. The columnar epithelium showed degenerative changes and there was a marked hyperemia of the lamina propria. The cellular reaction was mainly lymphocytic. The crypts of Lieberkuhm showed cystic dilations and hyperplasia of the lymph nodules.

All of the work mentioned has been carried out with weanling pigs, but since only one paper has been published on the pantothenic acid deficiency in baby pigs, it has been necessary to present the material to serve as a basis for comparison with work presented in this thesis.

Wiese et al. (1951) reported on pantothenic acid deficiency in baby pigs fed a synthetic milk diet. The symptoms reported were poor growth, loss of appetite, scours, coughing, loss of sucking reflex, a dark brown exudate around the eye, spastic gait, goose-steeping, alopecia, and low urinary excretion of pantothenic acid. In general, these symptoms agree with those observed in older pigs. However, post mortem examination failed to reveal any internal gross lesions. The deficient animals had little subcutaneous fat and the internal fat was lacking. The pigs used in the trials reported were started at two days of age and at fifty-six days the controls weighed 32 to 46 pounds.

It is well known that many interrelationships exist between the various nutrients. Work with pantothenic acid deficiency in rats by Nelson and Evans (1945), and Nelson et al. (1947) showed that high protein diets exerted a sparing effect on the pantothenic acid requirement. Work by Luecke et al. (1952) seems to indicate a similar effect in pigs started at 21 pounds.

Pathologists have made exhaustive studies of various vitamin deficiency states. With pigs deficient in pantothenic acid, workers have noted damage to the gut, consisting of atrophy and formation of ulcers. Berg et al.

(1949) working with pantothenic acid deficient rats observed duodenal ulcers in sixty per cent of the cases. It is suggested that pantothenic acid deficiency may be a contributing factor to duodenal ulcers in humans.

Ludovici et al. (1949) observed that in pantothenic deficient rats antibody response is slower and that thymus weights were lower. Later work by the same investigators (1951) showed that D-L-methionine has a significant sparing action upon the pantothenic acid requirement for antibody production.

Although none of the aforementioned research by Berg et al. (1949) and Ludovici et al. (1949, 1951) was connected directly with swine, more light is shed on the metabolism of pantothenic acid, and can be used to partially explain or at least for an initial theory upon some phenomena in pantothenic acid deficient pigs.

SYNTHETIC MILK

The development and use of synthetic milk for pigs is not new. At various times during the past twelve to fifteen years, papers have been published on synthetic milk diets for baby pigs.

In 1939, Wintrobe reported the use of a synthetic milk diet fed to young pigs two to three days of age. Final

weights of the pigs at eight weeks were poor and ranged from seven to eight kilograms.

McRoberts et al. (1944), Anderson et al. (1947) were other early research workers and reported fair to good growth on a milk made up largely of casein, sucrose, and lard. Green et al. (1947) artificially reared baby pigs and made some general observations on their habits. Bustad et al. (1947) reported no success in attempts to raise baby pigs from birth without colostrum milk.

Lehrer et al. (1949) raised forty-eight hour old pigs successfully on a special milk diet of casein, cerelose, lard, plus salt mixture and adequate vitamin supplementation. Since 1949, as much research has been published on the subject as in the previous ten years. In the future, more baby pig research will be done using synthetic milk diets as it permits a detailed study of the nutritional requirements and diseases of pre-weanling pigs. A more accurate estimate of genetic influences on the pigs ability to grow may be possible as maternal influences are reduced.

In a practical sense, the pigs can be raised free of parasites and be larger and more vigorous at eight weeks.

EXPERIMENTAL PROCEDURE

Three experimental trials were carried out. Experiments I and III were attempts to establish the requirement of pantothenic acid for baby pigs. In experiment II the protein-pantothenic acid interrelationship with baby pigs was investigated.

Duroc Jersey pigs, seventy-two hours old, were used in all cases. The pig's dams were fed the same basal diet. In experiments I and II, one litter was used in each case; in experiment III, pigs were chosen from two litters for vigor and uniformity. All pigs were lotted as fairly as possible according to weight, sex, and litter.

The pigs were raised in converted metabolism cages, equipped with a removable trough for easy cleaning. Originally the pigs were to be self-fed the synthetic milk diet by means of an overhead bottle, drained by a rubber tube fitting over a metal tube which ran into the trough. Such a system allowed too much milk in the trough for the pigs to drink at once, and souring occurred. To overcome this difficulty, the pigs were fed at approximately five hour intervals four times a day, according to their appetite at 8:00 A.M., 1:00 P.M., 6:00 P.M., and 11:00 P.M.

The baby pigs were starved for the first eight to ten hours of the experimental period, after which they readily learned to drink by dipping their noses into some warm milk placed in the trough. For the first two to three days, the milk was warmed slightly to prevent any digestive upsets.

The cages had removable screens and dropping pans to facilitate the separation of urine and feces. Heat lamps were provided for the first week, while room temperature was thermostatically controlled at 65° to 70° F.

The basal synthetic milk diet had the following composition:

Vitamin-free Casein - 30%
Cerelose - 37.4%
Lard - 26.6%
Salts - 6.0%
Added Vitamins

In experiment II, various levels of casein were used, with cerelose making up the difference when the casein level was lowered.

The salt mixture was a modified mixture of Phillips and Hart (1935):

NaCl	-	118.8 grams
K_2HPO_4	-	257.6 "
$CaHPO_4$	-	338.0 "
Ca Lactate	-	228.8 "
$MgSO_4.7H_2O$	-	35.4 "
$FeSO_4.7H_2O$	-	19.3 "
KI	-	.6 "
$MnSO_4.H_2O$	-	1.0 "
$ZnCl_2$	-	.2 "
$CuSO_4.5H_2O$	-	.2 "
$CoCl_2.6H_2O$	-	.1 "
		1,000.0 grams

All of the ingredients in the salt mixture were weighed on a gram balance and then mixed by hand. The salt mix was then placed in a ball mixer for twenty minutes in order to grind the coarser particles to a very fine powder so that the mix would go into solution easier, and pass through the homogenizer with less difficulty.

The milk was prepared in ten gallon milk cans. 29.5 liters of cold tap water was placed in each can and heated to 70° - 75° C. The casein was dissolved according to the method of Bird et al. (1935). Sodium bicarbonate equal to 4.75% of the weight of the casein was added to the heated water and then the casein was added slowly. The mixture was stirred constantly by use of an electric stirrer. When the casein was in suspension, the cerelose was added. This was followed by the addition of the lard containing the fat soluble vitamins A, D, E, and K. Portions of the salt mixture were placed in a Waring blender and then some of the hot solution from the milk cans was added and mixed with the salts to cause a suspension which was then added to the milk mixture in the cans. The final mixture was homogenized at approximately 3,000 pounds pressure. The B-vitamin solution previously made was added at the required levels after homogenization of the milk mixture. A separate calcium pantothenate solution was also added after homogenization at the required levels.

The vitamins added and the concentrations were as follows:

Vitamins	Mg. Per Kg. Milk
Thiamine	0.65
Riboflavin	0.65
Nicotinic Acid	2.50
Inositol	26.00
Choline	260.00
p-Aminobenzoic Acid	2.60
Pteroylglutamic Acid	0.052
Biotin	0.01
Pyridoxine	0.65
Alpha-tocopherol	1.00
2-methyl-1, 4-naphthaquinone	0.28
Vitamin A	2,000 I.U./kg.
Vitamin D	200 I.U./kg.

A total of 5400 grams of solids made up of casein, cerelose, lard, and salt mix, was added to each milk can. Thus each can contained approximately 35 kilograms of milk.

The B-vitamins were weighed on a beam balance and put into a twenty per cent alcohol solution. It was necessary to grind the vitamins inositol and p-Aminobenzoic Acid to a fine powder using a mortar and pestle before adding to the solution. A fortified cod liver oil containing known amounts of vitamins A and D was used. Vitamins E and K, required in much smaller amounts, were added to it. Vitamin K (Menadione, Merck) in the powder form, was added to some heated cod liver oil to facilitate its going into solution.

The lot treatments for experiments I, II and III were as follows:

Experiment I

Lot 1 - Basal (negative control)

Lot 2 - Basal + calcium pantothenate at 1 mg./100 gm. solids

Lot 3 - Basal + calcium pantothenate at 2 mg./100 gm. solids

Experiment II

Lot 1 - Low protein basal + calcium pantothenate at 1 mg./100 gm. solids

Lot 2 - Low protein basal + calcium pantothenate at 2 mg./100 gm. solids

Lot 3 - Low protein basal + calcium pantothenate at 3 mg./100 gm. solids

Lot 4 - High protein basal + calcium pantothenate at 3 mg./100 gm. solids

At the start of Experiment II the low protein level was 13.5 per cent. This was raised to 18 per cent after twelve days on trial. (The high protein level was the basal diet containing thirty per cent vitamin free casein.)

Experiment III

Lot 1 - Basal (negative control)

Lot 2 - Basal + calcium pantothenate at 1 mg./100 gm. solids

Lot 3 - Basal + calcium pantothenate at 1.5 mg./100 gm. solids

Lot 4 - Basal + calcium pantothenate at 2 mg./100 gm. solids

Lot 5 - Basal + calcium pantothenate at 2.5 mg./100 gm. solids

Experiments I and II were run for 20 days and experiment III for thirty-two days.

In all experiments the pigs were weighed at four day intervals.

Pantothenic acid assays were carried out on the prepared milk for all experiments. In experiment I, assays were also run on the blood and urine of the different lots.

The pantothenic acid assays were carried out microbiologically according to the method of Skeggs and Wright (1944). The samples were digested using the enzyme Mylase P as outlined by Buskirk et al. (1948).

