8-1931

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## SUMMARY


During the years 1925 to 1929 inclusive, the Nebraska Agricultural Experiment Station carried on a series of four experiments to study the importance of the antirachitic factors in the nutrition of swine. Vitamin D as obtained from cod-liver oil and the radiant-energy factor of sunlight were the sources of the antirachitic factors that received primary consideration. Emphasis was placed upon the study of the symptoms and lesions produced in pigs fed a rickets-producing ration.

Though results of the preliminary experiment, designated as Trial A, were somewhat inconclusive, they provided the foundation upon which the other trials were based. They demonstrated that the susceptibility of pigs to rickets had first to be proved by the actual production of the disease and by the careful study of the resultant lesions before the various phases of the problem could be investigated. Trial A further demonstrated the suitability of the pig as an experimental subject, and that growth was essential to the development of rickets.

In an effort to select a rickets-producing ration for pigs, yellow corn, soybean oil meal, blood meal, powdered skimmilk, and salt were used in Trials I, II, and III. This ration proved to be complete in all respects except the antirachitic factor.

Pigs denied exposure to direct sunlight and fed only the basal ration failed to grow and develop normally. The symptoms of nutritional disturbance were many and varied but similar throughout the three years' work. Although the abnormalities noted were not confined to any one part of the body, the most noticeable signs of rickets were found in the skeleton. The lesions produced in each trial conformed, for the most part, to those noted in small animals and in man, justifying the conclusion that the pigs confined indoors and receiving only the basal ration suffered from rickets.

The first rachitic symptoms appeared from five to eight weeks after the beginning of the experiment. Some pigs were affected sooner and to a much greater degree than others, indicating that nutritional reserve was a factor in determining the reaction of various pigs to the same ration. Age was responsible for differences only in that the older animals were able to withstand the deficiencies of the ration longer than the younger pigs. However, when the rachitic symptoms did appear in the older animals, a very rapid collapse resulted. Those pigs that "went down" invariably lost considerable weight and appeared very sick for several days immediately after going "off their feet". If death did not result at this time the pig would often lie around, unable to get to its feet for a period of several weeks and neither gaining nor losing any weight.

Growth, though necessary for the development of rickets, is controlled to a large extent by rickets, the severity of which shows a direct correlation with retarded growth. Because of the disposition of the pig, accurate body measurements are very difficult to obtain. Growth, however, can be measured quite accurately by gain in weight.

Ante-mortem examination of the pigs proved a valuable aid in diagnosis. However, bone changes other than deformity were not revealed clinically, and therefore post-mortem examination of the carcasses added to the above findings, revealing softened and deformed bones, the "rachitic rosary", joint erosion, fractures, and other characteristic abnormalities. Macroscopic study of the split bone revealed more accurately the exact nature of the lesions. Among the most common macroscopic findings was increased cartilagenous material, widening and separation of the epiphyseal lines, thin and softened bone shell, together with excessive amounts of fibrous or osteoid tissue.

With pigs, as with small animals and man, X-ray studies were of considerable value in interpreting the bone lesions caused by rickets. In addition to substantiating the findings of macroscopic and post-mortem examinations, radiographs demonstrated some bone lesions not revealed by the other studies. The X-ray, therefore, appears to be one of the best methods of identifying rickets in pigs. Macroscopic study of the bones may be dispensed with when the X-ray is available. However,

radiographs should not be relied upon when only the tibiae or ribs are studied, since they are less affected than the humeri. An X-ray picture of the humerus, however, can usually be depended upon to show evidence of bone lesions if any are present.

Without exception in Trials I and II, the antirachitic factors had a profound influence upon the calcium and inorganic phosphorus content of the blood. Sunlight and cod-liver oil in Trial I and sunlight alone in Trial II accounted for a blood calcium-phosphorus product more than twice as high as that shown by pigs receiving only the basal ration. In nearly every case those pigs suffering most from rickets showed the lowest plasma calcium and inorganic phosphorus content. When the rachitic pigs were supplied with the antirachitic factors, healing resulted and the calcium-phosphorus product of the blood rapidly returned to normal. (Trial I, Phase II.) The calcium-phosphorus product for normal pigs approaching maturity approximated that given for man by Howland and Kramer (24). It was noted that the plasma calcium and phosphorus of normal pigs increased from weaning until maturity. Studies made on the alkali reserve of the blood in Trial I indicate that acidosis may or may not accompany rickets and that analysis of the blood for alkali reserve is of little value in the diagnosis of rickets in pigs.

The basal ration used in these experiments, plus the antirachitic factors, produced bones significantly higher in ash content than did the basal ration alone in Trials I and II. Likewise, there existed a direct correlation between severity of rachitic conditions and lowered bone ash. The humerus was more affected than the tibia and the tibia more than the rib. However, these findings were not borne out in Trial III, indicating that ash analyses cannot always be relied upon to aid in the diagnosis of rickets. The fact that Trial III showed high bone ash in rachitic pigs might be explained as a redeposition of lime salts in the bone caused by the starvation of the pigs as a result of the severity of rickets.

Breaking-strength determinations on the tibiae revealed striking differences between normal and rachitic pigs. Without exception in each of the three trials, the strength of the tibiae from rachitic pigs was reduced more than half, those pigs most affected having the weakest bones. Though ash analyses revealed high bone ash in the rachitic pigs of Trial III, the strength of the tibiae was not increased. Breaking strength calculated per unit of bone area is a more reliable measure of breaking strength than the actual stress required to break the bone, since differences in the size of the bones and the thickness of the bone wall are taken into consideration.

*Trial I.*—Direct sunlight and one per cent of cod-liver oil added to the basal ration prevented rickets in pigs fed a ration deficient in vitamin D. Similarly the same antirachitic substances induced healing in pigs suffering from a severe form of rickets.

*Trial II.*—Direct sunlight prevented rickets in pigs fed a ration deficient in vitamin D, while another group of similar pigs on the same ration but denied exposure to direct sunlight developed severe rickets.

*Trial III.*—Cod-liver oil fed at the rate of 0.75 per cent of the basal mixture did not prevent mild rickets in pigs fed indoors on a ration deficient in vitamin D. When the cod-liver oil was increased to one per cent of the basal mixture, recovery of those pigs already affected took place and further trouble from rickets was prevented. Severe rickets developed in another group of similar pigs receiving the same ration but denied both direct sunlight and cod-liver oil.

# Studies of Rickets in Swine

WM. J. LOEFFEL, RAY R. THALMAN, F. C. OLSON, AND F. A. OLSON[1]

Efficiency demands that pigs reach market weight within the shortest space of time. It is a matter of common knowledge that a thrifty, well-fed pig will reach the desirable market weight of 200 pounds at six months of age. The major portion of pig rations is made up of cereal grains which are admittedly deficient, not only in mineral substances but also in the accessory substances which are recognized as equally important for the proper assimilation of the minerals present. This is particularly true during the portion of the year when pigs are confined in dry lot.

Most pigs are marketed at less than one year of age, in fact some as young as five and one-half months. At these ages, pigs are still making rapid skeletal growth and their bones lack the strength which comes with maturity. The carcass of the pig normally contains a higher proportion of fat than those of other meat animals. This means that the skeleton of the pig supports a proportionately larger weight than that of other domestic animals.

Naturally, then, pigs are subject to bone and joint disorders and abnormalities. Disturbances of mineral metabolism may sometimes be referred to as posterior paralysis, rheumatism, osteomalacia, and rickets. Regardless of what the disease may be called, the many symptoms are similar. Unless the ration is corrected or the causative factor removed, the usefulness of the animal is impaired or destroyed. While the disease may not be a direct cause of death, it may lower the vitality of the animal, thus increasing susceptibility to other diseases which ultimately cause death.

## REVIEW OF LITERATURE

Rickets, or rachitis, is a disease of growing bone. It is characterized by defective calcification. In embryonic life, structures appear which are later to develop into bones. Starting as simple connective tissue, a gradual change occurs, first to cartilage and this in time becomes bone due to a depo-

[1] This bulletin represents a summarization of three graduate theses by Fred C. Olson, Ray R. Thalman, and Fred A. Olson. Mr. Thalman carried on a fourth investigation after the completion of his graduate requirements and aided in the compilation of the results. Dr. L. Van Es of the Department of Animal Pathology and Hygiene by his counsel and assistance aided materially in carrying on this work. Dr. L. V. Skidmore made all ante-mortem and post-mortem examinations. Dr. E. W. Rowe of the Lincoln Clinic made and interpreted the radiographs. The members of the Agricultural Chemistry Department, Dr. M. J. Blish, Dr. C. W. Ackerson, and Mr. Rudolph Sanstedt, aided in formulating and conducting this investigation. Dr. J. S. Latta of the College of Medicine prepared some bone specimens for histological study. Prof. C. M. Duff of the Department of Mechanical Engineering aided with the breaking tests of bones.

sition of lime salts. In long bones, calcification starts at the center and proceeds towards the two ends. The shaft of a long bone is referred to as the diaphysis and the two ends or heads as the epiphyses. Where a diaphysis joins an epiphysis, a layer of cartilage, the epiphyseal line, is found. This persists until maturity. Lengthwise growth takes place at the epiphyseal lines. In normal bone, the bone-forming cells or osteoblasts lay down calcium salts, thus changing the rubber-like hyaline cartilage to bone.

In a rachitic condition, the normal amount of calcium salts is not laid down and, as a result, the bone contains an undue amount of connective tissue. The epiphyseal line persists and maturity is delayed. The bone lacks strength and, as a result, is frequently deformed. Nature attempts to strengthen the weak bones and joints with large amounts of white fibrous connective tissue, thus causing the abnormalities so generally associated with the disease.

It is recognized today that rickets may be caused by a lack of mineral matter, particularly calcium and phosphorus salts, by a disproportion between these substances, or by a lack of the accessory substances or vitamin necessary for bone formation. Exposure to the direct rays of the sun enables many animals to produce the necessary vitamin themselves.

That a seasonal variation exists in the prevalence of nutritional bone disorders was suggested by Kuhlman and Wilson (31) who state, "There is little doubt that the unthriftiness of winter pigs is often due to a rachitic condition." Bohstedt and co-workers (4) agree that rachitic symptoms in pigs are especially prevalent in winter or early spring.

Hart, Miller, and McCollum (14) found that the addition of 20 to 25 per cent of alfalfa and meat scraps to a grain ration prevented stiffness in pigs that were raised in confinement. Hart and Steenbock (15) were unable to maintain pigs over an extended period when grain and grain products were used as the sole ration. They also observed that a cereal ration led to increasing numbers of dead pigs at birth, the number of dead pigs increasing in the second and third farrowings. Maynard, Goldberg, and Miller (36) noted marked stiffness in pigs in confinement on a cereal and oil meal ration. However, no abnormalities developed in similar pigs on the same ration but allowed access to outdoor pens. Kernkamp (28) observed that the symptoms produced by a grain ration in winter feeding were very similar to those produced by a lack of sunlight, or vitamin D. He recommended the addition of minerals to the ration, which resulted in apparent recovery of the animals, together with normal growth. However, the addition of minerals to the ration failed to prevent disorder when fed to pigs confined indoors. The work

of Maynard (35) confirmed the findings of Kernkamp. After producing either stiffness or paralysis of the hind legs, he was successful in effecting recovery by the addition of precipitated bone meal and precipitated calcium carbonate to a grain ration. Further study on the effect of minerals in swine nutrition was carried on by Bohstedt and co-workers (5), who succeeded in preventing posterior paralysis by the use of ground limestone. However, they were unable to prevent slight stiffness and an occasional death by this addition to the ration.

Maynard, Goldberg, and Miller (36) produced results similar to those observed by Kernkamp and Bohstedt by feeding rations deficient in mineral matter. The failure of minerals in preventing stiffness, they believe, was due to the fact that the pigs were kept in barns and thus protected from the actinic rays of the sun. Livesay and Stillwell (33) reported success in bringing about recovery in swine suffering from rickets or stiffness by the addition of butterfat to the ration. Cod-liver oil also alleviated the condition. These observations suggested a possible vitamin or radiant-energy factor in the prevention of bone disturbances in swine.

Zilva, Golding, Drummond, and Coward (53) concluded that no definite relation existed between vitamin A and rickets in pigs. They suggested that the fact that pigs are "off their feet" is no sure sign of rickets.

For years the beneficial action of sunshine upon growth and development of animals has been recognized. Palm (47) as early as 1890 suggested sunshine as a curative agent for rickets. Finsen (11) in 1896 founded the Light Institute at Copenhagen, which has done much to organize our knowledge of heliotherapy.

Due to the privations suffered during the World War, rickets was very common in German children during the post-war period. Huldschinsky (25, 26) reported remarkable improvement in the condition of rachitic children after treating them by radiation with a quartz mercury vapor lamp and sunlight. Hess and Steenbock, to whom frequent references will be made, were among the pioneers in the study of light in its relation to rickets. They not only studied the effect of light upon the experimental subjects themselves but also irradiated oils and feeds with mercury vapor lamps, producing thereby substances that were protective against rickets.

Another of the early investigators in the field of rickets was Mellanby (41, 42), an English worker who carried on extensive investigations with puppies. He found that cod-liver oil, and, to a lesser extent, butterfat, cured rickets, while other fats and oils proved ineffective. He considered the vitamin A factor responsible for the cure and prevention of

rickets. That the latter conclusion was faulty was shown by McCollum and co-workers (44, 45), who in 1922 demonstrated that another fat-soluble vitamin, later designated as D, was responsible for the cure and prevention of rickets.

Thus we have hastily reviewed the pioneer work which served definitely to classify rickets as a deficiency disease rather than a disease due to heredity, gluttony, infection, or to disturbances of the endocrine glands as was once believed.

Hess (21) pointed out a direct correlation between mammalian rickets and hours of sunshine in world cities within the same zone.

Direct sunlight has recognized antirachitic qualities and a standard dose for the prevention of rickets in rats is known. Season, smoke, dirt, moisture in the atmosphere, and time of day affect the potency of the sun's rays (9). Investigators (19, 51) have also shown that window glass prevents the effective rays from passing through, since glass absorbs rays shorter than 330 millimicroms. Hess (21) states that "in order to be of any value in rickets, the ultra-violet rays must have a wave length not longer than 310 or possibly 313 millimicrons."

Steenbock, Hart, and Jones (50) conclude from chemical analyses of the blood, gross symptoms, and histological evidence, that sunlight is an important factor in the production of pork in northern latitudes. Other workers (10, 31, 37) have noted the beneficial effect of sunshine on pigs.

Some workers have been successful in irradiating food substances by means of the quartz mercury vapor lamp, and thereby increasing their antirachitic potency. Irradiated ergosterol is perhaps the most potent source of the vitamin factor responsible for proper mineral metabolism, although this substance has been little used as yet in the treatment of rickets in pigs.

Perhaps one of the most commonly used of the antirachitic food substances in swine nutrition is cod-liver oil. Investigators (5, 38, 32, 48) have observed that cod-liver oil added to the ration prevented rickets and induced healing when various minerals failed.

Howland and Kramer (23) showed that cod-liver oil when given to infants suffering from rickets caused an increase in the phosphorus content of the blood, and thus caused healing. Likewise, the administration of cod-liver oil to pigs suffering from stiffness was found by Bohstedt and co-workers (4) to raise the serum phosphorus to a normal level. While this suggested a relationship between a lowered serum phosphorus and rickets, the workers (4) agreed with Hart and Steenbock (16) that a lowered inorganic phosphorus con-

tent of the blood cannot always be relied upon as an index of rickets. Other investigators (1, 21, 24, 27, 46) supported the contention that a close correlation existed between a lowered inorganic calcium and phosphorus content of the blood and rickets. Hodgson (22) also suggests a relationship between rickets and acidosis.

The first experiment reported herein was started in the fall of 1925. It was planned to study the effect of the radiant-energy factor as obtained from sunlight, from the quartz mercury vapor lamp, from the photodynamic action of eosin, and from skin pigmentation on the growth and development of swine. However, many complications intervened, rendering the results inconclusive and unsatisfactory. Nevertheless it paved the way for the work which followed. The three subsequent trials were planned to ascertain specifically the part played by sunlight and vitamin D in the nutrition of swine. A second object in these experiments was to determine the exact nature of the symptoms and lesions produced in swine by a ration deficient in the antirachitic factor, or factors.

## EXPERIMENTAL METHODS

Each of the experiments reported in this bulletin was carried on during the winter months. Trials A, I, II, and III were begun November 7, 1925, December 4, 1926, November 26, 1927, and January 3, 1929, respectively. No definite date for closing the experiments was set, since it was planned to continue the project until nutritional disturbance appeared or the pigs reached market weight.

### EXPERIMENTAL ANIMALS

Fall-farrowed pigs were used in each of the experiments and in no case did the variation in age between the pigs used in any single trial exceed 25 days. The average age of the pigs was 90 days and the average weight 49 pounds. All pigs were produced in the Experiment Station herd and had received the same treatment during the suckling period.

With the exception of Trial A, in which there were six lots, all trials consisted of two lots. There were 10 pigs in each lot of Trials A, II, and III, and 12 in each lot of Trial I. Allotment was made, so far as practicable, according to sex, weight, and breeding. Before weaning, the pigs were castrated and vaccinated for hog cholera by the serum simultaneous method. The experiments were started as soon as possible after weaning.

### PREVIOUS TREATMENT OF PIGS

The pigs were farrowed in a central hog house in which they remained for approximately one week. They were then removed to a large lot and allowed to run with their dams.

While this lot contained a small amount of forage, in reality it was more nearly comparable to a dry lot. The pigs, along with their dams, received a ration of ground yellow corn, ground oats, shorts, tankage, linseed meal, steamed bone meal, and salt. In addition, they had access to alfalfa hay of good quality.

