

THE CALCIUM AND INORGANIC PHOSPHORUS CONTENT OF THE BLOOD SERUM OF SWINE¹

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INTRODUCTION

The object of this study was to ascertain the normal content of calcium and inorganic phosphorus in the blood serum of swine at various ages, and to determine the effect of a high and a low calcium diet on the amount of these elements. Bohstedt and his associates (3)² noted differences in these constituents in the blood of pigs when the calcium : phosphorus ratio varied greatly in the rations fed. Loeffel et al. (14) found wide differences between the maximum and minimum content of these two elements in the blood of the pig, and stated that the blood plasma of animals receiving the direct rays of the sun were higher in these elements than those maintained in the absence of direct sunlight. Hughes and Hart (10) reported an average content in the blood serum of swine of 11.77 mg of calcium and 8.53 mg of inorganic phosphorus in 103 samples taken at random in the California Experiment Station herd. Bethke, Edgington, and Kick (2) showed the serum calcium and inorganic phosphorus of pigs about 200 days of age to be 11.1 and 6.4 mg, respectively (average of lots 4, 5, 6, 7, 8, 9, and 10 of the third experiment). The average of some of the figures reported by Dunlop (6) are serum calcium, 11.32 mg and inorganic phosphorus in whole blood, 6.93 mg (average of 56 determinations of blood taken from hogs weighing from 50 to 200 pounds, in experiment 4, groups 1, 2, 3, and 6). Howland and Kramer (9) collected, summarized, and published much of the early work up to and including that of 1921 on the calcium and inorganic phosphorus content of the serum of plasma of domestic animals. In this summary it was shown that three workers had reported, respectively, the calcium content to be 8.7, 9.6, and 9.7 mg and one had reported the inorganic phosphorus to be 2.3 mg, in 100 cc of the blood serum of the pig.

PROCEDURE

Blood for analysis was secured by cutting off the tip of the tail and collecting from 50 to 60 cc in a small Erlenmeyer flask. It was necessary to kill the very small pigs to obtain a sample large enough for analysis. The blood was allowed to clot, the serum poured off, and then centrifuged. In a few cases where the sample was taken before the pig was allowed to suckle, the blood would not clot; in such cases it was impossible to determine the phosphorus. (It was impossible to obtain a clear solution after the addition of trichloroacetic acid.) The clear serum obtained after centrifuging was used for analysis. Serum calcium was determined by the Clark-Collip (4) modification of the Kramer-Tisdall method and the inorganic phosphorus was ascertained by the method of Fiske and Subbarow (7).

¹ Received for publication May 12, 1935; issued October 1936.

² Reference is made by number (italic) to Literature Cited, p. 278.

NORMAL SERUM CALCIUM AND INORGANIC PHOSPHORUS OF PIGS AT DIFFERENT AGES

Since the literature indicated that the serum calcium and inorganic phosphorus of the very young animals are greater than those of older and mature animals (an exception is found in the case of chickens as reported by Hughes, Titus, and Smits (11), it seemed advisable in attempting to determine the normal blood calcium and inorganic phosphorus of the pig to divide the life span into limited parts or units.

Examples of some of the diets fed, and the calcium and phosphorus content of 100 pounds of the feed mixture, are given in table 1.

TABLE 1.—*Ingredients of the various diets fed and their calcium and phosphorus content*

Composition of diet (pounds)	Calcium	Phosphorus
	<i>Percent</i>	<i>Percent</i>
Barley, 43; rice bran, 30; alfalfa, 20; tankage, 5; salt, 1; CaCO ₃ , 1.....	1.58	0.88
Barley, 88; tankage, 10; salt, 1; CaCO ₃ , 1.....	1.07	.63
Barley, 52; wheat middlings, 15; tankage, 10; linseed meal, 3; rice bran, 15; alfalfa, 5; salt, 1; CaCO ₃ , 1.....	1.28	.78
Barley, 58.5; wheat middlings, 25; wheat bran, 14; salt, 1; CaCO ₃ , 1.5.....	.69	.52
Barley, 93.5; casein, 1.5; salt, 1; CaCO ₃ , 1; cod-liver oil (5-10 cc daily).....	.50	.39

All pigs had free access to direct sunlight, some had access to pasture, and some were fed in dry lot.

While the diets were not all optimum for calcium and phosphorus as suggested by Simmonds (15), Bethke et al. (2), and Dunlop (6), the ratio of calcium to phosphorus in each case was in favor of calcium. Except for the last diet listed, the calcium and phosphorus were greater than the 0.65 g of calcium and 0.40 g of phosphorus in 100 g of the food mixture suggested by Simmonds as optimal for the rat. It contained slightly less phosphorus than that suggested by Bethke and his coworkers (2), but, the amount of vitamin D was optimum.

