SUPPLEMENTAL PANTOTHENIC ACID IN SMALL GRAIN RATIONS FOR SWINE

by

Bruce Douglas Owen

Department of Animal Science

University of Alberta

April, 1952

THE UNIVERSITY OF ALBERTA

SUPPLEMENTAL PANTOTHENIC ACID IN SMALL GRAIN RATIONS FOR SWINE

A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

FACULTY OF AGRICULTURE

DEPARTMENT OF ANIMAL SCIENCE

by

BRUCE DOUGLAS OWEN

EDMONTON, ALBERTA,

APRIL, 1952

SUPPLEMENTAL PANTOTHENIC ACID IN SMALL GRAIN RATIONS FOR SWINE

ABSTRACT

An investigation of the effects of pantothenic acid supplementation of small grain rations for Yorkshire swine has been conducted. The studies were prompted by the reported occurrence of deficiencies of this vitamin in swine fed rations based on corn. Growth trials during two generations, a reproduction and lactation experiment, and microbiological assays for pantothenic acid in the milk and blood of the animals involved in the reproduction and lactation trial were conducted. A ration, based on barley as it is the small grain lowest in pantothenic acid content, was used throughout so that control lots received as little pantothenic acid as is possible in a typical balanced Western Canadian ration.

Supplemental pantothenic acid had no effect on first generation growth, or on gilts between the time that they were weaned and the time they farrowed their first litters. However, indications were that supplemental levels of from three to twelve mg. per lb. of feed in gilts' rations may have had a detrimental effect on the birthweight of their offspring, and on the growth of the suckling pigs.

The results of microbiological assays, which were conducted following both water and enzymatic hydrolysis of samples, indicated that the pantothenic acid content of the blood and milk of the sows, and the blood of their suckling pigs was related to the content of the vitamin in the sows' ration. Values obtained for the pantothenic acid content

of sows' blood, pigs' blood and sows' milk ranged from 0.16 to 0.47, 0.85 to 3.70 and 5.50 to 16.23 γ per ml., respectively, following water hydrolysis of the samples. Corresponding values obtained following enzymatic hydrolysis of the samples ranged from 0.46 to 0.99, 1.37 to 4.42 and 5.09 to 14.76 γ per ml.

Since the basal rations contained sufficient amounts of pantothenic acid to promote normal growth and reproduction in Yorkshire swine, it has been concluded any balanced small grain ration of the type commonly fed in Western Canada should contain adequate amounts of the vitamin. It has been concluded also that unnecessary supplemental pantothenic acid in swine rations may result in certain harmful effects during the second generation.

ACKNOWLEDGMENTS

For the use of the facilities of the Department of Animal Science, sincere thanks are extended to Dr. L. W. McElroy, Professor of Animal Husbandry. I am particularly indebted to Dr. J. P. Bowland, Assistant Professor of Animal Husbandry, for initiation of the studies reported herein, and for his helpful advice and criticisms in connection with the carrying out of the research and the preparation of this manuscript. I wish also to acknowledge the invaluable advice with regards to the conducting of microbiological assays which was provided by Drs. D. R. Clandinin and A. R. Robblee of the Department of Animal Science, and by Miss Ruth Renner.

Sincere thanks are extended to Mr. Jack Francis, swine herdsman at the University Livestock Farm, who cared for the experimental animals.

Financial assistance received from Burns and Company, Limited, and from the General Research Fund, University of Alberta, is gratefully acknowledged.

TABLE OF CONTENTS

SUPPLEMENTAL PANTOTHENIC ACID IN SMALL GRAIN RATIONS FOR SWINE

INTRODUCTION

Pantothenic acid, one of the B-complex group of vitamins, has been shown in the past few years to be an essential factor in the metabolism of several species of animals, including swine. It is generally recognized that pantothenic acid, although widespread in various natural feedstuffs, can become a limiting factor in nutrition. Deficiencies of this vitamin, furthermore, may produce serious effects on the growth and thriftiness of the animals concerned.

Several investigators have reported pantothenic acid deficiencies in swine rations. However, when produced on diets of natural feedstuffs, the deficiencies have been confined to rations containing a relatively large proportion of corn. At the time of writing there has been little work done concerning the possibility of a pantothenic acid deficiency occurring on small grain rations such as are generally used in swine feeding in Western Canada. It may be noted that as wheat, oats and barley contain higher levels of this vitamin, such rations would generally contain a greater amount of pantothenic acid than rations formulated with corn as the chief constituent. Nevertheless, the possibility of a borderline deficiency of pantothenic acid occurring on a small grain ration must be considered, and it was to investigate this contingency that the studies reported herein were initiated.

The experiments were designed to investigate pantothenic acid requirements of Yorkshire swine fed a small grain ration, for growth

over two generations, and for reproduction and lactation. The control ration was supplemented with varying levels of calcium pantothenate. In addition, a study was made of the pantothenic acid content of the blood and milk of the lactating sows, and the blood of their suckling pigs.

The experiments reported in this thesis were carried out in the Department of Animal Science at the University of Alberta between May, 1950 and January, 1952.

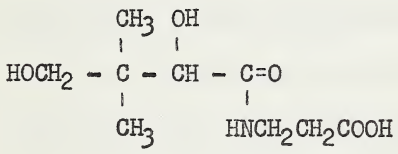
REVIEW OF LITERATURE

Pantothenic acid was first recognized as a growth stimulant for yeast. However, from the time of its discovery, in 1931 in a bios concentrate (78), the possibility that it would prove to be a vitamin required by animals was considered, and further investigation continued along two lines. Poultry nutritionists postulated an antidermatitis or filtrate factor for chicks, and chemical studies of this factor (66) (80) proceeded simultaneously with attempts to isolate and identify pantothenic acid (77). The various workers soon began to suspect that they were working towards identification of the same biological factor, and this proved to be the case, for soon after the announcement of the structure and synthesis of pantothenic acid (72) it was proven to be identical with the filtrate factor (36) (46) (81).

Pantothenic acid is an organic acid, soluble in water, and unstable to heat when in solution. It is also unstable to acids and alkalis. The formula is shown in Figure 1 (27). Pantothenic acid has been synthesized, and the vitamin is available commercially as the calcium salt, calcium pantothenate.

Figure 1

Pantothenic Acid



It may be noted that in recent years several analogues of the vitamin, such as panthenol, and N-methyl pantothenic acid, have been synthesized and studied (1) (17) (28) (42).

The biochemical role of pantothenic acid has been the subject of considerable investigation during the past few years. The vitamin is concerned in both plant and animal metabolism to a much greater extent than was originally thought. The most important physiological action of

pantothenic acid in the animal body is now considered to be its connection with vital processes which involve acetylation (51). Its particular role here is as a component of coenzyme A, which is required for one type of pyruvic acid oxidation (56) (57) (62). In addition to its role in acetylation it is considered likely that coenzyme A, and thus pantothenic acid, is involved in carbohydrate, fat and steroid synthesis. The vitamin functions in the control of respiration of widely different cells, and is concerned with the maintenance of the health of the spinal cord, nerves, adrenal glands, and of skin and fur (79). Pantothenic acid is believed to play a part in the prevention of certain types of anemia (13) and it is also thought that the vitamin has a role in the control of the water and salt balance of the body. Other possible functions are being investigated. Pantothenic acid is thought to be involved in the utilization of certain other vitamins, particularly riboflavin (79), and evidence has been put forward indicating interrelationships with biotin, folic acid and with vitamin B_{12} (83).

Pantothenic acid occurs as such, to a limited extent, in natural materials. A large proportion of the vitamin is present naturally in various conjugated or bound forms which have been studied quite extensively (8) (38) (54) (56) (82). It is generally believed that coenzyme A is the most widespread of pantothenic acid conjugates, and that it contains a large part of the cellular pantothenic acid. However, several other bound forms have been identified, one of these being the newly discovered L. bulgaricus factor (8) (38) (56) (82). The availability of bound forms of pantothenic acid for animals has not been extensively studied although a report by Lih et al. (41) has stated that all the known naturally occurring bound forms of pantothenic acid are available to the rat.

The importance of pantothenic acid has been studied from several points of view during the past few years through the medium of small animal experimentation. Melampy and Northrop (48), working with mice, found that animals on a pantothenic acid deficient ration survived only six to seven weeks. A deficiency resulted in weight losses in the heart, kidney and liver, but little, if any, in the brain. Deficiencies resulted also in a decrease in the tissue content of the vitamin, with the exception of the heart where an increase occurred. The average daily urinary and fecal excretion decreased from 80 to 96 percent after a six week period on a pantothenic acid deficient ration.

Carter et al. (13), studying pantothenic acid deficiencies in rats, have noted that a severe hypochromic anemia developed in about 60 percent of the cases. The anemia was characterized by a decrease in hemoglobin, erythrocytes and polymorphonuclear leucocytes. It was found during the course of these experiments that the administration of pantothenic acid would restore the blood to normal in a proportion of cases, but that treatment had to be begun early if it were to be successful.

Pilgrim et al. (62) have reported a decrease in pyruvate oxidation in the livers of pantothenic acid and biotin deficient rats as compared to normal animals. This was considered as an indication that these vitamins were components of the enzyme systems concerned with the metabolism of pyruvate. Olson and Kaplan (58) found that rats maintained on pantothenic acid free diets for periods up to nine weeks maintained normal coenzyme A content in their tissues for two to three weeks and then showed a gradual depletion to 35 to 40 percent of normal. Adding pantothenic acid to these diets increased the coenzyme A content of the tissues and the ability to utilize pyruvate.

A report by Supplee et al. (74) indicated weight losses and

dermatitis in rats deprived of pantothenic acid. Damage to the cortical layer of the adrenal glands has also been reported by these workers. Such damage, in turn, caused bleeding, wasting away and finally death of the tissue, and a cessation of the cortical hormone secretions which control the water and salt balance of the body. The observations of the latter workers are substantiated by Figge and Atkinson (19) who reported that partial dehydration of rats has produced the most conspicuous symptoms of pantothenic acid deficiency, i.e. porphyrin incrustations on the nose and fur.

The fact that pantothenic acid is required by poultry for growth, reproduction and maintenance of normal metabolism has led to considerable investigation of the vitamin in this field. Gillis, et al. (21) (22) (23) have established that pantothenic acid is required for hatchability in eggs. A deficiency of the vitamin resulted in embryonic mortality which was found to occur almost entirely during the last two or three days of incubation. It was found also that the hatchability of eggs from depleted hens returned to normal after one to two weeks of pantothenic acid supplementation.

Further effects of pantothenic acid deficiency in poultry have been studied in some detail by several workers. Ram (63) found, in experiments using pantothenate deficient synthetic diets, that the pantothenic acid deficiency syndrome was characterized by a specific type of dermatosis affecting the skin on the dorsal surface of the feet, corners of the mouth and eyelids. They also noted stunted growth, and retarded and rough feathering, as well as thymus involution, eroded gizzard lining, slight thickening of the proventriculus, and distended gall bladder.

Shaw and Phillips (68) have made neuropathologic studies of pantothenic acid deficient chicks, and have reported a widespread myelin and axon degeneration in the spinal cord. No accompanying degeneration in the peripheral nerves was noted in these studies. Tissue assay results reported by Snell et al. (70) have shown that in a pantothenic acid deficient state, the tissues contain only 30 to 70 percent as much pantothenic acid per gm. as do tissues from chicks receiving sufficient amounts of the vitamin to meet their requirements.

The actual requirement of chicks for pantothenic acid has been estimated by Jukes and McElroy (37) to be about one mg. per 100 gm. of diet. This requirement was found by these workers to be lowered somewhat when a preliminary depletion period was omitted. The requirement stated above has been generally substantiated in more recent investigations (29) (40). A level of 0.8 mg. per 100 gm. of diet has been found to be sufficient for normal hatchability. Turkey poults have been found to require a somewhat higher level of pantothenic acid in their diets than chicks (40).

Requirements have been established for certain other species. In their studies with mice, Melampy and Northrop (48) reported that 30 γ of d-calcium pantothenate per gm. of purified diet was adequate to maintain body weight and normal levels of the vitamin in the tissue of adult males. Unna (75) found the requirement of the rat to be approximately 80 γ per day.

There is, at present, some controversy concerning the role of pantothenic acid in human nutrition. However, although no deficiency symptoms have been reported a level of nine to 12 mg. per

2500 Calories is recommended for maintenance of a state of good health (24).

Ruminants do not appear to require a dietary source of pantothenic acid, as they are able to synthesize the vitamin in the rumen in sufficient quantities to meet their requirements (47) (49).