### HOUSING OF PIGS

The barn in which the various indoor groups of pigs were housed was constructed of hollow tile and lumber, having a large number of windows both on the north and south sides and also several rows of windows in the roof as shown by Figure 1. The doors of the barn were kept closed to eliminate the possibility of direct sunlight striking the pigs. In Trials A and I, to eliminate even the possibility of filtered sunlight striking the pigs, those windows in the vicinity of the experimental pens were given a coating of whitewash. The experi-

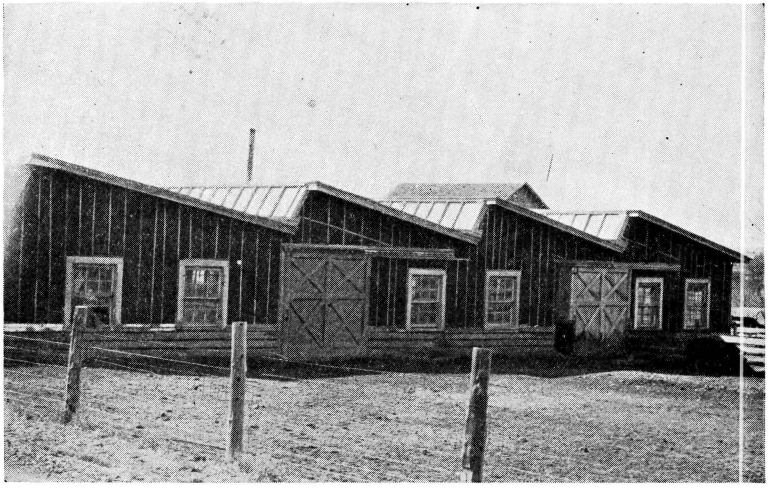

FIG. 1.—The barn used to house the pigs fed indoors

mental pens were located in the northeast end of the barn and consisted of 320 square feet each, 180 of which were used for bedding. The floor was of concrete, covered with wood in the bedded portion. Good, dry, clean straw was used for bedding, except in Trial A in which wood shavings were used. The pens were cleaned daily and kept as dry as possible.

The outside groups were located where full benefits of the sun could be obtained. With the exception of Trial I, in which smaller pens were used, the lots contained 468 square feet, including the sheltered portion. The *entire floor* was of

concrete and the sheltered portion was bedded with a liberal supply of dry, clean straw. The pens were cleaned daily and in good weather the feed trough was placed in the open portion of the pen.

### PRELIMINARY TRIAL A

In this investigation, it was planned to study the effect of the radiant-energy factor as secured from sunshine and the mercury vapor arc lamp and to study the photodynamic action of eosin. As an incidental project, the effect of skin pigmentation was to be studied since every lot contained white, black, and red pigs.

The trial began November 7, 1925, and continued 162 days, terminating April 17, 1926. The pigs were purebred Duroc Jerseys, Poland Chinas, and Chester Whites, allotted equally between the lots as to sex and breed. Six lots of ten pigs were used. At the beginning of the trial, the average age of the pigs was 68 days and the average weight 33 pounds. All lots were fed the same basal ration, the variables being confined to difference in treatment.

The treatment accorded the various lots was as follows:

Lot 1—Outdoors
Lot 2—Indoors but given one hour outdoors daily
Lot 3—Indoor check group (not exposed to sunshine)
Lot 4—Indoors, but feed treated with eosin
Lot 5—Indoors always but feed irradiated
Lot 6—Indoors always but pigs radiated daily with lamp

The pigs in Lot 1 were confined to an outside pen where they could obtain direct sunshine. Out of a possible 1,711.5 hours of sunshine, the pigs in this lot had available 1,040.5 hours, or 60.78 per cent, according to U. S. Weather Bureau records.

The pigs in Lot 2 were confined indoors but were driven to an outdoor concreted lot for one hour daily from 10 to 11 a. m. These pigs actually had an average of 0.66 hour of sunshine daily during the experiment.

Lots 3 to 6 inclusive were confined for the period of the experiment in the barn previously described. Lot 3 was the indoor check lot receiving no special treatment.

The feed supplied to Lot 4 was treated with eosin. This was done on experimental evidence, largely of German origin, that eosin made animals sensitive to sunlight. In Germany, a tax was assessed against barley used for malting purposes, although feeding barley was exempt from tax. To prevent the use of feeding barley for malting, it was denatured by staining with eosin. Feeders found that the denatured barley could be fed satisfactorily as long as the animals to which it was fed were kept out of direct sunlight. Direct sunshine produced a rather severe reaction in animals with unpig-

mented skins. Similar results were reported from feeding buckwheat.

The eosin used was Coleman and Bell, Eosin Y, yellowish (alcohol and water soluble). The amount used approximated the official German requirements for denaturing feeding barley and amounted to 0.0486 gram of eosin per pig daily. The eosin was dissolved in a pint of water and mixed with the ration at feeding time.

Lot 5 received the basal ration irradiated with a Cooper-Hewitt Uviarc Poultry Treater on a 220-volt circuit. Fifty pounds of the standard feed mixture was spread over 10 square feet of floor space. The feed was irradiated for 15 minutes at a distance of three feet. During the process, the feed was stirred frequently.

The pigs in Lot 6 were themselves treated with the quartz bulb mercury vapor lamp just mentioned. They were confined in a small pen four feet square and treated for 25 minutes daily, the lamp being four feet from the floor. Prior to treating pigs or irridiating feed, the lamp was burned for ten minutes.

In order to keep this trial as practical as possible, the ration was confined to the simple ration of yellow corn, tankage, and salt. To supply bulk, corn bran was included in the ration for the first 52 days, but was withdrawn at that time in an effort to increase the gains. The corn was coarsely ground and mixed with the other feeds to supply a nutritive ratio of 1:3.5 for the first 52 days; 1:4.39 for the second 20 days; and 1:5.35 for the last 90 days. The feed supplied approximated the requirements set forth by the Morrison (18) feeding standard. Salt comprised one per cent of the feed mixture. The ration presumably supplied all the pigs' nutrient requirements with the exception of the antirachitic factor.

### STUDIES MADE IN TRIAL A

The pigs were weighed regularly each week and measurements were taken every four weeks. The measurements taken were length of body from point of withers to root of tail, circumference of chest and rear flank, height at withers and hips, and depth through chest and rear flank.

At the close of the trial, April 17, 1926, detailed antemortem and post-mortem examinations were made by Dr. L. V. Skidmore. The tibia, sixth rib, humerus, and first dorsal vertebra were removed from each carcass and used for radiographic study, breaking-strength tests, ash determinations, and macroscopic study.

### OBSERVATIONS ON TRIAL A

Through no fault of those in charge, this trial became seriously complicated by disease. About two weeks after the beginning of the trial, 17 of the pigs showed unmistakable evidence of hog cholera, necessitating the revaccination of all the pigs. About the fifteenth week of the experiment, all lots were involved in a severe outbreak of pneumonia. On autopsy at the close of the experiment, 39 of the 45 pigs showed lesions of pneumonia, ranging from slight involvement to extensive purulent cases. A total of 17 pigs, or 28 per cent, died from disease before any nutritional disturbance made its appearance. Failure of growth was evident, several pigs weighing little more at the close than at the beginning. While rachitic symptoms appeared, they were not pronounced and varied in severity among the pigs of all lots. Ash analyses, macroscopic study, radiographs, and breaking-strength determinations of the bones revealed that a bone disturbance was present, yet the findings were not distinct. In no lot were all pigs free from abnormalities nor was there any lot in which all pigs were normal.

Though inconclusive, the results of this experiment suggested a close relationship between failure of growth and retarded rickets, and also that pigs are susceptible to bone disturbances resembling rickets in other animals.

The first trial emphasized in a striking way the need for a yardstick by which faulty bone growth could be accurately measured. The need was clearly seen for a project to determine what constituted normal and what characterized pathological bone development.

### RATIONS USED IN TRIALS I, II, AND III

From the results secured in the preliminary trial, it was doubtful whether rickets could be produced on a ration of yellow corn and tankage. Therefore in Trials I, II, and III, an effort was made to select a basal ration that would meet all the requirements for normal growth and fattening in swine, with the exception of the antirachitic factors, vitamin D and radiant energy. This basal ration was made up as follows:

| | *Pounds* |
|---|---|
| Yellow corn | 73.5 |
| Soybean oil meal | 15.5 |
| Blood meal | 4.5 |
| Dried skimmilk | 5.5 |
| Sodium chloride | 1.0 |
| TOTAL | 100.0 |

The chemical composition of the feeds used in compounding the basal mixture is given in Table 1. Yellow corn was

TABLE 1.—*Average analyses of feeds used in Trials I, II, and III—pounds in one hundred*

| Feed | Moisture | Crude protein | Carbohydrates | | Fat | Ash | | |
|---|---|---|---|---|---|---|---|---|
| | | | Nitrogen free ext. | Crude fibre | | Calcium | Phosphorus | Other than Ca. & P. |
| Yellow corn..... | 15.02 | 8.98 | 69.41 | 1.79 | 3.40 | 0.010 | 0.188 | 1.40 |
| Soybean oil meal.. | 10.47 | 48.20 | 24.12 | 5.00 | 6.15 | 0.316 | 0.655 | 6.06 |
| Blood meal..... | 8.55 | 81.11 | 1.46 | .... | 1.49 | 1.863 | 1.163 | 7.39 |
| Dried skimmilk... | 5.77 | 34.96 | 51.03 | .... | 0.38 | 1.282 | 0.892 | 7.86 |

selected, since investigators (5, 49) have shown that it is superior to white corn in supplying the vitamin A so essential to proper growth. It has also been proved that yellow corn contains vitamin B in sufficient quantities to meet the needs of growing pigs (49, 53). Henry and Morrison (18) state that soybean oil meal "is one of the very best protein-rich concentrates of plant origin for pork production." While little work has been done to determine the vitamin content of soybeans, it was assumed that this plant, like most cereals, is practically devoid of vitamin D. This assumption was borne out by the results of these trials.

Besides soybean oil meal, powdered skimmilk and blood meal were included in the basal mixture, thus giving proteins of both plant and animal origin. This was felt advisable since Maynard and Miller (39, 40) found that the protein from blood meal is superior to that from linseed meal in inducing calcification of the bones.

Skimmilk and buttermilk have long been recognized as valuable supplements to grain rations for pigs. In addition to its protein, skimmilk contains considerable mineral matter, yet it is apparently lacking in the factor responsible for proper mineral metabolism. Kuhlman and Wilson (31) found that buttermilk increased the gains of pigs on a corn-and-tankage ration but did not prevent stiffness, or rickets, in pigs that were not exposed to sunlight. These findings are strengthened by those of Golding (13) who fed dried skimmilk to pigs that were not exposed to sunlight on a ration of barley meal, animal charcoal, and chalk. Rapid growth resulted for a short period but the pigs were "off their feet" within three months. Histological examination showed true rickets in 8 of 10 pigs. Hess and Weinstock (20) conclude that milk does not aid in the prevention of rickets and, in fact, if consumed in large quantities may make rickets more probable.

While blood meal contains a high percentage of protein and phosphorus, it is low in calcium and may, therefore, be of questionable value in a ration where the vitamin D factor is being studied. However, it seems reasonable to assume that

the deficiency of blood meal in calcium would be of small consequence so long as the calcium content of the ration was not deficient. Some objection might be raised to its use in a ration of this sort. The variety and amount of protein provided in the ration should offset any deficiencies in quality of blood-meal proteins. The failure to produce rickets in Trial A with tankage precluded the use of that feed in these trials. Definite knowledge of the ash requirements of growing animals is limited. Armsby (2), basing his conclusions on the work of several investigators, gives the daily retention of calcium in a six-months-old pig as 0.084 gram, and of phosphorus as 0.051 gram. The amount that must be supplied in the daily ration will depend to some degree upon the availability of these substances.

In Trials I and III cod-liver oil was fed to one group of pigs as a source of vitamin D. In each of these trials pure Norwegian non-freezable cod-liver oil of the highest grade was used.

### FEEDING

The experimental animals were hand-fed twice daily, morning and evening. In all the trials a daily record of the feed consumed by lots was kept. Each lot received as much feed mixture as they would consume in two hours without waste. A check was made of all animals at feeding time and variation in appetite was noted. Water was available at all times in troughs.

### STUDIES MADE

As a means of studying growth, the pigs were weighed individually at weekly intervals. The initial and final weights given are averages of three weights taken at the same hour on consecutive days. In addition to being weighed, all pigs in Trials I and II were measured every 28 days. The measurements taken were length of body from point of withers to root of tail, circumference of chest and rear flank, and height at withers and hips. No measurements were taken in Trial III, since weight proved a more valuable criterion of growth than increase in skeletal measurements.

In an effort to study the symptoms accompanying nutritional disorder, detailed clinical examinations were made by Dr. L. V. Skidmore at intervals throughout the experiment and at the close.

Since rickets is closely associated with a decrease in the calcium and phosphorus in blood serum, blood samples were taken for analyses in Trials I and II. It was impossible to continue this study in Trial III. All bleeding was done from the tail. The samples were obtained before feeding in the morning. Time of bleeding seemed important, since the cal-

cium and phosphorus were found to increase just after feeding.

At the close of the trials, all experimental animals were slaughtered in the station abattoir and a careful post-mortem examination was made. After autopsy, the humerus, tibia, and sixth rib from each side of the carcass were removed. The bones from the left side were first X-rayed and then dried in an oven for chemical analysis. Drying was done at 80° to 90° C. The humerus from the right side was reserved for histological examination, and the sixth rib used for macroscopic study. The left tibia was used to determine the breaking strength and was later split for macroscopic study.

Olson's Improved Testing Machine with Dial Vernier beam was used in breaking the bones. Breaking strength was recorded as stress required to break the bone and as breaking strength per square inch of bone area. This work was done in the laboratory of the Department of Mechanical Engineering. The formula suggested by Professor C. M. Duff for calculating breaking strength on a square-inch basis was as follows:

$$S = \frac{P\,L}{4} \cdot \frac{d}{2} \cdot \frac{I}{.049\,(d^4 - d_1^4)}$$

S = unit fiber stress, pounds per square inch
P = load in pounds at middle
L = length in inches between supports
d = outside diameter of circle
$d_1$ = inside diameter of circle
I = moment of inertia, 4th power

### TRIAL I—SUNLIGHT AND COD-LIVER OIL

The object of this experiment was to study the effect of the antirachitic factors, vitamin D and solar radiation, on the growth and development of pigs. A further object was to study the exact nature of the lesions produced in pigs that were fed upon a rickets-producing ration as a basis of comparison for future trials.

The experiment was begun December 4, 1926, and was terminated August 23, 1927. It was divided into two phases, which terminated May 5, 1927, and August 23, 1927, respectively. Phase I included all pigs up to May 5, 1927, when six representative pigs from each lot were slaughtered for detailed study. The remaining pigs were carried through Phase II, which was introduced to study the extent of recovery when the antirachitic factors were supplied.

Two lots of 12 crossbred fall pigs averaging 87 days in age and 50 pounds in weight were used. Lot 1, the check lot, was

located outdoors where full benefits of the sunlight could be obtained. Lot 2 was located in the large hog barn previously described. The same ration was fed to both groups with the exception that Lot 1 received 450 c.c. or one pound of cod-liver oil to each 100 pounds of the basal mixture.

#### GROWTH—PHASE I

In view of the fact that some investigators (21, 46) have demonstrated that rickets and growth are closely related, the pigs used in this experiment were weighed every week and measured every four weeks. Table 2 gives the data obtained from these studies.

Although there was some variation in rate of gain between individuals within a single lot, only two pigs in Lot 1 failed to make satisfactory gains. Autopsy of these pigs failed to show any abnormality in one case but revealed a fractured mandible of long standing in the other. The bones of both pigs were of good quality, well formed, and exceptionally hard.

During the first five weeks of the trial, Lot 2 made as large gains as did Lot 1, but as the signs of nutritional disturbance progressed the rapidity and regularity of growth in Lot 2 decreased. By the close of Phase I, an average difference of 84 pounds per head existed between Lots 1 and 2. For Phase I, the average daily gain of Lot 1 was 1.02 pounds per pig as

TABLE 2.—*Growth data (Trial I)*

| Phase I | Lot 1—Basal ration + 1% cod-liver oil (outdoors) | | | Lot 2—Basal ration (indoors) | | |
|---|---|---|---|---|---|---|
| | Min. | Max. | Av. | Min. | Max. | Av. |
| Age at close (*days*) | 233 | 250 | 238 | 233 | 250 | 239 |
| Initial weight (*pounds*) | 32.33 | 72.00 | 49.91 | 32.00 | 69.66 | 49.38 |
| Gain in weight (*pounds*) | 57.67 | 227.00 | 154.82 | 29.67 | 132.00 | 41.36 |
| Average daily gain (*pounds*) | 0.38 | 1.50 | 1.02 | 0.19 | 0.87 | 0.47 |
| Increase in body measurements | | | | | | |
| Length of body (*cm.*) | 21.00 | 38.00 | 34.59 | 6.00 | 30.10 | 20.43 |
| Circumference | | | | | | |
| Chest (*cm.*) | 17.00 | 54.00 | 39.77 | 3.80 | 30.50 | 19.72 |
| Flank (*cm.*) | 18.00 | 56.00 | 40.59 | 4.00 | 28.00 | 15.86 |
| Height | | | | | | |
| At withers (*cm.*) | 14.17 | 27.70 | 22.32 | 6.80 | 15.40 | 11.33 |
| At hips (*cm.*) | 15.00 | 31.00 | 23.14 | 6.00 | 14.80 | 9.66 |
| **Phase II** | **Lot 1—Basal ration + 1% cod-liver oil (outdoors)** | | | **Lot 2—Basal ration + 1% cod-liver oil (outdoors)** | | |
| Age at close (*days*) | 343 | 359 | 348 | 343 | 359 | 353 |
| Initial weight (*pounds*) | 134 | 271 | 206.2 | 85 | 189 | 142 |
| Gain in weight (*pounds*) | 70.33 | 138.66 | 105.79 | 43.00 | 150.33 | 96.47 |
| Average daily gain (*pounds*) | 0.64 | 1.44 | 0.996 | 0.39 | 1.37 | 0.88 |
| Increase in body measurements | | | | | | |
| Length of body (*cm.*) | 10.00 | 16.00 | 12.60 | 12.00 | 20.00 | 15.25 |
| Circumference | | | | | | |
| Chest (*cm.*) | 14.00 | 28.00 | 19.80 | 8.00 | 90.00 | 35.00 |
| Flank (*cm.*) | 11.00 | 23.00 | 17.00 | 12.00 | 23.00 | 17.00 |
| Height | | | | | | |
| At withers (*cm.*) | 6.40 | 10.90 | 8.50 | 7.00 | 9.90 | 8.20 |
| At hips (*cm.*) | 3.50 | 7.20 | 5.68 | 6.00 | 10.30 | 8.82 |

against 0.47 pound for Lot 2. This difference seems significant and suggests that the rachitic condition of the pigs in Lot 2 probably was the causative factor. The difference is illustrated graphically in Figure 2.

