## Stations Circular No. 134

February 1951

639.73 V27mc ma. 134

C

\*

C

## EFFECT OF CERTAIN B-COMPLEX VITAMINS ON GESTATION AND LACTATION IN SWINE

M. E. Ensminger, R. W. Colby, and T. J. Cunha



Washington Agricultural Experiment Stations Institute of Agricultural Sciences The State College of Washington Pullman

## EFFECT OF CERTAIN B-COMPLEX VITAMINS ON GESTATION AND LACTATION IN SWINE<sup>1,2</sup>

M.E. Ensminger, R. W. Colby, and T. J. Cunha

#### INTRODUCTION

The high percentage of reproduction and lactation failures in sows, and the high mortality of small pigs, are problems of major concern to the hog producer. To help solve these problems, a great deal of research has been conducted. In addition to the many beneficial contributions to the livestock industry, many of the research findings from work with monogastric animals are applicable to human nutritional problems because of the similarity in the digestive tracts and size of body.

The Livestock Advisory Committee of the United States Department of Agriculture (33)\* recognized the magnitude of the pig-mortality problem and recommended investigation of the causes of death of young pigs. The Committee specifically proposed that such research should include nutrition projects directed toward the artificial production of deficiency symptoms in both the sow and litter. The Committee reported that an estimated 33 per cent of the annual pig crop dies between the farrowing and weaning dates, while an additional 10 per cent is lost before marketing age is attained. A part of these losses, the Committee stated, are attributed to infectious diseases or parasitism aggravated, in all probability, by faulty nutrition.

The purpose of the trials reported herein was to study the effects of the addition or deletion of certain B-complex vitamins and unknown factors in the ration of the gestating and lactating sow.

In Part I, the effects of the omission of thiamin, riboflavin, and choline from a purified ration were studied with regard to reproduction and lactation in brood sows.

<sup>1</sup>The assistance of Dean C. Lindley and Wilton H. Heinemann in the 1945 trials, L. K. Bustad and J. P. Bowland in the 1946 trials, R. F. Johnson in the 1947 trials, and H. W. Hodgskiss in the 1947 and 1948 trials is gratefully acknowledged. Dr. D. R. Cordy, College of Veterinary Medicine, performed the post-mortem and microscopic examinations.

<sup>2</sup>This work was made possible through grants from the Research Corporation, New York City, and from Armour and Company, Chicago. Dr. D. F. Green and Merck & Co., Rahway, New Jersey, supplied the crystalline B-vitamins. Dr. T. H. Jukes and Lederle Laboratories, Rockefeller Plaza, New York City, supplied folic acid. The vitamin A and D oil (Oladal) was obtained through Dr. E. B. Carter, Abbott Laboratories, Inc., North Chicago, Illinois.

\*Numbers in parentheses refer to "Literature Cited," p. 31.

In Part II, the effects of the addition of folic acid; a combination of folic acid, biotin, inositol, and para-aminobenzoic acid; and fish meal and alfalfa meal were studied. These were added to a purified ration containing all the other known dietary essentials for the pig.

In Part III, the effects of the omission of pantothenic acid and nicotinic acid from a purified ration were studied with regard to reproduction and lactation in brood sows.

## REVIEW OF LITERATURE

Adequacy of the ration fed swine during growth is very important, since it definitely affects the results obtained later during reproduction and lactation (13, 14). Research has shown the importance of adequate nutrition during gestation as a factor in promoting successful lactation (20, 28, 29). Other work (18,19) has shown that, after weaning, pigs from sows fed more nearly adequate rations during lactation manifested a residual effect of the higher nutritional regime. Investigations in recent years have added greatly to the knowledge of the nutritive requirements of the pig. However, the specific effects of certain B-complex vitamin deficiencies on reproduction and lactation by the sow have not definitely been established. A lack of some of these vitamins, or combinations of them, in the ration of the sow undoubtedly is responsible for a good portion of the small pig losses sustained yearly in this country.

Death losses of young pigs present a serious economic problem. Smith (43) estimated that only 60 per cent of the annual pig crop reaches the market.

#### PART I

## THIAMINE, RIBOFLAVIN, AND CHOLINE NEEDS OF SOWS FOR REPRODUCTION (1945-1946)

#### Materials and Methods

1945 tatala L

To the knowledge of the investigators, at the beginning of these trials, no work was reported in the scientific literature on the use of purified rations for sows during gestation and lactation. Accordingly, in a preliminary trial beginning in March, 1945, two sows were fed a purified ration supplemented with the six B-complex vitamins (thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, and choline). Later, during the spring and summer of the same year, the project was further enlarged by feeding eight additional sows on the same purified ration during gestation and lactation. However, with two sows in each group, thiamine, riboflavin, and choline were omitted. This study was repeated during the spring and summer of 1946, when eight more sows were used. In the latter trial, littler mates were used in each of the four lots, lessening the effects of genetic variation. In the preliminary trial, in which the adequacy of the basal ration was studied, two 8-month-old Chester White gilts were used. These two gilts were bred to the same Danish Landrace boar.<sup>3</sup> In the subsequent and larger study in 1945--in which either thiamine, riboflavin, or choline were omitted from the ration of groups of two gilts--eight 9-month-old Chester White gilts were bred to another Danish Landrace boar.

In the 1946 study, eight 8-month-old Chester White gilts were bred to still another Landrace boar. This boar was left with the gilts for the duration of two heat periods to try to assure conception. A chemical test of pregnancy in swine (41) was also run.

Before going on the purified ration, all gilts were fed the regular college herd ration of 20 per cent alfalfa meal and 5 per cent tankage, plus wheat, barley, cull peas, wheat bran, and minerals.

The gilts were kept on raised floors during the 1945 trials to guard against coprophagy. Neither they nor their litters showed any such tendency. During subsequent trials, raised floors were not used, but the concrete floors were carefully washed twice daily. As before, no tendency was shown towards coprophagy. Individual feeding stalls (17) were used, each gilt eating in the same stall at every feeding. This procedure was followed to carry out a modification of the "paired-feeding" technique (17, 38). Thus, the feed consumption of gilts 164 and 166 in the earlier pilot trial was kept approximately the same. As certain animals in the subsequent trials lost their appetite or became so erratic in their eating habits as to jeopardize the results of the entire group, the paired-feeding technique was altered.

Composition of the two different purified rations used was:

	Ration "A"	Ration "B"
Casein <sup>4</sup>	26.1%	20.0%
Sucrose	57.7	63.8
Lard	11.0	11.0
Mineral Mix	5.2	5.2
	100.0	100.0

<sup>3</sup>This cross was used simply because the animals were available as part of a crossbreeding program under way at this institution at the time this experiment was initiated.

<sup>4</sup>In the 1945 trials, the casein used was acid-washed, and was obtained through Mr. G. D. Turnbow, Vice President and General Manager, and Dr. Paul F. Sharp, Director of Research, Golden State Co., Ltd., San Francisco, California. In the 1946 trials, vitamin test casein, which was alcohol-extracted, was used, and obtained through General Biochemicals, Inc., Lab. Park, Chagrin Falls, Ohio. A lower amount of protein was used in ration "B," the choline-deficient ration, and in its control, since higher levels of protein would provide excess methionine from which methyl groups might be derived for the synthesis of choline from its precursor. The allotment of the gilts is shown in Table 1. The levels of vitamins fed to the gilts during the three trials are shown in Table 2.

	TABLE 1.	DISTRIBUTION	OF GILTS	IN 1945	AND 1946	TRIALS.
--	----------	--------------	----------	---------	----------	---------

Lot num	nbers		T'otal	*
1945	1946	Ration	no. gilts	Vitamins <sup>®</sup> fed or deleted
A (Pilot trial)	nte ogsår	"A"	2	Pilot trial 6 B- vitamins ** fed
(a (	(a (	"A"***	2	Controls6 B-vitamins fed
I( ( (b	I( (b	"B"****		Controls6 B-vitamins fed
II	II	"A"	w (1 <sup>4</sup> allata	Thiamine deleted from 6 B-vitamins
HOLDER	III	"A"	4	Riboflavin deleted from 6 B-vitamins
IV	IV	"B"	** 4	Choline deleted from 6 B-vitamins

Vitamins A, D, E, and K, fed in all cases.

\*\*Thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, and choline. \*\*\*Contains 26.1 per cent casein. 0

\*\*\*\*Contains 20.0 per cent casein.

In 1946, slight increases were made in vitamins A, D, and E. However, the rate of feeding the B-complex group of vitamins remained the same in both of the larger trials during 1945 and 1946.

The levels of the vitamins fed were in excess of the recommended allowances for growing pigs (26). The sucrose and lard were of commercial table grade. The crystalline B-complex vitamins were made into 20 per cent ethanol solutions and stored in dark amber bottles in a refrigerator. Two days' requirements of all vitamins were added to a small amount of feed on the top of the ration in the trough just before feeding time. This method made struggling with the animals unneccessary.