The serum calcium of 258 samples and the inorganic phosphorus of 232 samples of blood taken from normal pigs of various ages are shown in tables 2 and 3. These data are arranged to show any differences in the mean values for each age group. One determination of calcium in the blood taken from a pig immediately after the umbilical cord was severed is not shown. The serum calcium in this case was 17.32 mg, the highest for any normal pig recorded in these studies.

The range of the calcium for any one age is less than for the inorganic phosphorus. This is shown in the tables and is substantiated by the standard deviation for the means. The average content of serum calcium in these studies was 11.93 mg, which is slightly higher than the figure reported previously by the writer and Hart (10). In that instance only the blood of older pigs was examined, which would account for the difference. The calcium content of the blood of very young pigs (birth to 15 days) is higher than that of older hogs. The mean calcium content for all hogs over 70 days of age (216 determinations) was 11.65 mg, with a standard deviation of 0.69 ± 0.022 . The mean value for pigs 210 to 365 days of age is slightly higher than for pigs younger or older. In attempting to find an explanation for this

it was noted that in several instances blood samples had been taken just previous to or soon after farrowing. An average of 11 determinations of the serum calcium and inorganic phosphorus taken from sows at this stage were 12.94 and 7.62 mg, respectively. The calcium content of the blood is apparently higher and the inorganic phosphorus is lower just before and immediately after farrowing.

TABLE 2.—*Calcium content of the blood serum of normal pigs, the data being presented in the form of a distribution table*

Age of animals (days)	Samples of serum having indicated milligrams of calcium in 100 cc								Mean ¹ calcium in 100 cc	Standard deviation ¹
	9-10	10-11	11-12	12-13	13-14	14-15	15-16	Total samples		
	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Milli-grams</i>	
Birth to 15.....	0	0	0	0	5	9	6	20	14.44	0.62±0.066
15 to 70.....	0	0	6	12	4	0	0	22	12.40	.49±.050
70 to 120.....	1	5	30	10	1	0	0	47	11.64	.61±.043
120 to 210.....	1	7	38	12	0	0	0	58	11.57	.55±.036
210 to 365.....	1	3	28	15	3	0	0	50	11.85	.72±.045
365 to 730.....	0	6	19	8	2	0	0	35	11.72	.68±.055
730+.....	1	5	16	3	1	0	0	26	11.40	.66±.062
Total or average.....	4	26	137	60	16	9	6	258	11.93	.99±.029

¹ From original, not group data.

TABLE 3.—*Inorganic phosphorus content of the blood serum of normal pigs, the data being presented in the form of a distribution table*

Age of animals (days)	Samples of serum having indicated milligrams of inorganic phosphorus in 100 cc								Mean ¹ inorganic phosphorus in 100 cc	Standard deviation ¹
	5-6	6-7	7-8	8-9	9-10	10-11	11-12	Total samples		
	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Milli-grams</i>	
Birth to 15.....	0	0	0	7	2	5	0	14	9.37	0.77±0.098
15 to 70.....	0	4	2	7	3	3	1	20	8.61	1.31±.140
70 to 120.....	1	6	6	13	14	4	0	44	8.51	1.18±.085
120 to 210.....	1	3	7	20	14	3	3	51	8.66	1.20±.075
210 to 365.....	1	4	17	19	4	2	0	47	8.05	.96±.067
365-730.....	1	11	6	7	4	1	0	30	7.63	1.15±.100
730+.....	0	2	14	7	2	1	0	26	7.96	.86±.080
Total or average.....	4	30	52	80	43	19	4	232	8.34	1.19±.035

¹ From original, not group data.

While the inorganic phosphorus varied considerably for any one age, and while the value for very young pigs (birth to 15 days) is higher than for older hogs, the differences in the means are not so great as for the serum calcium. The mean value for inorganic phosphorus without the very young pigs (birth to 15 days) was 8.27 mg. Whether the higher content of serum calcium and inorganic phosphorus in the very young animal is due to the amount of these elements ingested from the milk of the mother, or whether high concentrations are mobilized in utero for the early growth of the body

tissues is not clear. The serum calcium continues to be relatively high during the entire suckling period.

Although calcium in the blood of the mother just before and soon after parturition was higher than normal, the maternal blood was not so high either in this element or in phosphorus as that of the newborn pig.

After the pig has attained an age of about 70 days (weaning time) there seems to be very little change in its serum calcium throughout life (except for periods immediately before and after farrowing). The inorganic phosphorus, which is higher at birth and for a short time thereafter, gradually decreases until maturity.