Evidence has been put forward indicating that the horse does require a dietary source of pantothenic acid. Pearson and Schmidt (60), in experiments carried on with Shetland ponies reported that about 38 γ. per kg. of liveweight per day is necessary for normal growth and health in the horse. Intestinal synthesis does meet a part of this requirement according to Maynard (47). The experiments of Pearson and Schmidt indicated that the amount of pantothenic acid excreted by the horse is definitely influenced by the amount ingested.

The role of pantothenic acid in swine nutrition has been studied extensively in the United States during the past several years. It has been shown repeatedly that this class of livestock requires a dietary source of the vitamin, and that a deficiency can have very serious effects. Hughes and Ittner (34), using a purified diet of sugar, casein, salts and vitamins other than pantothenic acid determined the minimum requirement of pantothenic acid for the growing pig. They found that the minimum requirement of pantothenic acid lies between 7.8 and 11.8 mg. per 100 lb. of pig daily. In the trial they added various levels of calcium pantothenate equivalent to 0, 3.6, 7.8 and 11.8 mg. of pantothenic acid per 100 lb. of pig daily, and used rate of growth and other deficiency symptoms as the criteria.

Hughes (32) reported that young pigs fed a diet deficient in pantothenic acid developed subnormal appetites, grew slowly, became thin and emaciated, showed a lack of coordination, and goose stepped. There was also some loss of hair, and autopsy showed gastritis and involvement

of the large intestine. McMillen et al. (50) have reported pantothenic acid deficiencies in swine fed a diet of corn, soybean oil meal, casein, minerals and vitamins other than pantothenic acid. Animals being fed this ration made slower and less economical gains than animals on a control ration of the same constituents plus 25 mg. of calcium pantothenate per lb. of ration. Deficient animals began goose stepping in seven weeks, and showed locomotor incoordination and myelin degeneration. Severe diarrhea was also present. Further experiments by McMillen et al. reported at the same time showed that a ration of 77 percent corn, 20 percent soybean oil meal and three percent of a mineral mix, plus a supplement of vitamins other than pantothenic acid was not satisfactory. This basal ration which produced deficiency symptoms contained, by microbiological assay, 4.21 mg. of pantothenic acid per pound. Blood levels of pantothenic acid in animals showing symptoms of a deficiency were found to be approximately two-fifths of those in animals receiving the same ration supplemented with calcium pantothenate.

In similar trials, Luecke et al. (45) found that growing pigs fed a basal ration of corn, casein, soybean oil meal, minerals and vitamins except pantothenic acid developed typical pantothenic acid deficiency symptoms, including poor growth, locomotor incoordination and myelin degeneration. The symptoms did not appear until the seventh week of the experiment. The same ration, with pantothenic acid added, produced good growth and increased the efficiency of feed utilization. A separate trial, using a basal ration of corn, casein and minerals, plus other known required vitamins produced more severe deficiency symptoms, and a severe diarrhea. Blood and urine analyses were carried out on the animals used in the experiments. Urinary excretion showed wide variation within lots, while blood levels of the vitamin were quite uniform within each

group. Large differences in favor of the supplemented lots were noted in both urinary excretion and blood levels.

Further work by Luecke et al. (44) with a ration of corn and soybean oil meal, minerals and required vitamins other than pantothenic acid illustrated definitely that the locomotor incoordination was a result of pantothenic acid deficiency. A group of pigs fed the basal ration plus all known required vitamins including pantothenic acid made good growth and was normal in all respects. Where pantothenic acid alone was added to the basal ration, poor growth and unthriftiness was noted, but there was no incoordination. The lot receiving all required vitamins except pantothenic acid showed a definite incoordination, and the other characteristic symptoms of a pantothenic acid deficiency. Growth differences attributable to pantothenic acid were significant, and blood levels of the vitamin in animals showing deficiency symptoms were about two-fifths of the normal levels of 1.0 to 2.0 γ per ml. of whole blood.

Wiese et al. (76) have produced a pantothenic acid deficiency in baby pigs, and have found the characteristic deficiency symptoms. Forty-eight hour old pigs were placed on an artificial milk diet of casein, cerelose, lard, salts and all known required vitamins except pantothenic acid. The deficiency produced was characterized by poor growth, loss of appetite, scours, lachrymation, dermatitis, coughing, loss of the suckling reflex, a dark brown exudate around the eyes, spastic gait, goose stepping and low urinary excretion of pantothenic acid. Adding one mg. of pantothenic acid to the ration of each pig daily stopped the scours and improved the appetite, but failed to improve any of the other symptoms. However, addition of from 10 to 20 mg. of pantothenic acid to the daily ration resulted in complete recovery, and a great improvement in appetite and growth rate.

The necessity of pantothenic acid for normal reproduction in swine has been indicated by Hodgskiss et al. (31). These workers have reported results of reproduction experiments with gilts which were fed a purified ration of 26.1 percent casein, 57.7 percent sucrose, 11.0 percent lard and 5.2 percent of a mineral mix. The ration was tested with supplements of all known necessary vitamins, and with all known necessary vitamins except pantothenic acid. On the ration which included all the vitamins, gilts conceived and farrowed, but some pigs were in the process of resorption when born. All of the pigs were weak, showed signs of a nervous disorder, and died within 36 hours of farrowing. Animals receiving the pantothenic acid deficient ration displayed a typical deficiency syndrome, i.e., loss of appetite, reduced water intake, goose stepping with the hind legs and an exudate on the skin. There was also diarrhea and rectal hemorrhage. The gilts conceived but showed no signs of pregnancy. Autopsy revealed partially resorbed, macerating fetuses in the uterine horns of the animals, as well as gastro enteritis in the gilts.

The complementary effects of certain other vitamins on the pantothenic acid requirement of swine, and the possibility of interrelationships of pantothenic acid with other vitamins have been the subject of several investigations in recent years. Ellis et al. (18) reported experiments with heated diets, which indicated that although calcium pantothenate greatly reduced the incidence and severity of locomotor incoordination in swine, pyridoxine seemed to be necessary for full prevention of the disorder. There was also some evidence that lack of choline was partially responsible for the symptoms. It was felt, however, that the rather frequent occurrence of locomotor incoordination in growing swine fed normal corn rations was due to borderline levels of pantothenic acid rather than of the other vitamins mentioned.

Briggs and Beeson (7) fed weanling pigs a practical, mixed animal and plant protein ration, supplemented with vitamin B_{12} and aureomycin. It was shown that while the simultaneous addition of riboflavin, niacin and pantothenic acid increased the average daily gain by ten percent, and decreased the feed consumed per 100 lb. gain by 13 percent, single additions of either riboflavin or calcium pantothenate failed to improve growth rate markedly, and exerted only a slight favorable effect on feed efficiency. The basal ration used in these experiments was a high quality one of yellow corn, soybean oil meal, fish meal, meat and bone scraps, cottonseed meal, alfalfa meal, minerals and vitamin A and D feeding oil. No diarrhea or any other B-complex deficiency symptoms were observed in any of the groups.

Bennison et al. (4) noted no significant interactions between pantothenic acid and vitamin B_{12}. However, experiments reported by Luecke et al. (43) have indicated that there may be a complementary effect here. The latter workers found that on an 18 percent protein ration, a combination of vitamin B_{12} and pantothenic acid did not prove in any way superior to either of the vitamins alone; but that on a 15 percent protein ration the combined feeding of vitamin B_{12} and pantothenic acid did yield some increase in rate of gain over that obtained with the same ration with either of the vitamins added singly.

Microbiological assays for pantothenic acid have been carried out by numerous workers in the field of vitamin research since the discovery of the importance of this vitamin. The accuracy of values obtained for the pantothenic acid content of various materials by the use of microbiological methods has been the subject of some controversy, and as a result of this quite a variety of methods has been proposed for releasing bound pantothenic acid to a form available to test microorganisms. As the vitamin is acid and alkali labile, the use of either acid or alkaline

hydrolysis procedures is totally impractical, so that the methods used must be confined to either water or enzymatic hydrolysis. Earlier procedures used included direct addition (3), water hydrolysis (3) (53), digestion with such enzymes as clarase or papain (53) or a mixture of papain and takadiastase (64).

Two hydrolysis procedures which have been proposed more recently are now being generally used, and are considered to be more effective than previous methods for releasing bound pantothenic acid. These are: digestion with mylase P, or digestion with a mixture of a liver enzyme and an alkaline phosphatase. The digestion procedure involving the use of mylase P has been and is being quite widely used (35) (9) (10). The method releases a considerable amount of bound pantothenic acid, and has a distinct advantage in that enzyme blanks are very low (10) as the pantothenic acid content of mylase P is negligible.

Although the procedure involving mylase P for digestion is much more effective than autolysis alone in releasing pantothenic acid from natural materials, the values obtained on extracts prepared in this manner are still somewhat below those obtained by chick assay of similar materials (26). The mixture of liver enzyme and alkaline phosphatase is therefore being tentatively recommended, as this procedure has been shown to permit a closer approximation to complete release of bound pantothenic acid than do other methods. However, no standardized procedure for the use of these two enzymes with various natural materials can yet be given (26), and it is still not certain that all of the pantothenic acid in natural materials is being released. A "blank" sample containing only the enzyme preparations is always run, and the pantothenic acid content found is subtracted from that found for the digested samples. The pantothenic acid content of the enzyme mixture is relatively high (55).

The organism L. casei $\mathcal{E}$ was used extensively as the test organism in the earlier assays for pantothenic acid (53) (61) (73), but it has been largely replaced in recent years by L. arabinosus 17-5. The nutritional requirements of this latter organism have been shown to be considerably simpler than those of L. casei (26). The basis of the assay media has been, in all cases reviewed, either hydrolyzed casein or alkali treated peptone properly supplemented to supply all necessary ingredients for growth of the test organism used (30) (53) (61) (69) (73).

Pantothenic acid values for a variety of feeds, and for the blood and milk of certain species have been determined by various workers. Reported values that have a relation to the present study are listed in Table 1. It may be noted that no evidence has been found (15) to indicate that any of the pantothenic acid in milk is in a form unavailable to L. casei or L. arabinosus.

Table 1

Reported Values for Pantothenic Acid Content of Some Natural Materials

Material Assayed	Pantothenic Acid Content			Source
		Average	Range	
Dehydrated alfalfa	γ/gm.	33.7	25.1 - 41.3	Blaylock et al. (5)
" "	"	27.9	23.0 - 36.0	Bauernfeind et al. (3)
Meat scrap	"	9.8	6.3 - 16.5	" " " (3)
Barley	"	7.2	5.2 - 10.3	" " " (3)
Soybean oil meal	"	13.7	11.3 - 14.8	" " " (3)
Cows' milk	γ/ml.	3.67	-	Pearson et al. (59)
Ewes' milk	"	3.66	-	" " " (59)
Cows' milk	"	-	2.5 - 5.5	Lawrence et al. (39)
Sows' milk				
5th day after parturition	"	1.9	-	Davis et al. (15)
15th " " "	"	2.9	-	" " " (15)
55th " " "	"	5.4	-	" " " (15)

EXPERIMENTAL

A series of experiments was carried out to determine whether the swine rations commonly used in Western Canada contained sufficient pantothenic acid for normal growth, reproduction and lactation. Barley, supplemented with a mixed protein supplement, minerals and vitamins A and D when necessary was used exclusively in the experimental rations. Barley is, generally speaking, lower in pantothenic acid content than either wheat or oats, so it was assumed that if an all barley ration proved adequate insofar as its content of the vitamin was concerned, the findings could be applied generally to small grain rations.

Experiment I - Pantothenic Acid Requirements for Growth and Fattening

Four lots of six purebred Yorkshire pigs from the University herd were started on experimental rations at an average weight of approximately 35 lb. The lots were made up on a littermate basis with as uniform as possible distribution as to weight and vigor, and with two males and four females in each lot. All pigs in this and subsequent experiments were treated with sodium fluoride to remove round worms (Ascaris lumbricoides) prior to going on experiment. The dams of the pigs were fed a basal ration of oats, barley and a 40 percent protein supplement, and vitamin A and D feeding oil during pregnancy and lactation. The pigs, during the period between weaning and going on experiment were fed a ration of oats, barley, alfalfa meal and the same protein supplement, which included tankage and fish meal.