Growth is necessary in order that rickets may develop. As the disease progresses, the affected animals lose their appetites and, as a result, lose weight. In this and in succeeding trials, the pigs showing the most severe rickets were consistently lighter in weight than the control pigs.

The data presented in Table 2 indicate a direct correlation between gain in body weight and increase in body measurements. Weight, however, appears to be a more reliable criterion of growth, since the disposition of the pig makes it difficult to obtain accurate measurements.

Feed consumption is recognized as a factor of importance in controlling gains and is, therefore, included in this study.

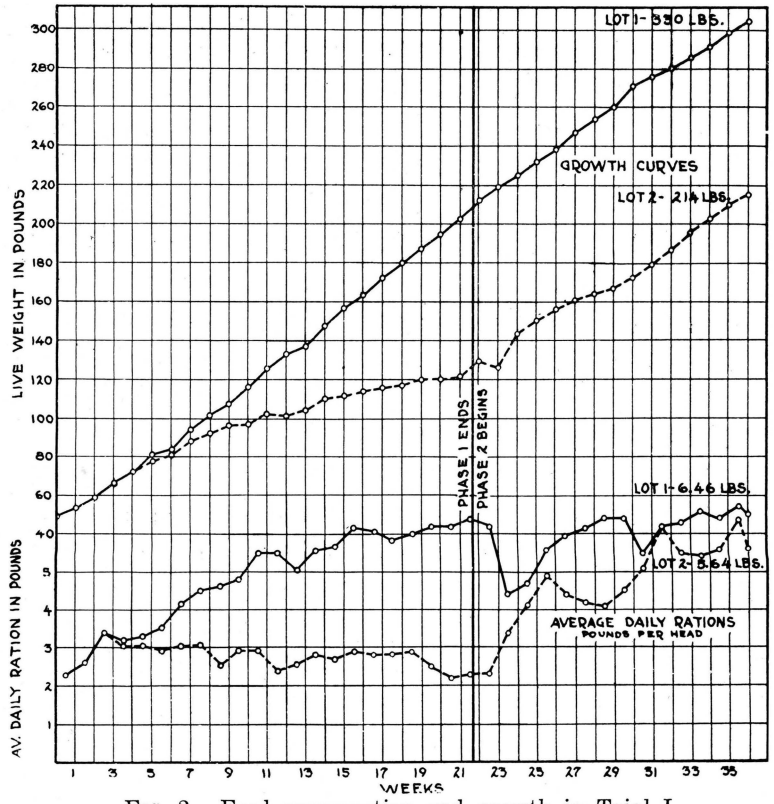


FIG. 2.—Feed consumption and growth *in Trial I*

Figure 2 gives the average daily feed consumption per pig computed by weekly averages. The feed consumption of both lots correlates closely with their respective growth curves.

No correlation existed between the growth curves of different pigs from the same litter, nor did sex show any relationship to growth. Skin pigmentation may possibly have been a factor since the three red pigs (Duroc X Tamworth) were first to show evidence of rachitic symptoms as indicated by retarded growth. However, breed comparisons cannot be made because of the limited number of pigs involved.

Pneumonia was the only disease, other than rickets, found when the animals were slaughtered. Most of these lesions were confined to the apical lobes and the disease was present in some pigs from both lots. Neither lot was free from round worms (*Ascaris lumbricoides*), which probably account for some of the lung involvement. However, neither pneumonia nor worms can account for the difference in growth between the lots, since some of the poorest individuals showed less involvement than the best.

#### GROWTH—PHASE II

At the beginning of Phase II (May 7, 1927), Lot 2 was removed to outside quarters and received the same ration and treatment that Lot 1 had received during Phase I. Thus Lot 2 received the radiant-energy factor, sunlight, as well as the antirachitic factor contained in cod-liver oil. No pig in Lot 2 was able to walk more than a few feet at the beginning of Phase II and as may be seen from Figure 2, growth was at a standstill.

During Phase II, Lot 1 continued to increase in weight at practically the same rate as during Phase I. The average daily gain amounted to 0.996 pound, only 0.024 pound less than during Phase I. This decrease was to be expected, since the pigs in Lot 1 were rapidly reaching maturity and were comparatively fat. In the case of Lot 2, a daily gain of 0.47 pound per pig in Phase I increased in Phase II to 0.88 pound, or practically 100 per cent. This was only 0.11 pound less per pig daily than the gain made by Lot 1 during this period. The physical condition of the pigs at the beginning of Phase II should also be taken into consideration in computing gains, since some time was needed for the pigs to recuperate sufficiently to regain their normal appetites. Had the gain been computed on the last 12 weeks of the experiment, it would have been equal to, if not greater than, the gain of Lot 1. It is evident that some recovery took place among the pigs of Lot 2, at least so far as growth was concerned.

The effect of recovery on skeletal growth is seen in Table 2. The height of the body of the pigs in Lot 2 increased faster

in proportion than in Phase I. The increase in Lot 1 was less than in Lot 2, probably because of two factors, that Lot 1 was fast reaching maturity and that in Lot 2 growth had been retarded in Phase I but was resumed in Phase II when the animals recovered from the dietary deficiency. From a study of the measurements, it appears that bone growth was resumed along with that of other body tissues.

Clinicians recognize that a sudden, prolonged exposure to direct sunlight causes a severe reaction to patients who have been confined indoors for extended periods. Nausea and other complications may develop. A similar reaction was observed at the beginning of Phase II when the pigs were first removed to outside quarters. All the pigs in Lot 2, except one, lost weight the first two weeks. In addition, the animals became feverish and constipated, showed signs of pneumonia, and breathed with difficulty. The condition became severe with several pigs and in one case death resulted.

With the exception of some slight irregularities, the feed consumption as shown in Figure 2 follows that of the growth chart quite consistently. Part of this irregularity can be attributed to weather conditions, since some of the animals were in good condition and consequently suffered much from the heat during parts of July and August. In spite of the heat, however, the feed consumption of Lot 2 increased from 15 pounds per pig weekly to 39.7 pounds, indicating at least that the appetites of the pigs in Lot 2 were fast approaching normal.

### ANTE-MORTEM EXAMINATION—PHASE I

As previously mentioned, a detailed clinical examination was made of every pig at intervals during the progress and at the close of the experiment. Those evidences of rickets observed by other workers received special attention. The results of the examination made at the close of the trial are given in Table 3.

As far as could be determined clinically, the pigs in Lot 1 were normal in all respects. In Lot 2 the first signs of nutritional disturbance appeared in the sixth week. Two weeks later all pigs in this lot were showing distinct peculiarities of gait. In ten weeks, three pigs were barely able to walk.

Although no paralysis developed during the experiment, several pigs in Lot 2 showed difficulty in controlling their posterior limbs, apparently because of a contraction or cramping of the muscles. In some cases the hind feet were placed beside the fore feet and well under the body. There was a general tendency for the pigs to cross their hind feet when standing and at the same time shift their weight from one foot to the other. When the pigs began to walk, extreme pain

TABLE 3.—*Summary of ante-mortem findings (Trial I, Phase I)*

| Findings | Cases observed | |
|---|---|---|
| | Lot 1—12 pigs Basal ration + C. L. O. (outdoors) | Lot 2—12 pigs Basal ration (indoors) |
| Stiff and stilty gait (cross-legged behind) | 0 | 6 |
| Unable to walk (crawls on knees) | 0 | 5 |
| Trembles when forced to move | 0 | 5 |
| Akenesia algera | 0 | 9 |
| Nervous and irritable | 0 | 8 |
| Knuckles over at knees | 0 | 5 |
| Front pasterns very upright | 0 | 9 |
| Hind pasterns very upright | 0 | 4 |
| Hind pasterns "down" (walks on dewclaws) | 0 | 4 |
| Legs bowed | 0 | 5 |
| Carpus and tarsus thickened | 0 | 9 |
| Stands with 4 feet together | 0 | 6 |
| Back extremely arched | 0 | 7 |
| Condition and musculature poor | 0 | 7 |
| Hair long, coarse, and rough | 0 | 10 |
| Skin hard, thick, and scurfy | 0 | 10 |
| Eyes bulging | 0 | 5 |
| Head abnormally large | 0 | 5 |
| Thorax contracted | 0 | 5 |
| Deaths | 1[1] | 1[2] |

[1]Pig destroyed on 77th day—unthrifty, pig typhoid.
[2]Pig died on 126th day—hog cholera.

was evident and a pronounced trembling occurred as if they were trying to walk on a slippery surface. The gait was characterized by a stiff, stilted, lateral motion.

The abnormalities that appeared in the bones as the experiment progressed were confined chiefly to the legs. In several cases a thickening of the pastern joints, knees, and hocks was noted. Some pigs showed extreme steepness of pasterns while others practically walked on their dewclaws. Bowed legs were common throughout Lot 2. Among other common findings were extreme arching of the back, emaciation, abnormally large skulls, and bulging eyes that exuded a mattery secretion but apparently did not impair the sight. The skin over the entire body became very thick and hard and covered with a black, greasy scurf, and the hair became long, harsh, and brittle.

All pigs in Lot 2 except two were unable to walk more than a few feet by the end of Phase I. One "went down" two days later and the other was slaughtered at the close of Phase I and although the latter was the best pig in Lot 1, it is evident from Table 4 that it was not normal.

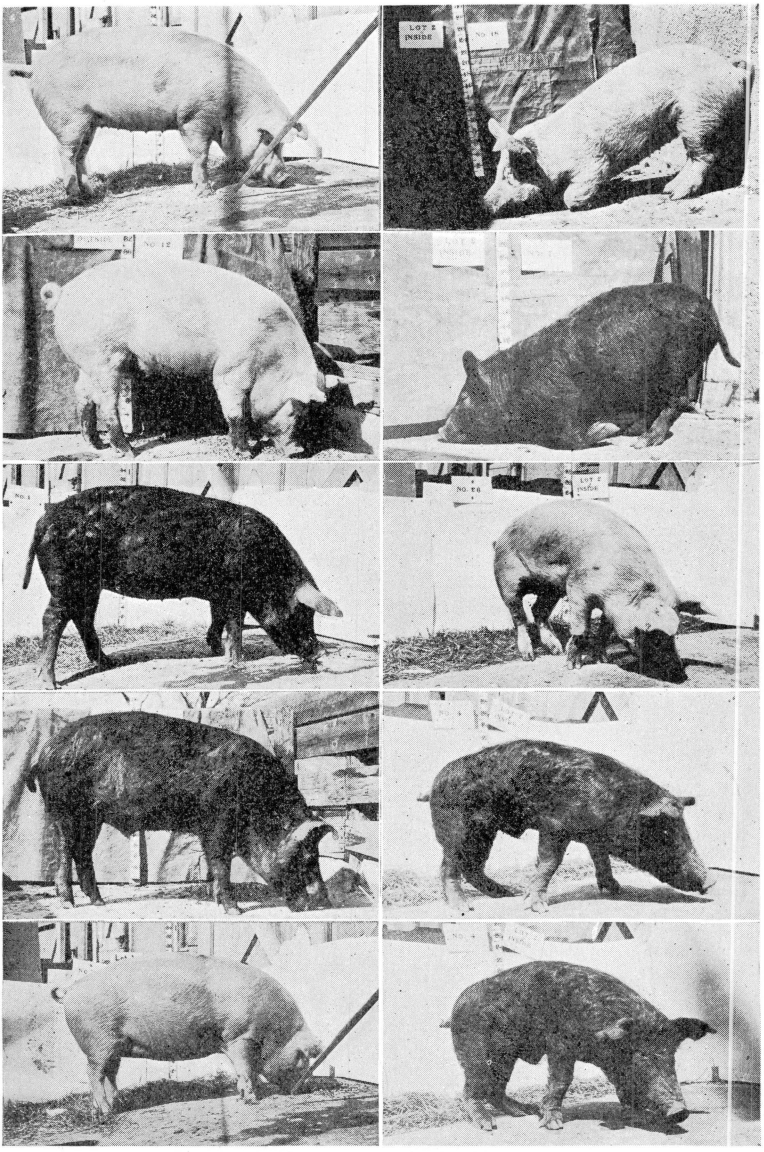


PLATE I

LOT 1, PHASE 1

Normal pigs were produced on the basal ration plus cod-liver oil and sunshine.

LOT 2, PHASE 1

Disaster followed where the basal ration was fed in the absence of sunlight. The pigs shown here were used in Trial I.

The clinical symptoms, as revealed by ante-mortem examination, showed no correlation between litter mates, sexes, or rates of growth. However, a direct correlation was noted between the time of "going down" and age, the older animals being the last affected. When rachitic symptoms appeared in the older animals, however, their complete collapse occurred, usually within two weeks.

### ANTE-MORTEM EXAMINATION—PHASE II

Within eight days after the beginning of Phase II, distinct signs of improvement were noted in Lot 2. In one pig this was quite slow, but by the end of the second month all pigs were walking and active. However, the thickening in the joints and the bowed legs never entirely disappeared. The general condition of the pigs improved greatly and the hair and skin took on a thrifty, mellow appearance. Clinical symptoms, as revealed by ante-mortem examination, are reported in Table 4. It will be noted that Lot I is reported as normal and in good condition.

TABLE 4.—*Summary of ante-mortem findings (Trial I, Phase II)*

| Findings | Cases observed | |
|---|---|---|
| | Lot 1—5 pigs Basal ration + C. L. O. (outdoors) | Lot 2—4 pigs Basal ration + C. L. O. (outdoors) |
| Slightly stiff and jerky gait | 0 | 2 |
| Knuckles over at knees | 0 | 1 |
| Front pasterns upright | 0 | 1 |
| Legs bowed | 0 | 2 |
| Carpus and tarsus thickened | 0 | 4 |
| Condition fair | 1 | 1 |
| Condition excellent | 4 | 3 |
| Deaths | 0 | 1[1] |

[1]Pig died on 161st day—pneumonia and pig typhoid.

### POST-MORTEM EXAMINATION—PHASE I

A careful autopsy was made of all animals slaughtered at the close of Phases I and II in an effort to verify clinical symptoms as well as to permit a detailed study of any abnormalities not revealed by ante-mortem examination. The autopsy included a study of the entire carcass and especially of the long bones and joints, the vertebrae, skull, mandibles, and teeth. The findings are reported in Table 5.

All pigs in Lot 1 were found normal with the exception of a fractured mandible in one pig. In Lot 2 ,however, no normal pigs were found. Most of them were thin in flesh, showing

TABLE 5.—*Summary of post-mortem findings (Trial I, Phase I)*

| Findings | Cases observed | |
|---|---|---|
| | Lot 1—6 pigs Basal ration + 1% C. L. O. (outdoors) | Lot 2—7 pigs Basal ration (indoors) |
| Marked softening of the skeleton | 0 | 7 |
| Thickening of the carpus and tarsus | 0 | 7 |
| Joint erosion | 0 | 4 |
| Excessive synovial fluid | 0 | 4 |
| Reddened and congested bones | 0 | 5 |
| Periostitis | 0 | 5 |
| Abnormal curvature of sacrum | 0 | 4 |
| Nephritis | 0 | 3 |
| Poor and flabby musculature | 0 | 5 |
| Emaciation | 0 | 3 |
| Loosened teeth | 0 | 3 |
| Deformed and fractured ribs | 0 | 7 |
| Enlarged costochondral junctions | 2 | 7 |
| Enlarged costovertebral junctions | 0 | 3 |
| Pneumonia (apical lobes) | 2 | 7 |
| Kidneys congested | 1 | 4 |
| Ascariasis | 2 | 4 |
| Fatty degeneration of liver | 0 | 3 |

deficient and flabby muscular development, although no significant abnormalities were noted in the viscera of any of the animals. A few round worms (*Ascaris lumbricoides*) were found in the pigs of both lots and also slight evidence of pneumonia in the apical lobes.

Several malformations of the joints were noted. A pronounced excess of cartilagenous material was found surrounding the joints and ends of the bones. In several cases an unusual amount of synovial fluid escaped when the joints were opened. Erosion of the articular surfaces was observed in several instances.

Beading of the ribs was found in every pig. This was most pronounced from the fourth to the eighth ribs. The costochondral junctions were most affected by beading but in some cases the costovertebral junctions also were enlarged. In one case pronounced costovertebral beading was noted with only slight costochondral involvement. Figure 3 shows the extent of the "rachitic rosary" in this pig. Fractures of the ribs were found, usually near the center of the shaft. In one pig 18 ribs showed fractures. In most cases the fractures were of long standing and healing had taken place, producing an enlarged callus which showed a marked deficiency of calcareous material.