EFFECT OF A HIGH AND LOW CALCIUM DIET ON SERUM CALCIUM AND INORGANIC PHOSPHORUS OF SWINE

EFFECT OF HIGH CALCIUM DIET

Bohstedt and his coworkers (3) showed that the addition of 2 percent CaCO_3 to a diet of white corn, wheat middlings, linseed meal, and salt resulted in a higher content of serum calcium in pig's blood than did the basal diet. Dunlop (6) reported increased calcium and decreased phosphorus in the serum of pigs fed rations containing 1.62 percent of calcium, with a calcium-phosphorus ratio of 1.0 to 0.34. Bethke et al. (2) found the inorganic phosphorus of swine as low as 4.5 mg in 100 cc of serum when the phosphorus in the ration was from 0.31 to 0.33 percent. Hjort (8) stated that calcium salts administered orally to dogs caused a marked increase in the serum calcium of normal animals. Kramer and Howland (13) showed that increasing the calcium in the diet of rats increased the serum calcium and depressed the phosphorus.

In the studies relating to the effect of a high calcium diet, two methods of administering the calcium were employed: (1) The pigs were fed diets to which calcium carbonate was added; (2) calcium salts in solution or in suspension were administered directly into the pig's stomach, by means of a funnel and rubber tube. The diet fed in the first series of high calcium tests consisted of barley, 55 percent; wheat middlings, 25 percent; wheat bran, 14 percent; and salt, 1 percent, to which was added 5 percent of CaCO_3 . The calcium content of this diet was 2.09 percent and the phosphorus 0.51 percent. The time that elapsed from the beginning of the high-calcium feeding to the taking of the first blood samples ranged from 4 hours to 7 days. A summary of the results is given in tables 4 and 5. A few of the extremely high calcium determinations are not included. These will be discussed briefly later.

The mean calcium content is nearly 3 mg higher and the inorganic phosphorus is about 2.5 mg lower than for normal pigs of the same age. None of the calcium determinations was below 12 mg and none of the inorganic phosphorus determinations was above 8 mg. There is a wide range in the values for serum calcium. Some of the higher ones were from pigs in which calcium salts were administered directly into the stomach.

TABLE 4.—*Calcium content of the blood serum of hogs fed a high calcium diet, the data being presented in the form of a distribution table*

Age of animal (days)	Samples of serum having indicated milligrams of calcium in 100 cc									Mean ¹ calcium in 100 cc	Standard deviation
	12-13	13-14	14-15	15-16	16-17	17-18	18-19	19-20	Total samples		
	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Milli-grams</i>	
70 to 120.....	4	7	0	1	0	0	0	0	0	12	0.75±0.103 1.83±.101
210 to 365.....	11	21	16	12	4	4	3	4	4	75	
Total or average..	15	28	16	13	4	4	3	4	87	14.53	1.80±.092

¹ From original, not group data.TABLE 5.—*Inorganic phosphorus content of the blood serum of hogs fed a high calcium diet, the data being presented in the form of a distribution table*

Age of animal (days)	Samples of serum having indicated milligrams of inorganic phosphorus in 100 cc							Total samples	Mean ¹ inorganic phosphorus in 100 cc	Standard deviation
	3-4	4-5	5-6	6-7	7-8	8-9				
	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Milli-grams</i>		
70 to 120.....	0	0	0	5	5	1	11	7.18	0.69±0.099 1.01±.063	
210 to 365.....	2	19	22	11	4	1	59	5.50		
Total or average...	2	19	22	16	9	2	70	5.77	1.15±.066	

¹ From original, not group data.

To determine the effect of suddenly increasing the calcium content of the ration, a group of six pigs 8 months of age were fed for a short time on a ration of barley, wheat middlings, wheat bran, and 1.5 percent of CaCO₃, and two pigs of the same age similarly bred were fed a normal diet and kept as controls. On April 30, 1932, the CaCO₃ of the experimental animals was increased to 5 percent. The calcium and phosphorus in the ration were, respectively, 2.09 and 0.51 percent. Blood samples were taken and analyzed, as shown in table 6.

A third series of experiments designed to show the effect of administering calcium salts directly into the stomach was carried out. Eight pigs about 3½ months of age were used. Some of them were weaned and some were not. In the morning of July 18, 1932, their average blood calcium and phosphorus were, respectively, 11.97 and 7.3 mg. At 4:30 in the afternoon, 25 g of CaCO₃ in 125 cc of water was administered to four of the pigs. The others were kept in the same pen as controls. At 8:30 a. m., July 19, blood samples were taken and examined. The average for the experimental animals was calcium, 31.68 mg, and phosphorus, 6.85 mg. The average for the control animals was calcium, 12.41 mg, and phosphorus, 7.98 mg. The blood serum calcium of the experimental animals ranged from 27.01 to 34.26 mg. None of the pigs was fed on July 19, the day of sampling. On July 20 at 8:30 a. m. another sample of blood was

taken. The average serum calcium of the experimental animals was 10.97 mg and that of the control animals 11.32 mg. During the 24-hour interim the excess serum calcium had disappeared from the blood.