To protect the ingredients against the development of rancidity, the rations were stored in a refrigerator, and only 2 or 3 days' feed requirements were mixed and on hand at any one time. TABLE 2. VITAMIN SUPPLEMENTS FED TO THE EXPERIMENTAL ANIMALS IN THE 1945 AND 1946 TRIALS, IDENTICAL LOTS I TO IV IN EACH YEAR.

dugble red.	A (Pilot	trial of	ment, gilles	hoors of	t to normalize	. At the ter
Vitamin		1945)	le constructed	945 and 1	946 Trials	bes siveRodl
supple-	During	During	Lot I	Lot II*	Lot III*	Lot IV*
ment bosh	gesta-	lacta-			stalbormmi :	
of Conner	tion	tion	on beatmits	were detr	se analyses	stamine, The
(2).15 13 3	mg.	mg.	mg.	mg.	mg.	mg.
Thiamine	0.22	0.25	0.19	None	0.19	0.19
Riboflavin	0.27	0.32	0.24	0.24	None	0.24
Niacin	1.09	1.25	0.94	0.94	0.94	0.94
Pantothenic						
acid	1.62	1.86	1.40	1.40	1.40	1.40
Pyridoxine	0.27	0.31	0.23	,0.23	0.23	0.23
Choline	23.12	26.66	20.00	20.00	20.00	None

Per sow daily (all lots):

Even so, because of the high cost of purified diets and the limited

	1945 trials		1946 trials	
Vitamin A	20,000 I.U.	(Fed as shark	26,000 I.U.	(Fed as perca-
		liver oil		morph liver
				oil)
Vitamin D	1,500 I.U.	(Viosterol)	3,800 I.U.	(Percamorph
	· · · ·			liver oil)
Vitamin E	150 mg.	(Mixed toco-	155 mg.	(Alpha-toco-
		pherols) <sup>3</sup>		pherol) <sup>4</sup>
Vitamin K	10 mg.	(Menadione) <sup>3</sup>	10 mg.	(Menadione) <sup>4</sup>
Vitamin C			150 mg.	(Ascorbic
				acid) <sup>5</sup>

\*These vitamin levels were fed during gestation and lactation.

<sup>1</sup>Amounts are daily per kg. body weight unless otherwise indicated.

<sup>2</sup>Bio-Vita 10 MA. Natural shark liver blend containing 10,000 I.U. vitamin A per g., supplied by T. R. Curruthers, Bio-products, Inc., Astoria, Oregon.

<sup>3</sup>Supplied by Dr. T. H. Jukes, Lederle Laboratories, Pearl River, New York.

<sup>4</sup>These vitamins and the B-complex vitamins were supplied by Dr. D. F. Green of Merck and Co., Inc., Rahway, New Jersey.

<sup>5</sup>Fed to only one gilt in each group.

¢,

L

A saturated solution of ferrous sulfate plus traces of copper and cobalt was swabbed daily on the udders of the gilts to prevent anemia in the suckling pigs.

At the termination of the experiment, gilts used in 1946 were slaughtered. Riboflavin and thiamin analyses were conducted on samples of heart, liver, kidneys, hams, shoulder, and loin. Samples (whole pig) of the small pigs which died at birth or immediately after were also analyzed for ribo flavin and thiamine. These analyses were determined according to the method of Conner and Straub (5) with modifications by Peterson et al. (39) and Bedford et al. (2).

## Results and Discussion

A summary of the gestation, farrowing, and lactation records of the individual sows is given in Table 3. Summaries of the thiamine and riboflavin analyses of the tissues of the gilts and the baby pigs in the 1946 trials are contained in Tables 4 and 5.

In these trials, only a limited number of animals were used. Thus the results should be considered as indicative rather than conclusive in nature. Even so, because of the high cost of purified diets and the limited data available where such feeds have been used, the comparison will be of value to investigators working on the vitamin needs of sows during reproduction and lactation.

0

Control Gilts - or (10081 Control Gilts

A total of six gilts were used as controls during the 2 years, including the pilot trial and the 1945 and 1946 trials. They received the purified ration supplemented with six B-complex vitamins.

Three of the six gilts placed on the control (or basal) ration did not conceive even though subject to the same breeding procedure as the others.

Apparently, the purified basal ration, when fed over a long period of time, is deficient in a factor or factors required to support satisfactory reproduction. The sow apparently stores this factor (s) for a long time, as evidenced by the normal litters raised to normal weaning weights when the sows were fed the purified ration for only 52 days prior to farrowing. The fact that long-time storage of factors needed for reproduction and lactation occurs in the sow has been conclusively shown by others (13, 14, 29).

Cunha et al. (13), Ross et al. (40) and Krider et al. (30) showed a need of an unknown factor or factors in reproduction and lactation of sows fed natural rations. Data (7, 12, 32) have shown that the addition of biotin, folic acid, para-aminobenzoic acid, and inositol does not help growth or efficiency

The strength of the strength o	Cain	No. of days on	Length of ges-	No. pigs	No. pigs	Av. birth	Wean- ing	No. pigs	Av. weaning	Initial wt. of	Av. daily gains to	Av. daily feed con-	Total wt. gain	Av. daily feed con-
Type of ration	No	hefore	neriod	rowed	dead	wt.,	day s	weaned	WL.,	gints,	lbc	during	or loss in	sumption
	NO.	parturi-	dave	roweu	ueau	ibs.	age		IDS.	IDS.	ibs.	gestation	lbc	loctation
		tion	uays									lbs.	ibs.	lbs.
1945 Trial	31												H	
Control	164	52	114	10	0	2.44	42	8	20.6	368	1.19	5.55	-66	5.29
Control	165	52	112	8	0	2.70	42	6	21.46	324	1.50	4.32	- 65	5.19
Control	434	(75 days l	before end	of norm	nal ge	station)		4		225		3.95		
Control	436	(75 days l	before end	of norm	nal ge	station)				210	13	3.29		
Thiamine-deficient	359	71	103	7	0	2.61	56	5	19.6	272	0.57	2.26	+21	4.63
Thiamine-deficient	403	74	105	9	0	1.98	56	7	19.46	238	0.74	2.22	-13	4.77
Riboflavin-deficient	338	68	98	10	6	1.96		0		260	0.13	1.96		
Riboflavin-deficient	357	71	110	8	5	1.96		0		267	0.04	2.00		
Choline-deficient	351	81	116	7	0	2.34	56	2	22.00	297	0.95	3.25		
Choline-deficient	402	84	114	11	0	2.38	56	9	17.28	229	1.08	3.24		
1046 Tricl		00		-						0 -				
1940 IFIAI	0											in 191		
Control	554	Did not f	farrow	100	1.	2.02	***	area.	1-	307	0	4.15**	Terr	
Control	576	140	114	10	7	2.40	12	1		285	0.99	3.87	+6	4.27
Thiamine-deficient	552	Did not f	farrow							294		3.83**		
Thiamine-deficient	571	.96	118	8	3	2.80		0		312	1.21	3.67		
Riboflavin-deficient	551	85	105	12	12	1.65		0	42	269	0.82	3.51		
Riboflavin-deficient	569			11*	11*	0.83		0.		315	0.45	3.39	1. All the second	
Choline-deficient	567	88	115	3	0	2.56	42	1	21.5	287	0.83	3.79	-1	4.33
Choline-deficient	574	91	114	11	1	2.40	42	1 .	21.0	310	0.88	3.81	- 27	4.66

TABLE 3. GESTATION AND LITTER RECORD.

\*Removed from the uterus when sow was slaughtered 2 weeks after normal date of farrowing.

\*\*Based on 90-day feed consumption.

8

e a

 $\chi_{n}^{(1)}$ 

**\*\*\*Weaned at this age to conserve feed.** 

an and the second second	ter etter strangen		and Typical Strength	Charles Charles and Charles	Compo-	<mark>a la constanti da constanta</mark> A		
Lot	No. animals	Heart	Liver	Kidney	site av. of inter- nal or-	Shoul- der	Loin	Ham
					gans			
			Tha	imine				
Control	2	4.56	2.73	3.08	3.46			7.83
Thaimine				. 1				
deficient	2	1.19	0.58	0.83	0.87	0.56	0.88	0.56
Riboflavin-	1	4.56	3.51	6.29	4.79	3.90	7.14	5.48
deficient	1**	4.56	3.00	3.16	3.57	7.78	12.36	9.07
Choline-								
deficient	2	3.43	1.63	2.89	2.65	5.32	6.39	7.12
			Rib	oflavin				
Control	2	14.37	31.36	20.57	22.10			2.82
Thiamine-				41 ·				
deficient	2	14.13	30.60	21.44	22.06	2.69	• 2.44	7.44
Riboflavin-	1	7.78	13.59	19.49	13.62	1.98	1.62	2.45
deficient	1**	17.61	83.59	30.45	43.88	2.38	4.17	5.68
Choline -								
deficient	2	12.66	20.66	20.79	18.04	2.57	3.21	2.45

5

T.

0

T.

# TABLE 4. AVERAGE THIAMINE\* AND RIBOFLAVIN\* ANALYSESOF GILTS USED IN 1946 TRIALS.

\*Analyses in micrograms per gram.

\*\*Analyses for sow which was turned out to pasture for 3 weeks before slaughter

TABLE 5. AVERAGE THIAMINE\* AND RIBOFLAVIN\* ANALYSESOF BABY PIGS IN 1946 TRIALS.

Lot	No. of pigs	Thaimine	Riboflavin	
Control	3	2.51	2.83	
Thiamine-deficient	2	0.34	2.55	
Riboflavin-deficient	2	2.65	1.08	
Riboflavin-deficient pigs	2	0.71	0.95	
Choleine-deficient	2	2.26	4.27	

\*Composite (whole pig) samples. Analyses in micrograms per gram.

of feed utilization in the young pig fed a purified ration. However, that does not eliminate the possibility that these vitamins may be needed for reproduction and lactation.

#### Thiamine-Deficient Gilts

In the 1945 trials on several occasions during the gestation period the gilts lost their appetites. Only after the injection (subcutaneously) of 35 or 40 milligrams of thiamine for several days did they eat normally. These injections had to be repeated in 8 to 10 days. The gilts had good appetites following parturition. Their litters generally had a weak-legged condition, including buck knees, cow hocks, spraddled hind legs, and cocked rear pasterns.

From the forty-second to the fifty-fourth day after birth, three pigs in each litter were given 9 milligrams of thiamine every second day. No apparent effect on growth or external appearance was obtained. Weaning weights were considerably below normal, and the hair coats and general appearance became increasingly rough toward the end of the suckling period.

In 1946, the gilts weren't given any injections of thiamine if they lost their appetites, but rather the depletion was allowed to run its course. In no case did the gilts go entirely off feed.