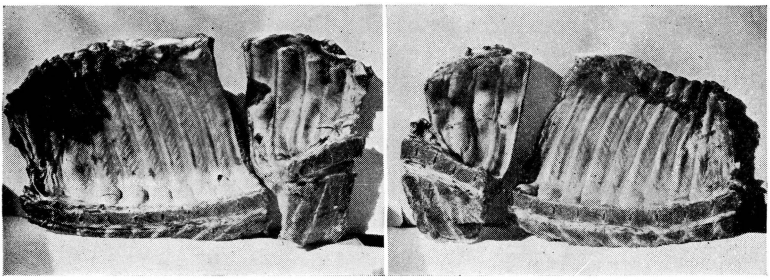

FIG. 3.—Enlarged costochondral and costovertebral junctions and fractured ribs were common in Lot 2

A pronounced softening of the bones was evident, especially of the larger ones. Besides softness, the long bones were not regular in contour and frequently the shaft was disproportionately short. In many cases the teeth were loose and imperfectly formed, yet no evidence of scurvy was found. These findings were common and existed in nearly all of the pigs of Lot 2 in varying degrees but were not common in any of the pigs of Lot 1.

#### POST-MORTEM EXAMINATION—PHASE II

In order to study the extent of recovery made by the pigs in Lot 2, post-mortem examination was carried on in Phase II as in Phase I. The results of these findings are reported in Table 6. As in Phase I, Lot 1 was normal. No evidence whatever was revealed that would account for the slow growth made by one pig.

TABLE 6.—*Summary of post-mortem findings (Trial I, Phase II)*

| | Cases observed | |
|---|---|---|
| | Lot 1—5 pigs Basal ration + C. L. O. (outdoors) | Lot 2—4 pigs Basal ration + C. L. O. (outdoors) |
| Slight softening of skeleton | 0 | 3 |
| Thickening of carpus and tarsus | 0 | 3 |
| Abnormal curvature of sacrum | 1 | 1 |
| Poor muscular development | 0 | 1 |
| Excessive synovial fluid | 0 | 1 |
| Bone deformity | 0 | 1 |
| Enlarged costochondral junctions | 0 | 4 |
| Enlarged costovertebral junctions | 0 | 1 |
| Ascariasis | 2 | 2 |
| Pneumonia | 0 | 3 |

Lot 2 showed many of the same abnormalities as in Phase I, although to a lesser degree. The bones were harder than in Phase I, yet softer than the bones of Lot 1 of either phase. The tendency toward recovery of the joints was apparent, though there was still an excess of cartilagenous material surrounding the articulations. In one pig, a large amount of a watery fluid was found surrounding the scapulo-humeral and humero-radial joints. The marked thickening of the pastern joints noted clinically in Phase I of pig No. 25, although present, was much less marked at the close of Phase II.

In general, it seems safe to conclude that some recovery had taken place and that the abnormal findings of Phase II were less frequent and less pronounced than in Phase I. However, complete recovery had not taken place at the close of the experiment and it is doubtful whether all evidence of deformity in the skeleton would ever wholly have disappeared.

### MACROSCOPIC PATHOLOGY OF THE BONES—PHASE I

Studies on the macroscopic pathology of the bones were made by Dr. L. Van Es of the Department of Animal Pathology and Hygiene. Since the right humerus was reserved for histological examination, it was necessary to confine these studies to the tibia and sixth rib. Neither of these bones is as satisfactory for this purpose as the humerus or femur, since they are smaller and mature earlier, and therefore do not show abnormalities to so marked a degree. It was found in Trial A that the humerus and femur frequently showed lesions while the tibia from the same pig was apparently normal. This agrees with the observations of Kernkamp (28).

As in previous studies, no abnormalities were found in the bones of Lot 1, while those of Lot 2 revealed several characteristic rachitic lesions. The softness noted on post-mortem examination was borne out in macroscopic study of the split bone. The bone wall was thinner than is normal and quite porous. The compact layer especially was found to be deficient. Beading and fractures of the ribs were common.

The evidence of rickets in the growing portion of the bone was more pronounced in the tibia than in the rib. The proximal extremity of the tibia in all cases seemed more affected than the distal end and in some cases only the proximal end was pathological. In the case of the rib, the reverse was true, the costochondral end being more affected than the costovertebral. It appears that pathological changes occur more frequently in the larger end where most growth takes place.

One of the most frequent bone lesions was a widening, and, in some cases, a detachment of the epiphyseal cartilage. The osteoid layer was also affected, being softened and widened

and, in some cases, showing a fibrous condition. Softness of the cancellated tissue was noted and occasionally the marrow cavity extended much farther than is normal. Erosion of the articular surfaces and excessive cartilagenous material around the ends of the bones were common, as will be noted in Table 7.

TABLE 7.—*Summary of macroscopic studies of split bone (Trial I, Phase I)*

| Findings | Cases observed | | | |
|---|---|---|---|---|
| | Lot 1 Basal ration + C. L. O. (outdoors) | | Lot 2 Basal ration (indoors) | |
| | Tibia | 6th rib | Tibia | 6th rib |
| Epiphyseal line widened | | | | |
| a—Proximal | 0 | 0 | 6 | 2 |
| b—Distal | 0 | 0 | 4 | 5 |
| Osteoid hemorrhagic | | | | |
| a—Proximal | 0 | 0 | 4 | 3 |
| b—Distal | 0 | 0 | 2 | 1 |
| Osteofibrosis | | | | |
| a—Proximal | 0 | 0 | 5 | 0 |
| b—Distal | 0 | 0 | 3 | 0 |
| Cancellated tissue soft | | | | |
| a—Proximal | 0 | 0 | 6 | 3 |
| b—Distal | 0 | 0 | 4 | 5 |
| Marrow cavity extended | 0 | 0 | 3 | 0 |
| Periosteal thickening | 0 | 0 | 5 | 6 |
| Shaft softened | 0 | 0 | 4 | 6 |
| Erosion of articular cartilages | 0 | 0 | 4 | 0 |
| Deformity | 0 | 0 | 2 | 6 |
| Number of bones | 6 | 6 | 7 | 7 |

MACROSCOPIC PATHOLOGY OF THE BONES—PHASE II

The findings in a macroscopic examination of the bones in Phase II are reported in Table 8, and, as will be noted, Lot 1 was normal except for a slight beading of the rib in one case and slight hyperemia in the epiphyseal cartilage of the tibia in another. The bones were hard and of good quality.

In Lot 2, recovery was apparently taking place as shown by harder and better calcified bones than in Phase I. However, recovery was not complete, as was evidenced by softer and less perfectly formed bones than those of Lot 1. One pig showed an enlarged and softened costochondral junction but a normal epiphyseal cartilage and a thin but hard bone shell with complete calcification in the osteoid layer.

TABLE 8.—*Summary of macroscopic studies of split bone (Trial I, Phase II)*

| Findings | Cases observed | | | |
|---|---|---|---|---|
| | Lot 1 Basal ration + C. L. O. (outdoors) | | Lot 2 Basal ration + C. L. O. (outdoors) | |
| | Tibia | 6th rib | Tibia | 6th rib |
| Epiphyseal line widened | | | | |
| a—Proximal | 1 | 1 | 4 | 1 |
| b—Distal | 0 | 0 | 0 | 2 |
| Osteofibrosis | | | | |
| a—Proximal | 0 | 0 | 0 | 0 |
| b—Distal | 0 | 0 | 0 | 1 |
| Cancellated tissue soft | | | | |
| a—Proximal | 0 | 0 | 1 | 0 |
| b—Distal | 0 | 0 | 0 | 1 |
| Shaft softened | 0 | 0 | 0 | 3 |
| Deformity | 0 | 0 | 0 | 2 |
| Number of bones | 5 | 5 | 4 | 4 |

ROENTGENOGRAPHY—PHASE I

Hess (21) states that "The Roentgen rays have been one of the significant factors in the recent advance in our knowledge of rickets." He further states that "With experience, one may draw histological deductions simply from the evidence obtained by the Roentgen rays." Along this same line, Kernkamp (28) noted marked evidence of calcium losses, thickened periosteum, and thinness of the compact portion of the bone wall in the bones of rachitic pigs by means of the radiographic plate.

Dr. E. W. Rowe of the Lincoln Clinic made and read the radiographs upon which the following studies are based. The bones used for X-raying were the tibia, humerus, and sixth rib from the right side. After the bones were dissected out, they were scraped clean of adherent tissue to prevent shadowing. The findings are reported in Table 9.

Rachitic lesions may be studied by X-ray from two angles: first, those changes that affect the joint, and secondly, those affecting the bones. In this study the joints had been destroyed, so the findings were confined to the long bones. However, autopsy revealed periarticular swelling in one or two cases, increase in joint fluid, and cartilagenous changes. Erosion was demonstrated in several cases both by the X-ray and macroscopic study. These changes affecting the joint were among the important rachitic lesions found in the pigs of Lot 2.

TABLE 9.—*Summary of X-ray findings (Trial I, Phase I)*

| Findings | Cases observed | | | | | |
|---|---|---|---|---|---|---|
| | Lot 1 Basal ration + C. L. O. (outdoors) | | | Lot 2 Basal ration (indoors) | | |
| | Humerus | Tibia | 6th rib | Humerus | Tibia | 6th rib |
| Widening epiphyseal line | | | | | | |
| a—Proximal | 0 | 0 | 0 | 5 | 5 | 2 |
| b—Distal | 0 | 0 | 0 | 3 | 2 | 5 |
| Separation epiphyseal line | | | | | | |
| a—Proximal | 0 | 0 | 0 | 3 | 3 | 1 |
| b—Distal | 0 | 0 | 0 | 1 | 1 | 4 |
| Increased condensation both sides epiphyseal line | | | | | | |
| a—Proximal | 0 | 0 | 0 | 7 | 7 | 2 |
| b—Distal | 0 | 0 | 0 | 6 | 5 | 4 |
| Erosion articular cartilages | 0 | 0 | 0 | 5 | 3 | 0 |
| Periostitis | 0 | 0 | 0 | 4 | 3 | 5 |
| Bone absorption | 0 | 0 | 0 | 5 | 3 | 4 |
| Bone atrophy, shaft | 0 | 0 | 0 | 6 | 4 | 3 |
| Thickening of cortex | 0 | 0 | 0 | 3 | 2 | 2 |
| Deformity | | | | | | |
| a—Proximal | 0 | 0 | 0 | 5 | 6 | 2 |
| b—Distal | 0 | 0 | 0 | 4 | 1 | 4 |
| c—Shaft | 0 | 0 | 0 | 5 | 2 | 6 |
| Bone fracture | 0 | 0 | 0 | 0 | 0 | 4 |
| Number of bones | 6 | 6 | 6 | 7 | 7 | 7 |

From a study of Table 9 it will be noted that X-ray findings were negative in Lot 1, agreeing with all studies made thus far. The slight beading shown at autopsy was not evident in the radiographs. In Lot 2, however, abnormalities were numerous and characteristic of rachitic lesions. Widening of the epiphyseal line was frequently noted. Increased condensation on both sides of the epiphyseal line was also common, as were bone atrophy, thickening of the cortex, deformity, erosion of the articular cartilages, decalcification, and absorption of lime salts. Thickening of the periosteum was also noted. Since it is impossible to show sufficient detail in reproductions of radiographs, they are not included in this publication.

In most cases the pathological findings in the tibia were less marked than in the humerus or the rib from the same pig and in no instance were the tibia and rib affected and not the humerus. Not only were the distal bones affected less than the proximal bones, but the distal ends were affected less than the proximal ends in all cases except the rib. Apparently rapidity of growth and bone abnormalities are closely related.

From a study of X-ray findings, Dr. Rowe was of the opinion that the pathological lesions were sufficient to warrant the diagnosis of rickets. He stated, "I think the pig bones under discussion show findings which are characteristic of rickets in the human being. I believe that from these findings the interpretation of the pathology found in rickets may be

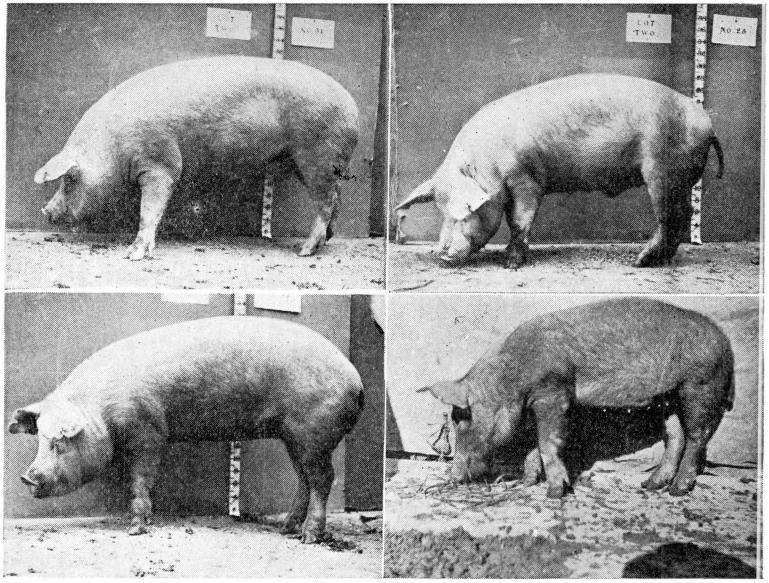


PLATE II

The pigs shown here are of Lot 2, Phase 2. The addition of cod-liver oil and exposure to sunshine brought a rapid improvement.

safely made. While not pathognomic in all cases, in many of them there is sufficient evidence to warrant the interpretation of rickets."

ROENTGENOGRAPHY—PHASE II

The only evidence of abnormality in Lot 1 at the close of Phase II was a very slight separation of the epiphyseal line in the proximal extremity of the humerus from pig No. 28, and evidence of a well-united fracture of the rib in pig No. 20, findings which are not sufficient to warrant calling the bones pathological.

That recovery was rapidly taking place in Lot 2 is evident as shown by recalcification, less widening of the epiphyseal cartilage, thicker bone walls, and fewer cartilagenous changes about the ends of the bones. The most common finding in this lot at the close of the trial was that of deformity; nearly every bone showed this condition to some degree. It appears that it would have been only a matter of time until active bone lesions would have disappeared, but it is doubtful whether the deformities ever would have. The findings revealed by X-ray study are presented in Table 10.

TABLE 10.—*Summary of X-ray findings (Trial I, Phase II)*

| Findings | Cases observed | | | | | |
|---|---|---|---|---|---|---|
| | Lot 1 Basal ration + C. L. O. (outdoors) | | | Lot 2 Basal ration + C. L. O. (outdoors) | | |
| | Humerus | Tibia | 6th rib | Humerus | Tibia | 6th rib |
| Widening epiphyseal line | | | | | | |
| a—Proximal | 0 | 0 | 0 | 2 | 3 | 0 |
| b—Distal | 0 | 0 | 0 | 1 | 0 | 3 |
| Separation epiphyseal line | | | | | | |
| a—Proximal | 1 | 0 | 0 | 0 | 0 | 0 |
| b—Distal | 0 | 0 | 0 | 0 | 0 | 0 |
| Increased condensation both sides epiphyseal line | | | | | | |
| a—Proximal | 0 | 0 | 0 | 0 | 2 | 0 |
| b—Distal | 0 | 0 | 0 | 2 | 1 | 1 |
| Bone absorption | 0 | 0 | 0 | 2 | 0 | 2 |
| Thickening of cortex | 0 | 0 | 1 | 0 | 0 | 0 |
| Deformity | | | | | | |
| a—Proximal | 0 | 0 | 0 | 2 | 1 | 0 |
| b—Distal | 0 | 0 | 0 | 0 | 0 | 0 |
| c—Shaft | 0 | 0 | 1 | 4 | 1 | 4 |
| Erosion articular surfaces | 0 | 0 | 0 | 2 | 0 | 0 |
| Number of bones | 5 | 5 | 5 | 4 | 4 | 4 |

### HISTOLOGICAL EXAMINATION OF THE BONES—PHASE I

Histological examination of the bones is perhaps the most accurate and reliable means of diagnosing rickets in pigs. Kernkamp (28) found that the sub-periosteal thickenings were composed of osteoid tissue, and the trabeculae were usually wide and not properly arranged. Collections of osteoblasts were seen along the edges of the trabeculae and the epiphyseal cartilages were often affected, being wider than normal and very irregular. In some cases, narrowed, very thin, and calcified spicules of the epiphysis and diaphysis were noted.

Evidence points (Korenchevsky, 29) toward the fact that rachitic and osteoporotic lesions are closely related and unless studied microscopically the differences confined to the cellular changes probably cannot be detected.

Dr. John S. Latta of the University College of Medicine made preparations for histological examination, which were read by Dr. L. Van Es. The results are reported in Table 11. The findings as reported for Lot 1 were negative, showing normal bones. In Lot 2, however, irregular epiphyseal lines and osteofibrosis were common. Defective calcification was evident in three instances. These studies strengthen the diagnosis of rickets indicated by the other studies made. Histological studies of the bones for Phase II are not available at this time.

### BLOOD ANALYSES—PHASE I

Numerous investigators have shown that the calcium and inorganic phosphorus in the blood plasma bear a close rela-

TABLE 11.—*Summary of histological findings (Trial I, Phase I)*

| Findings | Cases observed | |
|---|---|---|
| | Lot 1 Basal ration + C. L. O. (outdoors) | Lot 2 Basal ration (indoors) |
| | Humerus | Humerus |
| Osteofibrosis | 0 | 4 |
| Epiphyseal line irregular | 0 | 3 |
| Defective calcification | 0 | 3 |
| Number of bones | 5 | 4 |

tionship to rickets, and they regard blood analyses as of value in its diagnosis. Other workers advance the belief that blood analyses cannot always be relied upon as an index to rickets. In addition to a lowering of the calcium-phosphorus product, Hodgson (22) suggests a relationship of rickets to acidosis. Ackerson, Blish, and Mussehl (1), on the other hand, report little significant difference in the plasma bicarbonate of rachitic and non-rachitic chicks. In a study of the acid-base equilibrium, Hawk (17) gives the average normal value of carbon dioxide as 65 volume per cent, with an upper limit of 80 and a lower limit of 30 volume per cent in severe acidosis.