The trial was begun May 22, 1950. The control rations shown in Table 2 were fed in lot 1, with varying amounts of supplemental calcium pantothenate being added in the test lots.

Table 2

Control Rations - Experiment I

Feed		Period 1 40 to 110 lb	Period 2 110 to 200 lb
Barley	%	88.0	94.0
Supplement*	%	12.0	6.0
Protein**	%	13.8	12.6
Pantothenic acid**	γ/gm.		
water hydrolysis		6.0	5.9
enzymatic hydrolysis		7.8	7.1

*The protein, mineral supplement was made up as follows:

Meat scrap	50.0 lb.
Soybean oil meal	25.0 "
Alfalfa meal	15.0 "
Iodized salt	5.0 "
Ground limestone	5.0 "
	100 lb.

**Based on nitrogen analysis by the Kjeldahl method, and microbiological pantothenic acid assays.

The pantothenic acid content of feedstuffs used throughout the experiments is shown in Table 3. The feeds were assayed by the microbiological method of Ives and Strong (35) using L. arabinosus 17-5. Quite good agreement with values reported by other workers was noted. For details of the assay method employed see the Appendix.

Table 3

Pantothenic Acid Content of Feedstuffs Used

Feed	Pantothenic Acid Content	
	Water Hydrolysis γ/gm.	Enzyme Hydrolysis γ/gm.
Barley	4.3 - 7.0	4.6 - 7.4
Soybean oil meal	16.6 - 17.4	16.8 - 21.9
Alfalfa meal	20.0 - 34.0	21.2 - 32.0
Meat scrap	3.2 - 7.3	5.8 - 10.6

The rations used in experiment I were supplemented with the levels of calcium pantothenate shown in Table 4. The calcium pantothenate was

accurately weighed and added to the ration in the form of a soybean oil meal premix.

Table 4

Levels of Supplementary Calcium Pantothenate* - Experiment I

Lot no.	Ration and Supplement
1	Control
2	" + 3 mg. calcium pantothenate per lb. of feed.
3	" + 6 mg. " " " " " "
4	" + 12 mg. " " " " " "

*The calcium pantothenate used in these experiments was supplied through the courtesy of Merck and Company, Limited.

During period 1 up to 110 lb. in weight each lot was fed daily 48 ml. of a 1200 A - 200 D vitamin feeding oil spread evenly over the dry morning feed. The pigs were hand fed three times daily from the start of the experiment until they reached an average weight of 110 lb., and twice daily thereafter until reaching a weight of 200 lb. They were fed all they would clean up in 30 minutes, and water was added after each feeding at the rate of two and one half lb. per lb. of feed. The animals were housed indoors, and did not have access to either pasture or soil. Pens were kept as clean as possible in order that access to feces be kept at a minimum. Feed was weighed to each lot at each feeding, and suitable records were kept.

The pigs were weighed individually at 14 day intervals, and more frequently as they approached market weight. At an average weight of 200 lb., the two males, and one female in each lot were marketed. Dominion Government grades were determined on each carcass of the experimental pigs slaughtered. The remaining three females in each lot were retained for use in the reproduction experiment.

Hemoglobin levels in the blood of all pigs were determined at the

beginning of the experiment, and at six and twelve weeks thereafter*. The animals were watched carefully for any symptoms of a deficiency.

Experiment II - Pantothenic Acid Requirements for Growth and Fattening

A second growth trial was commenced on Sept. 26, 1950, for the purpose of checking results obtained in the previous experiment. The design and procedure followed were the same as used in Experiment I, with the following exceptions: The allotment was not made so as to include four females and two males in each lot, as all the animals used in this trial were marketed upon reaching a weight of 200 lb. Pigs in this and all subsequent experiments were self-fed. The upper level of pantothenic acid supplement used in Experiment I (12 mg. of calcium pantothenate per lb. of feed) was eliminated, and replaced by a 4.5 mg. per lb. level. Thus, the rations used in Experiment II were supplemented as shown in Table 5.

Table 5

Levels of Supplementary Calcium Pantothenate - Experiment II

Lot no.	Ration and Supplement
1	Control
2	" + 3.0 mg. calcium pantothenate per lb. of feed.
3	" + 4.5 mg. " " " " " "
4	" + 6.0 mg. " " " " " "

The protein and pantothenic acid content of the control rations varied from those of Experiment I, and were as shown in Table 6.

*Oxyhemoglobin determined colorimetrically on the Evelyn Colorimeter.

Table 6

Protein and Pantothenic Acid Content of Rations - Experiment II

Nutrient	Period 1 40 to 110 lb.	Period 2 110 to 200 lb.
Protein, %	15.3	14.0
Pantothenic acid, γ/gm.		
water hydrolysis	6.2	5.0
enzymatic hydrolysis	7.4	5.2

Experiment III - Pantothenic Acid Requirements for Reproduction and Lactation

Three gilts from each lot in Experiment I were retained, and fed the same ration, supplemented with the same level of calcium pantothenate that they had received during period 2 of the growth trial. They were bred when they reached nine months of age, during December, 1950 and January, 1951. One animal in lot 1 failed to conceive, and one of those in lot 2 died subsequent to breeding. The autopsy report on the latter gilt indicated that death was due to internal hemorrhage. No pathogen was isolated.*

At midgestation, the level of protein supplement in the ration was increased from six to nine percent. Average protein and pantothenic acid contents of the rations were as shown in Table 7.

Table 7

Protein and Pantothenic Acid Content of Rations - Experiment III

Nutrient	Period 1 To midgestation	Period 2 To end of lactation
Protein, %	13.8	14.6
Pantothenic acid, γ/gm.		
water hydrolysis	4.9	5.2
enzymatic hydrolysis	5.2	5.5

*The cooperation of Drs. G. Wilton and G. Weir of the Provincial Veterinary Laboratory in carrying out post-mortem examinations is acknowledged.

The gilts were weighed at the time of breeding, immediately preceding farrowing, and just after farrowing. The animals farrowed between April 16 and May 19, 1951, and individual weights of the pigs in each litter were recorded at birth and at one, three, six and eight weeks of age.

Blood and milk samples were obtained from each sow at one and six weeks postpartum. Blood samples were taken also from two pigs, one of each sex, which were selected as representative of each litter, at one and six weeks after birth. The method of obtaining blood samples was similar to that used by Carle and Dewhirst (12), and milk samples were obtained by the use of oxytocin in a method similar to the one employed by Braude et al. (6). These samples were assayed microbiologically for pantothenic acid using a procedure similar to that of Ray et al. (64).

The pigs were weaned at eight weeks of age, and selection of the animals to be used in Experiment IV was made.

Experiment IV - Second Generation Study of Pantothenic Acid Requirements for Growth and Fattening

The animals allotted to the four lots of six pigs each in this third feeding trial were selected from the litters of sows which had received the same levels of supplementary pantothenic acid throughout their lives as the lots were to receive in this experiment. The lots were made up with as uniform as possible distribution as to sex, weight and vigor. The rations and levels of calcium pantothenate used, and other experimental procedures were the same as in Experiment I. In this instance, however, no provision was made for retaining any of the animals for further experiments.

It was found necessary to terminate this trial at the end of the first period. Shortly after the pigs had reached 110 lb. in weight several of them contracted an infection, and began to lose weight. They soon became emaciated to such an extent that it was considered unfeasible to continue the experiment.

Autopsies were carried out on several animals. A typical post-mortem examination of one of the affected animals revealed the following: The apices of the lungs were consolidated; the stomach and intestines were chronically inflamed, with round worms being noted; and there was pericarditis. Also noted were abscesses on the tail and right hind leg. The affected organs were cultured and two pathogenic organisms isolated. A Pasteurella suiseptica was isolated from the lungs, and Corynebacterium pyogenes from the leg and tail lesions. The round worm infestation in this animal was probably due to its extreme weakened condition, as the parasites had not been found prevalent in two other affected animals which had been autopsied previously, and all animals had been treated for worms prior to going on experiment.

Experiment IV was initiated July 23, and terminated on October 3, 1951. The average protein and pantothenic acid content of the ration used was as shown in Table 8.

Table 8

Protein and Pantothenic Acid Content of Ration - Experiment IV

Nutrient	Period 1 40 to 110 lb.
Protein, %	16.3
Pantothenic acid, γ/gm.	
water hydrolysis	6.2
enzymatic hydrolysis	6.6

Microbiological Assays

The pantothenic acid content of the blood and milk samples obtained from the sows and young pigs used in Experiment III was determined microbiologically. Assays were conducted on a semimicro scale in which the final volume was 2 ml. The composition of the basal medium used in the assays was similar to that described by Riesen et al. (65). So far as is known, this type of medium, wherein the casein or peptone is replaced by a solution of pure amino acids has not been used previously for the assay of pantothenic acid. The medium was found very satisfactory, and yielded extremely low blanks.

Two procedures were used in the preparation of samples for assay i.e. water and enzymatic hydrolysis. The latter procedure involved digestion of the sample with a fungal enzyme, mylase P. Blood and milk samples were prepared for assay by a method based on that used by Ray et al. (64) with several modifications being made to adapt the method to the purposes of this assay. All samples were assayed in duplicate, and values reported were obtained in at least two replicate assays. Recoveries were run along with the samples at regular intervals, and were in almost all cases between 90 and 110 percent.

Details of the hydrolysis procedures used, and of the assay method are described in the Appendix.

RESULTS

The growth, reproduction, lactation and assay results for the various experiments are reported in the following pages.

Experiment I

The rate of gain, feed consumption and feed efficiency data for Experiment I are shown in Tables 9, 10 and 11.

Table 9

Rate of Gain, Feed Consumption and Feed Efficiency during Period 1 (40 to 110 lb.) - Experiment I

Lot No.	1	2	3	4
Treatment	Control Ration	Control + calcium pantothenate		
		3 mg/lb feed	6 mg/lb feed	12 mg/lb feed
No. pigs in lot	6	6	6	6
Av. no. days fed	79	79	72	72
Av. initial weight, lb.	36.3	35.8	36.2	36.0
Av. final weight, lb.	111.3	115.3	110.0	111.0
Av. daily gain, lb.	0.95	1.01	1.03	1.04
Av. daily feed consumed:				
Barley, lb.	3.06	3.17	3.21	3.21
Supplement, lb.	0.42	0.43	0.44	0.44
Total, lb.	3.48	3.60	3.65	3.65
Feed required per 100 lb. gain:				
Barley, lb.	322	315	313	308
Supplement, lb.	44	43	43	42
Total, lb.	366	358	356	350

During the growing period to 110 lb. there appeared to be a slight trend toward increased gain and feed efficiency favoring the lots receiving the calcium pantothenate supplemented rations. As may be seen in Table 9, the average daily gains increased consistently, although very slightly, with increasing amounts of the vitamin in the ration. The same general trend was indicated for over-all feed efficiency. The differences in

average daily gain were not statistically significant in any instance.

Table 10 illustrates rate of gain, feed consumption and feed efficiency during the second or finishing period of Experiment I.

Table 10

Rate of Gain, Feed Consumption and Feed Efficiency during Period 2 (110 - 200 lb.) - Experiment I

Lot No. Treatment	1 Control Ration	2 Control + calcium pantothenate 3 mg/lb feed	3 Control + calcium pantothenate 6 mg/lb feed	4 Control + calcium pantothenate 12 mg/lb feed
No. pigs in lot	6	6	6	6
Av. no. days fed	54	50	59	61
Av. initial weight, lb.	111.3	115.3	110.0	111.0
Av. final weight, lb.	202.5	198.2	203.2	200.3
Av. daily gain, lb.	1.68	1.66	1.58	1.47
Av. daily feed consumed:				
Barley, lb.	6.27	6.39	5.91	5.85
Supplement, lb.	0.40	0.41	0.38	0.37
Total, lb.	6.67	6.80	6.29	6.22
Feed required per 100 lb. gain:				
Barley, lb.	373	386	374	397
Supplement, lb.	24	25	24	26
Total, lb.	397	411	398	423

A reversal of the trend seen in period 1 occurred during the finishing period, at least insofar as average daily gains were concerned. This indicated that, if pantothenic acid was of any value in increasing daily rate of gain, the effect manifested itself prior to the finishing period. The situation with regard to feed efficiencies during this period was erratic with no trend being established.

Results for the complete feeding period of Experiment I are shown in Table 11.