Since the young pigs farrowed by these gilts appeared to be in an advanced stage of the thiamine-deficiency symptoms noted in the previous year, greater depletion of thiamine (20 days longer on the purified diet) and a possible lack of another factor or factors (same as shown to be lacking in gilts fed control ration for a long period of time) could account for the complete mortality of the pigs. The failure of the gilts to lose their appetite, as in the previous years, seems inconsistent and cannot be explained from present data.

Post-mortem examination of the small pigs showed hemorrhage of the stomach, yellowish areas in the liver, hyperemia of the kidneys and lymphatic system, and petechial hemorrhages of the heart. On microscopic examination, kidneys showed hyperemia and hemorrhage, and the liver cells showed a pale cytoplasm with no obvious droplets or granules.

Tissue analyses showed that gilts on the thiamine-deficient ration were very low in stored thiamine. Low thiamine storage also occurred in their young pigs. The average thiamine content of the ham samples (biceps femoris) of these gilts was 0.56 microgram per gram, compared with 7.83 micrograms per gram for the control gilts. Heinemann et al. (23) obtained values of 1.64 micrograms of thiamine per gram of ham tissue (biceps femoris) in young thiamine-deficient pigs. There appeared to be no change in the riboflavin content of the gilts or small pigs compared with the content of this vitamin in the control of sows and litters.

## **Riboflavin-Deficient Gilts**

The riboflavin-deficient gilts in both trials developed almost a complete loss of appetite on numerous occasions after 38 to 54 days on the deficient ration. Large doses (35 to 40 milligrams) of thiamine were injected subcutaneously during these periods of appetite loss with apparent beneficial results. Gilts 551 and 569 in the 1946 trials were brought back on feed after a partial loss of appetite by injections of 35 to 40 milligrams of thiamine for 2 or 3 days, after which their appetite remained normal for several days. Similar treatment on gilts 338 and 357 in the 1945 trials produced the same result. In other words, the gilts fed the riboflavin-deficient ration seemed to require more thiamine in the ration than did any of the other groups of sows.

With the riboflavin-deficient gilts, no pigs born alive survived more than 20 to 48 hours. The longer the period of depletion, the more severe the deficiency symptoms became.

Two of the pigs in the litter of gilt 338 had enlarged forelegs (due to gelatinous edema in the connective tissue). Both were stillborn. One had a severe umbilical hernia but lived for 48 hours. The other pigs in this litter appeared normal.

The litter of gilt 357 appeared normal at birth, but all were too weak to suckle. Sow 551 gave birth to twelve hairless, dead pigs. Seven pigs had enlarged forelegs. None of these latter seven showed enlargement of the bone.

1

The enlargement of the forelegs in these 1945 and 1946 trials resembles that described in pigs by Warwick, Chapman, and Ross (45). They suggested that it is possibly due to inheritance. However, it is difficult to attribute this condition to inheritance in this experiment. All gilts in a given year's experiment were bred to the same boar. In both years' work, only the riboflavindeficient gilts had pigs with enlarged front legs. In the 1946 trials, litter mate gilts were included in the four lots. Again, only gilts on the riboflavin-deficient ration had pigs with enlarged front legs. Some pigs showed a generalized edema (Fig. 1). On post-mortem, all pigs showed some of the following: pale kidneys, mottled with congested areas and yellow granular accumulations; yellow friable livers; red areas in the stomach; and excess body fluid. On microscopic examination, the following were observed: hemorrhage and marked hyperemia of the kidneys, hemorrhage and edema in the lymph nodes, numerous coarse granules and cytoplasm of the liver cells, and marked edema of the subcutis of the legs.

The litter removed from the uterus of sow 569 14 days after her normal farrowing date was also hairless. This was apparently not due to their stage of development. The fetuses appeared to have died at about the normal time for parturition and had begun to atrophy. Two pigs showed edema of the front legs, and one showed generalized edema.



Fig. 1. Three pictures on the left are from litters of riboflavindeficient sows. Upper left, litter from riboflavin-deficient sow 551--note enlarged front legs and generalized edema in some pigs. All pigs were born hairless. Center left, three pigs from riboflavin-deficient sow 338--note enlargement of legs in two pigs, compared with normal pig. No pigs were hairless. Lower left, litter from riboflavindeficient sow 569. This litter was in the process of resorption. All pigs were hairless. The pictures on the right are of pigs representing leg weaknesses obtained with sows on rations where choline or thiamine was omitted.

0

13

It is difficult to explain why hairless pigs were obtained from the riboflavin-deficient sows only during the 1946 trials. The hairlessness would appear not to be due to a lack of iodine, since none of the other sows getting the same source and approximately the same amount of iodine farrowed hairless pigs. The hairlessness might be due to a more severe deficiency of riboflavin, since the sows in 1946 were kept on the riboflavin-deficient ration longer than were the sows in 1945.

The riboflavin-deficient gilts gave birth to pigs very low in riboflavin. The one riboflavin-deficient gilt on which analyses were conducted was considerably below the control gilts in riboflavin storage. The other riboflavindeficient gilt was on pasture 3 weeks between farrowing and slaughter, and thus her analyses are not directly comparable. It is of interest to note that in 3 weeks' time on pasture the riboflavin storage of this gilt rose far above that of the control gilts. There seems to be no definite interrelationship between thiamine and riboflavin in the analyses obtained. In the case of the riboflavin-deficient gilt which began to resorb her litter, the pigs were very low in thiamine, as well as riboflavin. The other litter was normal in thiamine content.

#### Choline-Deficient Gilts

All four of these gilts farrowed at the end of a normal gestation period. With the exception of the weak leg condition apparent in most of their pigs, fairly good litters were obtained from sows 351 and 402. Half of these pigs were given injections of choline every second day from the forty-second to fifty-sixth days. No benefit was derived from these injections insofar as appetite or increased gains in weight were concerned. Their weaning weights were much below normal. Some of them were rough in appearance, becoming increasingly so with age.

In the litters of sows 567 and 574, a shaking of the body and head due to some muscular or nervous incoordination was observed. In the two pigs which lived, this shakiness continued until about the fourth week and then disappeared. Kinked tails were in evidence in both litters. Cunha et al. (13) observed similiar kinked tails in pigs from sows fed a natural ration deficient in certain factors.

Four pigs in the litter of sow 574 had spraddled hind legs. Two pigs in this litter were very sensitive to handling. There was very heavy mortality in both litters, and only one pig in each litter survived past 72 hours. The weaning weights of the two surviving pigs were higher but of no significance, since they were the only pigs suckling each sow. A rough hair coat developed as they became older. These two pigs were carried on a choline-deficient ration for approximately 70 days after weaning (10). They developed a very rough hair coat, and an autopsy showed very fatty livers.

Post-mortem and microscopic examinations of the small pigs farrowed by the choline-deficient gilts showed fatty livers, congestion and petechial hemorrhages, and yellow granular accumulations in the kidneys. Two pigs showed inflamed vulvi.

Analyses of thiamine in the tissues of the choline-deficient gilts showed a somewhat lower amount of thiamine in the internal organs, compared with the control gilts. There was very little difference in the riboflavin content of the tissues. In the young pigs, thiamine was the same as, and riboflavin content was higher than, that of the pigs from the control gilts.

## PART II

## SUPPLEMENTING A PURIFIED RATION FOR BROOD SOWS WITH FOLIC ACID, BIOTIN, PARA-AMINOBENZOIC ACID, FISH MEAL, AND ALFALFA MEAL (1947-1948)

## Materials and Methods

Fifteen Landrace-Chester White crossbred gilts from a crossbreeding program being conducted at this station were used for this study. Prior to being placed on the purified ration, all the gilts had been maintained on the regular herd ration. The average weight of the gilts when started on the experiment was 278 pounds. These gilts were mated to Chester White boars that had been previously proven fertile and were being maintained on the same breeding ration. The gilts were mated during four successive heat periods, if necessary, in an effort to ensure conception.

1

The composition of the purified basal ration, shown in Table 6, is the same as used in Part I of this trial.

berebgered,	Lo	ots 1, 2, and 3	Lot 4	Lot 5	od, this shake
		As an annual an	in post intern	and there are a	TRANS OF THE PARTY
Casein		26.1%	23.2%	22.75%	ig at strat bosh
Sucrose		57.7	45.6	56.05	
Lard		11.0	11.0	11.0	Bour pigs in
Minerals **		5.2	5.2	5.2	
Alfalfa		and <u>La</u> ndreader in	15.0	d ship ying be	
Fish meal		ter	e egic <u>s</u> ichee	5.0	and in the second
					dene vord stere
		100.0%	100.0%	100.0%	e omsoad yod?

TABLE 6. COMPOSITION OF THE RATIONS FED IN 1947-1948.

\*Vitamin test casein, alcohol-extracted, was used; obtained through General Biochemicals, Inc., Lab. Park, Chagrin Falls, Ohio.

\*\*Mineral mix used was the same as shown by Ensminger et al. (16)

The gilts in lots 1, 2, and 3 received this ration, but the amount of casein was altered in the rations containing 15 per cent alfalfa (lot 4) or 5 per cent fish meal (lot 5) to equalize the protein content of all rations. Alfalfa meal and fish meal were used as possible sources of an unknown factor(s). The average feed consumption during gestation was 4.80 pounds daily. During lactation, the feed was limited to 6 pounds daily to secure a more accurate comparison of the different rations. In some instances immediately after farrowing or when the gilts were temporarily off feed, they would not consume this much feed. Usually, however, they would have consumed considerably more.