The normal calcium, phosphorus, and alkali reserve for pigs is not known. Blood analyses should serve at least three purposes: first, as an aid in diagnosing rickets; second, as an indication of the severity of the disease; and third, to arrive at a normal calcium and phosphorus blood level for pigs. With these objectives in mind, blood studies were carried out in this experiment.[2]

All bleeding was done from the tail of the pig. For the calcium and phosphorus determinations about 25 c.c. of blood was collected. Sodium citrate was used to prevent clotting. For the alkali reserve, the blood was collected in a small centrifuge tube under oil. Care was taken to leave about one-half inch of oil in the tube to prevent exposure of the blood to the air. The first analysis was made one month after the trial started, this being followed by two more analyses, two months apart.

[2] For the determination of phosphorus a combination of the Bell and Doisy (3), Briggs (6), and Briggs (7) methods were used. A Leitz colorimeter was employed in making the color comparisons. Calcium was determined by the method of Kramer and Tisdall (30). The plasma bicarbonate was determined by Van Slyke's method (52).

Little difference existed between lots in the calcium and phosphorus levels of the blood at the first analysis. From that time until the close, Lot 1 showed a steady increase in both calcium and phosphorus, while Lot 2 showed a decrease, the greatest decrease occurring from the fourth to the twelfth week. Although both changed, the calcium content was most affected. Expressed in milligrams per 100 c.c. of blood plasma, Lot 1 showed a calcium concentration of 12.27 mg. as against 6.83 mg. for Lot 2, a difference of 5.44 milligrams at the close of Phase I. The difference in the blood phosphorus between lots was 1.37 mg. or 3.85 mg. for Lot 1 as compared to 2.48 mg. for Lot 2.

The calcium-phosphorus product for Lot 1 at the close of Phase I ranged from 38 per 100 c.c. of plasma to 57, and Lot 2 ranged from 14 to 22. The average for Lot 1 was 47 and for Lot 2, 17, a difference of 30. Classified according to Howland and Kramer (24) every pig in Lot 2 showed a calcium-phosphorus product below 30, the zone in which rickets is to be expected. The fact that Lot 1 showed a calcium-phosphorus product of only 37 at the time of the first blood analysis and 47 at the close might be interpreted to mean that young pigs

TABLE 12.—*Blood analyses (Trial I)*

| Phase I | Lot 1—Basal ration + 1% cod-liver oil (outdoors) | | | Lot 2—Basal ration (indoors) | | |
|---|---|---|---|---|---|---|
| | Min. | Max. | Av. | Min. | Max. | Av. |
| Calcium per 100 c. c. plasma | | | | | | |
| Jan. 1, 1927 (*mg.*) | 9.30 | 12.30 | 10.22 | 8.50 | 11.30 | 9.82 |
| March 1, 1927 (*mg.*) | 10.30 | 12.90 | 11.62 | 6.20 | 9.20 | 7.55 |
| May 1, 1927 (*mg.*) | 10.90 | 13.70 | 12.27 | 6.00 | 8.00 | 6.83 |
| Inorganic phosphorus per 100 c. c. plasma | | | | | | |
| Jan. 1, 1927 (*mg.*) | 2.63 | 4.00 | 3.55 | 2.78 | 3.86 | 3.47 |
| March 1, 1927 (*mg.*) | 2.81 | 4.50 | 3.81 | 2.06 | 3.38 | 2.79 |
| May 1, 1927 (*mg.*) | 3.13 | 4.54 | 3.85 | 2.10 | 2.91 | 2.48 |
| Calcium-phosphorus product | | | | | | |
| Jan. 1, 1927 | 25 | 47 | 37 | 25 | 40 | 34 |
| March 1, 1927 | 33 | 55 | 44 | 15 | 31 | 21 |
| May 1, 1927 | 38 | 57 | 47 | 14 | 22 | 17 |
| **Phase II** | **Lot 1—Basal ration + 1% cod-liver oil (outdoors)** | | | **Lot 2—Basal ration + 1% cod-liver oil (outdoors)** | | |
| Calcium per 100 c. c. plasma | | | | | | |
| May 1, 1927 (*mg.*) | 11.10 | 13.30 | 12.14 | 6.00 | 7.20 | 6.80 |
| July 1, 1927 (*mg.*) | 12.10 | 14.20 | 13.04 | 10.50 | 13.00 | 12.02 |
| August 1, 1927 (*mg.*) | 11.80 | 13.00 | 12.26 | 10.90 | 12.60 | 12.00 |
| Inorganic phosphorus per 100 c. c. plasma | | | | | | |
| May 1, 1927 (*mg.*) | 3.13 | 4.10 | 3.68 | 2.10 | 2.78 | 2.17 |
| July 1, 1927 (*mg.*) | 3.55 | 4.24 | 3.76 | 2.38 | 3.70 | 3.26 |
| August 1, 1927 (*mg.*) | 3.55 | 3.82 | 3.71 | 2.96 | 3.38 | 3.26 |
| Calcium-phosphorus product | | | | | | |
| May 1, 1927 | 38 | 50 | 45 | 14 | 20 | 16 |
| July 1, 1927 | 44 | 54 | 49 | 25 | 48 | 40 |
| August 1, 1927 | 42 | 50 | 45 | 32 | 44 | 39 |

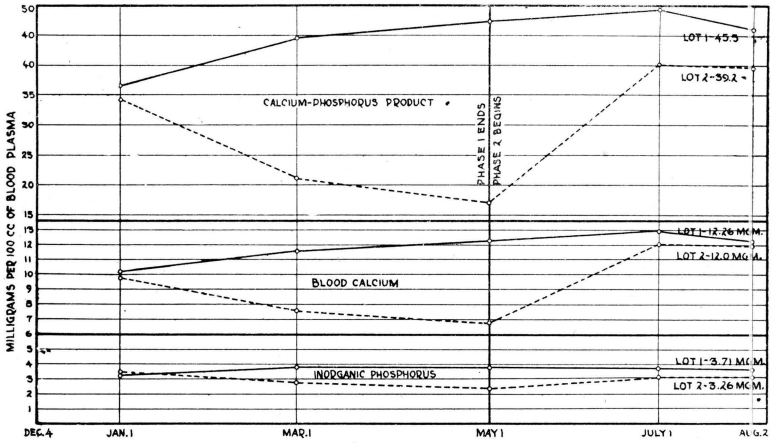


FIG. 4.—Blood analyses, Trial I

show a slightly lower product than older pigs. No doubt both lots were normal at the beginning of the trial. The results of the analyses are reported in Table 12 and in Figure 4.

The results from a study of the alkali reserve of the blood though not conclusive are interesting. As shown by Table 13, the difference between lots in plasma bicarbonate was 5.56 volume per cent at the time of the first analysis. Apparently during the first month of the experiment the carbon dioxide content of the blood was more affected than the calcium and phosphorus. The second and third analyses showed an increase in Lot 1 while Lot 2 remained constant until the third analysis, which showed an increase of 3 volume per cent. This increase cannot be explained. However, Lot 2 was still 6.41 volume per cent below Lot 1, which might be interpreted as evidence of acidosis in Lot 2.

These results do not agree with those of Hawk (17). If the blood of Lot 1 was a fair sample of normal pig blood, then the concentration of carbon dioxide in pigs' blood is lower than in men's. If the concentration is the same, then only two pigs showed a normal volume percentage of plasma bicarbonate, four pigs were on the border line of mild acidosis, thirteen pigs showed mild acidosis, and three pigs moderate to severe acidosis. It is very doubtful, however, that this was the case.

In general, these studies on the blood showed a significant lowering of the calcium and phosphorus in the blood of the pigs from Lot 2. The results also indicate that the calcium-phosphorus blood level of normal pigs near maturity is not greatly different from the blood level of man, as given by

TABLE 13.—*Blood analyses (Trial I)*

| Phase I | Volume per cent of plasma bicarbonate | | |
|---|---|---|---|
| | Jan. 1, 1927 | March 1, 1927 | May 1, 1927 |
| Lot 1—Basal ration+1% cod-liver oil (outdoors) | | | |
| Minimum | 35.20 | 35.6 | 43.20 |
| Maximum | 50.00 | 61.8 | 54.40 |
| Average | 46.16 | 49.28 | 49.98 |
| Lot 2—Basal ration (indoors) | | | |
| Minimum | 31.40 | 29.60 | 37.60 |
| Maximum | 47.50 | 50.40 | 52.20 |
| Average | 40.60 | 40.6 | 43.57 |
| **Phase II** | **May 1, 1927** | **July 1, 1927** | **Aug. 1, 1927** |
| Lot 1—Basal ration+1% cod-liver oil (outdoors) | | | |
| Minimum | 45.00 | 41.70 | 44.40 |
| Maximum | 54.00 | 48.80 | 50.40 |
| Average | 49.58 | 45.60 | 47.86 |
| Lot 2—Basal ration+1% cod-liver oil (outdoors) | | | |
| Minimum | 38.50 | 51.30 | 48.20 |
| Maximum | 52.20 | 56.20 | 54.00 |
| Average | 46.10 | 53.02 | 50.35 |

Howland and Kramer (24). The carbon dioxide content of the blood does not appear to be reliable as an index of rickets.

#### BLOOD ANALYSES—PHASE II

During Phase II, the calcium-phosphorus blood level in Lot 1 continued to increase, though slightly, during the first two months, while in Lot 2 it doubled during the same time. Why this increase in Lot 2 was not continued from the ninth to the fourteenth weeks cannot be explained unless the calcium and phosphorus were rapidly being taken up by the bones and body tissue, thus preventing an excess in the blood until a normal balance was restored in those organs.

The results of the blood analyses for alkali reserve are shown in Table 13. The same tendency for the plasma bicarbonate to increase at first and then remain constant is noted as in the case of the calcium and inorganic phosphorus. The curve of Lot 1, on the other hand, indicates that some disturbing factor was at one time present, causing a sudden decrease in alkali reserve. The following increase, however, indicates that this condition was temporary. Why Lot 2 was so much higher than Lot 1 cannot be explained.

In general, the blood analyses of Phase II indicate that the calcium and phosphorus have a tendency to return to normal

when the antirachitic factors are supplied. With this recovery there was also an increase in the volume percentage of the plasma bicarbonate in the blood.

### CHEMICAL ANALYSES OF THE BONES—PHASE I

The ash content of the bones from rachitic animals has been used by many investigators as an index of defective mineral metabolism. Hess (21) states that "Analysis of the bones is a simple and valuable method of ascertaining the changes which have been brought about by rickets. The significant characteristics of rachitic bones are their relative increase in water, their decrease in total ash, and lower content of calcium and phosphorus. Instead of ash bearing a normal ratio to organic matter of 3 to 2, the ratio may be reduced to 4 to 1." Several other investigators (4, 8, 12, 34, 39) have noted a lowered ash content in the bones of pigs showing rachitic symptoms. A strong positive correlation was also noted by these same workers between ash content and breaking strength of the bones.

Although the ash content of the bones and rickets often show a close correlation, Hart [3] makes the statement and is confirmed by Hess (21) that care must be taken in interpreting the results obtained from a study of the ash content. In case pigs have lost their appetites and are really starving, there may be a redeposition of lime in the bones, and consequently an ash analysis may be deceptive.

TABLE 14.—*Percentage of ash in humerus, tibia, and sixth rib (fat and water free) (Trial I, Phase I)*

| | Humerus | Tibia | 6th rib |
|---|---|---|---|
| Lot 1—Basal ration + 1% cod-liver oil (outdoors) | | | |
| Minimum | 47.66 | 51.66 | 44.99 |
| Maximum | 58.60 | 59.75 | 54.35 |
| Average | 54.18 | 56.85 | 51.42 |
| Lot 2—Basal ration (indoors) | | | |
| Minimum | 36.10 | 39.55 | 39.72 |
| Maximum | 51.26 | 51.52 | 50.58 |
| Average | 44.29 | 47.74 | 44.78 |

Some investigators (4, 8, 39) have noted a lowering in the bone ash from rachitic animals, though the variations in the methods used by different workers prevents a comparison of their results. These investigators are agreed, however, that the calcium-phosphorus ratio remains constant, thus eliminating the necessity of analyzing the bone for each of these elements.

[3] Personal communication.

In this study, ash determinations were made on the humerus, sixth rib, and tibia from the left side of each pig. The bones were cleaned of adherent flesh, dried, crushed, and extracted with ether and the entire bone then ground into flour. Analyses based upon duplicate gram samples of this material are reported in Table 14.

Trial A, the preliminary trial, indicated that the different bones from the same skeleton may vary considerably in the percentage of ash. This observation was borne out in this trial, since the average ash of the tibia in Lot 1 was 2.67 per cent higher than that of the humerus. The ribs showed 2.76 per cent less ash than the humerus and 5.43 per cent less than the tibia. The differences in the ash content between the bones of the same skeleton in Lot 1 were not the same as were noted in Lot 2. The tibiae of Lot 2 showed an average ash content of 3.45 per cent more than the humeri. If the bones from Lot 1 were normal, as indicated by previous studies, then the difference in ash content between different bones of the same skeleton was altered under the disturbing influence of rickets.

Among the three sets of bones studied, the humerus was most affected, the tibia second, and the rib was least. The humeri of Lot 2 showed an average ash content 9.89 per cent less than the humeri of Lot 1. The tibiae showed an average reduction in ash of 8.11 per cent and the ribs 6.64 per cent. Apparently the long bones are most affected in ash content by a rachitic condition. The later maturity of the humerus probably explains why that bone is more affected than the tibia. The ash content of the bones showed a correlation with the severity of the nutritional disorder. No correlation existed between ash content of the bones and age or breeding within the limits of this trial. On account of the destruction of the bones from Phase II in an accidental fire in a drying oven, ash determinations for that phase were not secured.

#### BREAKING STRENGTH OF THE TIBIAE—PHASE I

In view of the differences in bone ash between Lots 1 and 2, differences in breaking strength would be expected. To see if any correlation existed, the tibia from the right side of each pig was tested for breaking strength. Since great variation existed in the size and the thickness of the bone wall, the breaking strength was calculated on the basis of a square inch of bone area. In this way the results were made directly comparable and the quality of bone substance was considered. The strength required to break the bone as well as the breaking strength per square inch are given in Table 15.

The tibiae from Lot 1 showed an average breaking strength of 21,735 pounds per square inch of bone area, or 2.17 times

TABLE 15.—*Breaking strength of the tibia (Trial I)*

| Phase I | Pounds per square inch of bone area | Pounds required to break bone |
|---|---|---|
| Lot 1—Basal ration +1% cod-liver oil (outdoors) | | |
| Minimum | 17,805 | 395 |
| Maximum | 32,765 | 710 |
| Average | 21,735 | 577 |
| Lot 2—Basal ration (indoors) | | |
| Minimum | 7,323 | 120 |
| Maximum | 13,678 | 340 |
| Average | 10,010 | 221 |
| Phase II Lot 1—Basal ration +1% cod-liver oil (outdoors) | | |
| Minimum | 15,542 | 708 |
| Maximum | 21,547 | 1,020 |
| Average | 19,224 | 849 |
| Lot 2—Basal ration +1% cod-liver oil (outdoors) | | |
| Minimum | 10,587 | 300 |
| Maximum | 15,291 | 740 |
| Average | 12,552 | 527 |

that of Lot 2. As would be expected, some variation existed between individual bones within a single lot, yet the lowest strength in Lot 1 was 17,805 pounds. The stress required to break the tibia from different pigs in Lot 1 varied from 395 to 710 pounds. However, the bone that broke at 395 pounds showed a higher strength per square inch of bone area than did the bone that broke at 635 pounds. This was due entirely to a difference in thickness of the bone wall.

The breaking strength per square inch of bone area in Lot 2 varied from 7,323 to 13,678 pounds, with an average of 10,010 pounds. As noted in Table 15, no tibia in Lot 2 showed within 5,000 pounds as high a breaking strength per square inch of bone area as the weakest bone in Lot 1. The same difference as in Lot 1, between strength per square inch and stress required to break the bone, was noted in Lot 2. However, these differences tended to compensate for each other, since comparative averages are closely related. For instance, the strength per square inch of bone area was found to be 2.17 times as great in Lot 1 as in Lot 2. The stress required to break the bone was 2.61 times as great in Lot 1 as in Lot 2. It is apparent that the strength per square inch of bone area is the preferable method of measuring breaking strength, since differences in size as well as those due to thickness of bone wall are taken into consideration.

### BREAKING STRENGTH OF THE TIBIAE—PHASE II

At the close of Phase II, the average breaking strength of the tibiae from Lot 1 was less than at the close of Phase I. However, the difference between the two averages is less than between different bones from the same lot. Because of the larger size, more stress was required to break the bones at the close of Phase II than at the close of Phase I, yet, as previously noted, the "strongest" bones were not necessarily the strongest when strength was calculated on a unit area basis.

In Phase II the difference in breaking strength between the lots decreased, partly because of the decrease in the strength of Lot 1. However, the strength of the bones in Lot 2 increased from 10,010 pounds per square inch to 12,552 pounds. The stress increased from 62.71 pounds to 95.58 pounds, partly because of the increase in size of the bones. In Phase I, the tibiae of Lot 1 were 2.17 times as strong as those in Lot 2, but in Phase II this difference had decreased to 1.54. The average stress required to break the bones decreased in practically the same proportion.

From these studies it seems safe to conclude that some healing of the rickets occurred during Phase II. Apparently less time is required for gross pathological lesions to disappear than for the bone to regain its normal strength.

### DISCUSSION—PHASE I

The basal ration plus one per cent of cod-liver oil and sunlight produced normal growth and development in Lot 1. The failure of Lot 2 can be attributed to the absence of the antirachitic factors. Vitamin A was not lacking in the ration, since all pigs in Lot 2 grew rapidly until signs of rickets became pronounced, while Lot 1 continued to grow throughout the entire experiment. It seems evident, therefore, that growth, though necessary for the development of rickets, is controlled to a large extent by rickets. The severity of the disease shows a direct correlation with the retardation of growth. Differences due to age, sex, weight, or breeding within the limits of this experiment seemed unimportant in their relation to growth.