TABLE 6.—Calcium and inorganic phosphorus in 100 cc of serum of a group of pigs 8 months of age when the calcium in the diet was suddenly increased ¹

Date of sampling	Samples taken	Calcium	Inorganic phosphorus	Date of sampling	Samples taken	Calcium	Inorganic phosphorus
	<i>Number</i>	<i>Milli-grams</i>	<i>Milli-grams</i>		<i>Number</i>	<i>Milli-grams</i>	<i>Milli-grams</i>
May 2.....	4	17.28	5.79	May 19.....	6	14.25	5.61
May 3.....	6	16.35	5.37	May 24.....	6	14.32	5.69
May 4.....	6	16.54	4.39	June 15.....	6	13.52	6.75
May 11.....	6	15.35	4.96				

¹ 2 controls fed a normal diet had an average of 12.40 mg calcium and 7.51 mg phosphorus per 100 cc serum for the same period.

The evidence as reported in table 4 shows definitely that the serum calcium of the pig can be increased by giving a large quantity of calcium in the diet. As measured by the calcium content of the serum, pigs show a rapid response either to feeding a high calcium diet, or to having calcium salts administered directly into the stomach. While the feeding of a high calcium diet causes an immediate rise in the serum calcium and while continuous feeding of such a diet results in the calcium remaining above normal, after a time the calcium appears to decrease slowly. Apparently the body attempts to return the serum calcium and inorganic phosphorus to normal values but is unable to accomplish this as long as a marked excess of calcium is furnished. Kramer and Howland (13) have postulated that a serious high concentration of calcium and phosphorus in the blood is avoided by excretion of the excess of the kidneys, the intestines, or by the deposition of part of these materials as insoluble calcium phosphate in the bones. A single dose of a calcium salt introduced indirectly into the stomach causes a sudden rise, which is followed by a relatively rapid fall in the serum calcium, extending over a period of not more than 24 hours.

That there is a reciprocal relation of the serum calcium and inorganic phosphorus of the blood of the pig under most conditions seems certain. Under normal conditions the blood calcium and inorganic phosphorus fluctuate round a certain mean; but as the calcium content of the diet is raised the serum calcium of the blood increases and at the same time the inorganic phosphorus decreases.

EFFECT OF LOW CALCIUM DIET

As early as 1920 Kramer and Howland (12) showed that the calcium of the blood serum of children affected with tetany was far below that of normal adults, or that of placental blood. Six years later Bohstedt et al. (3) reported that pigs fed diets containing less than 0.10 percent of calcium had lower blood serum calcium than those fed from 0.50 to 0.80 percent. In 1930 Loeffel and his coworkers (14) found that pigs fed yellow corn, soybean-oil meal, blood meal, dried skim milk, and salt, indoors, had lower blood serum calcium and

inorganic phosphorus than those fed the same ration outdoors. Hughes and Hart (10) in 1932 reported a decrease in the serum calcium of two sows fed a ration deficient in calcium. Kramer and Howland (13) showed that when the phosphorus in the diet of rats was above normal (except when an adequate amount of calcium was fed to counteract the effect), or when the calcium concentration was reduced to a minimal value, there resulted a decrease in the calcium and an increase in the organic phosphorus in the serum of the rat. Benjamin and Hess (1) in tests with young rats on a high calcium diet, and Templin and Steenbock (16) in tests with mature rats on a low calcium diet, found that the serum calcium of the animals fed a basal diet was lower than that of animals fed the same diet to which had been added various antirachitic agents such as cod-liver oil, ultraviolet irradiation, and irradiated ergosterol.

The object of the experiments described below was to determine whether rations having an inadequate amount of calcium would effect a change in the calcium and inorganic phosphorus content of the blood serum of pigs of different ages, and whether the physical well-being of the experimental animals would be affected. The rations fed were as follows:

Barley, 99 percent; salt, 1 percent.

Barley, 99 percent; sodium bicarbonate, 1 percent.

Barley, 60 percent; wheat middlings, 25 percent; wheat bran, 14 percent; and salt, 1 percent.

The percentage of calcium and phosphorus in the first two diets was, respectively, 0.091 and 0.385, while the third ration contained 0.095 percent of calcium and 0.53 percent of phosphorus; the ratio of calcium to phosphorus being roughly 1:4.0 and 1:5.5.