The basal ration for the control gilts in lot 1 consisted of the purified ration plus vitamins A, D, E, K, and C, and the six B-complex vitamins listed in Table 7. Folic acid was added to the basal ration in lot 2. The gilts in lot 3 received the basal ration, including the six B-complex vitamins, plus folic acid, biotin, inositol, and para-aminobenzoic acid. Alfalfa meal, at a 15 per cent level, was added to the basal ration in lot 4. In lot 5, the gilts were fed herring fish meal at a 5 per cent level. The rate at which the vitamins were fed is shown in Table 7. The vitamins were mixed and fed the same as in Part I. As a precaution against the development of anemia in the suckling pigs, the gilts' udders were swabbed daily with a saturated solution of copper and iron.

Daily per kilogram	ppearance d by othe		
of body weight, all			D 1.11
lots, during gesta-			Per sow, daily,
tion and lactation			all lots
state erent i braseres	mg.	nas ita mana onen aste	Lee lot 2 gale (okyol
Thaimine	0.19	Vitamin A	26,000 I.U.
Riboflavin	0.24	Vitamin D	3,800 I.U.
Nicotinic acid	0.94	Vitamin E	155 mg.
Pyridoxine	0.23	Vitamin K	10.5 mg.
Calcium pantothenate	1.40	Vitamin C	150.0 mg.
Choline	20.00		a obtained when the follow
Per 100 grams of feed		Lot 2	Lot 3
Folic acid		200 micrograms	200 micrograms
Inositol			200 mg.
P-aminobenzoic acid		-attan and south attant	20 mg.
Biotin		and the set whe way of the	30 micrograms

\*Dr. D. F. Green and Merck and Company, Rahway, New Jersey, supplied biotin and other B-complex vitamins. The cooperation of Dr. T. H. Jukes and Lederle Laboratories, Pearl River, New York, in supplying folic acid is acknowledged. The vitamin A and D oil (Oladal) was supplied through E. B. Carter, Abbott Research Laboratories, North Chicago, Illinois.

TABLE 7. VITAMINS ADDED TO THE RATIONS FOR VARIOUS LOTS OF GILTS<sup>\*</sup>

Q2

## Results and Discussion

A summary of the gestation and farrowing data on the individual sows is shown in Table 8. Table 9 shows the lactation data.

This discussion of results is based on preliminary trials with a limited number of animals. These results should not be regarded as definite conclusions but as foundations for further investigations.

Unfortunately, two gilts in the control lot failed to conceive after four heat periods. Examination of the reproductive organs of gilt 501, lot 1, revealed cystic, dialated Fallopian tubes. The ovaries also were cystic but active. Gilt 435, lot 1, appeared normal. Gilt 438, in the alfalfa lot, failed to conceive, due possibly to cystic ovaries. One gilt in the fish meal lot aborted about two-thirds of the way through the gestation period. She was negative to the Bang's test. All remaining gilts conceived and farrowed within normal limits of the 114-day gestation period.

#### Litter Size and Strength of Pigs

#### Controls

The only gilt that farrowed in the control lot had a litter of nine pigs. Three were stillborn and another lived only 27 hours. Autopsy of the four dead pigs showed yellow concretions in the kidney, many bladder and kidney petechial hemorrhages, and a generally underdeveloped appearance. Yellow concretions in the kidneys of small pigs have also been found by others (4, 13, 35).

#### Lot 2

The lot 2 gilts (basal plus folic acid) all farrowed, averaging 10.33 pigs per litter. Only the gilts in the lot receiving alfalfa had a higher percentage of strong pigs.

Allgeier et al. (1), in experiments with rats fed synthetic rations, showed that folic acid given only during pregnancy and lactation has some beneficial effect. However, a more pronounced effect on both reproduction and lactation was obtained when the folic acid supplement was given from weaning through gestation and lactation.

#### Lot 3

The gilts in lot 3 (fed biotin, inositol, para-aminobenzoic acid, and folic acid plus the basal ration with the six B-complex vitamins) did not perform so well as the gilts in lot 2 (basal plus folic acid only), using litter size and strength of pigs as the criteria. Therefore, it appears that folic acid is the

and the second		1		190	2			3			4	14	0,6	5	a w	-
Lot No.	*	Basal	L'ANNA I	Ba	.sal + fol	ic acid	15	Basal +	10 B's	Ba	sal + al	falfa	Basa	l + fisl	h meal	
No. of gilts farrowed		1	0		3	14	17	3			2		3	2		
No. of gilts sterile		2			. 0			0			1			1'	*	
Sow no.	435	414	501	421	436	504	408	437	503	438	482	512	486	484	428	
Days on purified ration before farrowing	151**	80	130**	102	72	110	94	74	98	94**	95	98	88**	109	77	
Initial wt. per gilt	261	255	268	215	267	279	300	225	300	217	240	281	256	255	257	
Av. daily feed consumption	4.91	4.96	4.80	4.49	4.99	5.06	4.90	4.72	4.90	4.27	4.77	4.67	4.78	4.83	4.97	
Av. daily gain until farrowing	1.05	1.73	1.25	1.38	1.67	1.41	1.13	1.38	1.37	1.18	1.14	1.38	1.27	1.53	1.68	1
No. of pigs farrowed per litter	0	9	0	9	9	13	7	8	6	0	8	11	0	7	7	
Av. birth weight of pigs - lbs.	0	2.61	0	2.63	2.89	2.10	3.21	2.70	2.00	0	2.62	2.82	0	2.78	2.60	
% of pigs farrowed strong				88.88	100		100	37.50	16.66		50.00	72.72		28.57	57.14	
medium		44.44		13		30.77		25.00			25.00	27.28			42.86	
weak		11.11	0.51.65			69.23	•1 · · ·	37.50	33. 33	12				71.43		
dead		44.44		11.11	4		•	J	50.00		25.00					
a to the second s																

1 - 6

.

TABLE 8. GESTATION RECORD.

¢ . . . ¢ ¥

2

CI

\*Aborted two-thirds of the way along in gestation.

6

¢ .

. 0

 $q_{k,i}^{(n)})$ 

\*\*Days gilts were fed purified ration, did not farrow.

	1		2			3	and the second second second	4		5	5
	Basal	Basal	+ folic	acid	В	asal + 1	0 B's	Basal +	- alfalfa	Basal -	+ fish meal
No. sow	414	421	436	504	408	437	503	482	512	484	428
No. days on ration											
before farrowing	80	102	72	110	94	74	98	95	98	109	77
Av. no. pigs farrowed								*			
per litter	9	9	9	13	7	8	6	8	11	7	7
Av. no.pigs weaned per											
sow farrowing	5	8	8	4	6	7	2	6	11	2	7
% pigs weaned	55.55	88.88	88.89	69.23	85.75	87.50	33.33	75	100	28.57	100
Av. 21-day wt. per											
pigs (lbs.)	11.68	10.56	11.69	12.19	11.53	13.14	12.25	12.08	11.32	11.50	10.91
Av. 35-day wt. per											
pigs (lbs.)	17.25	15.47	14.39	18.19	16.00	17.54	18.12	19.79	15.88	14.00	15.50
Av. 56-day wt. per											ż
pigs (lbs.)	25.00	23.59	21.37	23.62	21.08	26.75	29.25	29.96	21.34	15.25	24.43
Final wt. of litter minus								,			
wt. loss of sow (lbs.)	93	108.75	113	34	86.50	103.25	78.50	85.75	89.75	55.5	115.75
Av. daily ration fed sow							•				
and litter (lbs.)	5.97	5.86	5.95	5.86	5.91	5.88	5.52	5.92	5.89	5.91	5.54
% pigs dead at birth	33	0	0	38.46	0	0	66.66	12.50	0	71.43	0
Died 1 - 3 days	11.11	11.11	0	30.77	0	12.50	0	12.50	0	0	0
4 - 21 days	0	0	0	0	0	0	0	0	0	0	0
22 - 56 days	0	0	11.11	0	14.25	• 0	0	0	0	0	0
Wt. loss by sow											
during lactation (lbs.)	82	80	58	60.50	40	84	-20	94	145	-25	56

\* \* Y'

AN"

important one of these four vitamins; and that biotin, inositol, and paraaminobenzoic acid do not have to be added to the ration under the conditions of this experiment. This is in line with work by Lindley and Cunha (32) and Cunha et al. (8).

#### Lot 4

Gilts on the basal ration plus alfalfa rated second to those in lot 2 (basal plus folic acid) in the number of pigs farrowed per litter, and first in the percentage of strong pigs farrowed.

Since there was no great difference in the results obtained between lots 2, 3, and 4, and since alfalfa is a good source of folic acid, it is reasonable to assume that alfalfa at a 15 per cent level supplied the folic acid requirement of the sow for reproduction.

It is difficult to determine whether or not alfalfa was supplying some unknown factor or factors for the sow, as has been postulated by Ross et al. (40), and Krider et al. (30). Cunha (6), Spitzer and Phillips (44), Hartman et al. (22), and Bowland et. al. (3) showed that alfalfa contains an unknown factor or factors for the rat. To show a need for the unknown factor or factors in alfalfa, gilts need first to be depleted (15, 16). The fact that these gilts were not previously depleted may account for alfalfa not giving more of a response than that obtained with the basal ration plus folic acid.

Certainly this work indicates that folic acid is needed by the sow for reproduction and lactation. Cunha et al. (9) recently showed that folic acid needs to be added to the ration of the young growing pig for hemopoieses when fed for 22 weeks on the same basal ration used in this sow work.

#### Lot 5

With the exception of the control gilts, gilts on the basal ration plus fish meal (lot 5) had the lowest percentage of strong pigs.

The quality of fish meal varies considerably and has been shown (31) to be a poor source of folic acid, which may account for the low percentage of strong pigs in this lot. Also, recent work (11) has shown that addition of 7.5 per cent herring fish meal was of considerable benefit in improving a basal ration of wheat, barley, cull peas, alfalfa, and minerals for reproduction and lactation with the sow; whereas 5 per cent of the same fish meal was of no benefit. Therefore, it may be that not enough fish meal was used in this trial.