Table 11

Rate of Gain, Feed Consumption and Feed Efficiency during Experiment I

Lot No. Treatment	1 Control Ration	2 Control + calcium pantothenate 3 mg/lb feed	3 Control + calcium pantothenate 6 mg/lb feed	4 Control + calcium pantothenate 12 mg/lb feed
No. pigs in lot	6	6	6	6
Av. no. days fed	133	129	131	133
Av. initial weight, lb.	36.3	35.8	36.2	36.0
Av. final weight, lb.	202.5	198.2	203.2	200.3
Av. daily gain, lb.	1.25	1.26	1.28	1.24
Av. daily feed consumed:				
Barley, lb.	4.36	4.42	4.43	4.41
Supplement, lb.	0.41	0.42	0.41	0.41
Total, lb.	4.77	4.84	4.84	4.82
Feed required per 100 lb. gain:				
Barley, lb.	350	351	347	357
Supplement, lb.	33	34	32	33
Total, lb.	383	385	379	390

The rate of gain, feed consumption and feed efficiency of all lots were similar throughout the overall growing and finishing period. However, it appeared that there might have been a slight beneficial effect from the added vitamin in the earlier stages of growth as indicated in Table 9.

As a result of the average daily gain picture, shown in Experiment I, it was decided that the 12 mg. per lb. level of supplementation would be deleted from Experiment II, and replaced with one of 4.5 mg. per lb. Any possible beneficial effects of the vitamin on growth appeared to have been obtained on a supplemental level not higher than six mg. per lb. of calcium pantothenate.

Table 12 shows the average hemoglobin content of the blood of the pigs used in Experiment I. Allotment was made prior to determining hemoglobin levels so that they did not enter into distribution of the pigs.

Table 12

Hemoglobin Levels - Experiment I

Lot No. Treatment	1 Control Ration	2 Control + calcium pantothenate 3 mg/lb feed	3 Control + calcium pantothenate 6 mg/lb feed	4 Control + calcium pantothenate 12 mg/lb feed
Hemoglobin content of blood:				
at commencement of trial, gm./100 ml.	10.1	9.9	7.9	10.4
6 wks. after commencement of trial, gm./100 ml.	9.9	9.2	9.4	9.4
12 wks. after commencement of trial, gm./100 ml.	12.2	10.4	11.7	12.6

It may be noted from Table 12 that at no time did the supplemental pantothenic acid have any effect on hemoglobin levels.

Experiment II

The results of Experiment II, a trial conducted to verify results obtained in Experiment I are shown in Tables 13, 14 and 15.

Table 13

Rate of Gain, Feed Consumption and Feed Efficiency during Period 1 (40 to 110 lb.) - Experiment II

Lot No. Treatment	1 Control Ration	2 Control + calcium pantothenate 3 mg/lb feed	3 Control + calcium pantothenate 4.5 mg/lb feed	4 Control + calcium pantothenate 6 mg/lb feed
No. pigs in lot	6	6	6	6
Av. no. days fed	62	62	62	57
Av. initial weight, lb.	40.8	40.2	40.1	40.4
Av. final weight, lb.	112.2	109.7	107.5	108.3
Av. daily gain, lb.	1.15	1.12	1.09	1.19
Av. daily feed consumed:				
Barley, lb.	3.36	3.33	3.32	3.50
Supplement, lb.	0.46	0.45	0.45	0.48
Total, lb.	3.82	3.78	3.77	3.98
Feed required per 100 lb. gain:				
Barley, lb.	292	297	305	294
Supplement, lb.	40	41	42	40
Total, lb.	332	338	347	334

As may be seen from Table 13, there were no evident effects of supplementing the basal ration with calcium pantothenate. Both the average daily gain and the feed required per 100 lb. gain were similar in all lots. It was therefore evident that the possible trend in daily gain and feed efficiency, which had been noted in period 1 of the first trial, was due to chance only.

Results during the finishing period from 110 to 200 lb. are shown in Table 14.

Table 14

Rate of Gain, Feed Consumption and Feed Efficiency during Period 2 (110 - 200 lb.) - Experiment II

Lot No.	1	2	3	4
Treatment	Control Ration	Control + calcium pantothenate		
		3 mg/lb feed	4.5 mg/lb feed	6 mg/lb feed
No. pigs in lot	6	6	6	6
Av. no. days fed	64	66	67	63
Av. initial weight, lb.	112.2	109.7	107.5	108.3
Av. final weight, lb.	198.3	195.0	202.7	197.3
Av. daily gain, lb.	1.35	1.29	1.41	1.41
Av. daily feed consumed:				
Barley, lb.	6.01	5.50	5.73	6.06
Supplement, lb.	0.38	0.35	0.37	0.39
Total, lb.	6.39	5.85	6.10	6.45
Feed required per 100 lb. gain:				
Barley, lb.	444	427	406	429
Supplement, lb.	28	27	26	27
Total, lb.	472	454	432	456

Although the two lots receiving the highest levels of calcium pantothenate did make the best gains during period 2, the fact that there were no significant differences, and that lot 2 made slower gains than the controls must be taken as ample indication that differences between lots were due to chance. The same applies to feed efficiencies, even though the three lots receiving supplemental calcium pantothenate did require slightly less feed per 100 lb. gain.

Table 15 summarizes results for the entire feeding period of the second growth trial.

Table 15

Rate of Gain, Feed Consumption and Feed Efficiency during Experiment II

Lot No. Treatment	1 Control Ration	2 Control + calcium pantothenate 3 mg/lb feed	3 Control + calcium pantothenate 4.5 mg/lb feed	4 Control + calcium pantothenate 6 mg/lb feed
No. pigs in lot	6	6	6	6
Av. no. days fed	126	128	129	120
Av. initial weight, lb.	40.8	40.2	40.1	40.4
Av. final weight, lb.	198.3	195.0	202.7	197.3
Av. daily gain, lb.	1.25	1.21	1.26	1.31
Av. daily feed consumed:				
Barley, lb.	4.70	4.45	4.58	4.84
Supplement, lb.	0.42	0.40	0.41	0.43
Total, lb.	5.12	4.85	4.99	5.27
Feed required per 100 lb. gain:				
Barley, lb.	375	369	365	370
Supplement, lb.	34	33	32	33
Total, lb.	409	402	397	403

As in the previous trial, the calcium pantothenate had no effect on rate of gain, feed consumption or feed efficiency. It would appear that normal variation accounted for any slight differences which occurred during either of the trials.

It may be noted here that, of those pigs which were sold commercially 50 percent of the carcasses graded A and 50 percent graded B_1 in Experiment I, and 86 percent graded A and 14 percent graded B_1 in Experiment II. No differences in the grades between lots were obtained.

Experiment III

Three gilts were retained from each of the four lots in Experiment I, and managed and fed as indicated previously. Each animal was

weighed at the time of breeding, just prior to farrowing and again immediately after farrowing. Average weights for the gilts in the four lots are shown in Table 16.

Table 16

Average Weight of Gilts - Experiment III

Lot No. Treatment	1 Control Ration	2 Control + calcium pantothenate 3 mg/lb. feed	3 Control + calcium pantothenate 6 mg/lb. feed	4 Control + calcium pantothenate 12 mg/lb. feed
No. of sows in lot	2	2	3	3
Av. weight when bred, lb.	316.5	316.0	307.3	306.3
Av. " prior to farrowing, lb.	420.0	475.0	411.7	406.7
Av. " after farrowing, lb.	360.0	422.5	370.0	363.3
Av. gain during gestation, lb.	103.5	159.0	104.4	100.4
Av. loss at farrowing, lb.	60.0	52.5	41.7	43.4

The gains made by the gilts in the four lots were quite uniform during the interval between the end of Experiment I and the date of breeding. The animals in lot 2 gained somewhat faster during the gestation period than did those in lots 1, 3 and 4 with average gains in lot 2 being slightly greater than is normally desired in the first gestation period. Decreases in weight after farrowing were normal in all cases. There was no indication that the level of supplemental calcium pantothenate had any effect whatsoever on the gains made by the animals between the time they reached a weight of 200 lb. and the time they farrowed.

Table 17 illustrates the average litter size for individual sows, and averages for each of the four lots.

Table 17

Number of Pigs per Litter - Experiment III

Lot No.	1		2		3		4	
Treatment	Control Ration		Control + calcium panthothenate 3 mg/lb. feed		6 mg/lb. feed		12 mg/lb. feed	
No. of pigs in litter	Born	Weaned	Born	Weaned	Born	Weaned	Born	Weaned
Sow A	10	10	11	11	11	11	8	3
Sow B	4	3	7	4	9	8	11	9
Sow C	-	-	-	-	8	8	11	8
Av. no. pigs per litter	7	6.5	9.0	7.5	9.3	9.0	10.0	6.7

It may be seen that the average number of pigs born per litter increased somewhat with increasing levels of pantothenic acid in the sows' rations. However, the only notable difference occurred between lots 1 and 2, and this difference must be attributed to the fact that one of the litters in lot 1 was unusually small. This fact, in turn, cannot be attributed to a lack of pantothenic acid, as the other litter in the same lot was one of the largest.

The percentage survival in the four lots varied to such an extent that there was no indication of an effect of supplemental calcium pantothenate on survival at weaning time. The percentages of the pigs born which were surviving at weaning time were 93, 83, 97 and 67 percent for lots 1, 2, 3 and 4 respectively.

Table 18 indicates the average weight per pig in the various litters at different stages during the suckling period. Analysis of variance was carried out on birth and weaning weights.

Table 18

Average Weight per Pig During the Suckling Period - Experiment III

Lot No.	Treatment	Sow No.	Av. wt. per pig in litter at: birth	1 wk.	3 wk.	6 wk.	8 wk.
			lb.	lb.	lb.	lb.	lb.
1	Control ration	A	3.1	5.9	13.1	18.9	25.1
		B	2.8	5.4	17.6	22.6	38.0
	Av. for lot		3.0	5.8	14.4	19.9	28.1
2	Control + 3 mg. calcium pantothenate/lb. feed	A	3.1	5.8	12.7	19.6	25.5
		B	3.0	6.1	16.2	20.8	24.1
	Av. for lot		3.1	5.9	13.9	20.0	25.1
3	Control + 6 mg. calcium pantothenate/lb. feed	A	3.0	5.7	11.5	16.3	21.8
		B	2.4	4.5	8.6	13.7	21.1
		C	3.1	6.5	11.3	19.9	23.1
	Av. for lot		2.8	5.6	10.6	16.6	22.0**
4	Control + 12 mg. calcium pantothenate/lb. feed	A	2.7	4.8	11.6	19.0	22.0
		B	2.0	4.4	8.7	14.4	18.2
		C	2.4	4.4	7.6	12.4	16.1
	Av. for lot		2.3**	4.5	8.6	14.3	17.9**

**Highly significant differences between these and corresponding weights in the control lot based on analysis of variance carried out according to the method of L.P.V. Johnson,"An Introduction to Applied Biometrics".

Table 18A*

Analysis of Variance

Variance due to:	Birth Weights D.f.	Mean Square	F	Weaning Weights D.f.	Mean Square	F
Groups	3	2.83	10.11**	3	249	5.93**
Individuals	86	0.28		71	42	
Total	89			74		

*It is recognized that the use of the individual weights of the pigs in the various litters in each lot as independent measures of treatment effect is subject to criticism. For this reason no definite conclusions have been drawn from the results of the analysis of variance on this particular data.

The table illustrates a definite trend towards decreasing average weights with increasing levels of pantothenic acid in the dams' rations. Even when due consideration is given to the fact that one of the two litters in lot 1 was unusually small, which resulted in the average weights of the pigs in that litter being greater than those of any of the other animals in the experiment, the fact that highly significant differences were found indicates a possible treatment effect. However, the recognized weaknesses of the statistical analysis as applied to these particular data permit no definite statement on this point.

Experiment IV

As has been previously stated, it was found necessary to terminate this trial at the end of period 1, so that data are available only for the growth period to an average weight of 110 lb. The results obtained are shown in Table 19.