TABLE 7.—Calcium content of the blood serum of hogs fed a low calcium diet, the data being presented in the form of a distribution table

Age of animals (days)	Samples of serum having indicated milligrams of calcium in 100 cc										Mean ¹ calcium in 100 cc	Standard deviation
	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	Total samples		
15 to 70.....			1	3	0	4	2	9	1	20	10.21	1.63±0.174
70 to 120.....	1	3	6	2	1	2	6	4	-----	25	8.47	2.17±.207
120 to 210.....				1	2	10	18	15	1	47	10.47	.87±.061
210 to 365.....			1	1	3	2	10	-----	-----	17	9.56	1.09±.125
365 to 730.....			4	4	3	0	4	7	-----	22	9.27	1.92±.196
730+.....	2	13	12	10	9	5	10	6	1	68	7.99	2.05±.118
Total or average.....	3	16	24	21	18	23	50	41	3	199	9.13	2.02±.068

¹ From original, ifot group data.

A summary of the findings in these tests is given in tables 7 and 8. The mean value for serum calcium of 199 determinations of blood serum of pigs of all ages was 9.13 mg; the mean of 182 determinations for inorganic phosphorus was 8.44 mg. The value of calcium in this case was more than 2.50 mg less than the normal serum calcium of pigs of the same ages. The serum calcium of 68 samples of

blood taken from animals 2 years old and older is considerably below the mean. This may be accounted for in part by the fact that several older animals were on a low calcium diet for a long period. Of particular interest is the fact that the inorganic phosphorus of young pigs fed a low calcium diet, some of which were still with their mothers, was much lower than that of older hogs, and was far below the normal for pigs of the same age (p. —). Likewise the inorganic phosphorus for hogs 2 years old or over was higher than the average, while the average of the serum calcium for the same individuals was lower.

TABLE 8.—*Inorganic phosphorus content of the blood serum of hogs fed a low calcium diet, the data being presented in the form of a distribution table*

Age of animal (days)	Samples of serum having indicated milligrams of inorganic phosphorus in 100 cc										Mean ¹ inorganic phosphorus in 100 cc	Standard deviation
	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	Total samples		
	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Milligrams	
15 to 70.....	2	3	8	3	1	1	1	1	1	18	6.54	1.21±0.136
70 to 120.....	1	2	7	5	4	1	1	1	1	22	7.45	1.60±.163
120 to 210.....			5	9	9	8	8	1		40	8.61	1.36±.103
210 to 365.....		1	1	3	5	5	1			16	8.51	1.19±.142
365 to 730.....		1	3	3	11	2	1	1		² 23	8.49	1.79±.178
730+.....			4	11	11	22	10	3	1	³ 63	9.17	1.40±.084
Total or average...	3	7	28	34	41	39	21	6	1	182	8.44	1.66±.059

¹ From original, not group data.

² 1 determination of 14.15 mg included but not shown in the table.

³ 1 determination of 13.55 mg included but not shown in the table.

As would be expected, the serum calcium and the inorganic phosphorus varied within wide limits. Many of the blood samples were taken soon after the animals were placed on calcium-deficient rations, and therefore some of the samples, though only a small proportion, were about normal, whereas in certain extreme cases they were below 5 mg. The same wide variations are found in the inorganic phosphorus; however, except for pigs under 7 months old most of the results are above the average for normal pigs of the same age. The statements concerning wider variations than for normally fed pigs is borne out by the standard deviation from the mean.

When the older animals were low in serum calcium for a long time it was very difficult to get them pregnant, and they often failed to conceive. This condition may have been due to a deficiency of vitamin A or to the combination of an insufficient quantity of this vitamin and calcium in the diet. Of those that did farrow many had weak undersized pigs that failed to grow normally, apparently because of a lack of normal milk production by their mothers.

The records of several individuals fed calcium-deficient diets for long periods follow.

Two sows, born in September 1929 and bred in November 1930, were fed a basal diet consisting of barley, 60 percent; wheat middlings, 25 percent; wheat bran, 14 percent; and salt, 1 percent. Such a diet contains a relatively small amount of calcium but probably enough

phosphorus for normal growth, and physical well-being. It is low in vitamin A. Both sows farrowed in March 1931, and one farrowed again in October 1931. The other was due to farrow in December of the same year but did not. Neither farrowed thereafter, though fertile boars were put into the pens with them. Serum calcium and inorganic phosphorus were first determined before the sows farrowed in March. Since the records of these two sows are very similar, only one (for 7-F-29) is given in detail (table 9).