Number and Percentage of Pigs Weaned

The number and percentage of pigs weaned per litter was highest in the litters in the alfalfa lot. This may indicate that alfalfa was supplying some factor or factors that were not supplied by the other rations. Two of the gilts in the folic acid lot weaned a high number and percentage of pigs, but the third gilt (No. 504) did not do so well. The addition of biotin, inositol, paraaminobenzoic acid, and folic acid to the basal ration in lot 3 was of no benefit over folic acid alone, indicating that folic acid is probably the essential vitamin of the four.

Results with gilts in the fish meal lot were highly variable in number and percentage of pigs weaned. The gilt in the control lot weaned five of the seven pigs farrowed.

## Weaning Weights

As shown in Table 9, there was a great deal of variation in the weaning weights of the litters within each lot. This is to be expected, since the number of pigs per litter varied considerably, as did the length of time the gilts were fed the purified ration.

Since the results obtained with the gilts in lot 2 (basal ration plus folic acid) were approximately the same as those obtained in lot 3 (basal ration plus folic acid, biotin, inositol, and para-aminobenzoic acid), it appears that the beneficial effect was derived from folic acid. Bowland et al. (3) showed that rat lactation was improved by adding folic acid to a purified diet.

One sow in the fish meal lot weaned seven pigs averaging 24.43 pounds at 56 days. This compares favorably with the average weaning weights of the other lots. The other sow in lot 5 gave very poor results by weaning only two pigs that averaged 15.25 pounds at 56 days. These poor results can largely be attributed to the poor lactation of the sow, especially during the last month of lactation. Gains of the young pigs were nearly the same as those in the other lots for the first 3 weeks. After that they made very slow gains, became thin, and died shortly after weaning. As in the alfalfa lot, a dark exudate developed on their skin. The fact that one of the sows in this lot produced a fair litter and the other a very poor litter may be due partly to individual sow differences, as well as to the fact that the sow producing the poorer results had been on the purified ration 32 days longer than the other sow.

These results may indicate that as the experiment progressed, the body reserves of some known or unknown required dietary essential (s), either not supplied by fish meal or not supplied in sufficient amounts, may have been nearly depleted in the sow on the ration for the longer period of time. Since fish meal is a very poor source of folic acid (31), and since folic acid has shown indications of improving reproduction and lactation, this may have been a factor in influencing the results with the litters from gilts in this lot. Also, the fish meal level may not have been high enough.

The only control lot sow to farrow was on the purified ration for only 80 days, a shorter perod of time than most of the other sows. Therefore, she may have retained some essential factor or factors from her previous ration, and her body stores may not have been depleted in this length of time. Work with natural rations (13, 15) showed that the ration fed gilts during growth definitely affects the results obtained later during reproduction and lactation. Ensminger et al. (16) found that the longer gilts were kept on this purified ration, the poorer the results obtained, due to a depletion of a known or unknown factor or factors. Russell et al. (42) obtained complete reproductive failures with gilts that were grown and maintained on a purified ration plus supplements of the six B-complex vitamins, para-aminobenzoic acid, and 10 grams of dried liver per pig per day for periods up to 469 days. This is in line with results obtained from this trial and that of Ensminger et al. (16), in that the poorest results were obtained from the gilts fed the purified rations the longest. The above data indicate that the sow stores for a considerable time an essential metabolite(s) needed for reproduction and lactation.

#### PART III

## OMISSION OF NICOTINIC AND PANTOTHENIC ACIDS FROM PURIFIED RATIONS FOR BROOD SOWS (1948-1949)

#### Materials and Methods

Fifteen gilts were used. Thirteen were Landrace-Chester White crossbred gilts, the same breeding as those used in Parts I and II. These gilts were divided into three lots of three each, and two lots of two each.

In addition, two purebred Chester White gilts were used, one in each of the two lots with two crossbred gilts. This made three gilts in each of the five lots. One of these lots, using a Chester White and two crossbred gilts, was used as a control. The other was the corresponding deficient lot. This was necessary because of a shortage of crossbred gilts.

Prior to being placed on the purified ration, all of the gilts had been maintained on the regular college herd ration. Average weight of the gilts beginning the experiment was 292 pounds.

The gilts were fed and handled as in Part I. Also, the amounts of vitamins fed were the same as shown in Table 7, except when a vitamin was omitted from the ration. Since biotin, inositol, and para-aminobenzoic acid showed no benefit in the 1947 trials, they were omitted from all of the rations used.

The gilts in lots 1, 3, and 5 received the same purified ration used in Part I, consisting of casein 26.1 per cent, sucrose 57.7 per cent, lard 11 per cent, and minerals 5.2 per cent. In lots 2 and 4, this ration was modified to reduce the protein content by reducing the amount of casein used and replacing it with an equal amount of sucrose. A lower level of casein was used in this ration (nicotinic-acid deficient and its control) since 26.1 per cent casein in the ration furnishes enough tryptophane (a precursor of nicotinic acid) to prevent nicotinic-acid deficiency symptoms from developing, even though this vitamin was omitted from the ration. This ration was made up of casein 18 per cent, sucrose 65.8 per cent, lard 11 per cent, and minerals 5.2 per cent.

The basal ration for the control gilts in lot 1 consisted of the purified ration (26.1 per cent casein) plus vitamins A, D, E, K, and C; folic acid; and the six B-complex vitamins (thiamine, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, and choline). Casein content of the ration was reduced to 18 per cent for lot 2, but the vitamin supplement used was the same as for lot 1.

The gilts in lot 3 (26.1 per cent casein) received the same ration as the lot 1 gilts, except that nicotinic acid was not added to the vitamin supplement. In lot 4, the gilts received the same ration as those in lot 2 (18 per cent casein), except no nicotinic acid. Pantothenic acid was omitted from the vitamin supplement fed to the gilts in lot 5; otherwise, the ration was identical to that used in lot 1.

#### Results and Discussion

A summary of gestation and farrowing data on the individual sows is shown in Table 10. Table 11 shows lactation data.

This discussion of results is based on observations with a limited number of animals on an apparently inadequate basal ration. Therefore, the deficiency symptoms which developed in this trial were undoubtedly complicated deficiencies rather than a single vitamin deficiency.

All gilts in this trial farrowed within normal limits of the 114-day gestation period, with the exception of the gilts on the pantothenic acid-deficient ration.

#### Litter Size and Strength of Pigs

#### Lot 1

Even though the control gilts in lot l appeared normal and farrowed normally, all pigs were either dead and macerating, or died within 36 hours after birth (Fig. 2).

The pigs that were born alive were affected with some type of nervous disorder which caused them to shake continuously until death. An autopsy of these young pigs revealed some excess clear fluid in the peritoneal cavity and pericardial sac. Some of the kidneys from the various pigs showed numerous petechiae, and the lymph nodes were normal to often reddened and prominent. The brain appeared normal in all the pigs examined that were affected with this nervous disorder.



Ċ

ő

C

0.

O

1

Fig. 2. Litter farrowed by control gilt 146. Two were dead at birth and in the process of resorption. Six small, weak pigs died very soon after birth. Two lived for 6 to 8 hours.



Fig. 3. Litter farrowed by gilt 66 on a ration containing no niacin. At birth, seven were in the process of resorption, two were dead but normal in appearance, and two were alive but died within 24 hours.

Lot No.		1 Bas 26.1% c	al asein	11	2 Basal 18% casein		Niacin- 26.1%	3 deficient casein	Niacin 189	4 -deficie % casein	nt I	Pantothe def	5 enic aci icient	d-
No. gilts farrowed		3			3		3		3	•	11	0		11
Sow no.	146	160	152	120	29 80	148	66	149	118	139	25	69	105	75
Days on purified ration before farrowing	82	86	87	86	89 83	85	91	89	90	87	83	88	75	47
Initial wt. per gilt	291	304	260	287	320 233	272	290	268	260	330	320	315	340	286
Av. daily gain until farrowing	1.65	1.35	1.72	1.83	1.35 2.13	1.43	1.10	1.37	1.61	1.66	1.32	0.19	0.66	0.00
No. pigs farrowed per litter	10	10	8	9	99	10	11	4	6	12	9	11*	15*	9*
Av. birth wt. of pigs - lbs.	1.9	2.2	2.2	2.5	2.5 3.5	1.8	1.2	1.4	1.2	2.6	3.2	-3	321	8
% pigs farrowed - strong	**			15- 0	<del>-13</del> * 9					33. 33	100	-== 2	tt.	r
medium	0	<u></u>	2-	55.55	55.55	**	0	0	0	33. 33	50		14	4.
weak	80	90	87.50	22.22	22.22 100	60	18.18			8.33		* .		
dead normal						. ð								
appearing dead				11.11	11.11	20	18.18	50	33.3	8, 33				
macerating	20	10	12.50	11.11	11.11	20	63.63	50	66.6	16.66		100	100	100

#### TABLE 10. GESTATION RECORD.

8

\* Macerating feti found in utero on autopsy.