Table 19

Rate of Gain, Feed Consumption and Feed Efficiency during Period 1 (40 to 110 lb.) - Experiment IV

Lot No. Treatment	1 Control Ration	2 Control + calcium pantothenate 3 mg/lb feed	3 Control + calcium pantothenate 6 mg/lb feed	4 Control + calcium pantothenate 12 mg/lb feed
No. pigs in lot	6	6	6	6
Av. no. days fed	58	58	72	72
Av. initial weight, lb.	41.3	41.5	42.5	40.0
Av. final weight, lb.	109.8	110.0	115.5	113.5
Av. daily gain, lb.	1.18	1.18	1.01	1.02
Av. daily feed consumed:				
Barley, lb.	3.24	3.19	3.23	3.25
Supplement, lb.	0.44	0.44	0.44	0.45
Total, lb.	3.68	3.63	3.67	3.70
Feed required per 100 lb. gain:				
Barley, lb.	275	270	319	319
Supplement, lb.	37	37	43	43
Total, lb.	312	307	362	362

As may be noted in the table, lots 1 and 2, and lots 3 and 4 respectively were practically identical as to rate of gain, feed consumption and feed efficiency. Once again no definite differences were noted among the various lots. The fact that lots 3 and 4 made slower and less efficient gains may be explained, in part at least, by the fact that the acute infection which resulted in the termination of the experiment may have been already present in a chronic form, and was having an adverse effect on the pigs. It was the animals in these two lots which were most extensively involved. As pigs from the litters in lots 3 and 4 were weaned at lighter weights than those from lots 1 and 2, they may have been less thrifty at the time of allotment.

Microbiological Assays

Tables 20 and 21 summarize the results of assays carried out on blood samples obtained from sows and baby pigs in Experiment III.

Table 20

Pantothenic Acid Content of Blood Samples as Determined by Microbiological Assay, Following Water Hydrolysis

Lot No.	1	2	3	4
Treatment	Control Ration	Control + calcium pantothenate 3 mg/lb. feed	6 mg/lb. feed	12 mg/lb. feed
Time after parturition	1 wk / 6 wks	1 wk / 6 wks	1 wk / 6 wks	1 wk / 6 wks
	γ/ml	γ/ml	γ/ml	γ/ml
Sow A	0.18 / 0.27	0.18 / 0.20	0.23 / 0.32	0.24 / 0.53
Pig 1F*	1.79 / 0.43	3.10 / 0.58	2.24 / 0.71	2.72 / 0.79
Pig 2M*	3.48 / 0.98	3.19 / 0.78	4.30 / 0.81	4.33 / 0.90
Av. of 2 pigs	2.63 / 0.71	3.14 / 0.68	3.27 / 0.76	3.52 / 0.84
Sow B	0.15 / 0.27	0.27 / 0.27	0.40 / 0.47	0.41 / 0.35
Pig 1F	1.79 / 1.41	1.41 / 0.61	7.22 / 1.78	2.66 / 1.81
Pig 2M	1.98 / 1.13	1.75 / 1.44	4.28 / 1.02	2.15 / 1.48
Av. of 2 pigs	1.89 / 1.27	1.58 / 1.03	5.75 / 1.40	2.40 / 1.65
Sow C			0.24 / 0.29	0.39 / 0.55
Pig 1F			1.61 / 1.30	5.63 / 0.91
Pig 2M			1.83 / 1.61	4.74 / 1.14
Av. of 2 pigs			1.72 / 1.46	5.19 / 1.02
Av. of all sows	0.16 / 0.27	0.23 / 0.24	0.29 / 0.36	0.35 / 0.47
Av. of all pigs	2.26 / 0.99	2.36 / 0.85	3.58 / 1.20	3.70 / 1.17

*F indicates female, and M indicates male in this and following tables.

Table 21

Pantothenic Acid Content of Blood Samples as Determined by Microbiological Assay, Following Enzymatic Hydrolysis

Lot No. / Treatment	1 Control Ration		2 Control + calcium pantothenate 3 mg/lb. feed		3 Control + calcium pantothenate 6 mg/lb. feed		4 Control + calcium pantothenate 12 mg/lb. feed	
Time after parturition	1 wk	6 wks	1 wk	6 wks	1 wk	6 wks	1 wk	6 wks
	γ/ml	γ/ml	γ/ml	γ/ml	γ/ml	γ/ml	γ/ml	γ/ml
Sow A	0.55	0.69	-	0.63	0.57	0.73	0.67	0.98
Pig 1F	2.70	0.90	3.56	0.98	2.94	1.18	3.37	1.19
Pig 2M	4.52	1.28	4.09	1.13	5.59	1.16	5.60	1.25
Av. of 2 pigs	3.61	1.09	3.82	1.05	4.27	1.17	4.48	1.22
Sow B	0.38	0.81	0.61	0.76	0.58	0.94	0.61	0.92
Pig 1F	2.66	2.40	2.10	1.23	8.68	2.37	3.32	2.52
Pig 2M	2.37	1.76	2.03	2.15	4.63	1.47	2.42	1.97
Av. of 2 pigs	2.51	2.08	2.06	1.69	6.66	1.92	2.87	2.25
Sow C					0.47	0.66	0.59	1.08
Pig 1F					2.39	1.73	7.03	1.40
Pig 2M					2.29	2.14	4.99	1.46
Av. of 2 pigs					2.34	1.93	6.01	1.43
Av. of all sows	0.46	0.75	0.61	0.70	0.54	0.78	0.62	0.99
Av. of all pigs	3.06	1.58	2.94	1.37	4.42	1.68	4.45	1.63

As may be seen by comparing Tables 20 and 21, enzymatic digestion of the samples gave consistently higher values for pantothenic acid, and the increase in values obtained appeared to be considerably greater in the case of sows' blood than in the blood of the young pigs. It may be noted also that the pantothenic acid content of the sows' blood increased as the stage of lactation advanced, while the content of the vitamin decreased in the blood of the young pigs as they grew older. There was no indication that the sex of the young pigs had any influence on the pantothenic acid content of their blood. Although blood levels tended to correlate with levels of pantothenic acid in the ration, significant differences between

lots were not present insofar as the content of pantothenic acid in the blood was concerned. The above mentioned points will be discussed later.

Results of the assays carried out on milk samples obtained from the sows used in Experiment III are shown in Table 22. Values obtained in assays following both water and enzymatic hydrolysis are included. Analysis of variance was carried out on values obtained for pantothenic acid in samples of milk following water hydrolysis, but not on those obtained following enzymatic hydrolysis.

Table 22

Pantothenic Acid Content of Milk Samples as Determined by Microbiological Assay

Lot No.	Treatment	Sow No.	Time after parturition: 1 wk. water hydrolysis γ/ml.	1 wk. enzyme hydrolysis γ/ml.	6 wk. water hydrolysis γ/ml.	6 wk. enzyme hydrolysis γ/ml.
1	Control ration	A	3.99	3.84	3.63	3.97
		B	7.01	6.34	8.75	6.95
	Av. for lot		5.50	5.09	6.19	5.46
2	Control + 3 mg. calcium pantothenate/lb. feed	A	6.24	5.71	6.66	6.99
		B	5.92	6.03	7.45	6.15
	Av. for lot		6.08	5.87	7.06	6.57
3	Control + 6 mg. calcium pantothenate/lb. feed	A	7.81	7.17	9.30	8.87
		B	9.36	10.19	13.23	11.48
		C	7.18	8.13	9.90	7.70
	Av. for lot		8.12	8.50	10.81*	9.35
4	Control + 12 mg. calcium pantothenate/lb. feed	A	10.14	9.17	17.93	17.15
		B	8.19	9.30	15.78	14.15
		C	10.54	10.78	14.98	12.98
	Av. for lot		9.62*	9.75	16.23**	14.76

*Significant difference between this and corresponding value in the control lot.
**Highly significant difference between this and corresponding value in the control lot.

Table 22A

Analysis of Variance

Variance due to:	1 week D.f.	1 week Mean Square	1 week F	6 weeks D.f.	6 weeks Mean Square	6 weeks F
Groups	3	8.84	5.17*	3	53.0	11.75**
Individuals	6	1.71		6	4.51	
Total	9			9		

The results shown indicate that the pantothenic acid content of the sows' ration had a very definite effect on the amounts of the vitamin present in the milk, as the milk levels increased consistently and quite markedly with the amount of calcium pantothenate added to the basal ration. It may be seen also that the pantothenic acid content of the sows' milk increased as the lactation period progressed. A possible explanation for the low values obtained on the milk samples following enzymatic hydrolysis will be discussed later.

Table 23 summarizes the results of Experiment III.

Table 23

Summary of Results - Experiment III

Time of parturition	1 wk.				6 wk.			
Lot No.	1	2	3	4	1	2	3	4
Treatment	Control Ration	Control + calcium pantothenate			Control Ration	Control + calcium pantothenate		
		3mg/lb feed	6mg/lb feed	12mg/lb feed		3mg/lb feed	6mg/lb feed	12mg/lb feed
No. of litters	2	2	3	3	2	2	3	3
Av. no. pigs/litter	7.0	9.0	9.0	9.0	7.0	8.5	9.0	7.7
Av. wt./pig, lb.	5.8	5.9	5.6	4.5	19.9	20.0	16.6	14.3
Pantothenic acid content of:								
Sows' blood, γ/ml.								
water hydrolysis	0.16	0.23	0.29	0.35	0.27	0.24	0.36	0.47
enzyme hydrolysis	0.46	0.61	0.54	0.62	0.75	0.70	0.78	0.99
Sows' milk, γ/ml.								
water hydrolysis	5.50	6.08	8.12	9.62	6.19	7.06	10.81	16.23
enzyme hydrolysis	5.09	5.87	8.50	9.75	5.46	6.57	9.35	14.76
Pigs' blood, γ/ml.								
water hydrolysis	2.26	2.36	3.58	3.70	0.99	0.85	1.20	1.17
enzyme hydrolysis	3.06	2.94	4.42	4.45	1.58	1.37	1.68	1.63

DISCUSSION

A consideration of the results of the first generation growth trials in Experiments I and II, which are outlined in Tables 9 to 15, leads to the conclusion that the control ration used contained adequate amounts of pantothenic acid. No signs of a pantothenic acid deficiency occurred in the control lots, and no response that even approached significance occurred as a result of supplementing the ration with calcium pantothenate. As has been mentioned, some indication of a beneficial effect of calcium pantothenate supplementation was obtained during the earlier growing period of Experiment I. However this was not significant and was not substantiated in any way throughout the remainder of this experiment, or in Experiment II. Daily gain, feed consumption and feed efficiency differences were nothing more than would be expected due to normal variation.

Because of the fact that little work has been done concerning pantothenic acid deficiencies in small grain rations it is rather hard to compare the results obtained in these experiments with those obtained by other workers. However, it may be noted that Briggs and Beeson (7), using a high quality, well balanced ration based on corn found that calcium pantothenate failed to improve growth rate markedly and exerted only a slight favorable effect on feed efficiency.

It is notable also that pantothenic acid deficiencies are easily produced, and symptoms are quite obvious, in cases where rations are definitely deficient (44) (45) (50). This fact may be considered as lending strength to the inference, made on the basis of the results of Experiments I and II, that the control ration used contained sufficient pantothenic acid to meet the growth requirements of the animals concerned.

It may also be stated, at this point, that as barley contains less pantothenic acid than the other small grains it appears unlikely that typical balanced rations based on wheat or oats would be deficient in this vitamin.

As may be seen in Table 12 the hemoglobin determinations which were carried out gave no indication that the level of pantothenic acid in the ration had any effect on hemoglobin levels in the blood of the pigs. This may possibly be further evidence to the effect that the control ration used contained adequate amounts of the vitamin, for, although lowered hemoglobin levels are not necessarily characteristic of a pantothenic acid deficiency, they have been reported (13) (14).

The gilts retained for use in Experiment III gained evenly and normally during the prebreeding period. The fact that the two remaining gilts in lot 2 as is illustrated in Table 16 gained more rapidly than those in lots 1, 3 and 4 during the gestation period was considered as being due largely to chance but possibly, to some extent at least to the fact that there may have been less quarreling and competition for feed than in the other lots. There were three animals in each of the other lots throughout most of the gestation period, as the unbred animal in lot 1 was kept with the other two in the hope that she would conceive in time for use in the experiments. As further indicated in Table 16, weight decreases in all lots at the time of farrowing were normal, and did not differ to any extent. It may therefore be said that supplemental pantothenic acid had no visual effect on the experimental animals between the time they reached a weight of 200 lb. and the beginning of their first lactation.