TABLE 9.—Calcium and inorganic phosphorus in 100 cc of blood serum of sows 7-F-29, 6-F-29, and 32-F-30 while on the diets indicated

SOW 7-F-29¹

Diet	Date of sampling	Calcium	Phosphorus
	1931		
		<i>Milligrams</i>	<i>Milligrams</i>
	Mar. 7	11.35	9.19
	Mar. 16	10.90	8.76
	Apr. 9	11.00	10.10
	June 3	7.55	11.19
	July 24	6.50	8.88
Basal, from Nov. 10, 1930, to Dec. 20, 1931.....	July 31	6.80	8.62
	Oct. 12	6.29	10.37
	Oct. 13	6.50	9.47
	Oct. 15	5.05	9.76
	Nov. 9	5.75	9.70
	Nov. 10	4.75	10.22
	Dec. 19	5.00	9.14
Basal, plus 1.5 percent of CaCO ₃ from Dec. 20, 1931, to Jan. 5, 1932....	Dec. 21	6.35	8.13
	Dec. 23	6.85	8.81
	Dec. 28	7.90	8.83
	1932		
Basal, from Jan. 5-14, 1932.....	Jan. 4	9.18	8.96
	Jan. 7	6.23	10.15
	Jan. 11	5.11	10.94
Basal, plus 1.5 percent of CaCO ₃ from Jan. 14-27, 1932.....	Jan. 15	7.91	10.51
Basal, from Jan. 27 to Feb. 8, 1932 (no sunlight).....	Jan. 25	9.78	7.97
	Feb. 8	6.94	10.30
	Feb. 15	7.05	10.52
Basal, plus 6 drops of viosterol daily from Feb. 8 to Mar. 29, 1932.....	Mar. 1	8.22	10.57
	Mar. 8	8.21	-----
	Mar. 25	8.52	-----
	Apr. 20	10.61	7.77
Basal, from Mar. 29 to Dec. 22, 1932.....	May 15	7.25	8.42
	June 14	6.06	9.20
	July 11	6.55	8.89
	Sept. 13	8.00	9.76
	Dec. 22	8.48	12.55

SOW 6-F-29

	1931		
	Oct. 15	10.10	7.08
	Nov. 9	6.15	9.14
	Nov. 10	4.95	9.52
	Dec. 9	5.55	10.00
Basal, from June 14, 1931, to Feb. 18, 1932 (no sunlight).....	Dec. 19	5.90	8.04
	1932		
	Jan. 11	5.56	9.49
	Feb. 8	7.46	9.14
	Feb. 15	5.72	7.87
	Feb. 18	7.25	² 11.62

¹ This sow farrowed a normal litter of 8 pigs Mar. 15, 1931, that weighed 22 pounds. The pigs were weaned May 15, of the same year.

² The blood sample taken immediately after death.

TABLE 9.—Calcium and inorganic phosphorus in 100 cc of blood serum of sows 7-F-29, 6-F-29, and 32-F-30 while on the diets indicated—Continued

SOW 32-F-30³

Diet	Date of sampling	Calcium	Phosphorus
Normal herd ration.....	1931	Milligrams	Milligrams
	{ July 31		
	{ Dec. 19	11.45	7.15
	1932		
Basal (barley, 60 percent; wheat middlings, 25 percent; wheat bran, 14 percent; and salt, 1 percent) from Dec. 19, 1931, to Oct. 4, 1932 (no sunlight).	{ Apr. 21	12.02	7.19
	{ May 9	7.19	8.91
	{ May 15	6.51	8.29
	{ May 23	6.55	8.00
	{ May 31	7.53	8.29
	{ June 14	7.43	9.41
	{ July 11	6.45	8.12
{ Oct. 4	5.30	9.47	

³ On Apr. 9, 1932, farrowed 10 normal pigs; late in May became very nervous; by June 14 all the pigs had died.

Not until these sows lactated did their blood calcium show a marked reduction below normal. One of them showed a lack of coordination of the hindquarters when the pigs were weaned and for a time was unable to get up alone, but she gradually regained her strength and after 5 days was able to walk again. The serum calcium of sow 7-F-29 became very low in the fall of 1931. The addition of 1.5 percent of CaCO_3 resulted in an immediate but slow rise in the serum calcium. A return to the basal diet caused a decrease in the calcium content of the serum at once. The giving of 6 drops of viosterol daily in the absence of sunlight caused a gradual rise in the serum calcium. A change back to the basal ration, however, resulted in a lowering of the serum calcium during the summer. By late December 1932 both sows were in such bad condition that they were killed. Neither was pregnant.

When the pigs from these sows were weaned in May 1931, they were fed the same basal ration as their mothers, and this diet was continued until August 5. At no time after weaning did the pigs appear normal. Their condition was poor, their hair was dry and curly, and they seemed to have an abnormal desire for water. After August 5, they were divided into two groups. One group was fed the basal diet and the other was fed the basal diet to which was added 1.5 percent of CaCO_3 . Neither group did well, and by September 13 only one pig in each group was alive. The rest of the pigs had died either of pneumonia or what seemed to be necrotic enteritis. The serum calcium and inorganic phosphorus of these pigs were below normal for animals of their age. From September 13, 1931, to January 7, 1932, the two pigs still alive were fed the basal ration plus 1.5 percent of CaCO_3 and each was given 10 cc of cod-liver oil daily. They were finally turned out to pasture, fed a normal diet, and marketed.