Digestion Procedure for Synthetic Milk

Pipette 5 ml. of milk and 5 ml. of distilled water into a 50 ml. Erlenmeyer flask. Add .2 ml. of glacial acetic acid, 1 ml. of 1 N NaOH, and 1 gm. of mylase-P. Incubate 2 to 3 hours at 50° C. Dilute to 100 ml., and filter. Collect the filtrate in a 125 ml. Erlenmeyer flask and store in the refrigerator. Assay levels for milk - dilute 1 to 10 and use 1, 2, 3, ml. levels.

Preparation of the Standard Solution

The stock standard (50 ug./ml.) is diluted to give a solution containing 1 γ /ml. of calcium pantothenate. The 1 γ /ml. solution is then diluted 5 - 250 to give a concentration of .02 γ /ml.

γ/tube of Ca. Pantothenate	Mls. of Standard	Mls. of Water
.00	0.0	5.0
.01	0.5	4.5
.02	1.0	4.0
.03	1.5	3.5
.04	2.0	3.0
.06	3.0	2.0
.08	4.0	1.0
.10	5.0	0.0

Milk Samples

The digested milk samples are so diluted with distilled water that 1 ml. of the dilution will contain .01 - .03 γ/ml. of calcium pantothenate. Duplicate levels of 1, 2, and 3 ml. are set up for each sample, and the volume is made to 5 ml. with distilled water.

Basal Medium

Use double strength Difco medium. The basal medium for 20 tubes (100 ml.) consists of the following amounts of stock solution:

Casein hydrolysate 100 mg./ml.	10 ml.
l-cystine 4 mg./ml.	5 ml.
d-l-tryptophane 4 mg./ml.	10 ml.
Thiamine 200 ug./ml.	1 ml.
Riboflavin 100 ug./ml.	2 ml.
Nicotinic acid 100 ug./ml.	2 ml.
Pyridoxine 200 ug./ml.	2 ml.
Adenine, guanine, uracil 1 mg./ml.	1 ml.
Xanthine 1 mg./ml.	1 ml.
p-Aminobenzoic Acid 100 γ/ml.	.2 ml.
Biotin .5 γ/ml.	1 ml.
Salts A	1 ml.
Salts B	1 ml.
Glucose	4 gms.
Sodium Acetate (anhydrous)	4 gms.

The mixture is adjusted to pH 6.6 - 6.8, and diluted to 100 ml.

5 ml. of the media is added to each sample tube. Autoclave for 15 minutes at 15 pounds pressure at 250° F. Inoculate each tube with a saline suspension of Lactobacillus arabinosus. Incubate for 72 hours and titrate the acidity with 0.1 N NaOH. Refer the results to the graph prepared from the standard solution.

Blood Pantothenic Acid Assay

Weigh 1 gm. of mylase-P into a 50 ml. Erlenmeyer flask, add 10 ml. of the acetate buffer and 2 ml. of oxalated blood. Incubate for 3 hours at 50° C. Autoclave for 3 minutes to destroy the enzyme, cool, dilute to 50 ml. and filter.

In experiment I, blood samples were obtained at the conclusion of the experiment. These were obtained by use of a hypodermic needle inserted slightly ahead of the sternum of the pig to obtain the blood from the anterior venous sinus. The blood so collected was quickly transferred to tubes containing oxolate and agitated to prevent clot formation.

Urine Pantothenic Acid Assay

Since pantothenic acid exists in the free state in urine, no digestion is required. Proceed with the assay the same as for the synthetic milk.

In experiment I, the urine was collected over a twelve hour period at the conclusion of the trial. 5 cc. of toluene and 5 cc. of glacial acetic acid were placed in a gallon bottle, and placed beneath the collecting pan, which was part of the cage equipment.

Milk Pantothenic Acid Assay

Milk samples were collected after the pantothenic acid solution had been added and mixed with the milk. About 20-25 ml. of milk were placed in a 50 ml. Erlenmeyer flask from which the required volume of milk for assay was taken.

Pathological Studies

In order to study the pathology of pantothenic acid deficient baby pigs, all the pigs which received no pantothenic acid in experiments I and III, were autopsied. A total of five pigs were used for pathological studies, two from experiment I, and three from experiment III. All of the pigs used for pathological studies were approximately four weeks of age with the exception of one which was left on experiment until eight weeks of age for therapeutic and recovery studies. Sections of various tissues were removed and examined microscopically for lesions.

RESULTS

Each experiment will be treated separately to avoid confusion. The pathology of all the pantothenic acid deficient pigs will be discussed together rather than making separate mention in experiments I and III.

Experiment I

Table 1. Results of Experiment I[1]

Lot	Calcium Pantothenate Supplement in mg./100 gm. Solids	No. Pigs	Av. Initial Wt.	Av. Final Wt.	Av. Daily Gain	Dry Matter per lb. gain
			lbs.	lbs.	lbs.	lbs.
1	None[2]	2	3.08	8.69	0.28	1.25
2	1	3	3.49	11.55	0.42	0.97
3	2	3	3.16	15.66	0.61	0.94

[1]The level of protein in the synthetic milk was 27 per cent on the dry matter basis.

[2]The two pigs in lot 1 remained on the basal diet beyond the 20 day experimental period until more severe deficiency symptoms developed.

The pigs receiving no pantothenic acid were observed to develop a severe diarrhea within two weeks and a "goose-stepping" gait within three to four weeks. Some growth was made up to three weeks but after this the pigs usually lost weight. The pigs were unkempt in appearance and had a very impaired appetite. After thirty six days on experiment

one of the deficient pigs was injected for three alternate days with one hundred mg. of pantothenic acid and placed on milk containing 2 mg. of pantothenic acid per 100 grams of dry matter. At the time of the injection the pig was losing weight and was in an advanced state of pantothenic acid deficiency. After pantothenic acid therapy there was a marked and almost immediate improvement in appetite, appearance and rate of growth. Although there was some improvement in locomotion, recovery was not at all complete. The response of this pig indicated conclusively that pantothenic acid deficiency in the basal ration of this pig was responsible for his condition.

Figure 1 illustrates the average growth of the various lots. The three pigs receiving 2 mg. of pantothenic acid per 100 grams of dry matter gained significantly better than the three receiving 1 mg. of pantothenic acid per 100 grams of dry matter. Although none of the pigs receiving the lower level exhibited noticeable deficiency symptoms, one of them occasionally scoured. They consumed less milk than the lot at the higher level, but because of a much lower rate of gain they were slightly less efficient.

Pantothenic Acid Assay Results

The pantothenic acid assay results for the synthetic milk in most cases indicated that the pantothenic acid

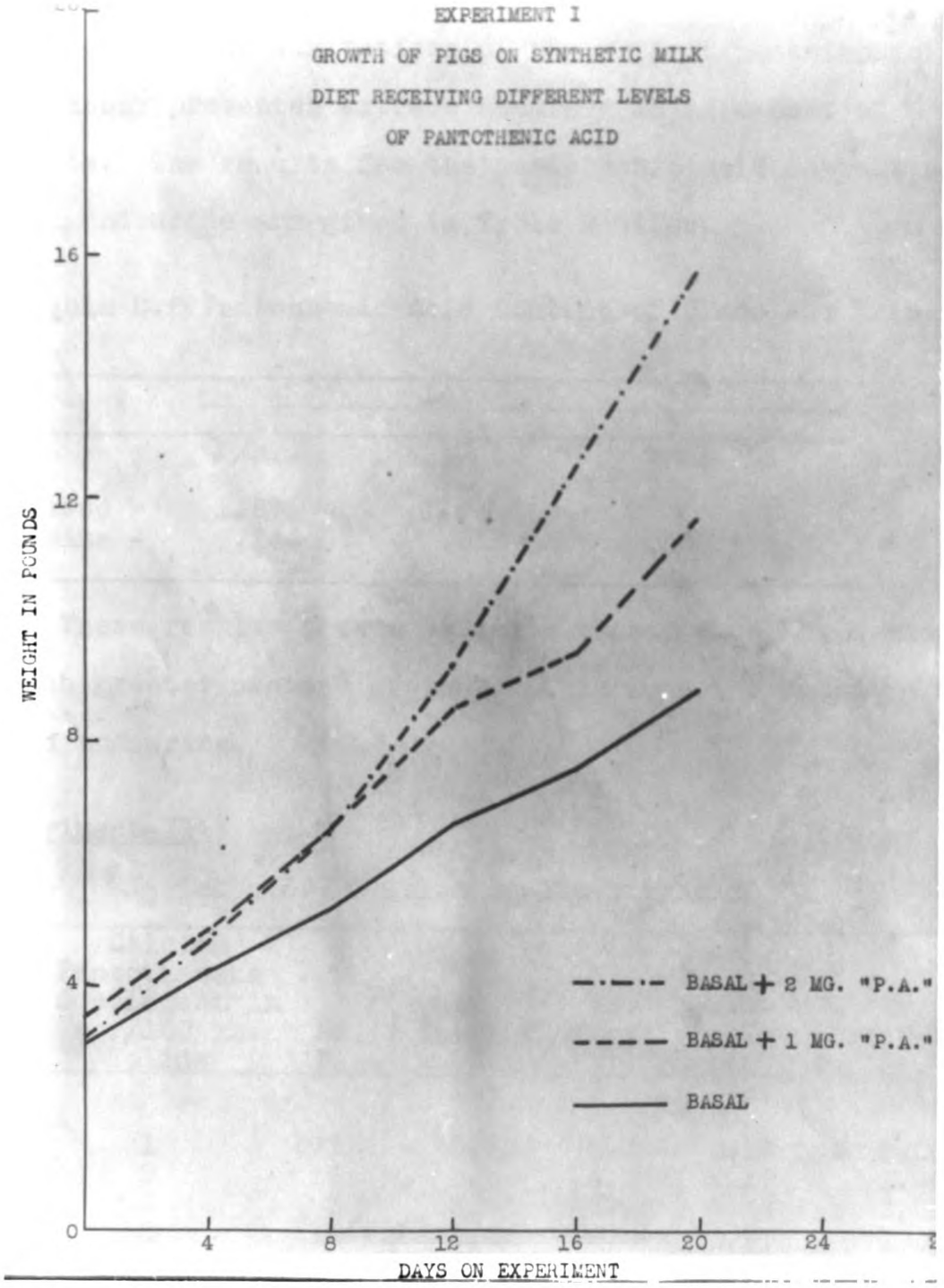
EXPERIMENT I
GROWTH OF PIGS ON SYNTHETIC MILK
DIET RECEIVING DIFFERENT LEVELS
OF PANTOTHENIC ACID
WEIGHT IN POUNDS
16
12
8
4
0
4
8
12
16
20
24
DAYS ON EXPERIMENT
BASAL + 2 MG. "P.A."
BASAL + 1 MG. "P.A."
BASAL

Figure 1

content of the milk to be close to the level of pantothenic acid added. The limitations of the present pantothenic acid assay prevented extreme accuracy in agreement of results. The results for the pantothenic acid content of blood and urine are given in Table 2 below.