The average number of pigs farrowed as is shown in Table 17, appeared to increase with the level of calcium pantothenate in the

ration. However, when consideration is taken of the fact that one of the litters in lot 1 was unusually small, the differences are no greater than would have been expected due to normal variation. Likewise, the number of pigs surviving at weaning time was unaffected by the amount of pantothenic acid in the dams' ration. The mortality rate was unusually high in one litter in lot 4, but normal variation may be considered to have accounted for all differences as there was no significant trend.

The average weights of the suckling pigs showed a definite downward trend, with the weights decreasing as the level of calcium pantothenate in the sows' rations was increased. As may be seen in Table 18, statistical analysis showed that there may have been a treatment effect involved. The highly significant differences in birth weights, between lots 1 and 4, and in weaning weights, between lots 1 and 3 and lots 1 and 4 indicated that in some way, the higher levels of supplemental calcium pantothenate being added to the dams' rations may have had a detrimental effect on the well-being of their offspring.

Tables 20 and 21 show that blood levels of pantothenic acid tended to increase in both sows and young pigs with the level of the vitamin in the sows' ration. This trend appeared to be quite regular, although several values were obtained for individual animals which were considerably out of line, and none of the differences were statistically significant. Values obtained following enzymatic digestion of samples were higher, as may be noted by comparing tables 20 and 21, than those obtained following water hydrolysis. This would indicate that some of the blood pantothenic acid exists in a bound form, which observation agrees with work reported by Wright (82). The increase in values obtained

was considerably greater for sows than for young pigs, which possibly indicates that a greater proportion of bound pantothenic acid existed in the blood of the older animals. It may be noted also that the pantothenic acid level in the blood of the sows increased, as lactation progressed, while that in the pigs decreased. A possible explanation for these "reverse" trends is that the high level found in the young pigs at one week of age had come about as a result of prenatal depletion of the sows in order to build up the levels of the young pigs. While the sows were able to increase their levels after farrowing, the young pigs were unable to maintain the high levels per ml. of blood from the amounts available to them in the milk. Although there are no comparable results of other workers with which to compare these findings, a similar occurrence takes place in the case of certain other blood constituents of swine. The work of Grummer et al. (25) shows a similar trend with several other vitamins.

Results of assays conducted on milk samples, as shown in Table 22, indicated that the level of pantothenic acid in the sows' ration was reflected in milk levels of the vitamin. The milk levels in lot 4 at one and six weeks, and in lot 3 at six weeks after parturition were higher by either a significant or highly significant degree than the respective levels in the control lot. Throughout the other lots there was a definite trend toward increased levels of the vitamin in the milk as the level in the ration increased. Differences in milk levels of pantothenic acid tended to be wider than corresponding differences in the blood. This was not surprising since the blood tends to remain reasonably constant in the level of most nutrients, while secretions and places of storage of various constituents may vary widely in content of

that same nutrient. It was also noted that an increase in milk pantothenic acid levels occurred between one and six weeks after farrowing, an increase which was more marked as the level of the vitamin in the ration was increased. This was considered logical, since the percentage of the vitamin which was being ingested that was utilized for maintenance would be fairly constant, and the quantity of milk being produced had probably dropped. Hughes and Hart (33), in summarizing the literature, have noted that maximum milk production in sows occurs three to four weeks after parturition, following which there is a drop.

The fact that the enzyme values were quite consistently lower than those obtained following water hydrolysis is a feature of the milk assay results which is difficult to explain. The differences, in most cases were greater than those encountered between duplicates of either kind of hydrolysis, so that normal variation is not the explanation. It should be noted here that other workers (15) (39) have reported that little or none of the pantothenic acid in milk is in a bound form, so that the water hydrolysis values obtained in these experiments may be considered as maximum. This would indicate that there was perhaps some slight destruction of the vitamin, or of one of its active analogues, by the mylase P.

The results of Experiment IV indicated that supplemental pantothenic acid was of no benefit in the growing ration used in these experiments, even after the pigs were fed for two generations on rations containing a basal level of approximately six γ per gm. of the vitamin based on microbiological assay. For growing pigs, the National Research Council (52) recommends 4.7 mg. per lb. of feed which is equivalent to 10.4 γ of pantothenic acid per gm. of feed. As has been previously

stated, the infection which made termination of the experiment necessary possibly had some effect on the results obtained during the experimental period, but apart from this there was no indication of anything other than normal variation in daily gains, feed consumption and feed efficiency. As it would be difficult or impossible to compound a natural Western Canadian ration with levels lower than this, it appears likely that supplemental pantothenic acid is unnecessary for proper growth and finishing of Yorkshire swine.

In summing up this discussion, it may be said that, on the basis of results of experiments reported herein, supplemental pantothenic acid was unnecessary in the basal rations used for growth and fattening, and for reproduction at least insofar as good health of the sows during gestation and lactation was concerned. Furthermore, those results pertaining to the well-being of the offspring of the sows in Experiment III would indicate that supplemental amounts of the vitamin were unnecessary, and perhaps detrimental. The baby pigs whose dams had received the higher levels of supplemental pantothenic acid were significantly lighter at birth than those in the control lot, and these differences were magnified during the lactation period, when the animals suckling sows which were receiving a calcium pantothenate supplemented ration ingested considerably greater amounts of the vitamin.

The particular manner in which this seemingly detrimental effect was brought about is unknown. It is possible that some type of vitamin imbalance was created, for such situations are known to occur (20). While the reports of other workers (7) indicate that multiple vitamin supplements including pantothenic acid may be of considerable benefit, our experiments have shown that excessive amounts of unnecessary single vitamins may produce harmful effects when fed over an extended period of time.

SUMMARY AND CONCLUSIONS

The investigations reported herein of pantothenic acid supplementation of small grain rations for Yorkshire swine were prompted by the reported occurrence of deficiencies of this vitamin in swine fed rations based on corn. Barley was used as a basis for formulating the control rations, as it has been shown to be the small grain lowest in pantothenic acid content. Varying levels of supplemental calcium pantothenate were fed during the course of the experiments, to determine if the addition of the vitamin had any supplemental effect on the basal ration.

The experiments included two generation growth trials, and a reproduction and lactation experiment. In addition, a study was made by means of microbiological assays, of the pantothenic acid content of the blood and milk of the sows, and the blood of young pigs selected from each litter in the reproduction trial.

The two first generation growth trials, as well as the second generation growth trial yielded results which indicated that no benefit was to be obtained from supplementing the control ration used with calcium pantothenate. The same indications were noted in the reproduction experiment, at least insofar as the effects of the supplement on the behavior of the gilts and the size of litters were concerned. Furthermore, there was indication that the pantothenic acid content of the dams' rations, when considerably higher than usual in a natural ration, may have had an adverse effect on the birth and weaning weights of the pigs in their litters.

The results of the microbiological assays illustrated the fact that the pantothenic acid content of the blood and milk of the sows, and the blood of the suckling pigs was related to the content of the vitamin in the sows' ration. This was particularly true in the case of the milk,

where it was found that supplemental calcium pantothenate fed to the sows resulted in quite a marked increase in the milk levels of the vitamin.

From the investigation which has been carried out, the following conclusions may be drawn:

1. The basal rations of barley plus protein and mineral supplements, and a vitamin A and D supplement when necessary contained sufficient pantothenic acid to promote normal growth and reproduction in Yorkshire swine.

2. Any well balanced small grain ration of the type commonly fed in Western Canada will normally contain adequate amounts of this vitamin, since the all barley control ration used contained as low a level of pantothenic acid as is likely to be present in a natural ration of this type.

3. The amount of pantothenic acid ingested by the sows influenced the content of this vitamin in their blood and milk, and in the blood of their offspring.

4. There was a trend to indicate that relatively large amounts of supplemental pantothenic acid in the sows' rations may have had a detrimental effect on the birth weight and rate of gain of their offspring during the suckling period.

LITERATURE CITED

1. BARNETT, J. W. and F. A. ROBINSON. Analogues of pantothenic acid. Biochem. J. 36: 357, also 364, 1942.

2. BAUERNFEIND, J. C., L. C. NORRIS and G. F. HEUSER. The pantothenic acid requirement of chicks. Poult. Sci. 21: 142, 1942.

3. BAUERNFEIND, J. C., L. C. NORRIS and G. F. HEUSER. The pantothenic acid content of feedstuffs as determined by microbiological assay. Poult. Sci. 21: 136, 1942.

4. BENNISON, R. W., D. CATRON and H. M. MADDOCK. Effect of antibiotics and vitamin B_{12} on the pantothenic acid requirement of growing-fattening swine. J. An. Sci. 10: 1038, 1951.

5. BLAYLOCK, L. G., L. R. RICHARDSON and P. B. PEARSON. Vitamin content of fresh, dehydrated and field cured alfalfa. Poult. Sci. 29: 692, 1950.

6. BRAUDE, R., K. KON and S. Y. THOMPSON. A note on certain vitamins in sows' colostrum. J. Dairy Res. 14: 414, 1946.

7. BRIGGS, J. E. and W. M. BEESON. The supplementary value of riboflavin, calcium pantothenate and niacin in a practical mixed animal and plant protein ration containing B_{12} and aureomycin for weanling pigs in drylot. J. An. Sci. 10: 813, 1951.

8. BROWN, G. M., J. A. CRAIG and E. E. SNELL. Relation of the Lactobacillus bulgaricus factor to pantothenic acid and coenzyme A. Arch. Biochem. 27: 473, 1950.

9. BUSKIRK, H. H., A. M. BERGDAHL and R. A. DELOR. Enzymatic digestion of samples for microbiological assay of pantothenic acid. J. Biol. Chem. 172: 671, 1948.

10. BUSKIRK, H. H. and R. A. DELOR. The use of mylase P in the preparation of natural materials for microbiological pantothenic acid assay. J. Biol. Chem. 145: 707, 1942.

11. CANNON AUTOMATIC DISPENSOR-TITRATOR. International Instrument Co., 729 South Harvard Blvd., Los Angeles 5, California.

12. CARLE, B. W. and W. H. DEWHIRST. A method for bleeding swine. J. Am. Vet. Med. Assn. 101: 495, 1942.

13. CARTER, C. W., R. G. MacFARLANE, J. R. P. O'BRIEN and A. H. T. ROBB-SMITH. Anemia of nutritional origin in the rat. Biochem. J. 39: 339, 1945.

14. COLBY, R. W. Biotin-pantothenic acid interrelationship and enteritis in the pantothenic acid deficient pig. J. Am. Vet. Med. Assn. 113: 589, 1948.

15. DAVIS, V. E., A. A. HEIDEBRECHT, R. MacVICAR, O. B. ROSS and C. K. WHITEHAIR. The composition of swine milk II. Thiamine, riboflavin, niacin and pantothenic acid content. J. Nutr. 44: 17, 1951.

16. DEYOE, G. P. and J. L. KRIDER. Raising Swine. McGraw-Hill Book Company Incorporated, 1952.

17. DRELL, W., and M. W. DUNN. Inhibition of lactic acid bacteria by pantothenic acid analogues. J. Am. Chem. Soc. 70: 2057, 1948.

18. ELLIS, N. R., L. L. MADSEN and C. O. MILLER. Pantothenic acid and pyridoxine as factors in the occurrence of locomotor incoordination in swine. J. An. Sci. 2: 365, 1943.

19. FIGGE, F. H. J. and W. B. ATKINSON. Relation of water metabolism to porphyrin incrustations in pantothenic acid deficient rats. Proc. Soc. Exp. Biol. Med. 48: 112, 1941.

20. FREAR, D. E. H. Agricultural Chemistry, Volume I, D. Van Nostrand Company Incorporated, 1950.

21. GILLIS, M. B., G. F. HEUSER and L. C. NORRIS. The need for pantothenic acid and an unidentified factor in reproduction in the domestic fowl. Poult. Sci. 20: 460, 1941.

22. GILLIS, M. B., G. F. HEUSER and L. C. NORRIS. The need for pantothenic acid and an unidentified factor in reproduction in the domestic fowl. J. Nutr. 23: 153, 1942.

23. GILLIS, M. B., G. F. HEUSER and L. C. NORRIS. The pantothenic acid requirement of hens for reproduction. Poult. Sci. 26: 540, 1947.

24. GORDON, E. S. Biological Action of the Vitamins. E. A. Evans, Editor, University of Chicago Press, Chicago, 1942.

25. GRUMMER, R. H., C. K. WHITEHAIR, G. BOHSTEDT and P. H. PHILLIPS. Vitamin A, vitamin C and niacin levels in the blood of swine. J. An. Sci. 7: 222, 1948.