() . i () () . . ()

2

4

, RY

TABLE II, LACIATION RECOR	<b>FABLE</b>	JE 11.	LACT	<b>TATIOI</b>	NRE	CORD
---------------------------	--------------	--------	------	---------------	-----	------

1				2			3				4			
Lot no.		Basa	1	H	Basal		Niacin	-deficie	ent	Nia	acin-defi	cient		
	26.1% casein			18% casein			26.1% casein			18% casein				
No. sow	146	160	152	120	29	80	148	66	149	118	139	25		
No. days on ration														
before farrowing	82	86	87	86	89	83	85	91	89	90	87	87		
No. pigs farrowed			-											
per litter	10	10	8	9	9	9	10	11	4	6	12	9		
No. pigs weaned per														
sow farrowing	Ó	0	0	5	2	0	0	0	0	0	6	7		
% pigs weaned	0	0	0	55.55	22.22	0	0	0	0	0	50.0	77.77		
Av. 21-day wt per pig				10.9	13.5					981 9 <del>0</del> - 10	8.75	10.1		
Av. 35-day wt.per pig				17.1	32						13.5	15.0		
Av. 56-day wt. per pig				23.2	35						21.3	25.8		
Final wt. of litter														
minus wt. loss of sow				71	70						64	86		
Av. daily ration fed									,					
sow and litter-lbs.	30		DW300	5.96	5.94	588	320	_329	90 <u>0</u>	130_1	5.95	5.98		
% pigs dead at birth	20	10	12.50	22.22	22.22	0	40	81.81	100	100	25	0		
Died 1 - 3 days	80	90	87.50	22.22	33.33	100	60	18.18			8.33	11.11		
4 - 21 days	74	e 1	891 00	-120	22.22				11	15	8 60	70 <del>0</del> - 12		
22 - 56 days											16.66	11.11		
Wt. loss of sow														
during lactation				45	5	**	1001				64	95		
Lot No.														

.

22

-

.8

6 0 6 6 6 6

0 . 0 0 . 0

Although the purified ration used for this trial contained adequate protein and all the known required minerals and vitamins, it alone does not appear to be adequate for satisfactory reproduction and lactation, as shown by previous work at this station by Ensminger et al. (16) with swine, and Bowland et al. (3) in work with rats. In addition to the work reported in this bulletin, Hogan and Anderson (24) and Russell et al. (42) also reported unsatisfactory results when using purified rations for reproduction in swine.

Apparently, there are some unknown dietary essentials which are required for normal reproduction and lactation in swine. Biotin, inositol, and paraaminobenzoic acid were not included in the vitamin supplement for this ration because they have been shown to be of no benefit for the pig (37, 46) and did not appear to lend any support to the purified ration for sows reported in Part I.

Although the gilts in the control lot were fed a ration similar to that reported in Part II, they did not do nearly so well in reproduction and lactation. This variation in results may be due partially to the individual variations in the requirements of the gilts and also to the fact that natural rations vary considerably in their composition from year to year. Since these gilts were maintained on natural rations until the experiment was initiated, they may not have had as much storage of the unknown factor or factors (postulated to be required for satisfactory reproduction and lactation) as gilts in previous trials. Sows have the proven ability to store factors needed for reproduction and lactation for a considerable length of time. (6, 14, 29).

#### Lot 2

All the control gilts in lot 2, receiving 18 per cent casein, farrowed litters heavier than those farrowed by the control gilts receiving 26.1 per cent casein. One litter was affected with a nervous disorder similar to that observed in lot 1, whereas each of the other two litters possessed one macerating and one stillborn pig. However, in no case were the litter records satisfactory.

#### Lot 3

The gilts in lot 3 received the same ration as the control lot receiving 26.1 per cent casein, except that nicotinic acid was not included in the vitamin supplement. All of the pigs farrowed by gilts in lot 3 were either stillborn or dead within 24 hours. None of the gilts or litters in this lot showed nicotinic-acid deficiency. Since this ration contained a rather high percentage of casein, it is highly improbably that lack of nicotinic acid in the ration was a contributing factor to the results obtained. Wintrobe et al. (49) and Luecke et al. (34) were unable to produce marked symptoms of a nicotinic-acid deficiency in pigs when a high protein level ration was used. Therefore, very likely the protein level included in the ration for this trial was sufficient to furnish adequate tryptophane for conversion to nicotinic acid to supply the needs of the gilts.

The gilts in lot 4 received the same ration as the control gilts on the 18 per cent casein ration (lot 2), except that nicotinic acid was omitted from the vitamin supplement. All gilts in this lot farrowed, with a wide variation in results (Table 11). One gilt (No. 118) gave birth to six dead pigs.

Gilt 139 gave birth to twelve pigs--nine alive and three in the process of resorption.

Of the nine pigs born alive, one was very weak and died when about 24 hours old. The remainder of the pigs were medium to strong and appeared normal, except that two of them had scrotal hernias. This probably has no nutritional significance. All of the eight remaining pigs grew normally for almost 3 weeks. At this time, two of them began to grow very slowly, became listless, and the tips of their ears turned a dark red color. They became very thin and weak and died when 45 and 52 days old, respectively. Each one weighed only 10 pounds at the time of death, which was about the same as their weights at 3 weeks of age. An autopsy showed no abnormalities. Six of the pigs in this litter lived through the suckling period of 56 days.

Gilt 25, a Chester White, farrowed a litter of nine apparently normal pigs. All were alive at birth and seven lived through the suckling period. This was the only litter in this year's trial where all the pigs were born alive and at the same time free of the nervous disorder. All this litter was strong at birth. The average birth weight was 3.2 pounds. One of the young pigs was crushed by the sow at 2 days of age and another died at 4 weeks of age. Seven pigs lived through the suckling period of 56 days.

## Lot 5

The three gilts receiving the pantothenic acid-deficient ration, lot 5, developed a loss of appetite after 24 or 36 days on this ration. This was the first deficiency symptom observed; however, this was rather erratic. After refusing all or part of their feed for a few days, they would regain their appetite and consume all of the feed allowed them for 1 to 2 weeks and then go off feed again. This continued for about the first 7 weeks of the experiment, after which they never regained normal appetites. The inanition became progressively worse as the trial progressed, until the gilts were losing weight rapidly when they were removed from the trial in 47 to 88 days. As less feed was consumed, they also drank less water. Although no quantitative measurements were made of the water consumed, a conservative estimate would be that it was about one-half that consumed by the control group.

Gilts 69 and 75 developed a severe diarrhea accompanied by severe rectal hemorrhages in 34 and 44 days, respectively. The diarrhea and hemorrhage became so severe in gilt 75 after 47 days on trial that she was removed and slaughtered for autopsy. The autopsy reports will be discussed later. The other gilts in this lot, 69 and 105, were carried on the experiment until after their normal farrowing dates. The diarrhea of gilt 69 was intermittent, stopping and reoccurring at about 3-week intervals after the initial onset. The first attack occurred after 34 days on the deficient ration. The first attack was also accompanied by rectal hemorrhages which did not appear in the subsequent attacks. The other gilt, No. 105, developed a mild intermittent diarrhea; however, unlike the other two gilts, it did not become severe and was not accompanied by hemorrhages.

The characteristic goose-stepping with the hind legs was first noticed after the gilts had been on the deficient ration for about 2 months. This developed gradually and became more pronounced as the trial progressed. As this gait abnormality became worse, the gilts became listless and greatly restricted their activity. The diarrhea, inanition, listlessness, and goosestepping with the hind legs are very similar to deficiency symptoms observed in younger pigs by others (9, 21, 25, 27, 36, 47, 48).

L



Fig. 4. Gilt 75, fed a pantothenic acid-deficient ration. All gilts on this ration developed a diarrhea, and two also developed severe rectal hemorrhages. Other characteristic pantothenic acid-deficiency symptoms developed also.

In addition to the above pantothenic acid-deficiency symptoms shown by these three gilts, one of them, gilt 75 (Fig. 4), was knuckled over one of her knees, appeared weak in the back, and had a slight increase in body temperature. These gilts also had a dark exudate on the skin, especially prevalent around the eyes.

As mentioned earlier, gilt 75 was slaughtered and autopsied before she had been on the deficient ration for a sufficient time to reach her normal parturition date. The autopsy revealed hemorrhagiconecrotic cecocolitis, gastroenteritis, and catarrhal of stomach and small intestine. The uterine nucosa was pink in color, and nine dead macerating feti were found in the uterine horns. The feti were in an advanced stage of resorption, varied from 2.5 to 3.5 inches long, and were brown in color.

The other two gilts receiving this pantothenic acid-deficient ration remained on the trial until shortly after their normally expected parturition dates. They showed no mammary development or any other external signs of pregnancy and did not farrow. Shortly after their normal gestation periods, they were sacrificed and autopsy examinations made. These two gilts, 69 and 105, showed no evidence then of enteritis or of the rectal hemorrhages which had occurred previously in gilt 69. The digestive and reproductive tracts appeared normal, except for the eleven and fifteen dead and macerating feti found in the uterine horns of gilts 69 and 105, respectively. These feti were similar in size and appearance to those found in gilt 75.

Since these last two gilts were started on the experiment at a later date than gilt 75, and cold weather had set in, the gilts were bedded in wheat straw. They ate considerable quantities of the straw, which may partially explain the fact that their deficiency symptoms did not become so severe as in the gilt that was started earlier and did not have access to straw. It may have been that the ingested straw furnished additional surface area required by bacteria for their growth and reproduction and, hence, intestinal synthesis of vitamins. However, if this is true, there was not enough synthesis to prevent characteristic pantothenic acid-deficiency symptoms from developing; but it may have prevented the symptoms from becoming as severe as in the gilt that did not have access to straw. One other explanation may be that some vitamins were obtained from eating the straw which may have had small amounts of grain or other materials in it. Precautions were taken, however, to remove any visible foreign matter from the straw before it was placed in the pen.

5

Even though all the gilts receiving the pantothenic acid-deficient ration were pregnant, they showed no mammary development or other signs of pregnancy, and they did not farrow. This may indicate a possible interrelationship between pantothenic acid and the production or effect of the preparturition hormones. Some of the gonadotropic hormones responsible for normal maternal- and fetal-placental development may be affected by pantothenic acid.

#### Number and Percentage of Pigs Weaned

As mentioned previously the gilts in lot 1 (basal 26.1 per cent casein) and in lot 3 (nicotinic acid-deficient ration 26.1 per cent casein) either gave birth to dead pigs or all of the pigs died within 36 hours. The gilts in lot 5 (pantothenic acid-deficient ration) did not farrow but rather were resorbing their litters. Of the gilts on the basal ration containing 18 per cent casein (lot 2) two, 120 and 29, raised litters with five and two pigs, respectively, living through the suckling period. Gilt 120 weaned five pigs, or 55.55 per cent of the pigs farrowed.