Table 2. Pantothenic Acid Content of Blood and Urine

	Lots		
	1	2	3
	γ/ml.	γ/ml.	γ/ml.
Blood -	1.875	1.94	2.70
Urine -	.144	.218	.537

These results show a definite trend, with Lot 3 showing a much greater content of pantothenic acid (γ /ml.) in both blood and urine.

Experiment II

Table 3. Results of Experiment II[1]

Lot	Calcium Pantothenate Supplement in mg./100 gm. Solids	No. Pigs	Av. Initial Wt.	Av. Final Wt.	Av. Daily Gain	Dry Matter Per Lb. Gain
			lbs.	lbs.	lbs.	lbs.
1	1	2	3.38	6.56	0.16	2.08
2	2	2	3.18	6.63	0.17	1.90
3	3	3	2.82	6.19	0.16	1.59
4	3	2[2]	2.58	10.07	0.37	1.09

[1]Protein increased from 13.% to 18.0% on the 12th day in lots 1, 2 and 3.

[2]Originally the lot contained 3 pigs, however, 1 pig died suddenly after 1 day on trial so his initial weight was not included in the lot average. Post mortem examination showed lesions similar to a Vitamin E deficiency.

Recent work by Luecke et al. (1952) seems to indicate that the higher protein levels exert a sparing effect on the pantothenic acid requirement of the pig. Trial two, with baby pigs failed to reveal any significant differences between lots. Figure 2 illustrates the growth for the different lots in this experiment. Apparently, the protein level was too low for the first twelve days of the twenty day experimental period to meet the requirements for normal growth. A slight improvement in growth was noted when the protein content of the milk was increased after twelve days. However, all pigs in the three lots receiving the lower protein diet remained thin and grew poorly as contrasted to the two pigs receiving the higher level of protein and pantothenic acid. At the conclusion of the experiment, the two pigs in the high protein lot were much healthier and thriftier in appearance than the others and were growing much more rapidly.

Different protein levels and a longer experimental period would provide a better opportunity to study the protein-pantothenic acid interrelationship for baby pigs if such exists.

EXPERIMENT II

GROWTH OF PIGS ON SYNTHETIC MILK DIET RECEIVING DIFFERENT LEVELS OF PROTEIN AND PANTOTHENIC ACID

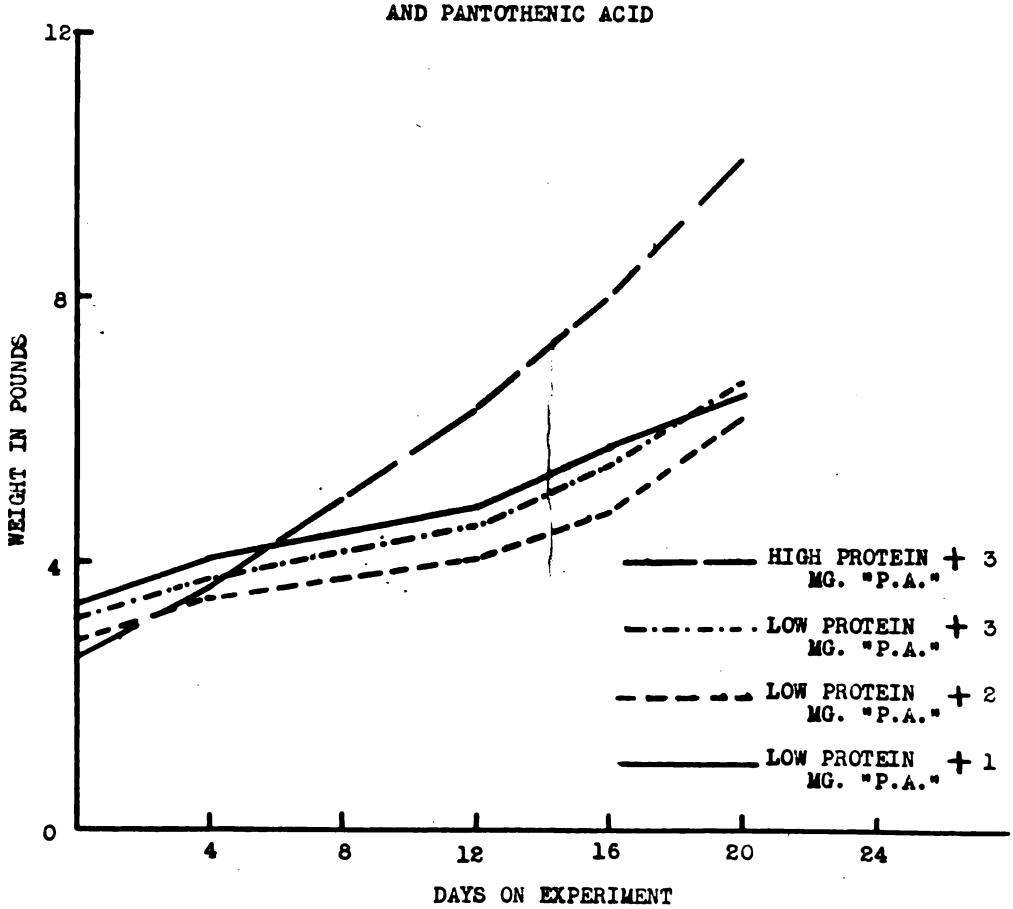

Figure 2

Experiment III

Table 4. Results of Experiment III

Lot	Calcium Pantothenate Supplement in mg./100 gm. Solids	No. Pigs	Av. Initial Wt. lbs.	Av. Final Wt. lbs.	Av. Daily Gain lbs.	Dry Matter Per Lb. Gain lbs.
1	0	3	3.19	8.95	0.17	2.12
2	1	3	3.27	17.29	0.44	1.17
3	1.5	3	3.19	15.42	0.38	1.19
4	2	3	3.17	17.61	0.45	1.12
5	2.5	3	3.19	19.31	0.50	1.09

All of the lots receiving pantothenic acid gained significantly better than the basal lot. However, the results of this experiment were rather inconsistent and navel infection which affected all lots to varying degrees within the first week of the trial contributed to part of this inconclusiveness. To overcome this navel infection all of the pigs were injected with penicillin. The pigs seemed to recover but in some cases growth response seemed slow, and never did reach the levels attained in experiment I. Part of this difference however, might have been attributed to genetic differences between litters and experiments.

All the deficient pigs became very thin, rough in appearance, and lost weight after three weeks on trial. They became progressively weaker and finally unable to

walk. One pig did exhibit a "goose-step". Like the deficient pigs in experiment I diarrhea developed in two to three weeks and persisted throughout the remainder of the trial.

Cultures of various tissues of all three deficient pigs were positive for bacteria. This factor could be one reason for the extreme weakness and resulting death of the three deficient pigs.

Figure 3 illustrates the growth for different lots in the experiment.