Ante-mortem and post-mortem examination of the experimental animals as well as macroscopic study of the bones, revealed a normal group of pigs in Lot 1 and in Lot 2 a group of pigs showing the characteristic rachitic lesions as observed in small animals and in man. The ash content of the bones and the breaking strength of the tibia confirmed the findings of previous examinations. The bones of Lot 1 were not only harder and higher in ash content but they were more than twice as strong as the bones of Lot 2.

Blood analyses are apparently a good index of rickets in pigs. Those animals showing severe signs of the disease also

showed a significant decrease in blood calcium and phosphorus. The calcium-phosphorus product of the blood plasma for Lot 2 was less than half of that of Lot 1. The product for normal pigs nearing maturity agreed quite closely with that given for man by Howland and Kramer (24). Though Lot 2 showed a reduction in plasma bicarbonate, it is doubtful that the difference between lots was of great significance. The carbon dioxide in the blood was lower for normal pigs than Hawk (17) gives for man, thus indicating that rickets is not necessarily accompanied by acidosis.

In general, Lot 2 showed the characteristic symptoms of rickets and it was evident that those symptoms were due to the absence of vitamin D and radiant energy, either one of which might have prevented the disease.

After the examination of the carcasses had been completed and the bones removed for detailed study, the meat was sold for human consumption. It is interesting to note that the lard made from the pigs of Lot 1 was returned by all purchasers because of the pronounced flavor of cod-liver oil. The meat also had a strong fishy taste, which made it objectionable to many. A similar condition is often experienced with fowls which have been fed cod-liver oil. It is generally recommended that the use of oil be discontinued ten days before slaughter. This was done in Phase II and no objectionable flavor was noted.

#### DISCUSSION—PHASE II

At the close of the second phase of the experiment, some recovery was noted in many of the abnormalities found in the pigs from Lot 2 during Phase I. The breaking strength of the bones increased and the blood analyses showed a calcium-phosphorus product only slightly below normal. A fire in the laboratory destroyed the bones which were to have been used for ash studies. Even without these studies, it is evident that healing occurred in Lot 2 during Phase II.

### TRIAL II—SUNLIGHT AS A PREVENTIVE OF RICKETS

In Trial II an effort was made to study the effect of sun-

TABLE 16.—*Growth data (Trial II)*

| | Lot 1—Basal ration (outdoors) | | | Lot 2—Basal ration (indoors) | | |
|---|---|---|---|---|---|---|
| | Min. | Max. | Av. | Min. | Max. | Av. |
| Age at close (*days*) | 203 | 220 | 214 | 211 | 216 | 215 |
| Initial weight (*pounds*) | 36.33 | 67.50 | 52.12 | 39.50 | 61.00 | 52.40 |
| Gain in weight (*pounds*) | 123.17 | 221.67 | 166.54 | 21.25 | 71.42 | 49.50 |
| Increase in body measurements | *cm.* | *cm.* | *cm.* | *cm.* | *cm.* | *cm.* |
| Length of body | 22.00 | 35.00 | 27.90 | 7.00 | 21.00 | 12.72 |
| Circumference | | | | | | |
| Chest | 24.00 | 44.00 | 33.65 | 7.00 | 20.50 | 14.17 |
| Flank | 21.00 | 44.00 | 29.95 | −3.50 | 11.00 | 5.28 |
| Height | | | | | | |
| At withers | 15.00 | 26.00 | 17.75 | 10.00 | 14.50 | 11.50 |
| At hips | 18.00 | 26.00 | 21.45 | 4.50 | 11.50 | 8.02 |

shine in swine nutrition. It differed from Trial I in that no cod-liver oil was used, sunlight being the sole source of the antirachitic factors. In addition, some further data were sought on the exact nature of the rachitic lesions produced in pigs fed a ration complete in all respects except vitamin D.

The experiment started November 26, 1927, and continued until April 3, 1928, a total of 129 days. Two lots of ten pure-bred Duroc Jersey pigs from the Experiment Station herd averaging 86 days in age and 52 pounds in weight were used in this investigation.

The basal ration was the same as was used in Trial I. Lot 2 was located inside the large hog barn previously described and Lot 1 was given the run of an outdoor pen where full benefits of the sun could be obtained. Experimental technique was the same as in Trial I.

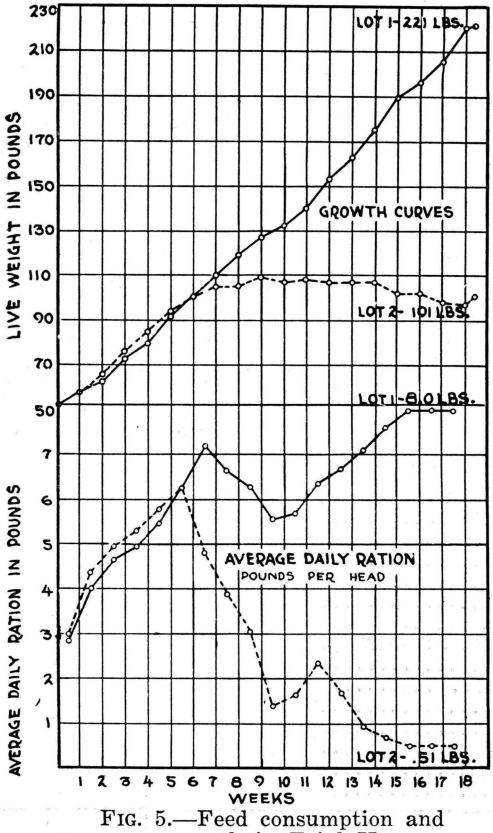


FIG. 5.—Feed consumption and growth in Trial II

### GROWTH

From the growth data presented in Table 16 and by Figure 5, it will be noted that sunlight exerted a marked influence on the growth of the experimental animals. The pigs in the outside lot made an average gain of 167 pounds, while the group kept inside gained only 49.5 pounds. The daily gain per pig in Trial II was practically the same as that made in Trial I.

During the first few weeks of the project, the lot kept inside (Lot 2) gained more rapidly than did Lot 1. In five weeks, Lot 2 averaged 5 pounds per pig heavier than Lot 1, probably because of the extremely cold

weather prevailing at that time. Lot 2 was sheltered from the cold and was not yet suffering from rickets. After the fifth week, the gains in Lot 2 decreased with each succeeding week until the pigs were actually losing weight. At the close of the trial, Lot 2 weighed exactly the same as at the end of the seventh week. Not only did the pigs in Lot 2 fail to make any net gain after the seventh week, but their growth curves as shown in Figure 5 are marked by great irregularity from week to week. Lot 1, on the other hand, continued to gain at approximately the same rate each week until the close of the trial, overtaking Lot 2 in the sixth week. When the experiment closed, Lot 1 averaged 219 pounds and Lot 2 averaged 102 pounds.

Skeletal growth, like gain in weight, was influenced by sunlight as indicated in Table 17. The greatest difference in growth between lots, as shown by measurements, was in circumference of chest and rear flank. The least difference was in height, that at the withers showing a gain of less than half of the height at the hips. Lot 1 gained in height more at the hips than at the withers, while the reverse was true in Lot 2. The increase in height of Lot 1 was due to normal growth and the failure to do likewise in Lot 2 can be attributed to the abnormal posture of the pigs at the close of the trial. The rachitic pigs were very steep in the front pasterns, thus increasing their height at the withers and decreasing it at the

TABLE 17.—*Summary of ante-mortem findings (Trial II)*

| Findings | Cases observed | |
|---|---|---|
| | Lot 1—10 pigs Basal ration (outdoors) | Lot 2—10 pigs Basal ration (indoors) |
| Stiff and stilty gait (cross-legged behind) | 0 | 7 |
| Unable to walk (crawls on knees) | 0 | 1 |
| Trembles when forced to move | 0 | 4 |
| Akenesia algera | 0 | 8 |
| Nervous and irritable | 0 | 6 |
| Knuckles over at knees | 0 | 2 |
| Front pasterns very upright | 0 | 6 |
| Hind pasterns very upright | 0 | 3 |
| Hind pasterns "down" (walks on dewclaws) | 0 | 3 |
| Legs bowed | 0 | 4 |
| Carpus and tarsus thickened | 0 | 8 |
| Stands with 4 feet together | 0 | 3 |
| Back extremely arched | 0 | 6 |
| Condition and musculature poor | 0 | 4 |
| Hair long, coarse, and rough | 0 | 3 |
| Thorax contracted | 0 | 5 |
| Deaths | 0 | 2[1] |

[1]One pig died on 59th day of acute pneumonia—the other destroyed on 94th day—paralysis.

hips since the hind feet were thrust forward under the body. The measurements taken in circumference of chest and rear flank were affected considerably by the fatness of the pig. Many of the pigs in Lot 2 were emaciated and in addition showed atrophy of the loin muscles. Length of body appeared to be the most accurate of the body measurements. Though there was a direct correlation between gain in weight and gain in body measurements, the former appears to be preferable as an index of growth.

Though both lots were given all the feed that they would consume without waste, Lot 1 showed an average daily feed consumption of 6.08 pounds per head as compared with 3.02 pounds for Lot 2. The difference in the appetites between the two lots became apparent during the seventh week of the experiment, at which time both lots were consuming slightly over six pounds per head daily. From that time until the close of the trial the feed consumption of Lot 2 showed a downward trend and Lot 1 a general upward trend. At the close, Lot 1 was consuming 8 pounds and Lot 2 only 0.8 pound per pig daily. The loss of appetite, due to the severity of the rachitic symptoms, no doubt was responsible for the difference in feed consumption between lots. The pain evident upon the movement of the rachitic pigs may also have been a factor in reducing feed consumption in Lot 2.

In Trial II, as in Trial I, growth continued at a normal rate in Lot 1 but was far below normal in Lot 2, and as the rachitic lesions became increasingly severe, growth decreased. The feed consumption of both lots can be compared with their respective growth curves (Figure 5). Sunlight, therefore, without doubt influenced growth by stimulating normal calcium and phosphorus metabolism and thereby affecting the health and thrift of the animal, whetting his appetite and increasing his gains.

### ANTE-MORTEM EXAMINATION

Lot 1 did not show any clinical symptoms of any nutritional disturbance during the experiment or at its close. The pigs were in a healthy, thrifty condition, having made good gains the entire period. The opposite was true in Lot 2, every pig showing clinical symptoms of rickets. The evidences of rickets, as shown clinically, were in many cases identical with those noted in Trial I. The outstanding symptoms in this trial were lameness and stiffness of all the animals in Lot 2. They were nervous and irritable, probably because of the evident pain when forced to move. Bowed legs, thickened hocks and wrists, contracted thorax, and bulging eyes were common findings as shown by Table 17. In some cases the extreme steepness of the front pasterns was so marked that the pigs

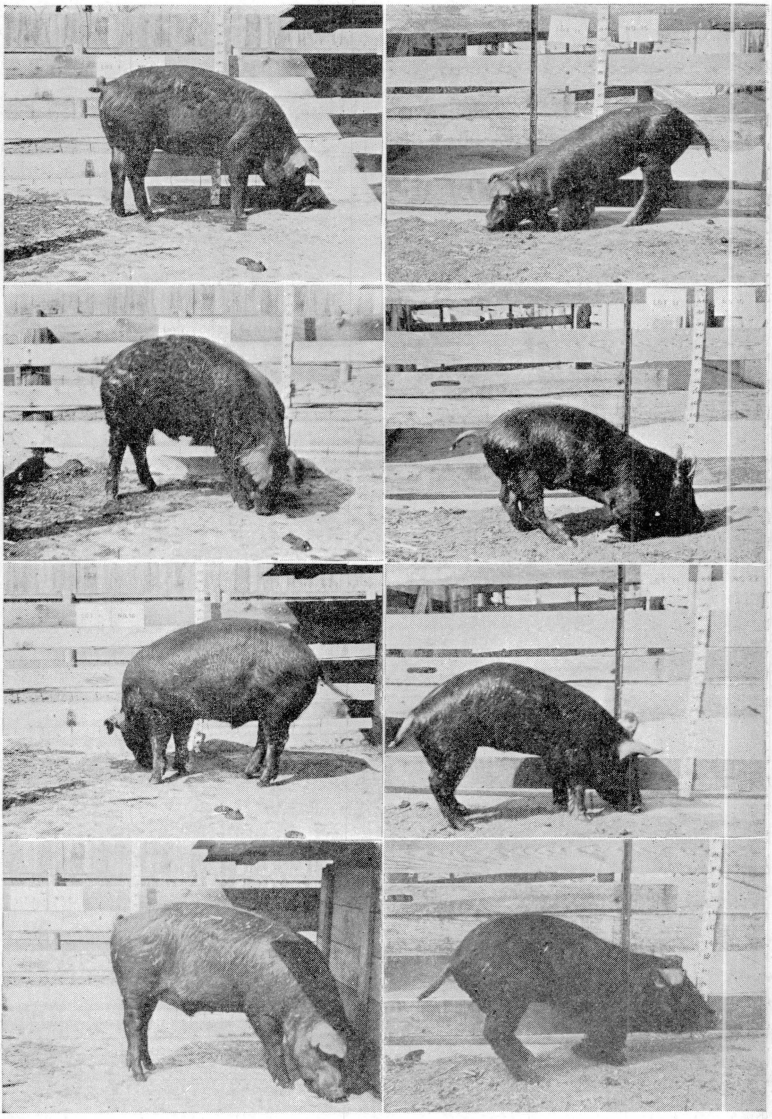

PLATE III

The pigs shown here were used in Trial II. Direct sunshine made the difference. Those to the right were kept indoors.

appeared to "knuckle over" and as a result crawled on their knees. Muscular development was poor in all of the animals, especially in the lumbar region, although this may have been due in part to emaciation. The hair was long, coarse, and dry, and the skin was thickened and scurfy in many instances.

The results of ante-mortem examination in this trial are in close agreement with those of other investigators and with the results obtained in Trial I. It is evident from a comparison of Lots 1 and 2 that sunlight exerted a profound influence on the nutrition of the animals in this experiment and in general prevented the occurrence of clinical signs of rickets.

### POST-MORTEM EXAMINATION

With few exceptions, the pigs in Lot 1 appeared normal at autopsy. These exceptions were slightly enlarged costochondral junctions found in several pigs. The fourth to the ninth junctions were most frequently involved. It is difficult to say just what significance should be attached to these findings, since apparently normal, well-fed pigs that have had access to sunlight often show similar findings. If these lesions are marked and frequent, it would seem that they probably indicate abnormal mineral metabolism. The only other abnormality found in the pigs of Lot 1 was apical pneumonia. In no case was the pneumonia severe and it is quite certain that the lung lesions had no relation to malnutrition. Neither lot was free from round worms (*Ascaris lumbri-*

TABLE 18.—*Summary of post-mortem findings (Trial II)*

| Findings | Cases observed | |
|---|---|---|
| | Lot 1—10 pigs Basal ration (outdoors) | Lot 2—9 pigs Basal ration (indoors) |
| Marked softening of skeleton | 0 | 8 |
| Thickening of carpus and tarsus | 0 | 8 |
| Joint erosion | 0 | 7 |
| Reddened and congested bones | 0 | 7 |
| Periostitis | 0 | 6 |
| Abnormal curvature of sacrum | 0 | 7 |
| Poor and flabby musculature | 0 | 5 |
| Emaciation | 0 | 3 |
| Loosened teeth | 0 | 3 |
| Deformed and fractured ribs | 1 | 6 |
| Enlarged costochondral junctions | 6 | 8 |
| Enlarged costovertebral junctions | 0 | 5 |
| Pneumonia | 6 | 8 |
| Kidneys congested | 1 | 6 |
| Fatty degeneration of liver | 0 | 5 |
| Degeneration of heart | 0 | 2 |
| Pleuritis and abdominal adhesions | 0 | 3 |
| Ascariasis | 4 | 5 |

*coides*) which may have accounted for some of the lung lesions.

All the pigs in Lot 1 carried considerably more fat than those in Lot 2. The thickness of the subcutaneous fat measured at the fifth lumbar vertebra in Lot 1 ranged from 1½ to over 2 inches, and in Lot 2, from practically nothing to 1¼ inches. The muscles of the pigs in Lot 2 lacked firmness.

As revealed in Table 18, the abnormalties found in the pigs of Lot 2 were many and varied. The articular cartilages of the various long bones were found to be eroded and roughened in many instances. Possibly this fact accounts, in part, for the lameness and stiffness of the pigs confined indoors. The skulls and mandibles were soft in most instances. The ribs were characterized by many enlargements; both the costochondral and costovertebral junctions were involved. The vertebral processes were very soft, and a marked curvature of the spinal column in the region of the sacrum was frequently noted. This condition was indicated in the live animal by an abnormally high arched back. The spinal column was normal in all cases. No fractures or dislocations were found which might cause partial impingement of the spinal cord. At autopsy, it was found that the distal end of the

TABLE 19.—*Summary of macroscopic studies of split bone (Trial II)*

| Findings | Cases observed | | | |
|---|---|---|---|---|
| | Lot 1 Basal ration (outdoors) | | Lot 2 Basal ration (indoors) | |
| | Tibia | 6th rib | Tibia | 6th rib |
| Epiphyseal line widened | | | | |
| a—Proximal | 0 | 0 | 6 | 1 |
| b—Distal | 0 | 0 | 4 | 6 |
| Osteoid hemorrhagic | | | | |
| a—Proximal | 0 | 0 | 3 | 0 |
| b—Distal | 0 | 0 | 2 | 3 |
| Osteofibrosis | | | | |
| a—Proximal | 0 | 0 | 4 | 0 |
| b—Distal | 0 | 0 | 2 | 4 |
| Cancellated tissue soft | | | | |
| a—Proximal | 0 | 0 | 5 | 0 |
| b—Distal | 0 | 0 | 3 | 5 |
| Marrow cavity extended | 0 | 0 | 2 | 0 |
| Periosteal thickening | 0 | 0 | 2 | 4 |
| Shaft softened | 0 | 4 | 7 | 6 |
| Erosion of articular cartilages | 0 | 0 | 3 | 0 |
| Deformity | 0 | 1 | 0 | 8 |
| Fracture | 0 | 0 | 0 | 2 |
| Number of bones | 10 | 10 | 8 | 8 |

ilium had failed to ossify in one case. A large amount of fibrous tissue had formed which evidently was not able to support the weight of the pig.