26. GYÖRGY, P. Vitamin Methods, Volume I, Academic Press Incorporated, 1950.

27. HARROW, B. Textbook of Biochemistry. Fourth Edition, W. B. Saunders Company, 1946.

28. HEGSTED, D. M. Activity of panthenol as pantothenic acid in promoting chick growth. Proc. Soc. Exp. Biol. Med. 69: 571, 1948.

29. HEGSTED, D. M. and T. R. RIGGS. The pantothenic acid requirements of chicks receiving a purified diet. J. Nutr. 37: 361, 1948.

30. HOAG, E. H., H. P. SARETT and V. H. CHELDELIN. Use of Lactobacillus arabinosus 17-5 for microassay of pantothenic acid. Ind. Eng. Chem., Anal. Ed., 17: 60, 1945.

31. HODGSKISS, H. W., M. E. ENSMINGER, R. W. COLBY and T. J. CUNHA. Inadequacy of purified diets for reproduction by swine with observations on an added deficiency of pantothenic acid. J. An. Sci. 9: 619, 1950.

32. HUGHES, E. H. Pantothenic acid in the nutrition of the pig. J. Ag. Res. 64: 185, 1942.

33. HUGHES, E. H. and G. H. HART. Production and composition of sows' milk. J. Nutr. 9: 311, 1935.

34. HUGHES, E. H. and N. R. ITTNER. The minimum requirement of pantothenic acid for the growing pig. J. An. Sci. 1: 84, 1942.

35. IVES, M. and F. M. STRONG. Preparation of samples for the microbiological assay of pantothenic acid. Arch. Biochem. 9: 251, 1946.

36. JUKES, T. H. The pantothenic acid requirement of the chick. J. Biol. Chem. 129: 225, 1939.

37. JUKES, T. H. and L. W. McELROY. Observations on the pantothenic acid requirement of chicks. Poult. Sci. 22: 438, 1943.

38. KING, T. E., I. G. FELS and V. H. CHELDELIN. Pantothenic acid studies VI. A biologically active conjugate of pantothenic acid. J. Am. Chem. Soc. 71: 131, 1949.

39. LAWRENCE, J. M., B. L. HERRINGTON and L. A. MAYNARD. The nicotinic acid, biotin and pantothenic acid content of cows' milk. J. Nutr. 32: 73, 1946.

40. LEPKOVSKY, S., F. H. BIRD, E. H. KRATZER and V. S. ASMUNDSON. The comparative requirements of chicks and turkey poults for pantothenic acid. Poult. Sci. 24: 335, 1945.

41. LIH, H., T. E. KING, H. HIGGINS, C. A. BAUMANN and F. M. STRONG. Growth promoting activity of bound pantothenic acid in the rat. J. Nutr. 44: 361, 1951.

42. LINDSAY, R. P. and V. H. CHELDELIN. Pantothenic acid studies VII. N-methyl pantothenic acid. J. Am. Chem. Soc. 72: 828, 1950.

43. LUECKE, R. W., J. A. HOEFER and F. THORPE, Jr. Relationship of pantothenic acid and vitamin B_{12} in the growing pig. J. An. Sci. 10: 1054, 1951.

44. LUECKE, R. W., W. N. McMILLEN and F. THORPE, Jr. Further studies of pantothenic acid deficiency in weanling pigs. J. An. Sci. 9: 78, 1950.

45. LUECKE, R. W., F. THORPE, Jr., W. N. McMILLEN and H. W. DUNNE. Pantothenic acid deficiency in pigs fed diets of natural feedstuffs. J. An. Sci. 8: 464, 1949.

46. LYTHGOE, B., T. F. MacRAE, R. H. STANLEY, A. R. TODD and C. E. WORK. The identity of the liver filtrate factor with pantothenic acid. Biochem. J. 34: 1335, 1940.

47. MAYNARD, L. A. Animal Nutrition, Third Edition, McGraw-Hill Book Company Incorporated, 1951.

48. MELAMPY, R. M., and L. C. NORTHROP. Effect of diet on the pantothenic acid content of adult mouse tissues, urine and feces. Arch. Biochem. 30: 1, 1951.

49. McELROY, L. W. and H. GOSS. A quantitative study of vitamins in the rumen content of sheep and cows fed vitamin-low diets. IV Pantothenic acid. J. Nutr. 21: 405, 1941.

50. McMILLEN, W. N., R. W. LUECKE and F. THORPE, Jr. Pantothenic acid deficiency in swine on diets of natural feedstuffs. J. An. Sci. 7: 529, 1948.

51. MITCHELL, P. H. A Testbook of Biochemistry, Second Edition, McGraw-Hill Book Company, Incorporated, 1950.

52. NATIONAL RESEARCH COUNCIL, Bul. II, Recommended Nutrient Allowances for Swine, Revised August, 1950. Washington, D.C.

53. NEAL, A. L., and F. M. STRONG. Microbiological determination of pantothenic acid. (Further Studies) Ind. Eng. Chem., Anal. Ed. 15: 654, 1943.

54. NEILANDS, J. B., H. HIGGINS, T. E. KING, R. E. HANDSCHUMACHER and F. M. STRONG. Concentration of bound pantothenic acid. J. Biol. Chem. 185: 335, 1950.

55. NEILANDS, J. B. and F. M. STRONG. The enzymatic liberation of pantothenic acid. Arch. Biochem. 19: 287, 1948.

56. NOVELLI, G. D., N. O. KAPLAN and F. LIPMANN. The liberation of pantothenic acid from coenzyme A. J. Biol. Chem. 177: 97, 1949.

57. NOVELLI, G. D. and F. LIPMANN. Bacterial conversion of pantothenic acid into coenzyme A (acetylation) and its relation to pyruvic oxidation. Arch. Biochem. 14: 23, 1947.

58. OLSON, R. E. and N. O. KAPLAN. The effect of pantothenic acid deficiency upon the coenzyme A content and pyruvate utilization of rat and duck tissues. J. Biol. Chem. 175: 515, 1948.

59. PEARSON, P. B. and A. L. DARNELL. The thiamine, riboflavin, niacin and pantothenic acid content of the colostrum and milk of the cow and ewe. J. Nutr. 31: 51, 1946.

60. PEARSON, P. B. and H. SCHMIDT. Pantothenic acid studies with the horse. J. An. Sci. 7: 78, 1948.

61. PENNINGTON, E., E. E. SNELL and R. J. WILLIAMS. An assay method for pantothenic acid. J. Biol. Chem. 135: 213, 1940.

62. PILGRIM, F. J., A. E. AXELROD and C. A. ELVEHJEM. The metabolism of pyruvate by liver from pantothenic acid and biotin deficient rats. J. Biol. Chem. 145: 237, 1942.

63. RAM, T. A histopathologic study of chicks deficient in pantothenic acid. Poult. Sci. 28: 425, 1949.

64. RAY, S. N., W. C. WEIR, A. L. POPE and P. H. PHILLIPS. Studies on the concentration of some B-vitamins in the blood of normal and cobalt deficient sheep. J. Nutr. 34: 595, 1947.

65. RIESEN, W., D. R. CLANDININ, C. A. ELVEHJEM and W. W. CRAVENS. Liberation of essential amino acids from raw, properly heated and overheated soybean oil meal. J. Biol. Chem. 167: 143, 1947.

66. RINGROSE, A. T., L. C. NORRIS and G. F. HEUSER. The occurrence of a pellagra-like syndrome in chicks. Poult. Sci. 10: 166, 1931.

67. SAUBERLICH, H. E. and C. A. BAUMANN. Effect of dietary protein upon amino acid excretion by rats and mice. J. Biol. Chem. 166: 417, 1946.

68. SHAW, J. H. and P. H. PHILLIPS. Neuropathologic studies of pantothenic acid, biotin and folic acid complex deficiencies in the chick. J. Nutr. 29: 107, 1945.

69. SKEGGS, H. R. and L. D. WRIGHT. The use of Lactobacillus arabinosus in the microbiological determination of pantothenic acid. J. Biol. Chem. 156: 21, 1944.

70. SNELL, E. E., D. PENNINGTON and R. J. WILLIAMS. The effect of diet on the pantothenic acid content of chick tissues. J. Biol. Chem. 133: 559, 1940.

71. SNELL, E. E. and A. N. RANNEFIELD. Vitamin B_6 group III. The vitamin activity of pyridoxal and pyridoxamine for various organisms. J. Biol. Chem. 157: 475, 1945.

72. STILLER, E. T., S. A. HARRIS, J. FINKELSTEIN, J. C. KERESZTESY and K. FOLKERS. Pantothenic acid VIII. The total synthesis of pure pantothenic acid. J. Am. Chem. Soc. 62: 1785, 1940.

73. STRONG, F. M., R. E. FEENEY and A. EARLE. Microbiological assay for pantothenic acid. Ind. Eng. Chem., Anal. Ed. 13: 566, 1941.

74. SUPPLEE, G. C., R. C. BENDER and O. J. KAHLENBERG. Interrelated vitamin requirements: kidney damage, adrenal hemorrhage and cardiac failure correlated with inadequacy of pantothenic acid. Endocrin. 30: 355, 1942.

75. UNNA, K. Pantothenic acid requirement of the rat. J. Nutr. 20: 565, 1940.

76. WIESE, A. C., W. P. LEHRER, Jr., P. R. MOORE, O. F. PAHNISH and W. V. HARTWELL. Pantothenic acid deficiency in baby pigs. J. An. Sci. 10: 80, 1951.

77. WILLIAMS, R. J., C. M. LYMAN, G. H. GOODYEAR, J. H. TRUESDAIL and D. HOLADAY. Pantothenic acid, a growth determinant. J. Am. Chem. Soc. 55: 2912, 1933.

78. WILLIAMS, R. J. and J. H. TRUESDAIL. The use of fractional electrolysis in the fractionation of the "bios" of Wildiers. J. Am. Chem. Soc. 53: 4171, 1931.

79. WOODS, R. Bordens' Review of Nutrition Research 8(2): 1947.

80. WOOLLEY, D. W., H. A. WAISMAN and C. A. ELVEHJEM. Studies on the structure of the chick antidermatitis factor. J. Biol. Chem. 129: 673, 1939.

81. WOOLLEY, D. W., H. A. WAISMAN and C. A. ELVEHJEM. Nature and partial synthesis of the chick antidermatitis factor. J. Am. Chem. Soc. 61: 977, 1939.

82. WRIGHT, L. D. The state of pantothenic acid in the blood. J. Biol. Chem. 147: 261, 1943.

83. YACOWITZ, H., L. C. NORRIS and G. F. HEUSER. Evidence for an interrelationship between vitamin B_{12} and pantothenic acid. J. Biol. Chem. 192: 141, 1951.

APPENDIX

Procedures Used in the Microbiological Assay for Pantothenic Acid

Preparation of Samples

Feedstuffs:

The procedure used for the enzymatic hydrolysis of feedstuffs was similar to that of Ives and Strong (35). The same general procedure was followed for the water hydrolysis, omitting the digestion with the enzyme.

Enzymatic hydrolysis

An amount of the finely divided sample estimated to contain between three and ten γ of pantothenic acid was suspended in 50 ml. of distilled water. The suspension was adjusted to pH 6.8 to 7.0 and autoclaved for 15 minutes at 121° C. After the mixture had reached room temperature, an amount of mylase P equivalent to 1/10 of the estimated dry weight of the sample was added to the flask. The contents were buffered with two ml. of 2.5 M sodium acetate, and the pH lowered to 4.8 with 1 N hydrochloric acid. The mixture was then layered with toluene, covered and incubated for 24 hours at 50° C. At the end of the incubation period the volume of the digest was increased to 200 ml. with distilled water, and it was filtered through a fluted Whatman No. 12 filter paper, several filterings being used when necessary, until a clear filtrate was obtained. A 50 ml. portion of this filtrate was then neutralized, and further diluted to a convenient concentration for assay.

Water hydrolysis

As in the case of enzymatic hydrolysis, an amount of the finely

divided sample estimated to contain between three and ten γ of pantothenic acid was suspended in 50 ml. of distilled water. The suspension was adjusted to pH 6.8 to 7.0 and autoclaved for 15 minutes at 121° C. After the mixture had reached room temperature, the contents were buffered with two ml. of 2.5 M sodium acetate, and the pH lowered to 4.8 with 1 N hydrochloric acid. The volume of the digest was then increased to 200 ml. with distilled water, and it was filtered through a fluted Whatman no. 12 filter paper, repeatedly when necessary, until a clear filtrate was obtained. A 50 ml. portion of this filtrate was then neutralized and diluted to convenient concentration for assay.