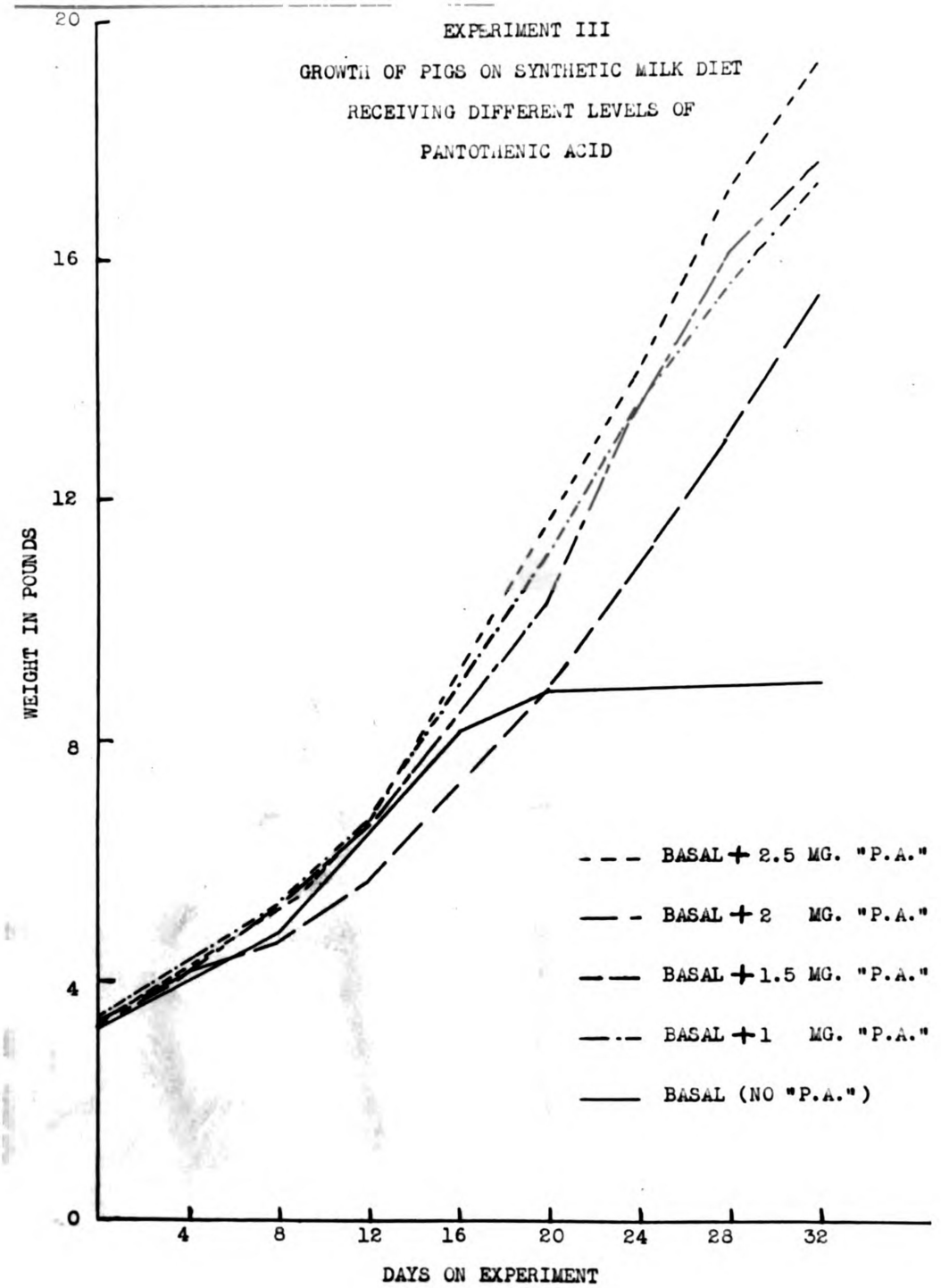
EXPERIMENT III
GROWTH OF PIGS ON SYNTHETIC MILK DIET
RECEIVING DIFFERENT LEVELS OF
PANTOTHENIC ACID
WEIGHT IN POUNDS
0
4
8
12
16
20
4
8
12
16
20
24
28
32
DAYS ON EXPERIMENT
BASAL + 2.5 MG. "P.A."
BASAL + 2 MG. "P.A."
BASAL + 1.5 MG. "P.A."
BASAL + 1 MG. "P.A."
BASAL (NO "P.A.")

Figure 3

LESIONS OF PANTOTHENIC ACID DEFICIENCY PRODUCED IN BABY PIGS

Gross Pathology

The characteristic gross pathology of pigs showing pantothenic acid deficiency was confined to the intestinal tract and particularly to the colon and cecum. The walls of these two organs were edematous and thickened. Frequent haemorrhage and congestion were also observed. The lumen of the colon was filled with a yellowish tenacious mucous, sometimes covering up a fibrinonecrotic membrane, and fecal material clung to the mucosa in the majority of cases. The mucosa was wrinkled, showed congestion and haemorrhages were prominent on the ridges. The wall of the duodenum and jejunum were thickened and edematous but were not congested or hemorrhagic like the colon or cecum.

Two of the deficient animals showed moderate dehydration and a rough scaly skin and hair coat. Fatty infiltration of the liver was not a prominent picture in these deficient pigs. In two cases an early bronchopneumonia was present. The kidneys were congested in a majority of the cases.

Microscopic Pathology

In the histopathologic studies, lesions were not confined entirely to the digestive system but were present in the nervous system as well. The characteristic lesions

in the nervous system were found in the sciatic and femoral nerves and in the dorsal root ganglia supplying those nerves.

In the dorsal root ganglion cells karyolysis, chromotolysis, vacuolar degeneration and lack of Nissl substance were found. The sciatic and femoral nerves were degenerated, fragmented and demyelinized. No microscopic alterations were observed in the spinal cord or brain.

As evident from the gross pathologic findings, extensive changes were observed in the intestinal tract and particularly in the large bowel. A diffuse hyperemia, haemorrhage and necrosis with ulceration were found. There was a mucoid degeneration and a ballooning of the glands in the mucosa. Many of these ballooned glands showed polymorphonuclear leucocytes with other inflammatory debris in the crypts. Abscesses in the solitary lymphoid follicles in the submucosa of the colon were also observed. There was an increase in connective tissue in the tunica propria and submucosa along with an infiltration of lymphocytes, neutrophils and macrophages. It was interesting to note that the plexuses of Auerbach in the colon and ileum manifested a swelling and vacuolar degeneration of the cell bodies. The lesion seemed to separate the longitudinal and circular layers of muscle.

The skin was slightly hyperkeratosed and the stratum mucosum was slightly atrophic with loss of a few intracellular bridges.

In one pig the zona glomerulosa of the adrenal gland was very thin and deficient in some areas. Haemorrhage was observed in the cortex of another adrenal gland. No consistent findings were observed.

The livers showed varying degrees of cloudy swelling and fatty infiltration but none were of a marked degree. The kidneys were involved to the same extent as the livers, showing cloudy swelling and fatty degeneration of the tubules. However, congestion and haemorrhage in the medulla were manifested in some sections. The epithelial cells of the visceral layer of Bowman's capsule were swollen, of the cuboidal type and were prominent at the periphery of the glomerular tuft.

Sections from the tongue revealed necrosis and an early inflammatory process around the circumvallate papillae.

CONCLUSIONS

The pantothenic acid requirement of the baby pig appears to be between 1.0 and 2.0 mg. per 100 grams of dry matter (4.5 to 9 mg./lb.). The National Research Council's recommendation for fifty pound pigs is 4.5 mg. per pound of feed. This being the case, it appears that the baby pig requirement is slightly higher.

Although other data (Luecke *et al.* 1952) seemed to indicate that a high protein level exerted a sparing effect on the pantothenic acid requirements of weanling pigs, the data presented in this paper failed to show any such interrelationship for baby pigs. However, the limitations of the trial carried out are discussed and suggestions made for a more exhaustive study of the problem.

Pantothenic acid deficiency of baby pigs was characterized by the development of a severe diarrhea within two weeks and locomotor incoordination within three to four weeks. At all times the pigs were rough in appearance, had poor appetites and usually failed to gain weight after three weeks of age.

The pathologic findings involving the intestinal tracts of pigs on a diet lacking pantothenic acid were in accordance with the findings of previous workers with

the possible exception of the vacuolar degeneration of the cells comprising the plexus of Auerbach. The lesion observed in these intrinsic plexuses might be associated with the diarrhea seen in pantothenic acid deficiency of swine. Further study is necessary to substantiate this. From earlier observations of intestinal tracts of older pigs on pantothenic acid deficiency, the enteritis present in these baby pigs seemed more severe in nature than that seen in the more mature animals.

In this study, the pantothenic acid deficient pigs showed loss of myelin, and degeneration of the dorsal root ganglion cells at three and one-half weeks of age. This length of time in which lesions developed is a considerably shorter period of time than is usually reported.

BIBLIOGRAPHY

1. Anderson, G. C., and A. G. Hogan. Adequacy of Synthetic Rations for Swine. Journal of Animal Science. 6 (1947), p. 482.

2. Ansbacher. para-Aminobenzoic acid. A Vitamin. Science. 93 (1941), p. 164.

3. Berg, B. N., T. F. Zucker, and L. M. Zucker. Duodenal Ulcers Produced on a Diet Deficient in Pantothenic Acid. Proceedings Society for Experimental Biology and Medicine. 71 (1949), pp. 324-326.

4. Bird, E. W., H. W. Sadler, and C. A. Iverson. The Preparation of a Non-Dessicated Sodium Caseinate Solution and the Use in Ice Cream. Iowa Agricultural Experiment Research Bulletin. 187 (1935), pp. 177-208.

5. Buskirk, H. H., A. M. Bergdahl, and R. A. Delor. Enzymatic Digestion of Samples for Microbiological Assay of Pantothenic Acid. Journal of Biological Chemistry. 172 (1948), p. 671.

6. Bustad, L. K., W. E. Ham, and T. J. Cunha. Preliminary Observations on Using a Synthetic Milk for Raising Pigs from Birth. Journal of Animal Science. 6 (1947), p. 484.

7. Chick H., T. F. Macrae, A. J. P. Martin and C. J. Martin, The Water Soluble Vitamins Other Than Aneurin (Vit. B_1), Riboflavin, and Nicotinic Acid Required by the Pig. Biochemical Journal. 32 (1938), pp. 2207-2224.

8. Daft, F. S., W. H. Sebrell, S. H. Babcock Jr., and T. H. Jukes. Effect of Synthetic Pantothenic Acid in Adrenal Hemorrhage Atrophy and Necrosis in Rats. Public Health Report. 55 (1940), pp. 1333-1337.

9. Doyle, L. P. Posterior Paralysis in Swine. Journal of the American Veterinary and Medical Association. 90 (1937), p. 656.

10. Ellis, N. R., L. L. Madsen and C. O. Miller. Pantothenic Acid and Pyridoxine as Factors in the Locomotor Incoordination in Swine. Journal of Animal Science. 2 (1943), p. 365.

11. Elvehjem, C. A., C. J. Koehn Jr. Studies on Vitamin B_2 (G). Journal of Biological Chemistry. 108 (1935), pp. 709-728.

12. Green, W. W., H. H. Burgeman and L. M. Winters. Artificial Rearing of Baby Pigs. Journal of Animal Science. 6 (1947), p. 146.

13. Hanson, L. E. Locomotor Incoordination in Pigs Fed Good Rations. Journal of Animal Science. 2 (1943), p. 375.

14. Hogan, A. G., S. R. Johnson. Inadequacy of the Concentrates Commonly Used in Swine Feeding. Missouri Agricultural Experiment Station Bulletin 321. (1940).