Gilt 29, lot 2, farrowed nine pigs--seven alive one macerating, and one stillborn. Three died within 24 hours. One pig died 6 days after birth and another died when 7 days old. Two pigs, or 22.22 per cent, in this litter lived through the suckling period.

One of the gilts in lot 4 (18 per cent casein nicotinic acid-deficient ration) farrowed a litter of dead pigs. The other two gilts in this lot, however, weaned a higher number and percentage of pigs than any of the other lots of gilts reported in this year's trial. Gilt 139 weaned six pigs, or 50 per cent of the pigs farrowed. Gilt 25, a Chester White, weaned seven pigs, or 77.77 per cent.

On the basis of present data, it is difficult to explain the fact that the control group receiving 18 per cent casein in the ration produced better results than the control group receiving 26.1 per cent. Since similar results were obtained, however, with similar rations without nicotinic acid supplement, this may indicate that the requirement for the unknown factor or factors may increase when higher levels of protein are incorporated in the rations.

#### Weaning Weights

The small number of pigs weaned in this trial greatly limits the information received. Weaning weights were quite variable, which may partially be explained by the fact that the number of pigs raised by the gilts varied from two to seven. Also, the limited feed allowance of 6 pounds of ration per day for sow and litter would tend to reduce the normal weaning weights of the pigs in the larger litters.

Gilt 120, lot 2 (18 per cent casein) raised five pigs until weaning at 56 days of age. The weaning weights varied from 22 to 26 pounds and averaged 23.2 pounds per pig. These young pigs remained normal throughout the suckling period, except for the development of a dark exudate on the skin. The cause of this exudate has not been determined, but it has been observed by Cunha et al. (9) that a similar mange-like skin condition gradually disappeared when pigs were turned out on pasture. They stated that possibly a lack of some nutrient or nutrients in the ration caused this abnormal skin condition.

Gilt 29, a Chester White in lot 2, lost seven of her nine pigs but raised the remaining two through the suckling period. They appeared normal in every respect and weighed 34 and 36 pounds at weaning time. These pigs were considerably heavier at weaning than the pigs in the other litters, which is to be expected because of the small number in this litter. Gilt 139, lot 4 (nicotinic acid-deficient ration), raised six of her twelve pigs through the suckling period. These young pigs varied in weight from 17 to 27 pounds, or an average of 21.3 pounds. The limited feed intake of 6 pounds daily for the sow and litter will undoubtedly partially account for the slower than normal gains made by the pigs in this litter. An exudate developed on the skin of the gilt and litter, similar to that mentioned previously. Other than the exudate, subnormal weaning weights, and the two scrotal hernias, all of the pigs weaned in this litter appeared normal.

Gilt 25, lot 4 (18 per cent casein, nicotinic acid-deficient ration), raised seven of her nine pigs through the suckling period of 56 days. The average weaning weight of the seven pigs in this litter was 25.8 pounds. Even though this gilt was on the lower protein nicotinic acid-deficient ration, she raised the largest litter; and the pigs from this litter weighed more than the pigs from two other litters with smaller numbers of pigs. In addition, this gilt was the only one in the trial that farrowed a litter with no dead or weak pigs.

These facts indicate that there is quite a variation of either storage or individual requirements for some unknown factor or factors.

## SUMMARY AND CONCLUSIONS

The data reported herein are based on results obtained from preliminary studies with a limited number of animals on a purified ration. Therefore, the results should be considered indicative rather than conclusive.

C?

In all parts of these trials, a basal ration composed of 26.1 per cent casein, 57.7 per cent sucrose, 11 per cent lard, 5.2 per cent minerals, vitamins A, D, E, K, and C, and six B-complex vitamins (thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, and choline) was nutritionally inadequate for satisfactory reproduction and lactation for the sow.

Some factor or factors, either one or more of the other known B-complex vitamins not included in the purified ration used, or some unknown factor(s), is essential for reproduction and lactation in swine. The storage of this factor or factors in the sow must be sufficient to carry her for a long period of time, as evidenced by the good record made by the two control gilts which were fed the purified basal ration (supplemented with the fat soluble vitamins A, D, E, and K, and the six B-complex vitamins) only during the last 52 days of gestation and through a lactation period of 42 days. Complete failure in lactation was obtained when the same ration was fed for 140 days prior to farrowing.

In Part I of these trials, the depletion of any one of the three B-complex vitamins (thiamine, riboflavin, or choline) resulted in unsatisfactory reproduction and lactation. Although these results most likely are all complicated by an incomplete control ration, under the conditions of these experiments the following results were obtained:

- 1. Some loss of appetite, but not consistent;
- 2. Efficient feed utilization obtained during pregnancy;
- 3. Parturition 9 and 11 days prematurely in two cases;
- 4. High birth mortality in litters;
- 5. Weak leg condition in pigs at birth; and
- 6. Unthrifty pigs and subnormal weaning weights.
- (B) Gilts fed ration with no riboflavin added:
  - 1. Erratic or, at times, a complete loss of appetite in pregnant gilts which was restored by injections of thiamine;
  - 2. Poor gains;
  - 3. Parturition 4 to 16 days prematurely;
  - 4. One case of death of fetus in advanced stage with resorption in evidence;
  - 5. All pigs either dead at birth or within 48 hours;
  - 6. Enlarged front legs, due to gelatinous edema in the connective tissue in nine pigs, and generalized edema in many pigs; and
  - 7. Two hairless litters born.
- (C) Gilts fed ration with no choline added:
  - 1. Appetite of sows good;
  - 2. Gilts farrowed on schedule;
  - 3. At birth, many pigs showed weak leg conditions such as those observed on thiamine-deficient ration;
  - 4. Muscular incoordination observed in some pigs;
  - 5. Heavy pig mortality and subnormal weaning weights; and
  - 6. Fatty livers in pigs.

Cases of hernia, kinked tail, enlarged forelegs, and liver and kidney abnormalities occurred in pigs farrowed by various sows receiving the purified rations. The use of litter mate gilts in the 1946 trials and the normal appearance of the pigs from the control gilts seem to eliminate genetic variation as a cause of these abnormalities. The weak leg condition and general weakness at birth of pigs was so prevalent that it appeared to be nutritional. It would seem that much of the variation in severity of abnormalities could be attributed to differences in the length of time the sows were fed the purified ration and, therefore, to variation in the amount of depletion of the factor(s) involved.

The addition of folic acid alone (Part II) to the basal ration appeared to improve reproduction somewhat. Although it also seemed to aid in lactation, the effect was not so pronounced as it was in reproduction.

Results obtained from addition of folic acid, biotin, inositol, and paraaminobenzoic acid to the basal ration were no better than results obtained from addition of folic acid alone. Therefore, indications are that folic acid is the important vitamin of those four, and that biotin, inositol, and paraaminobenzoic acid do not need to be added to a purified ration for sows under the conditions of this trial.

It is difficult to determine whether or not the addition of alfalfa meal to the basal ration was supplying some unknown factor or factors needed by the sow. Alfalfa appeared to have more effect on increasing milk production than any of the other supplements added to the basal ration, using appearance and weaning weights of the litters as criteria. Since alfalfa is a good source of folic acid, good quality alfalfa meal at a 15 per cent level in the ration may supply the sow's folic acid requirements.

t

The results obtained with fish meal were highly variable. More work will have to be done before much can be stated concerning its value. Since folic acid appeared to aid with reproduction and lactation in the lots where it was fed, and since fish meal is a poor source of this vitamin, it is possible that the lack of folic acid in this ration may partially account for the poor results obtained.

In Part III of these trials, folic acid was included along with the other vitamins in the supplement for the basal ration in Part I. This ration was also found to be inadequate for satisfactory reproduction and lactation when two levels (18 per cent and 26.1 per cent) of casein were used in the basal ration.

It was not possible to produce any apparent nicotinic acid deficiency when either 26.1 or 18 per cent casein was included in the ration.

The omission of pantothenic acid produced a pantothenic acid-deficiency syndrome characterized by loss of appetite, reduced water intake, goosestepping with hind legs, diarrhea, and--in two instances--rectal hemorrhages. In one case, this was very severe. Although all of these pantothenic aciddeficient gilts were pregnant, they did not farrow or show any signs of pregnancy. This may indicate some hormone interrelationship. An autopsy of these gilts revealed macerating feti in the uterine horns in all cases. Hemorrhagiconecrotic cecocolitis, gastroenteritis, and catarrhal of the stomach and small intestine were also observed.

#### LITERATURE CITED

- Allgeier, Ann Miriam; Sica, Albert J.; Mirone, Leonora; Panzorella, Frank P.; and Cerecedo, Leopold R. "Studies on Reproduction and Lactation in Rats and Mice on Synthetic Diets." Federation Proceedings. 7:283, 1948.
- Bedford, C. L.; McGregor, M.; and Wilcox, E. G. "National Cooperative Project on Conservation of Nutritive Value of Foods." <u>Washington</u> Progress Notes on Nutrition Research. 1:12, 1944.
- Bowland, J. P.; Ensminger, M. E.; and Cunha, T. J., "Need for an Unidentified Factor or Factors for Growth, Reproduction, and Lactation of Rats Fed Purified Rations." Archives of Biochemistry. 16:257-262, 1948.
- 4. Bustad, L. K.; Ham, W. E.; and Cunha, T. J. "Preliminary Observations on Using a Synthetic Milk for Raising Pigs from Birth." Archives of Biochemistry. 17:249-260, 1948.
- Conner, R. T., and Straub, G. J. "Determination of Thiamine by the Thiochrome Reaction." <u>Industrial and Engineering Chemistry</u> (Anal. Ed.). 13:380, 1941.
- Cunha, T. J. "A Study of Nutritional Deficiencies of Corn-Soybean Oil Meal Rations for Swine and Rats." Unpublished Ph.D. thesis, University of Wisconsin, 1944.
- Cunha, T. J.; Bustad, L. K.; Ham, W. E.; Cordy, D. R.; McCulloch, E. C.; Conner, D. H.; Woods, I. F.; and McGregor, M. A. "Nutritional Significance of Folic Acid, Para-Aminobenzoic Acid and Erythrocyte Maturation Factor for the Growing Pig." Journal of Animal Science. 5:407, 1946.