Blood and Milk:

The procedure used for the preparation of blood and milk samples was based on that described by Ray et al. (64). Several modifications were made to adapt the procedure to the requirements of this assay.

Enzymatic hydrolysis

A two ml. sample of blood was measured into a 50 ml. Erlenmeyer flask, and to it was added eight ml. of a 0.25 M sodium acetate solution (at pH 4.5) and ten ml. of distilled water. The mixture was then autoclaved for 15 minutes at 121° C. After the mixture had reached room temperature, an amount of mylase P equivalent to 1/10 of the estimated dry weight of the sample was added to the flask, and the mixture, after layering with toluene, was incubated at 50° C for 24 hours. At the end of the incubation period, the mixture was filtered through a fluted Whatman No. 12 filter paper, several filterings being used when necessary, until a clear filtrate was obtained. Following neutralization a five ml. aliquot was taken and extracted in a 125 ml. separatory funnel with two - ten ml. portions of ether, after which it was diluted to a convenient concentration

for assay.

The ether extraction, it was found, eliminated the "drifting" which was observed to give considerable trouble when ether extraction was not carried out. It should also be noted that in all cases where milk samples were being prepared, a ten ml. aliquot was taken for ether extraction and subsequent dilution. In the latter case 20 ml. portions of ether were used in the extractions.

Water hydrolysis

As described under enzyme hydrolysis, a two ml. sample of blood was measured into a 50 ml. Erlenmeyer flask, and to it was added eight ml. of a 0.25 M sodium acetate solution (at pH 4.5) and ten ml. of distilled water. The mixture was then autoclaved for 15 minutes at 121° C. After it had reached room temperature, the mixture was filtered through a fluted Whatman no. 12 filter paper, repeatedly when necessary, until a clear filtrate was obtained. Following neutralization, a five ml. aliquot was extracted in a 125 ml. separatory funnel with two - ten ml. portions of ether, after which it was diluted to convenient concentration for assay.

Details on the Method of Pantothenic Acid Assay

The medium used, and the assay technique followed were similar to that employed by Riesen et al. (65).

Preparation of Inoculum

The organism L. arabinosus 17-5 was used throughout, and was maintained by transferring every two weeks to fresh stabs, incubating for 24 hours and then storing in the refrigerator.

To prepare inoculum the bacteria were transferred from stab to broth culture and incubated for 24 hours at 37° C. The liquid cultures were then centrifuged, and the supernatant liquid decanted. The bacteria were washed and recentrifuged with three - five ml. portions of sterile physiological saline before being resuspended in a 20 ml. portion of the saline. A further dilution of the suspension was made before inoculation, which was carried out using a small hypodermic syringe, in order that blanks be kept at a minimum. Aseptic technique was used throughout.

The broth cultures and stabs contained the ingredients in the amounts given in Table 1.

Table 1

Composition of Liquid and Solid Broth Culture Media

Ingredient	Liquid	Solid
Salts B solution	0.5 ml.	0.5 ml.
K_2HPO_4	0.5 ml.	0.5 ml.
Vitamin solution (including calcium pantothenate)	2.0 ml.	2.0 ml.
Glucose	2.0 gm.	2.0 gm.
Tryptone	1.0 gm.	1.0 gm.
Yeast extract	0.5 gm.	0.5 gm.
Sodium acetate	1.0 gm.	1.0 gm.
Agar	-	1.5 gm.

Make up to 100 ml. with water and tube.

Basal Medium

As has been previously stated, the composition of the basal medium used in the assay was similar to that used by Riesen et al. (65)

As in their method:

(1) The amino acids were included at the maximum levels recommended by the Wisconsin Board.

(2) Xanthine was included in the medium.

(3) Pyridoxamine was used exclusively in place of pyridoxine, as it has been reported by Snell and Rannefield (71) that it is doubtful if lactic acid bacteria can utilize unchanged pyridoxine.

(4) Choline and inositol were omitted.

The medium differed from that used by Riesen et al. (65) in that proline was included in the medium.

Composition of Basal Medium

The composition of stock solutions for the basal medium is indicated below:

1. Salts A.

KH_2PO_4	-	10 gm.
K_2HPO_4	-	10 gm.

Make up to 100 ml. with distilled water.

2. Salts B.

$MgSO_4 \cdot 7H_2O$	-	200 mg.
$FeSO_4 \cdot 7H_2O$	-	10 mg.
$MnSO_4 \cdot 4H_2O$	-	10 mg.
NaCl	-	10 mg.

Make up to 100 ml. with distilled water.

3. Mixture of purines and pyrimidine.

Adenine sulfate.$2H_2O$	-	100 mg.
Guanine.HCl.$2H_2O$	-	100 mg.
Uracil	-	100 mg.

Make up to 100 ml. with water and to get into solution, warm and add one ml. concentrated HCl.

4. Xanthine

Xanthine	-	100 mg.

To 75 ml. warm water add 100 mg. xanthine. Add drop by drop concentrated NH_4OH until dissolved. Cool and make up to 100 ml. with distilled water.

5. Vitamin solution

Thiamine hydrochloride	-	12.5 mg.
Pyridoxamine dihydrochloride	-	5.0 mg.
Riboflavin	-	12.5 mg.
Nicotinic acid	-	25.0 mg.
Para-aminobenzoic acid	-	2.5 mg.
Biotin	-	25 γ
Folic acid	-	250 γ

Pantothenic acid was omitted from the vitamin solution.

To dissolve folic acid add two or three drops of concentrated NH_4OH. To dissolve riboflavin add two ml. of glacial acetic acid. Make up to 250 ml. with distilled water and warm to insure that all vitamins are in solution.

6. Amino acid solution

Table 2 shows the amount of each amino acid required for 120 ml. of a complete stock solution.

Table 2

Composition of Amino Acid Solution

Amino acid	Weight/120 ml. gm.
l(-) leucine	0.5
dl(-) isoleucine	1.0
dl(-) valine	1.0
l(-) cystine	1.0
dl(-) methionine	1.0
dl(-) tryptophane	1.0
l(-) tyrosine	0.5
dl(-) phenylalanine	1.0
l(+) glutamic acid	2.0
dl(-) threonine	1.0
dl alanine	1.0
dl asparagine	2.0
dl lysine HCl	2.0
l(+) arginine HCl	0.5
l(+) histidine $HCl.H_2O$	0.5
dl serine	1.0
l(-) proline	0.25
glycine	0.5

Dissolve in a small amount of water by heating and adding about 10 ml. concentrated HCl. Make up to 120 ml. with distilled water.

The basal medium was prepared from these stock solutions which were stored in the refrigerator. The amounts of each of the constituents used to make up sufficient basal medium for 100 assay tubes are shown in Table 4.

Table 4

Quantities of Stock Solutions used to Prepare Basal Medium Sufficient for a 100 Test Tube Assay

Constituent	Amount Used
Dextrose	4 gm.
Sodium acetate	4 gm.
Salts A	1 ml.
Salts B	1 ml.
Mixture of purines and pyrimidines	2 ml.
Xanthine	2 ml.
Vitamin solution	3 ml.
Amino acid solution	5 ml.

Neutralize to brom thymol blue and make up to 100 ml. with distilled water.

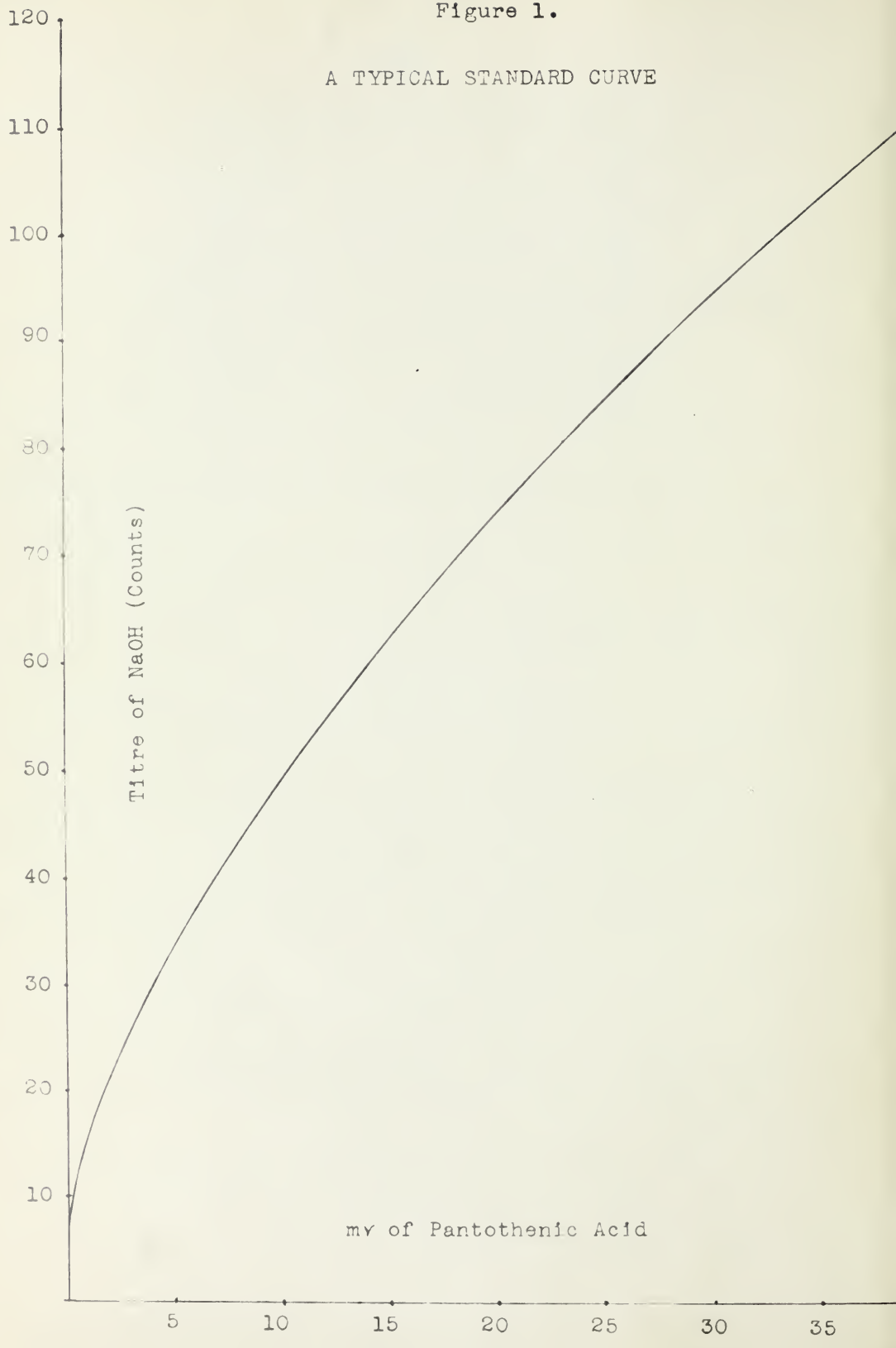
Preparation of Standards

A stock solution of calcium pantothenate containing 100 γ per ml. was made up in 0.05 M phosphate buffer at pH 6.8 to 7.0. The buffer solution consisted of 6.8 gm. KH_2PO_4 and 7.1 gm. Na_2HPO_4, dissolved in distilled water and made up to 1000 ml. This solution was diluted as required to give an assay range of from 0 to 40 mγ.

Assays were conducted on a semimicro scale in which the final volume was two ml. (67). Water and medium were added with a Cannon automatic dispensor (11) to 20 x 150 mm. bacteriological test tubes in racks provided with metal covers. The samples and standards were pipetted in by hand using a one ml. graduated pipette. Levels of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml. were assayed in each case. Water was then added to make up to one ml. and one ml. of basal medium was added to make the final volume of two ml. The tubes were then autoclaved for 12 minutes at 15

Figure 1.

A TYPICAL STANDARD CURVE



lb. pressure, cooled, inoculated and incubated at 37° C. for 72 hours.

The acid produced by the bacteria was then titrated with 0.1 N NaOH, using an automatic titrator (11) and brom thymol blue indicator.

Standard curves, a typical one of which is illustrated in Figure 1 were plotted, and from them the amount of pantothenic acid present in each sample was determined.