15. Hughes, E. H. Pantothenic Acid in Nutrition of the Pig. Journal of Agricultural Research. 64 (1942), pp. 185-187.

16. Hughes, E. H., N. R. Ittner. The Minimum Requirement of Pantothenic Acid for the Growing Pig. Journal of Animal Science. 1 (1942), p. 116.

17. Lehrer, W. P. Jr., P. R. Moore, A. C. Wiese, and O. F. Pahnish. A Synthetic Milk Ration for Baby Pigs. Journal of Animal Science. 8 (1949), p. 107.

18. Lipmann, F., N. O. Kaplan, G. D. Novelli, L. C. Tuttle, B. M. Guirard. Coenzyme for Acetylation, A Pantothenic Acid Derivative. Journal of Biological Chemistry. 167 (1947), p. 869.

19. Ludovici, P. P.,A. E. Axelrod, and B. B. Carter. Circulating Antibodies in Vitamin Deficiency States. The Pantothenic Acid Deficiency. Proceedings Society for Experimental Biology and Medicine. 72 (1949), pp. 81-83.

20. Ludovici, P. P.,A. E. Axelrod, and B. B. Carter. Circulating Antibodies in Vitamin Deficiency States. The Pantothenic Acid Sparing Action of D-L Methionine. Proceedings Society for Experimental Biology and Medicine. 76 (1951), pp. 670-672.

21. Luecke, R. W., F. Thorp Jr., W. N. McMillen, and H. M. Dunne. Pantothenic Acid Deficiency in Pigs Fed Diets of Natural Feedstuffs. Journal of Animal Science. 8 (1949), pp. 464-469.

22. Luecke, R. W., W. N. McMillen and F. Thorp Jr. Further Studies of Pantothenic Acid Deficiency in Weanling Pigs. Journal of Animal Science. 9 (1950), pp. 78-82.

23. Luecke, R. W., J. A. Hoefer and F. Thorp Jr. The Relationship of Protein to Pantothenic Acid and Vitamin B_{12} in the Growing Pig. Journal of Animal Science. 11 (1952), pp. 238-243.

24. McMillen, W. N., R. W. Luecke, and F. Thorp Jr. The Effect of Liberal B-Vitamin Supplementation on Growth of Weanling Pigs Fed Rations Containing a Variety of Feedstuffs. Journal of Animal Science. 8 (1949), pp. 518-523.

25. McRoberts, V. F., and A. G. Hogan. Adequacy of Simplified Diets for the Pig. Journal of Nutrition. 28 (1944), p. 165.

26. Mills, R. C., J. H. Shaw, C. H. Elvehjem and P. H. Phillips. Curative Effect of Pantothenic Acid on Adrenal Necrosis. Proceedings Society for Experimental Biology and Medicine. 45 (1940), pp. 482-484.

27. Nelson, M. M., H. M. Evans. Pantothenic Acid Deficiency and Reproduction in the Rat. Journal of Nutrition. 31 (1946), pp. 497-508.

28. Nelson, M. M., F. V. Nouhuys, and H. M. Evans. The Sparing Action of Protein on the Pantothenic Acid Requirement of the Rat. Journal of Nutrition. 34 (1947), p. 189.

29. Norris, L. C., A. T. Ringrose. Occurrence of Pellagra-like Syndrome in Chicks. Science. 71 (1930), p. 643.

30. Oleson, J. J., D. W. Woolley, and C. H. Elvehjem. Is Pantothenic Acid Essential for the Growth of Rats. Proceedings Society for Experimental Biology and Medicine. 42 (1939), pp. 151-153.

31. Phillips, P. H., and E. B. Hart. The Effect of Organic Dietary Constituents upon Chronic Fluorine Toxicosis in the Rat. Journal of Biological Chemistry. 109 (1935), p. 657.

32. Riggs, T. R. and D. M. Hegsted. The Effect of Pantothenic Acid Deficiency on Acetylation in Rats. Journal of Biological Chemistry. 122 (1948), p. 539.

33. Rohrman, E., G. E. Burget, and R. J. Williams. Pantothenic Acid Content of Animal Tissues. Proceedings Society for Experimental Biology and Medicine. 32 (1934), pp. 473-474.

34. Salmon, W. D., R. W. Engel. Pantothenic Acid in Hemorrhagic Adrenal Necrosis in Rats. Proceedings Society for Experimental Biology and Medicine. 45 (1940), pp. 621-623.

35. Sharma, G. L., R. L. Johnston, R. W. Luecke, J. A. Hoefer, M. L. Gray, Frank Thorp, Jr. A Study of the Pathology of the Intestine and Other Organs of Weanling Pigs When Fed a Ration of Natural Feedstuffs Low in Pantothenic Acid. American Journal of Veterinary Research. 13 (1952), pp. 298-305.

36. Shils, M. E., H. M. Seligman, and L. J. Goldwater. Pantothenic Acid and Conjugation Reactions in Vivo. Journal of Nutrition. 37 (1949), p. 227.

37. Skeggs, H. R. and D. L. Wright. The Use of Lactobacillus arabinosus in the Microbiological Determination of Pantothenic Acid. Journal of Biological Chemistry. 156 (1944), p. 21.

38. Supplee, G. C., R. C. Bender, O. J. Kohlenberg. Interrelated Vitamin Requirements. Kidney Damage, Adrenal Haemorrhage and Cardiac Failure Correlated with Inadequacy of Pantothenic Acid. Endocrinology. 30 (1942), pp. 355-364.

39. Wehrbein, H. Paralysis of Pigs. Journal of the American Veterinary and Medical Association. 49 (1916), p. 238.

40. Wiese, A. C., W. P. Lehrer Jr., P. R. Moore, O. F. Pahrish and W. V. Hartwell. Pantothenic Acid Deficiency in Baby Pigs. Journal of Animal Science. 10 (1951), p. 80.

41. Winters, R. W., R. B. Schultz, W. A. Krehl. Studies on Adrenal Cortex of Pantothenic Acid Deficient Rats. 4. Adrenal and Serum Cholesterol Levels. Proceedings Society for Experimental Biology and Medicine. 79 (1952), pp. 695-696.

42. Wintrobe, M. M. Nutritive Requirements of Young Pigs. American Journal of Physiology. 126 (1939), p. 375.

43. Wintrobe, M. M., J. L. Miller Jr., and H. Lesio. The Relation of Diet to the Occurrence of Ataxia and Degeneration in the Nervous System of Pigs. John Hopkins Hospital Bulletin. 67 (1940), pp. 377-404.

43. Wintrobe, M. M. Pantothenic Acid Deficiency in Swine. John Hopkins Hospital Bulletin. 73 (1943), p. 313.

44. Williams, R. J., C. M. Lysman, G. H. Goodyear, J. H. Truesdail, and D. Holaday. Pantothenic Acid - A Growth Determinant of Universal Biological Occurrence. Journal of American Chemical Society. 55 (1933), pp. 2912-2927.

45. Williams, R. J., H. H. Weinstock, Jr., E. Rohrmann, J. H. Truesdail, H. H. Mitchell, and C. E. Meyer. Pantothenic Acid-III Analysis and Determination of Constituent Groups. Journal of American Chemical Society. 61 (1939), pp. 454-457.

46. Woolley, D. W. Observations on the Multiple Nature of the Rat Filtrate Factor. Proceedings Society for Experimental Biology and Medicine. 43 (1940), pp. 352-354.

APPENDIX

Plate I - Metabolism Cage Used in Baby Pig Experiments.