The second litter of the other sow of the pair was farrowed October 8, 1931, and the pigs were weak at birth. This sow had little or no milk when the pigs were born; however, on December 12 five of

the seven pigs born were weaned. After weaning they were put on the same basal ration as their mother, and by February 1, 1932, all of them had died. The average serum calcium and inorganic phosphorus of these pigs were: On November 25, 1931, calcium, 7.53 mg and phosphorus, 6.32 mg; on December 12, 1931, calcium, 7.03 mg and phosphorus, 6.40 mg; and on February 10, 1932, calcium, 6.82 mg and phosphorus, 6.25 mg. These pigs, like the others, were far below normal in serum calcium and inorganic phosphorus. The milk of the mother was not tested for calcium or phosphorus, and it is therefore impossible to postulate that the calcium and phosphorus content of the mother's milk was not normal; however, the evidence indicates that such was the case.

The record of a third sow, 6-F-29, shown in table 9, is presented because some of her pigs apparently died of calcium tetany. She farrowed a normal litter in September 1930, and another litter in March 1931. On June 14, 1931, she was put on the same low calcium diet that had been given to sow 7-F-29, and kept away from the direct rays of the sun. On September 18, 1931, she farrowed eight pigs that appeared normal at birth. Like sow 7-F-29, her blood calcium was very low, particularly during the lactation period, and, generally speaking, as her serum calcium decreased her inorganic phosphorus increased. Of the pigs farrowed on September 18 only one was alive by December 21. The rest had died in convulsions. These pigs while apparently still in good condition would act in an abnormal manner, and finally would lie down, thrash about, their muscles would contract, their legs would extend straight out from their body, and death would ensue. Their muscles did not relax after death. The blood calcium of two of these pigs taken after death was, respectively, 4.65 and 7.40 mg in 100 cc of serum. The serum calcium of the latter pig taken 14 days previously was 5.95 mg. On December 8 the one pig that lived after weaning had a serum calcium and inorganic phosphorus content of 6.25 and 11.68 mg respectively. He failed to grow normally and in March 1932 was killed. On post-mortem examination it was found that one of his hip sockets was being resorbed.

The record of sow 32-F-30 is presented in table 9. On October 2, this pregnant sow seemed to be having nervous and muscular difficulties. A note taken from the original record reads: "She trembles all over, gasps for air, grunts loudly, lies down, jumps up suddenly, sits down with front legs stretched in front of her, and appears short of breath." On October 3 her udder developed rapidly, and on the morning of October 4, a blood sample was taken. By afternoon she was unable to rise. The next day she lay on her right side until about 11 a. m., when she began thrashing about and breathing hard. At 1 p. m. she was dead. On post-mortem examination, conducted a few minutes after death, six fetuses were removed and blood samples were taken. The fetuses were outwardly normal and weighed altogether 17.5 pounds. The sow had small infection areas in one lung and a slight necrosis of the small intestine. It is unlikely that either caused death; apparently she died of acute calcium tetany. The average serum calcium and inorganic phosphorus for five of the fetuses were, respectively, 19.58 mg and 17.24 mg.

Since the blood of these unborn pigs was taken after the death of the sow, asphyxiation may have influenced the quantity of inorganic phosphorus present. Collip (5) found that the inorganic phosphorus of asphyxiated fetuses was higher than that of normal fetuses and that asphyxiation caused a rise of about 1 mg in the blood of the dog.

The serum calcium of the mother taken on October 4 was less than one-third that of the fetuses taken the following day. Her inorganic phosphorus was only about one-half that of the fetuses she was carrying.

The following record shows the normal serum calcium of a sow and her litter during lactation. Sow 14-S-31 farrowed a litter of nine normal pigs April 9, 1932. The average of three samples of blood taken May 9, May 23, and June 14, 1932, was for the sow, serum calcium, 11.35 mg and inorganic phosphorus, 7.03 mg; for the pigs, serum calcium, 12.19 mg and the inorganic phosphorus, 7.86 mg.

SUMMARY

The mean serum calcium and inorganic phosphorus of the blood of normal pigs of all ages was found to be 11.93 and 8.34 mg respectively.

The serum calcium and inorganic phosphorus of the young pig was higher than for the older pig or the adult hog.

The serum calcium of the blood of the sow immediately before and after farrowing was higher, and the inorganic phosphorus slightly lower, than the mean for nonpregnant sows of the same age.

The administration of calcium salts directly into the stomach of the pig caused a temporary rise in the serum calcium.

Continuous feeding of a relatively large proportion of calcium carbonate in the diet caused a prolonged rise in the serum calcium.

As the serum calcium increased above the normal there was at the same time usually a decrease in the inorganic phosphorus.

Continuous feeding of a ration with a low calcium content over a long period of time resulted in a marked lowering of serum calcium.