In Lot 2 the abnormalities found in the thoracic and abdominal cavity were confined chiefly to slight fibrous degenerative changes, especially in the liver and spleen, and slight congestion in the iliac lymph glands. Evidences of apical and extensive pneumonia were found in several of the pigs of Lot 2, one having died of acute pneumonia.

#### MACROSCOPIC PATHOLOGY OF THE BONES

The results obtained from a macroscopic study of the tibia and sixth rib are given in Table 19. As noted in this table, no abnormalities were found in the tibiae of Lot 1. However, a slight softening of the rib was noted in five pigs from this lot. Immaturity could not have been responsible for the softness, since so little difference in age existed between the pigs of this lot.

In every case the pigs of Lot 2 showed a softening of the ribs, some of which was particularly pronounced. Widening of the epiphyseal line was another common finding and, as in Trial I, the distal end of the rib was most affected. Beaded, deformed, and fractured ribs were common. Where fractures occurred, healing had taken place by the formation of a large callus. A softening of the tibiae was also noted in Lot 2, due no doubt to imperfect calcification. The cancellated tissue appeared soft in many cases and the shaft softened and hemorrhagic.

These findings emphasize the contrast between Lots 1 and 2. Since sunlight was the only variable, we may conclude that it exerted an influence on bone development. Winter sunlight alone did not produce in Trial II as good bones as did the combination of sunlight and cod-liver oil in Trial I. No doubt the rachitic symptoms noted in Lot 2 of this trial were the same as those noted in Lot 2 of Trial I.

#### ROENTGENOGRAPHY

As in Trial I, X-ray findings in this experiment are in close agreement with those of clinical and post-mortem examinations and macroscopic study of the bones. There was but one abnormality found in the bones of the pigs of Lot 1, a healed fracture in good alignment on the rib of pig No. 1. In Lot 2, however, a great number of abnormalities indicating faulty bone formation were found in the humerus, tibia, and rib. A spreading or widening of the epiphyseal lines forming the so-called rachitic metaphysis was common. Numerous fractures in various stages of healing were found in the ribs from the pigs in Lot 2. These fractures were evidently due to fragility

TABLE 20.—*Summary of X-ray findings (Trial II)*

| Findings | Cases observed | | | | | |
|---|---|---|---|---|---|---|
| | Lot 1—Basal ration (outdoors) | | | Lot 2—Basal ration (indoors) | | |
| | Humerus | Tibia | 6th rib | Humerus | Tibia | 6th rib |
| Widening epiphyseal line | | | | | | |
| a—Proximal | 0 | 0 | 0 | 8 | 7 | 2 |
| b—Distal | 0 | 0 | 0 | 6 | 4 | 8 |
| Separation epiphyseal line | | | | | | |
| a—Proximal | 0 | 0 | 0 | 7 | 5 | 1 |
| b—Distal | 0 | 0 | 0 | 4 | 3 | 5 |
| Increased condensation both sides epiphyseal line | | | | | | |
| a—Proximal | 0 | 0 | 0 | 8 | 5 | 2 |
| b—Distal | 0 | 0 | 0 | 4 | 4 | 5 |
| Erosion articular cartilages | 0 | 0 | 0 | 7 | 0 | 0 |
| Periostitis | 0 | 0 | 0 | 8 | 2 | 3 |
| Bone absorption | 0 | 0 | 0 | 6 | 4 | 6 |
| Bone atrophy, shaft | 0 | 0 | 0 | 6 | 3 | 5 |
| Thickening of cortex | 0 | 0 | 0 | 4 | 2 | 1 |
| Deformity | | | | | | |
| a—Proximal | 0 | 0 | 0 | 6 | 6 | 3 |
| b—Distal | 0 | 0 | 0 | 4 | 1 | 4 |
| c—Shaft | 0 | 0 | 0 | 5 | 3 | 6 |
| Bone fractures | 0 | 0 | 1 | 0 | 0 | 6 |
| Number of bones | 10 | 10 | 10 | 9 | 9 | 9 |

of the bones, which was caused by a lack of proper lime salt deposition. As a result of the fractures, many cases of exostosis were revealed radiographically. Enlargements at the ends of the ribs noted on autopsy were verified by X-ray study. These enlargements were characteristic of the "rachitic rosary" as observed in small animals. Abnormal curvature of the ribs was common and probably accounts for the contracted thorax so frequently noted before death. Deformity was also present along the weight-bearing lines of the humerus and tibia from the pigs in Lot 2. This deformity was in the nature of excessive curvature and is typical of what would be expected, since clinical examination revealed several pigs with bowed legs. Other rachitic lesions as detected by X-ray in

TABLE 21.—*Blood analyses (Trial II)*

| | Lot 1—Basal ration (outdoors) | | | Lot 2—Basal ration (indoors) | | |
|---|---|---|---|---|---|---|
| | Min. | Max. | Av. | Min. | Max. | Av. |
| Calcium per 100 c. c. plasma | | | | | | |
| January 20, 1928 (*mg.*) | 11.41 | 13.01 | 12.30 | 6.38 | 7.88 | 7.20 |
| March 3, 1928 (*mg.*) | 10.87 | 13.27 | 12.38 | 6.47 | 8.02 | 7.31 |
| April 3, 1928 (*mg.*) | 11.57 | 13.82 | 12.88 | 6.23 | 8.53 | 7.38 |
| Inorganic phosphorus per 100 c. c. plasma | | | | | | |
| January 20, 1928 (*mg.*) | 2.91 | 3.91 | 3.39 | 2.38 | 3.40 | 2.95 |
| March 3, 1928 (*mg.*) | 3.33 | 4.25 | 3.75 | 2.16 | 3.57 | 2.78 |
| April 3, 1928 (*mg.*) | 3.74 | 4.29 | 4.02 | 2.08 | 3.05 | 2.53 |
| Calcium-phosphorus product | | | | | | |
| January 20, 1928 | 35 | 48 | 42 | 18 | 26 | 21 |
| March 3, 1928 | 36 | 54 | 47 | 17 | 27 | 20 |
| April 3, 1928 | 43 | 65 | 52 | 15 | 22 | 19 |

the humeri and tibiae of Lot 2, though varying in severity, were similar, if not identical, to those noted in Trial I and bear out the results secured in previous studies in this trial. The results are given in Table 20.

### BLOOD ANALYSES

The blood was analyzed three times during this experiment for calcium and inorganic phosphorus. The first analysis was made eight weeks after the trial was started; the second analysis four weeks later; and the third at the close. The results are presented in Table 21 and Figure 6.

Without exception the pigs in Lot 1 had a higher blood calcium content than did the pigs in Lot 2 at the time of the first analysis. The average difference was 5.10 mg. per 100 c.c. of blood plasma. Though the average inorganic blood phosphorus was 0.44 mg. lower in Lot 2 than in Lot 1, all the animals in Lot 2 were not lower than those in Lot 1. Neither was this true with the phosphorus when the second analysis was made, but at the close of the trial all pigs in Lot 2 were below those in Lot 1 in inorganic blood phosphorus.

As in Trial I, the blood calcium and inorganic phosphorus increased throughout the experiment in Lot 1. Lot 2 showed a decrease during this same time, most of which occurred during the first eight weeks. Especially was this true with the calcium, the decrease in inorganic phosphorus being more gradual and consistent.

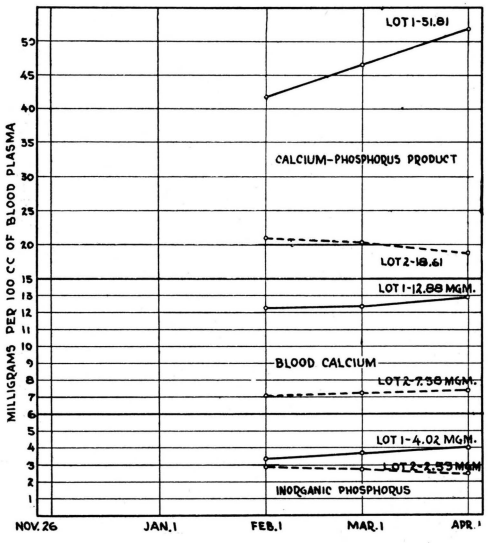


FIG. 6.—Blood analyses, Trial II

At the close of the experiment, the plasma of Lot 1 showed an average calcium content of 12.88 mg. and Lot 2 of 7.38 mg., a difference of 5.5 mg. per 100 c.c. The inorganic phosphorus concentration for Lot 1 was 4.02 mg. and for Lot 2, 2.53 mg., representing a difference of 1.49 mg. These differences appear to be significant and are in close agreement with the results of blood analyses in Trial I. The rise in the calcium content in Lot 1 during the last few weeks

of the experiment is probably due to the increase in the potency of the sun's rays during the spring months (51).

The increase in the calcium-phosphorus product of Lot 1, as in Trial I, probably indicates that the concentration of blood calcium and inorganic phosphorus for young pigs is less than for pigs nearing maturity. Figure 6 also indicates that the greatest changes in the blood levels took place during the first eight weeks of the experiment. This statement is made on the assumption that both lots were the same at the start of the trial. It appears sound in view of the uniformity in sex, age, breeding, and previous nutritional history. The difference between lots in calcium-phosphorus product at the time of the first, second, and final analyses was 21, 27, and 33 respectively. Lot 1 showed a calcium-phosphorus product at the close of 52, as compared to 19 for Lot 2. The concentration for both lots is only slightly above those of Trial I and indicates that the same nutritional disturbance affected the pigs of Lot 2 in both trials. Since sunlight was the only variable, it doubtless was responsible for the differences noted.

### CHEMICAL ANALYSES OF THE BONES

From ash analyses of the bones from the pigs in Lot 2, it is apparent that the mineral nutrition was disturbed in such a way as to lower the ash content of the skeleton. The percentages of ash in the bones studied from Lot 1 coincided closely with those of Trial I for normal pigs.

The average percentage of ash found in the humerus of Lot 1 was 54.95; in the tibia, 56.13; and in the rib, 50.96. This represented 10.29, 9.33, and 10.74 per cent respectively more ash than was found in similar bones from Lot 2. Though the difference was slight, the humeri were more affected than were the tibiae from the same animal. This agrees with the findings in Trial I and indicates that size and time of maturity are responsible for this difference in ash content between different bones from the same skeleton. It

TABLE 22.—*Percentage of ash and moisture in humerus, tibia, and sixth rib (Trial II)*

| | Humerus | | Tibia | | 6th rib | |
|---|---|---|---|---|---|---|
| | Ash[1] | Moisture | Ash[1] | Moisture | Ash[1] | Moisture |
| | Lot 1—Basal ration (outdoors) | | | | | |
| Minimum | 50.05 | 34.74 | 54.33 | 35.14 | 46.15 | 36.56 |
| Maximum | 57.37 | 38.51 | 57.24 | 40.26 | 55.51 | 45.78 |
| Average | 54.95 | 36.69 | 56.13 | 37.81 | 50.96 | 40.23 |
| | Lot 2—Basal ration (indoors) | | | | | |
| Minimum | 41.19 | 38.17 | 44.24 | 27.65 | 34.10 | 33.84 |
| Maximum | 47.69 | 50.32 | 50.50 | 45.25 | 47.43 | 54.93 |
| Average | 44.66 | 44.50 | 46.80 | 40.89 | 40.22 | 48.05 |

[1]Ash based on fat and water free sample.

will also be noted in Table 22 that the tibiae contained more ash than the humeri.

A study of the ash content of the ribs reveals an unusual situation, since the rib, a softer bone than either the humerus or the tibia, was more affected by the rachitic condition of the pig than either of the other bones. This is not in agreement with the results secured in Trial I. Why the ash in this case was so altered by rickets is not known. As might be expected, the rib showed less total ash than either of the other bones studied.

As an indication of the difference in the density and composition of the bone substance, the moisture content of the bones is reported. It will be noted that the rachitic bones contained more moisture than the non-rachitic. The highest percentage of moisture was found in the rib and the least in the tibia, showing that an inverse correlation existed between the ash and water content. It was very evident that the normal bones contained considerably more fat than the rachitic bones.

#### BREAKING STRENGTH OF THE TIBIAE

The observations made thus far, that sunlight exerted an influence on bone formation, are borne out in the study of the breaking strengths. Computed on a basis of a square inch of bone area, the average breaking strength of the tibiae from Lot 1 was 15,740 pounds as against 7,075 pounds for Lot 2. This difference seems significant, indicating a better calcified bone in the pigs of Lot 1. While variations occurred within each lot, Lot 2 without exception showed weaker bones than did Lot 1. On the basis of a square inch of bone area, the strength in Lot 1 was 2.22 times that of Lot 2. If the actual stress required to break the bone were used as a basis of comparison, then the bones of Lot 1 were 3.10 times as strong as those of Lot 2. As noted in Table 23, the tibiae from Lot 1

TABLE 23.—*Breaking strength of the tibia (Trial II)*

| | Pounds per square inch of bone area | Pounds required to break bone |
|---|---|---|
| Lot 1—Basal ration (outdoors) | | |
| Minimum | 12,701 | 460 |
| Maximum | 21,720 | 850 |
| Average | 15,740 | 713 |
| Lot 2—Basal ration (indoors) | | |
| Minimum | 5,254 | 155 |
| Maximum | 11,831 | 330 |
| Average | 7,075 | 230 |

broke at 713 pounds while those of Lot 2 broke at 230 pounds. Part of this is due to differences in the size of the bones and the thickness of the bone wall. This makes the comparison per unit of area more reliable as an index of strength.

In this trial, the tibiae from Lot 1 showed a breaking strength 5,995 pounds per square inch less than the tibiae from the same lot of Trial I. Likewise, Lot 2 of this trial showed weaker bones than Lot 2 in Trial I, the difference being 2,935 pounds. This difference can hardly be attributed to the slight difference in age that existed between the pigs of the two trials, but is probably due to the degree of calcification. Apparently sunlight alone did not produce as hard bones in Lot 1 as did a combination of sunlight and cod-liver oil used in Lot 1, Trial I. It appears that the pigs in Lot 2 in Trial II were suffering from more severe rickets than those of Trial I. However, breed may have had some influence on the relative strength of the bones. Bohstedt and co-workers (4) noted that Duroc pigs were very susceptible to bone abnormalities, an observation in accord with the limited amount of data secured in Trial I.

While the bones in this trial were not as strong as in Trial I, the relative difference between lots was practically the same in the two trials. The difference in breaking strength between lots was sufficient to warrant the conclusion that sunlight was responsible for the harder and stronger bones in Lot 1.

### DISCUSSION—TRIAL II

The conclusion drawn in Trial I, that pigs will not develop and grow normally in the absence of the antirachitic factors and that pigs are susceptible to rickets, the symptoms and lesions of which are similar to those in other animals, was again demonstrated in this trial.

As the pigs in Lot 1 attained a final average weight of 219 pounds, the basal ration apparently contained a sufficiency of the growth-promoting factor, vitamin A. This represented an average of 117 pounds per pig more gain than was made by Lot 2, the pigs denied exposure to sunlight. The severity of the rachitic condition of the pigs in Lot 2 was without doubt responsible for the difference between lots. Furthermore, all the pigs in Lot 1 survived the experimental period, while four pigs in Lot 2 died, all showing signs of severe rickets.

At the conclusion of the trial, the pigs in Lot 1 were normal in all respects in so far as could be determined. The pigs in Lot 2, however, showed many clinical symptoms of rickets in addition to many other abnormalities as shown by the various detailed studies.

The inorganic calcium and phosphorus content of the blood plasma was much lower in Lot 2 than in Lot 1. Throughout the trial, Lot 1 showed an increase in the calcium-phosphorus product, while Lot 2 showed a decided decrease. At the close of the trial, the product for Lot 1 was 52 compared to 19 for Lot 2.

That the pigs in Lot 1 had much harder and stronger bones than the pigs in Lot 2 is evident from breaking-strength determinations and ash analyses. The tibiae of Lot 1 were more than twice as strong as those of Lot 2 and the bones studied from Lot 1 showed a much higher percentage of ash than similar bones from the rachitic animals.

In general, it may be said that the abnormalities which occurred in the pigs of Lot 2 in this experiment could not be distinguished from those which occurred in Lot 2 of Trial I. The abnormalities found in these pigs were no doubt caused by severe rickets. In this experiment, sunlight alone apparently prevented rickets. This would justify the conclusion that sunlight appears to be important in the nutrition of swine on a ration low in vitamin D.

### TRIAL III—COD-LIVER OIL AS A PREVENTIVE OF RICKETS

The radiant-energy factor considered in Trials I and II played no part in the prevention of rickets in this experiment. The principal object in Trial III was to determine, if possible, to what degree the antirachitic vitamin contained in cod-liver oil would prevent rickets in pigs fed indoors on a rickets-producing ration. A further object was to make possible comparisons between sunlight and cod-liver oil, sunlight alone, and cod-liver oil alone in inducing normal mineral metabolism in pigs. In addition, it was felt desirable to make further inquiry regarding the exact nature of the lesions produced in rachitic hogs.

The experiment was begun January 3, 1929, and was terminated May 30, 1929, a total of 148 days. Purebred Berkshire, Hampshire, and Duroc pigs from the college herd, ranging in age from 94 to 119 days and averaging 63 pounds in weight, were used in this trial. There were two lots of ten each, both of which were confined to the large hog barn previously described and from which direct sunlight was excluded. The previous treatment and experimental routine were the same as that followed in Trials I and II.