Plate I

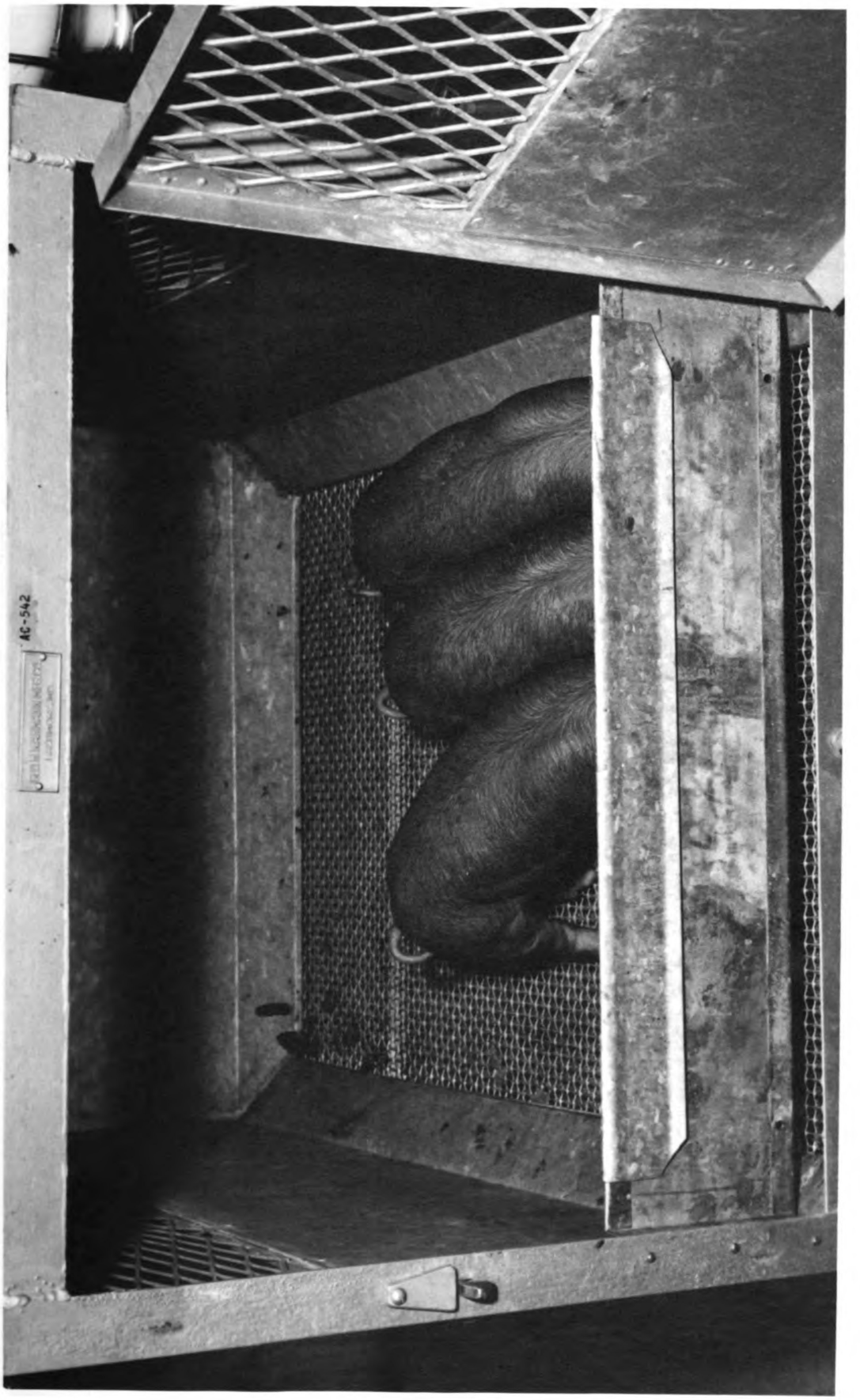

Plate II - Experiment I, Lot I - Negative Control.

Plate II

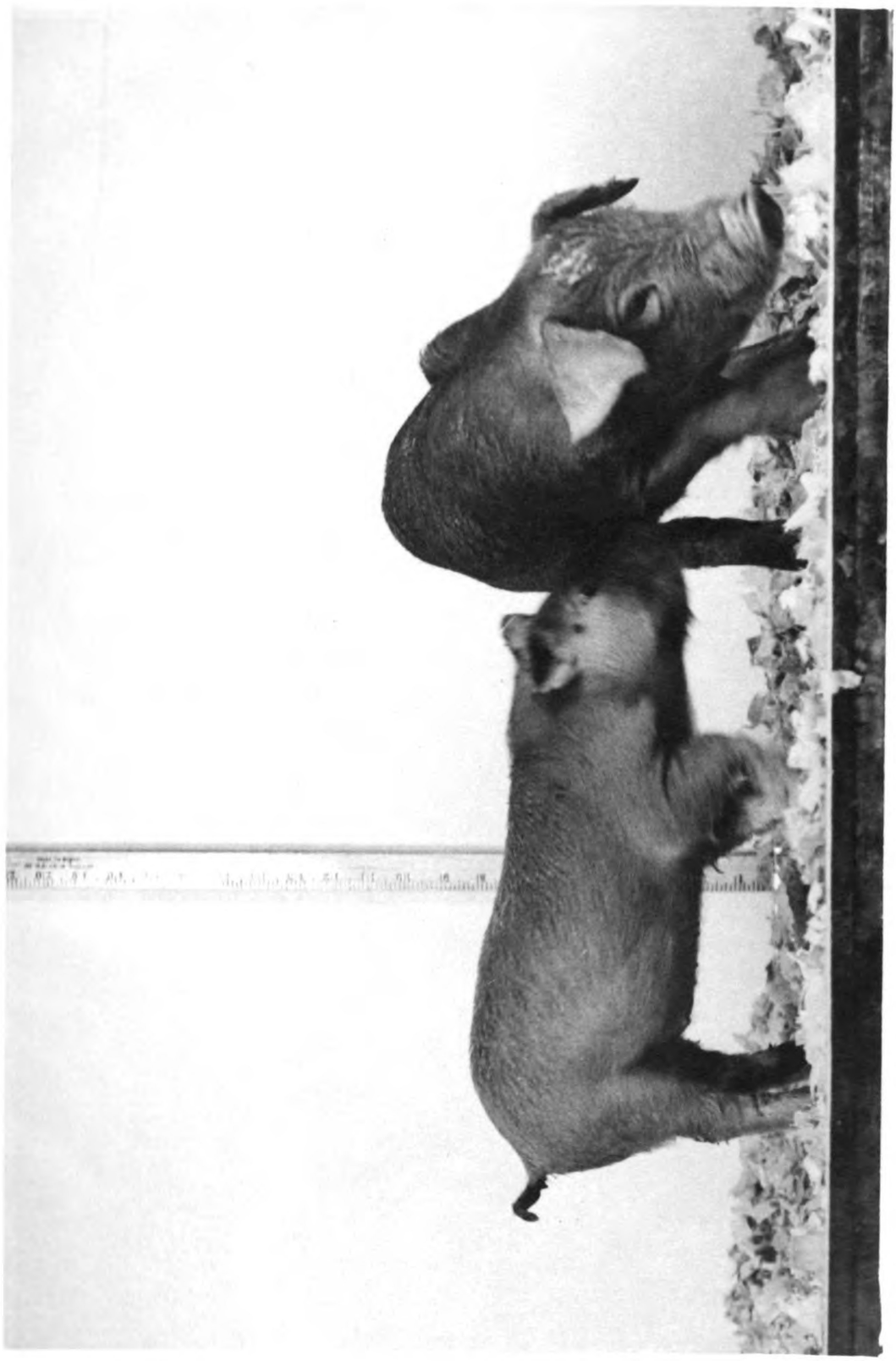

Plate III - Experiment I, Lot II - Receiving
Basal + 1 mg. of "P.A." /100 grams
of Solids.

Plate III

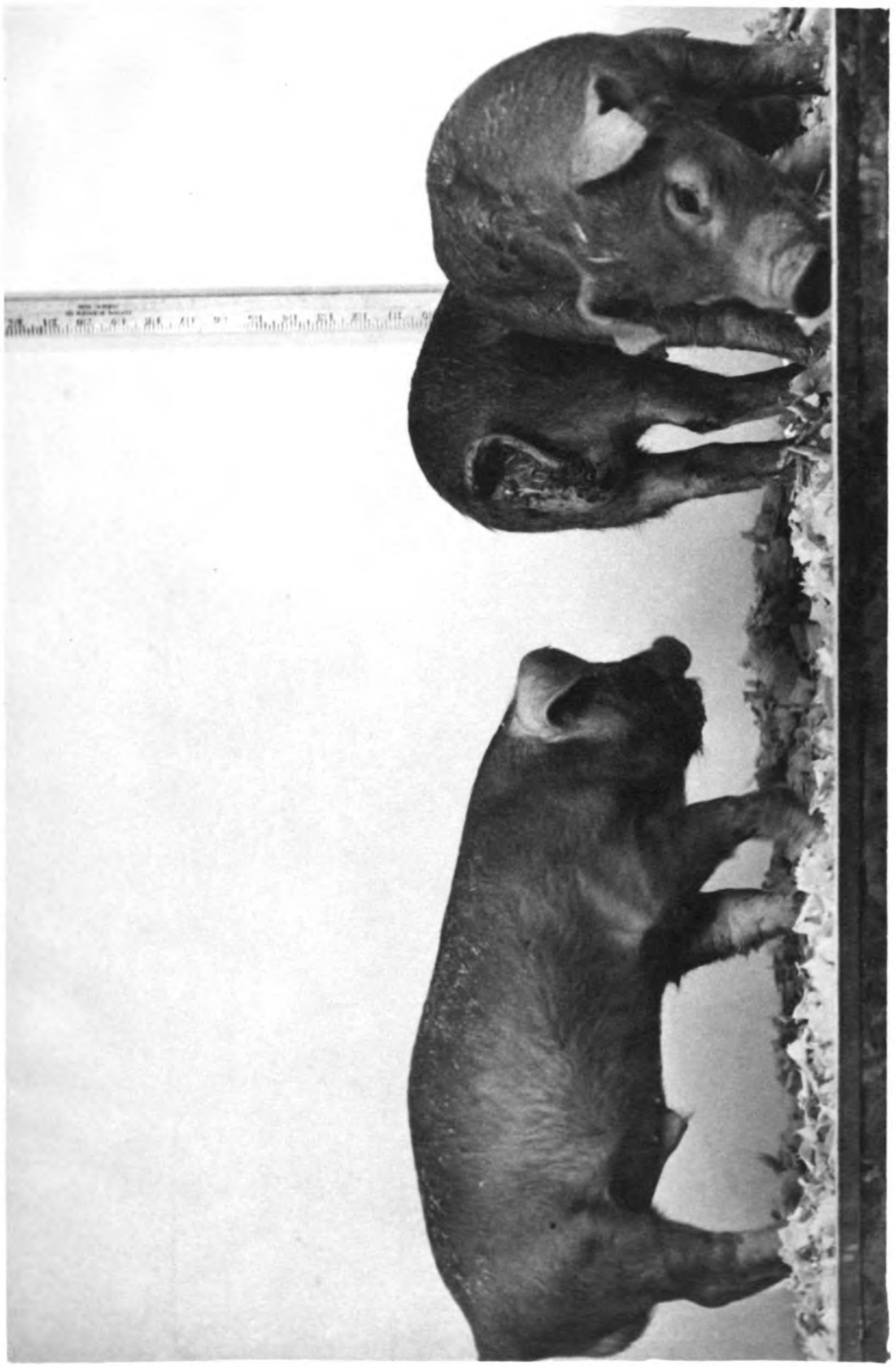

Plate IV - Experiment I, Lot III - Receiving Basal + 2 mg. of "P.A."/100 grams of Solids.