As the serum calcium decreased below the normal there was at the same time a fairly regular increase in the inorganic phosphorus in the serum of mature animals, but in young pigs there was a decrease.

Evidences of calcium tetany were manifested when the serum calcium became very low.

LITERATURE CITED

- (1) BENJAMIN, H. R., and HESS, A. F.
1933. THE FORMS OF THE CALCIUM AND INORGANIC PHOSPHORUS IN HUMAN AND ANIMAL SERA. I. NORMAL, RACHITIC, HYPERCALCEMIA, AND OTHER CONDITIONS. *Jour. Biol. Chem.* 100: 27-55, illus.
- (2) BETHKE, R. M., EDGINGTON, B. H., and KICK, C. H.
1933. EFFECT OF THE CALCIUM-PHOSPHORUS RELATIONSHIP OF THE RATION ON GROWTH AND BONE FORMATION. *Jour. Agr. Research* 47: 331-338. illus.
- (3) BOHSTEDT, G., BETHKE, R. M., ROBISON, W. L., and EDGINGTON, B. H.
1926. MINERAL AND VITAMIN REQUIREMENTS OF PIGS. *Ohio Agr. Expt. Sta. Bull.* 395, pp. 61-229, illus.
- (4) CLARK, E. P., and COLLIP, J. B.
1925. A STUDY OF THE TISDALL METHOD FOR THE DETERMINATION OF BLOOD SERUM CALCIUM WITH A SUGGESTED MODIFICATION. *Jour. Biol. Chem.* 63: 461-464.

- (5) COLLIP, J. B.
1927. SOME EFFECTS OF ASPHYXIA ON BLOOD CHEMISTRY. *Jour. Biol. Chem.* 74 (Amer. Soc. Biol. Chem. Proc. Ann. Meet. 21): xxviii-xxx.
- (6) DUNLOP, G.
1935. THE CALCIUM, PHOSPHORUS AND VITAMIN D REQUIREMENT OF SWINE. *Jour. Agr. Sci. [England]* 25: 22-49, illus.
- (7) FISKE, C. H., and SUBBAROW, Y.
1925. THE COLORIMETRIC DETERMINATION OF PHOSPHORUS. *Jour. Biol. Chem.* 66: 375-400.
- (8) HJORT, A. M.
1925. THE INFLUENCE OF ORALLY ADMINISTERED CALCIUM SALTS ON THE SERUM CALCIUM OF NORMAL AND THYREOPARATHYROPRIVIC DOGS. *Jour. Biol. Chem.* 65: 783-795, illus.
- (9) HOWLAND, J., and KRAMER, B.
1921. CALCIUM AND PHOSPHORUS IN THE SERUM IN RELATION TO RICKETS. *Amer. Jour. Diseases Children* 22: [105]-119.
- (10) HUGHES, E. H., and HART, H.
1932. CALCIUM AND PHOSPHORUS CONTENT OF THE BLOOD OF PIGS. *Amer. Soc. Anim. Prod.* (1931) 24: 274-277, illus.
- (11) HUGHES, J. S., TITUS, R. W., and SMITS, B. L.
1927. THE INCREASE IN CALCIUM IN HENS' BLOOD ACCOMPANYING EGG PRODUCTION. *Science (n. s.)* 65: 264.
- (12) KRAMER, B., and HOWLAND, J.
1920. METHOD FOR DETERMINATION OF CALCIUM IN SMALL QUANTITIES OF BLOOD SERUM. *Jour. Biol. Chem.* 43: 35-42.
- (13) ——— and HOWLAND, J.
1932. FACTORS WHICH DETERMINE THE CONCENTRATION OF CALCIUM AND OF INORGANIC PHOSPHORUS IN THE BLOOD SERUM OF RATS. SECOND PAPER. *Jour. Nutrition* 5: 39-60.
- (14) LOEFFEL, W. J., THALMAN, R. R., OLSON, F. C., and OLSON, F. A.
1931. STUDIES OF RICKETS IN SWINE. *Nebr. Agr. Expt. Sta. Research Bull.* 58, 67 pp., illus.
- (15) SIMMONDS, N.
1924. OBSERVATIONS ON REPRODUCTION AND REARING OF YOUNG BY THE RAT AS INFLUENCED BY DIET. *Amer. Jour. Hyg.* v. 4, Sept. sup., 108 pp.
- (16) TEMPLIN, V. M., and STEENBOCK, H.
1933. VITAMIN D AND THE CONSERVATION OF CALCIUM IN THE DIET. II. THE EFFECT OF VITAMIN D ON CALCIUM CONSERVATION IN ADULT RATS MAINTAINED ON LOW CALCIUM DIETS. *Jour. Biol. Chem.* 100: 209-224.