0

- Cunha, T. J.; Bustad, L. K.; Ham, W. E.; Cordy, D. R.; McCulloch, E. C.; Woods, I. F.; Conner, D. H.; and McGregor, M. A. "Folic Acid, Para-Aminobenzoic Acid and Anti-Pernicious Anemia Liver Extract in Swine Nutrition." Journal of Nutrition. 34: 173-188, 1947.
- Cunah, T. J.; Colby, R. W.; Bustad, L. K.; and Bone, J. F. "The Need for and Interrelationship of Folic Acid, Anti-Pernicious Anemia Liver Extract and Biotin in the Pig." Journal of Nutrition. 36:215-230, 1948.
- Cunha, T. J.; Hart, N. K.; and Ensminger, M. E. "Preliminary Observations on a Choline Deficient Ration for the Growing Pig." Unpublished data, Washington Agricultural Experiment Stations, The State College of Washington, 1946.

- 11. Cunha, T. J.; Hart, N.K.; and Ensminger, M. E. Unpublished data, Washington Agricultrual Experiment Stations, The State College of Washington, 1948.
- 12. Cunha, T. J.; Lindley, D. C.; and Ensminger, M. E. "Biotin Deficiency Syndrome in Pigs Fed Desiccated Egg White." Journal of Animal Science. 5:219, 1946.
- Cunha, T. J.; Ross, O. B.; Phillips, P. H.; and Bohstedt, G. "Further Observations of the Dietary Insufficiency of a Corn-Soybean Ration for Reproduction in Swine." Journal of Animal Science. 3:415-421, 1944.
- 14. Cunha, T. J.; Warwick, E. J.; and Ensminger, M. E. "Nutritional Adequacy of Cull Peas as a Protein Supplement for Growth, Reproduction and Lactation of Swine." Journal of Animal Science. 5:419, 1946.
- 15. Cunha, T. J.; Warwick, E. J.; Ensminger, M. E.; and Hart, N. K. "Cull Peas as a Protein Supplement for Swine Feeding." Journal of Animal Science. 7:117-126, 1948.
- 16. Ensminger, M. E.; Bowland, J. P.; and Cunha, T. J. "Observations on the Thiamine, Riboflavin and Choline Needs of Sows for Reproduction." Journal of Animal Science. 6:409-423, 1947.

125

13

- 17. Ensminger, M. E.; Heinemann, W. W.; Cunha, T. J.; McCulloch, E. C.; McGregor, M. A.; Thorning, W. M.; and Eastlick, H. L. <u>The Relation of Thiamine in the Ration of the Hog to the Amount of Thiamine and Ribo-flavin Deposited in the Tissues</u>. Washington Agricultural Experiment Stations Bulletin 468, 1944.
- Fairbanks, B. W.; Krider, J. L.; and Carroll, W. E. "Distiller's Byproducts in Swine Rations I. Creep-Feeding and Growing-fattening Rations." Journal of Animal Science. 3:39-40, 1944.
- 19. "Distillers' By-products in Swine Rations." Ibid. 4:420-429, 1945.
- 20. "Effect of Diet on Gestation-Lactation Performance of Sows." Ibid. 4:410-419, 1945.
- Follis, R. H., Jr., and Wintrobe, M. M. "A Comparison of the Effect of Pyridoxine and Pantothenic Acid Deficiencies on the Nervous Tissues of Swine." Journal of Experimental Medicine. 81:539-552, 1945.
- 22. Hartman, A. M.; Dryden, L. P.; Likely, G. D.; and Cary, C. A. "An Unidentified Nutrient in Food and Feeds." Journal of Animal Science. 5:404-405, 1946.

- 23. Heinemann, W. W.; Ensminger, M. E.; Cunha, T. J.; and McCulloch, E. C.
  "The Relation of the Amount of Thiamine in the Ration of the Hog to the Thiamine and Riboflavin Content of the Tissue." Journal of Nutrition. 31:107, 1946.
- 24. Hogan, Albert G., and Anderson, Gerald C. "Vitamins Required by Swine for Growth, With Some Observations on Reproduction." Journal of Nutrition. 36:437-449, 1948.
- Hughes, E. H. "The Role of Riboflavin and Other Factors of the Vitamin B-complex in the Nutrition of the Pig." Journal of Nutrition. 17:527-533, 1939.
- 26. Hughes, E. H.; Crampton, E. W.; Ellis, E. R.; and Loeffel, W. J. <u>Recommended Nutrient Allowances for Swine</u>. Report No. 11 by Committee on Animal Nutrition, National Research Council, Washington, D. C., 1944.
- 27. Hughes, E. H. "Pantothenic Acid in the Nutrition of the Pig." Journal of Agricultural Research. 64:185, 1942.
- 28. Krider, J. L.; Fairbanks, B. W.; and Carroll, W. E. "Effect of Diet on Gestation and Lactation Performance." Journal of Animal Science. 3:447, 1944.
- 29. Krider, J. L.; et al. "Sardine Condensed Fish Solubles and Rye Pasture for Sows During Gestation and Lactation." Journal of Animal Science. 5:256-263, 1946.
- Krider, J. L.; et al. "Preliminary Studies on Supplementing a Ration for Brood Sows With Folic Acid, Vitamins, and Vitamin Concentrates." Journal of Animal Science. 5:466-467, 1946.
- Lillie, Robert J., and Briggs, George M. "Biological Assay of Folic Acid Activity in Common Feedstuffs." <u>Poultry Science</u>. 26:289-294, 1947.
- 32. Lindley, D. C., and Cunha, T. J. "Nutritional Significance of Inositol and Biotin for the Pig." Journal of Nutrition. 32:47-59, 1946.
- Livestock Advisory Committee. Research and Marketing Proposals for the Livestock Industry. U.S.D.A. Report 1-50, 1947.

34. Luecke, R. W.; McMillen, W. N.; Thorp, F., Jr.; and Tull, Carolyn. "The Relationship of Nicotinic Acid, Tryptophane, and Protein in the Nutrition of the Pig." Journal of Nutrition. 30:395-412, 1947.

- Madsen, L. L.; Earle, I. P.; Hemmstra, Louis C.; and Miller, Charles O. "Acute Uremia Associated With Uric Acid Infarets in the Kidneys of Baby Pigs." American Journal of Veterinary Research. 5:262-273, 1944.
- 36. McMillen, W. N.; Luecke, R. W.; and Thorp, F., Jr. "Pantothenic Acid Deficiency in Swine on Diets of Natural Feedstuffs." Journal of Animal Science. 7:529, 1948.
- 37. McRoberts, V. F., and Hogan, A. G. "Adequacy of Simplified Diets for the Pigs." Journal of Nutrition. 28:165-174, 1944.
- Mitchell, H. H., and Beadles, J. R. "The Paired-Feeding Method in Nutrition Experiments and Its Application to the Problems of Cystine Deficiencies in Food Proteins." Journal of Nutrition. 2:225-243, 1930.
- 39. Peterson, W. J.; Brady, D. E.; and Shaw, A. O. The Thiamine Content of Pork Loin Muscles and the Retention During Cooking. North Carolina Agricultural Experiment Station, Progress Notes No. V, 1944.
- Ross, O. B.,; Phillips, P. H.; Bohstedt, G.; and Cunha, T. J. "Congenital Malformations, Syndactylism, Talipes, and Paralysis Agetans of Nutritional Origin in Swine." Journal of Animal Science. 3:406-414, 1944.
- Roth, S. Y.; Mayer, D. T.; and Bogart, R. "Pregnancy Diagnosis in Swine by Chemical Test." <u>American Journal of Veterinary Research</u>. 2:436-438, 1941.

1

- 42. Russell, Walter C.; Teeri, Arthur E.; and Unna, Klaus. "Growth and Reproduction of Swine on a Purified Diet." Journal of Nutrition. 35:321-322, 1948.
- 43. Smith, W. W. Pork Production, New York: The Macmillan Company, p. 66, 1937.
- 44. Spitzer, Robert R., and Phillips, Paul H. "Reproduction and Lactation Studied With Rats Fed Natural Rations." Journal of Nutrition. 32:631-639, 1946.
- 45. Warwick, E. J.; Chapman, A. B.; and Ross, Burr. "Some Anomalies in Pigs." Journal of Heredity. 1943.
- 46. Wintrobe, M. M. "Nutritional Requirements of Young Pigs." American Journal of Physiology. 126:375-378, 1939.
- Wintrobe, M. M.; Miller, W. H.; Follis, R. H., Jr.; Stein, H. J.; Mushatt, C.; and Humphreys, S. "Sensory Neuron Degeneration in Pigs. IV. Protection Afforded by Calcium Pantothenate and Pyridoxine." Journal of Nutrition. 24:345-366, 1942.

 Wintrobe, M. M.; Follis, R. H., Jr.; Alcayaga, Paul; Paulson, Moses; and Humphreys, Stewart. Pantothenic Acid Deficiency in Swine. Johns Hopkins Hospital Bulletin. 73:313-341, 1943.

\*

D

O × A ¢

S

G

P

 Wintrobe, M. M.; Stein, H. J.; Follis, R. H., Jr.; and Humphreys, Stewart. "Nicotinic Acid and the Level of Protein Intake in the Nutrition of the Pig." Journal of Nutrition. 30:395-412, 1945.