Plate IV

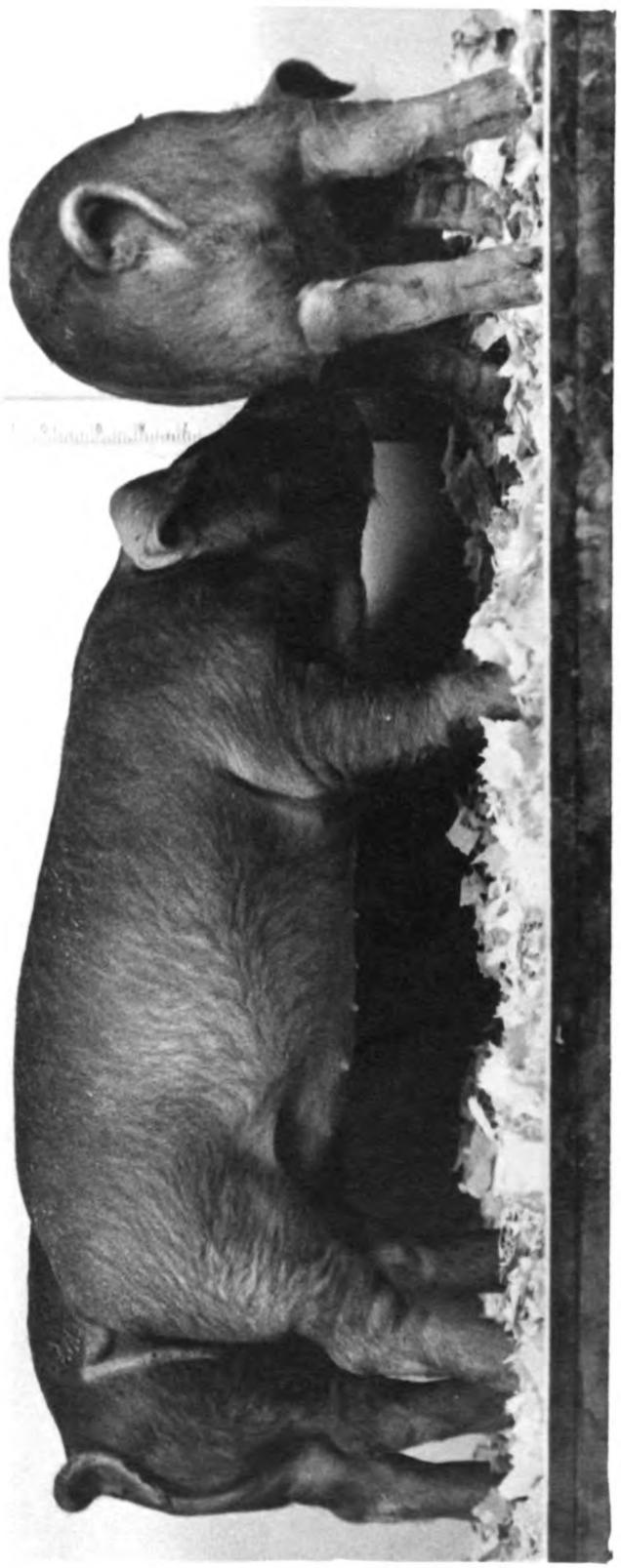

Plate V - Experiment I, Lot I, Pig No. 9 at 32 Days on Experiment showing a Severe Goose-step.

Plate V

Plate VI - Experiment I, Lot I, Pig No. 9 at 32 Days on Experiment. Note the extreme sickle hocks.

Plate VI

Plate VII - Vacuolar Degeneration of the Cell Body of the Neuron in a Dorsal Root Ganglion. H & E stain, 490x.

Plate VII

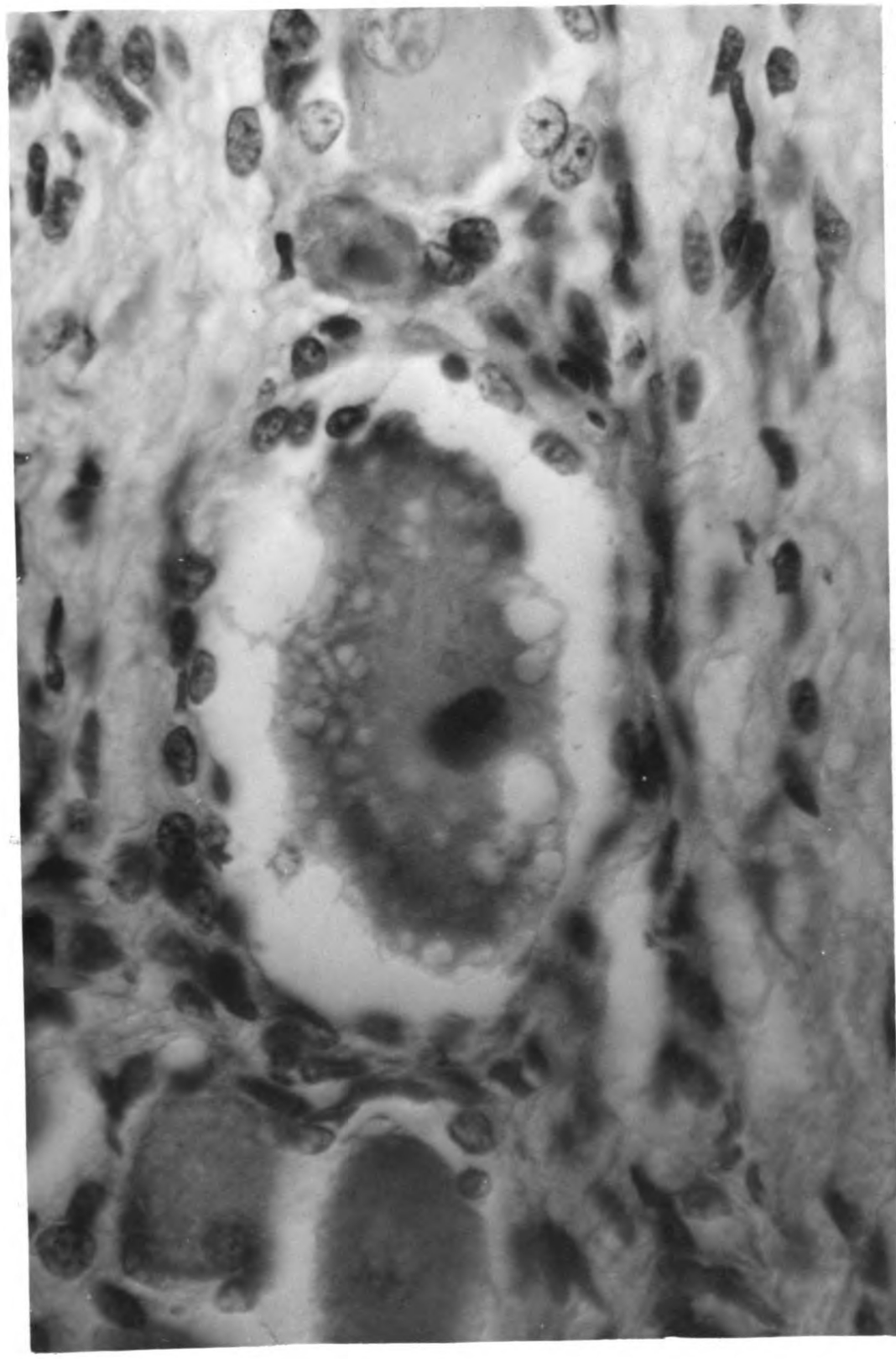

Plate VIII - Normal Sciatic Nerve, H & E stain,
490x.

Plate VIII

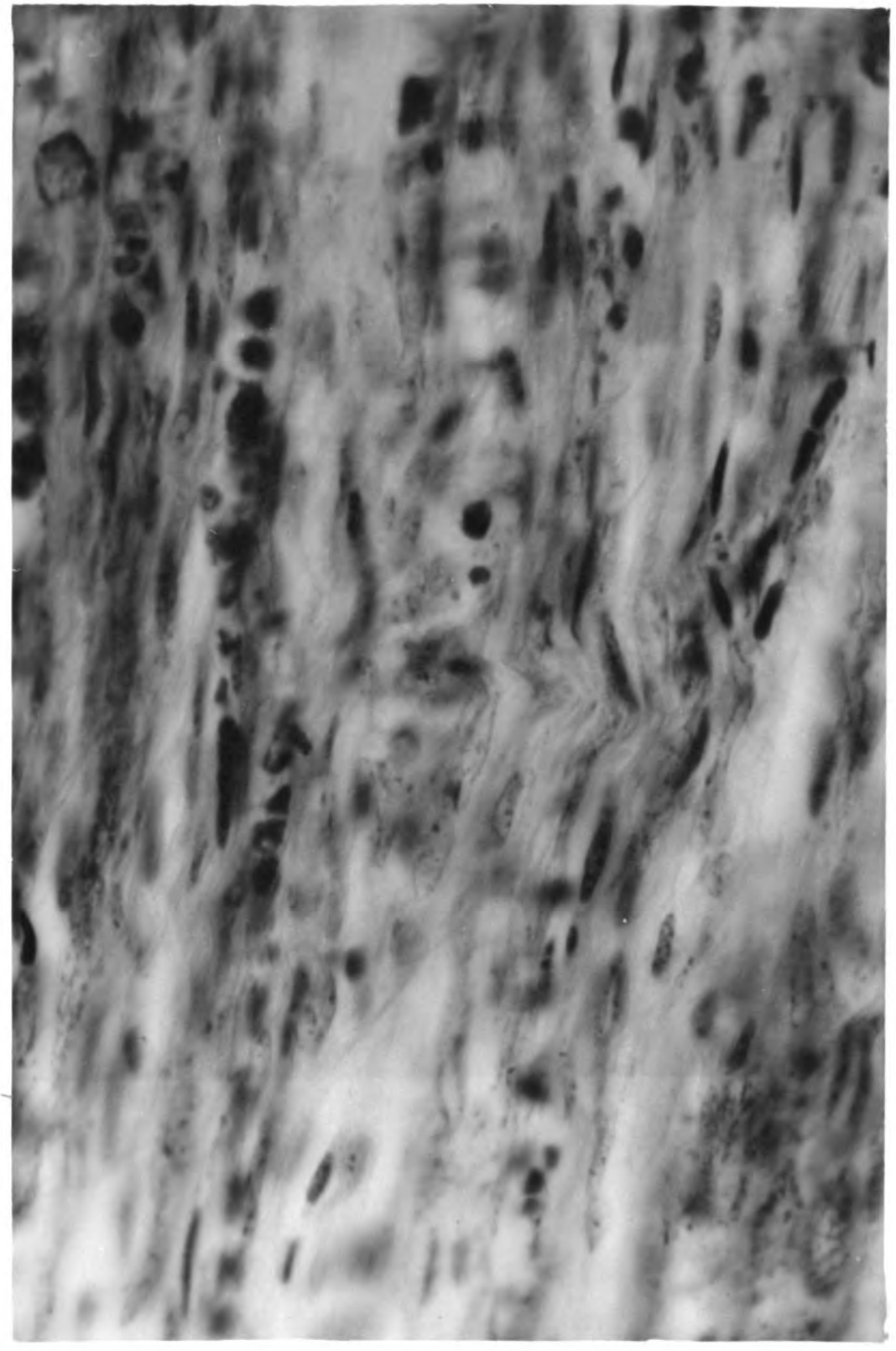

Plate IX - Sciatic Nerve showing Vacuolar Degeneration and Ballooning in the Myelin Layer of the Nerve Fiber. H & E stain, 490x.

Plate IX

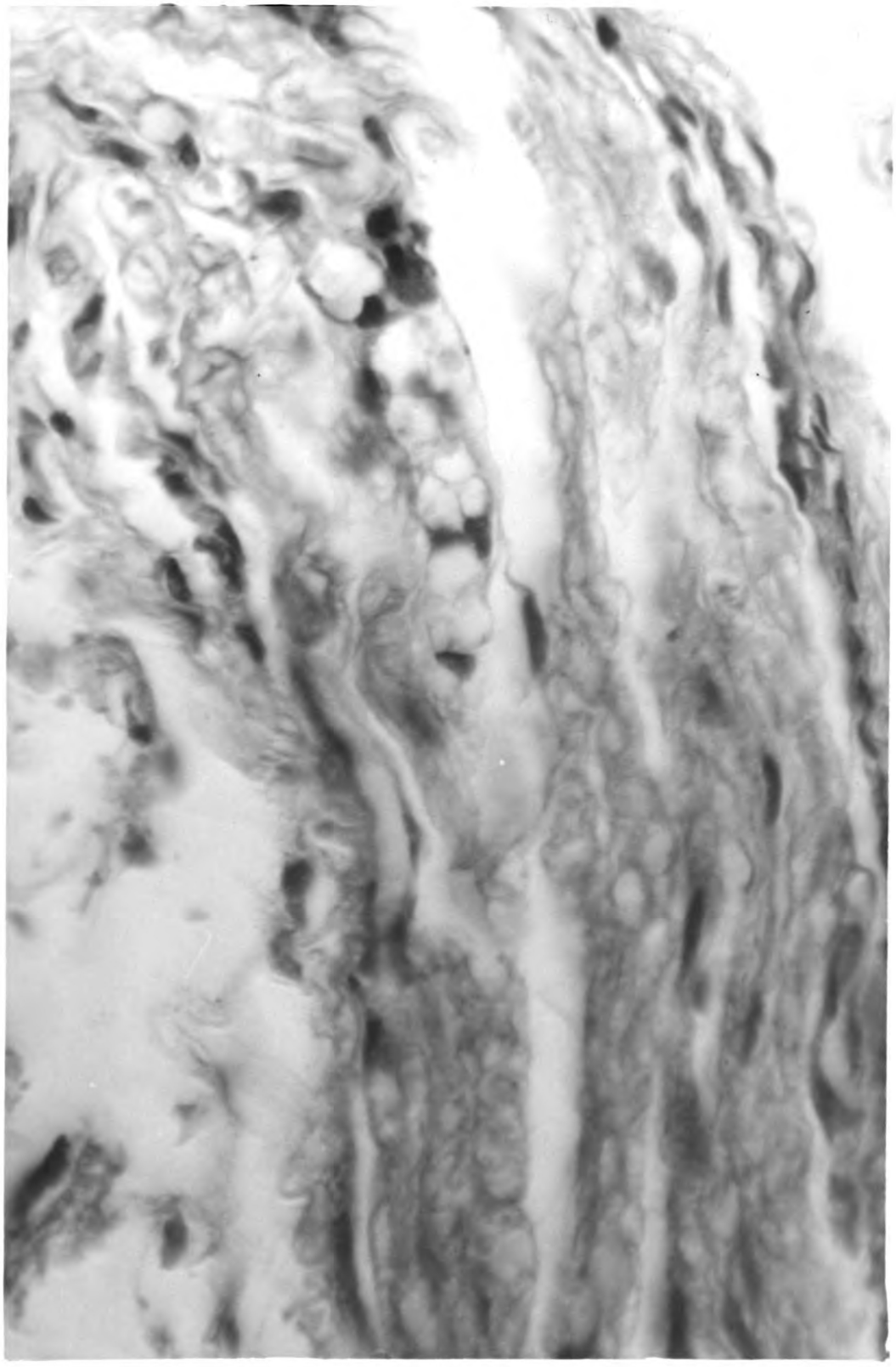

Plate X - Normal Sciatic Nerve, Weil stain, 490x.

Plate X

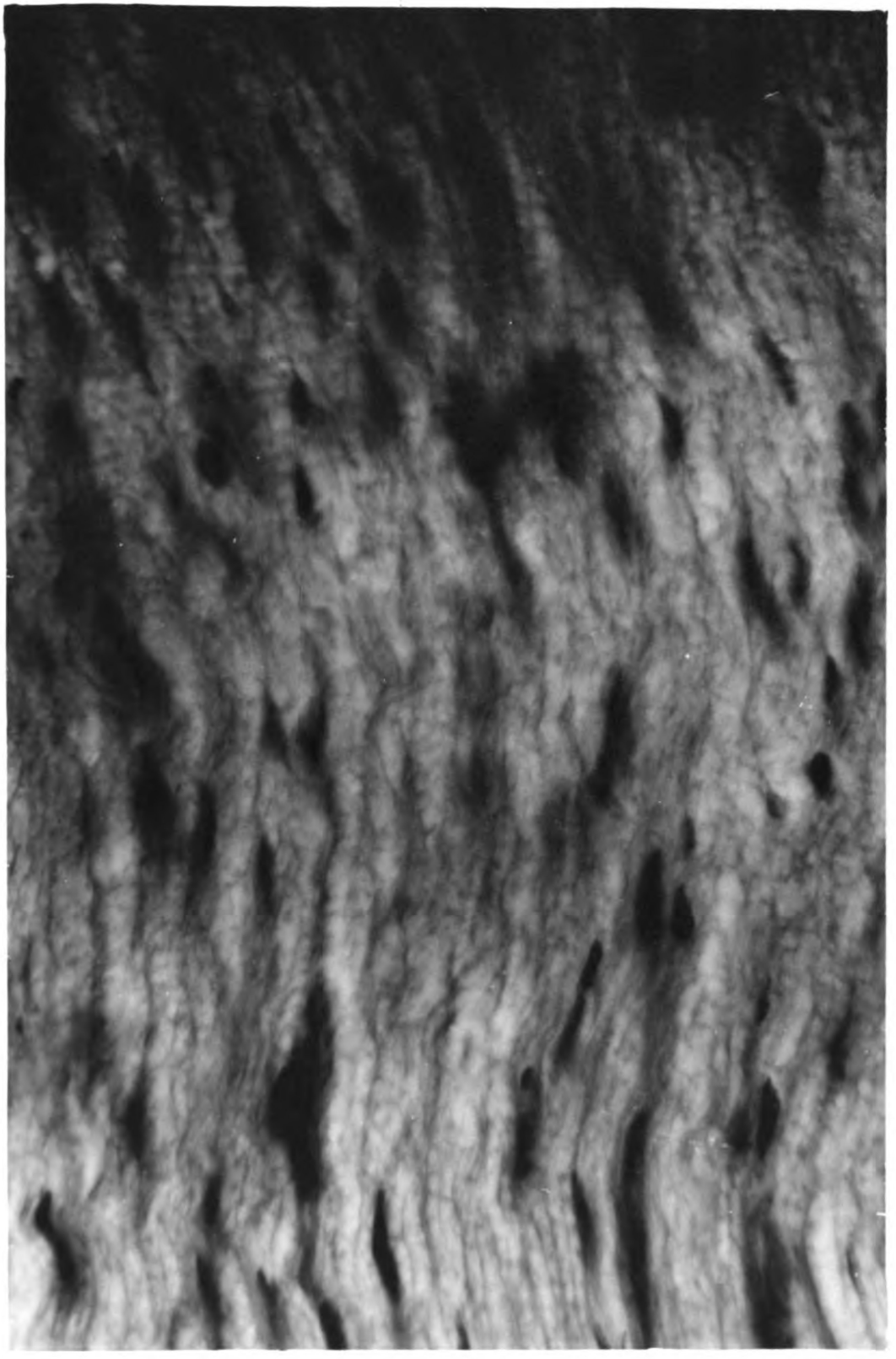

Plate XI - Sciatic Nerve showing Degeneration and Fragmentation of the Myelin Sheath, Weil stain, 490x.

Plate XI

Plate XII - Colon showing Vacuolar Degeneration and Swelling in the Plexuses of Auerbach, H & E stain, 430x.

Plate XII

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