The basal ration was identical with that used in previous trials. Lot 1 received the basal mixture plus cod-liver oil. Lot 2 received the basal mixture only. Cod-liver oil was used at the rate of one pound (450 c.c.) per 100 pounds of the basal mixture in Trial I. Since this was more than is usually recommended for small animals, it was decided to use less

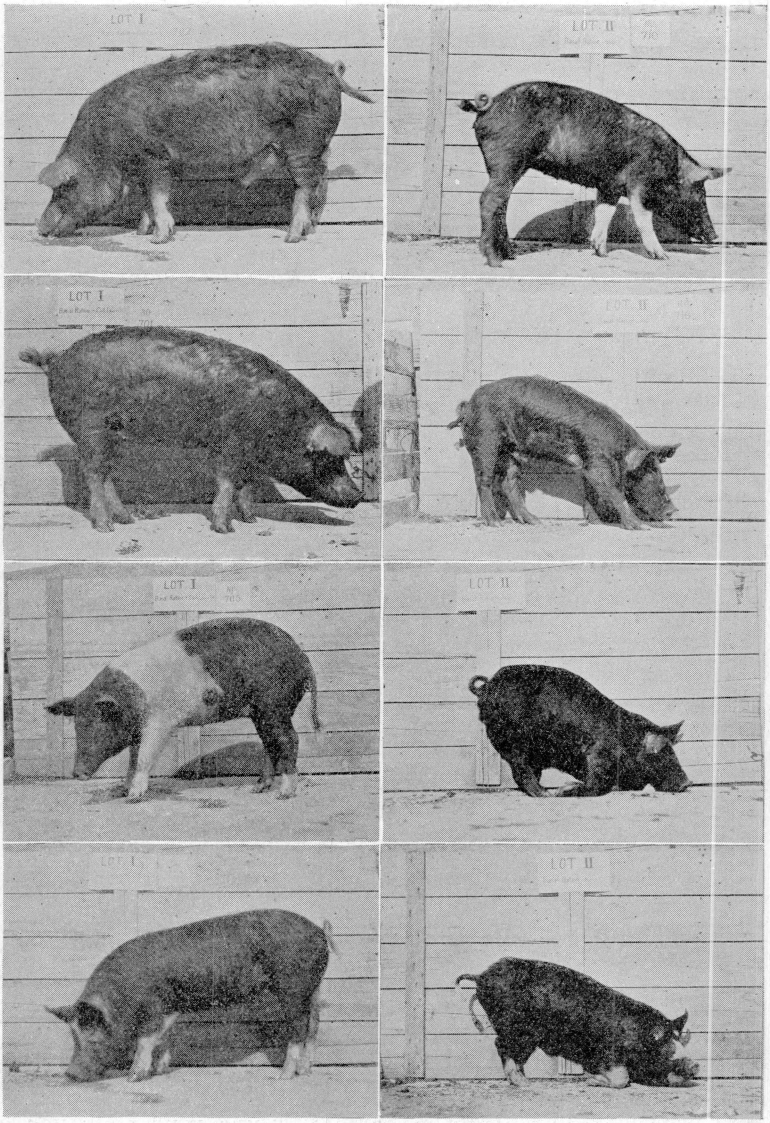


PLATE IV

These pigs were used in Trial III. Cod-liver oil protected pigs fed the basal ration indoors. Those to the right received no cod-liver oil.

cod-liver oil. Three-fourths of a pound (337.5 c.c.) was, therefore, used to each 100 pounds of basal mixture until March 25, 1929, at which time some of the pigs in Lot 1 began to show some rachitic symptoms. Since 0.75 pound proved inadequate, the amount was increased to one pound (450 c.c.), the same as that used in Trial I. Pure Norwegian non-freezable cod-liver oil of the highest grade was used in both trials and, as nearly as could be determined, the quality of the oil in both trials was identical.

The studies made in this experiment were the same as those of Trial II with two exceptions. It was not possible to make blood analyses in this trial and body measurements as a criterion of growth were discontinued because they appeared less reliable than gain in weight.

### GROWTH

The average gain in weight made by Lot 1 in this experiment was 0.58 pound per pig daily, which can hardly be considered as a satisfactory rate of growth for normal pigs (Table 24 and Figure 7). While this gain is three times as great as that made by Lot 2, it is approximately half that made by Lot 1 in the preceding trials. The slow growth made by Lot 1 in this experiment cannot be attributed to a lack of the growth-promoting factor in the ration because of the satis-

TABLE 24.—*Growth data (Trial III)*

| | Age at close | Initial weight | Gain in weight | Av. daily gain |
|---|---|---|---|---|
| Lot 1—Basal ration + cod-liver oil (indoors) | | | | |
| | *Days* | *Pounds* | *Pounds* | *Pounds* |
| Minimum | 242 | 49.66 | 40.34 | 0.27 |
| Maximum | 267 | 78.33 | 181.67 | 1.23 |
| Average | 261 | 62.86 | 86.70 | 0.59 |
| Lot 2—Basal ration (indoors) | | | | |
| | *Days* | *Pounds* | *Pounds* | *Pounds* |
| Minimum | 242 | 45.00 | 15.67 | 0.10 |
| Maximum | 267 | 76.66 | 57.00 | 0.39 |
| Average | 260 | 62.73 | 28.23 | 0.19 |

factory results in the two previous trials. In addition, Lot 1 of this trial received cod-liver oil, which presumably contains an abundance of vitamin A. It is possible, though doubtful, that the pigs at the beginning of the trial were not as thrifty as those previously used. Although no signs of severe rickets in Lot 1 occurred at any time during the experiment, several pigs did show evidences of being on the border line. It is quite probable, therefore, that mild rickets was responsible for decreased gains in this lot. This observation is strengthened by the fact that the gains increased after the amount of

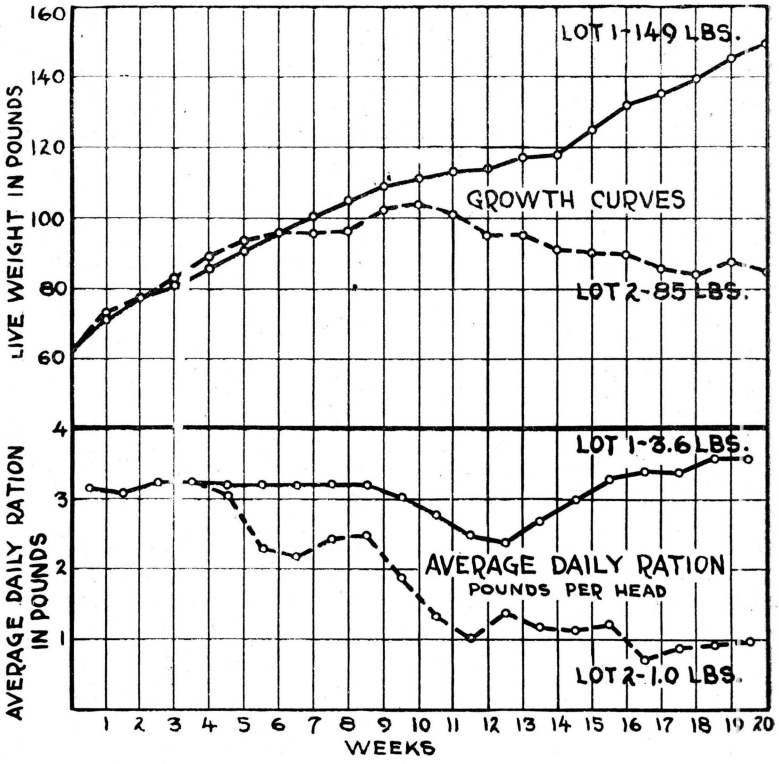


FIG. 7.—Feed consumption and growth in Trial III

cod-liver oil was increased from 0.75 to 1 per cent of the ration. The pigs in Lot 2 grew more rapidly than those in Lot 1 the first few weeks of the experiment (Figure 7). During the fifth week of the trial, the rapidity of gain in this lot decreased and by the sixth week Lot 1 was gaining more rapidly than Lot 2. After the sixth week the gains of Lot 2 never exceeded those of Lot 1 and from then on the difference in favor of Lot 1 increased. As in previous trials, the growth curve of Lot 2 was conspicuous because of the great irregularity in gains from week to week. At about the sixth week signs of rickets in Lot 2 became apparent, increasing in severity each succeeding week. Those animals most affected grew the least. These findings are in accord with those of previous trials.

The effect of the rachitic disturbances in Lot 2 was manifest in the feed consumption. At the start of the trial each pig was consuming 3.18 pounds of feed daily but as the experiment progressed the feed intake decreased until they were

taking less than half the original amount. On the other hand, the feed consumption of Lot 1 remained fairly constant throughout the trial, increasing only 0.42 pound per head daily. At the close, each pig was consuming 3.60 pounds, which is a light ration for eight-months-old pigs. This would indicate that some disturbing factor was present which influenced the appetites of the pigs in Lot 1. It is interesting to note, however, that while the feed consumption increased but little, the gains made by Lot 1 during the latter part of the trial were greater than at the beginning. This was especially true after the cod-liver-oil allowance was increased and the slight stiffness noted in this lot had disappeared. In general, it may be said that in Lot 2 a direct correlation between feed intake and rate of gain was shown.

### ANTE-MORTEM EXAMINATION

The first signs of rickets began to appear in Lot 2 about the fifth week of the experiment. At that time five pigs were showing stiffness in their gait, extremely arched backs, and a cramped appearance of the legs. At about the same time the first signs of rickets occurred, two pigs died of pneumonia. Although rachitic symptoms were present in both pigs, no

TABLE 25.—*Summary of ante-mortem findings (Trial III)*

| Findings | Cases observed | |
|---|---|---|
| | Lot 1—10 pigs Basal ration + C. L. O. (indoors) | Lot 2—8 pigs Basal ration (indoors) |
| Stiff and stilty gait (cross-legged behind) | 3 | 5 |
| Unable to walk (crawls on knees) | 0 | 3 |
| Akenesia algera | 0 | 7 |
| Nervous and irritable | 0 | 5 |
| Knuckles over at knees | 0 | 3 |
| Front pasterns very upright | 0 | 4 |
| Hind pasterns very upright | 0 | 2 |
| Hind pasterns "down" (walks on dewclaws) | 0 | 1 |
| Legs bowed | 1 | 5 |
| Carpus and tarsus thickened | 1 | 5 |
| Stands with 4 feet together | 0 | 2 |
| Back extremely arched | 0 | 4 |
| Condition and musculature poor | 0 | 5 |
| Hair long, coarse, and rough | 0 | 3 |
| Skin hard, thick, and scurfy | 0 | 3 |
| Eyes bulging | 0 | 3 |
| Thorax contracted | 0 | 4 |
| Deaths during experiment | 0 | 5[1] |

[1]Two pigs died on 41st day of pneumonia; one on 85th day—rickets, both femurs fractured; one on 130th day—rickets, pneumonia, emaciation; and one on 147th day—rickets and emaciation

evidence of pneumonia was shown upon examination the day before their death. From February 15 until the end of the trial, the pigs in Lot 2 grew steadily more rachitic. By the time the experiment had closed three more pigs had died because of rickets and the remaining five were barely able to walk.

Rachitic symptoms as noted clinically in this trial were, for the most part, identical with those described in Trials I and II. In view of this great similarity, it is felt unnecessary to elaborate further upon them. However, pneumonia was much more prevalent in this trial than in former trials. At times it seemed that the entire lot was suffering from an attack of acute pneumonia. Irritability, crooked legs, steep pasterns, enlarged heads, bulging eyes, stilty gait, pain, and rough, dead hair were common clinical evidences of rickets.

Although the pigs in Lot 1 did not make as rapid gains as were desired, they appeared to be in a thrifty condition for some time after the experiment started. At about the twelfth week of the trial, two pigs in Lot 1 showed a tendency toward a stiff, stilted gait and five pigs lacked thriftiness. Immediately following this, Lot 1 suffered an attack of pneumonia, from which all pigs recovered in the course of five or six days. However, the condition of several pigs indicated that they were on the border line of rickets if not already suffering from a mild form. It was at this time that the amount of cod-liver oil was increased to one per cent of the ration. A rapid recovery of the affected pigs took place following the increased cod-liver-oil allowance, and at the close of the trial no clinical symptoms of rickets were noted as shown in Table 25.

#### POST-MORTEM EXAMINATION

The differences in post-mortem findings of Lots 1 and 2 were quite marked as revealed by Table 26. For the most part, Lot 1 revealed few departures from the normal. The most common abnormality of the internal organs of Lot 1 was evidence of pneumonia in the lungs which most frequently affected the apical lobes. This condition was revealed in six pigs of Lot 1. Only one pig showed an involvement of the main lobes and in this case the upper one-third was affected. Extensive thoracic and abdominal adhesions were found in two pigs. Four pigs in Lot 1 showed evidence of the "rachitic rosary", that is, beading of the costochondral junctions. In some cases, several ribs on both sides were involved. One fractured rib was found in Lot 1. It is doubtful that much stress can be laid on beading, since this is a very common finding in carcasses of normal hogs. It is interesting to note, however, that in each case where beading occurred a slight softening of the bones was also noted, probably indi-

TABLE 26.—*Summary of post-mortem findings (Trial III)*

| Findings | Cases observed | |
|---|---|---|
| | Lot 1—10 pigs Basal ration + C. L. O. (indoors) | Lot 2—8 pigs Basal ration (indoors) |
| Marked softening of skeleton | 3 | 7 |
| Thickening of carpus | 2 | 7 |
| Joint erosion | 0 | 3 |
| Reddened and congested bones | 0 | 5 |
| Periostitis | 0 | 4 |
| Abnormal curvature of sacrum | 0 | 3 |
| Poor and flabby musculature | 2 | 4 |
| Emaciation | 2 | 5 |
| Loosened teeth | 0 | 3 |
| Deformed and fractured ribs | 1 | 5 |
| Enlarged costochondral junctions | 3 | 7 |
| Pneumonia | 3 | 7 |
| Kidneys congested | 1 | 2 |
| Cervical lymph glands congested | 0 | 3 |
| Fatty degeneration of liver | 0 | 3 |
| Degeneration of heart | 0 | 3 |
| Pleuritis and abdominal adhesions | 2 | 1 |
| Pericarditis | 1 | 1 |
| Fractured femur | 0 | 2 |

cating abnormal mineral metabolism. No loose teeth were found.

The autopsy revealed abnormalities in every pig of Lot 2. Most of the animals were thin in flesh, some to the point of emaciation, and showed poor and flabby muscular development. The lack of firmness of the muscle was pronounced in three pigs. Rib fractures were numerous, and a marked softening of the bones was evident. Especially was this true of the larger bones. Enlarged joints and deformed bones were common post-mortem findings, and practically every pig showed a marked excess of cartilagenous material surrounding the joints and ends of the bones. The carcass of one pig was condemned because of emaciation and another because of an excess of synovial fluid that escaped when the scapulohumeral joint was opened. Erosion of the articular surfaces of the bones was common, as was periostitis.

Few abnormalities of the digestive tract were found in the pigs from either lot. Slight degeneration of the kidneys and liver was noted in some cases. The lungs were quite frequently affected, showing evidence of pneumonia, particularly in the apical lobes. In two cases, degeneration of the heart and pericarditis were found. Extensive pleuritis and abdominal adhesions were noted in several cases.

### MACROSCOPIC PATHOLOGY OF THE BONES

Macroscopic study of the sixth rib and the tibia from the pigs of Lot 1 revealed that they were, in the main, normal. (Table 27.) In four cases the bone shell appeared to be thin, although the bones were hard and of good texture. The cancellated tissue and epiphyseal line seemed unaffected. However, one pig did reveal a defective rib, which was very soft throughout and showed a healed fracture of the shaft. It is interesting to note here that the tibia from the same pig was normal except for a thin bone shell. Probably this pig either had been, or was at the time, suffering from rickets. If the latter was true, then the disorder was undoubtedly in the early stages and had not yet seriously affected the tibia. In previous trials it was found that the humerus and ribs were affected by defective mineral metabolism sooner and to a greater degree than the tibia. Beading of the ribs, as mentioned in previous studies, was borne out by macroscopic examination, though to a lesser degree.

In the bones of Lot 2 only one tibia did not show a marked softness. In the other bones of this lot the cancellated tissue

TABLE 27.—*Summary of macroscopic studies of split bone (Trial III)*

| Findings | Cases observed | | | |
|---|---|---|---|---|
| | Lot 1 Basal ration + C. L. O. (indoors) | | Lot 2 Basal ration (indoors) | |
| | Tibia | 6th rib | Tibia | 6th rib |
| Epiphyseal line widened | | | | |
| a—Proximal | 0 | 0 | 5 | 0 |
| b—Distal | 0 | 1 | 2 | 7 |
| Osteoid hemorrhagic | | | | |
| a—Proximal | 0 | 2 | 4 | 0 |
| b—Distal | 0 | 2 | 2 | 4 |
| Osteofibrosis | | | | |
| a—Proximal | 0 | 1 | 5 | 0 |
| b—Distal | 0 | 2 | 3 | 4 |
| Cancellated tissue soft | | | | |
| a—Proximal | 1 | 1 | 6 | 8 |
| b—Distal | 1 | 3 | 4 | 5 |
| Marrow cavity extended | 0 | 0 | 3 | 0 |
| Periosteal thickening | 0 | 2 | 2 | 4 |
| Shaft softened | 1 | 3 | 6 | 8 |
| Erosion of articular cartilages | 0 | 0 | 4 | 0 |
| Deformity | 0 | 2 | 0 | 5 |
| Fracture | 0 | 3 | 0 | 3 |
| Number of bones | 10 | 10 | 8 | 8 |

was affected as well as the compact layer of the bone wall. In addition to softness, the bone wall was extremely thin in most cases. Beading of the distal ends of the ribs was very common, as were fractures of the shaft. In several bones a widening of the epiphyseal cartilage was found.

From a macroscopic study of the split bones, it appears that the pigs in Lot 2 were suffering from severe rickets. The findings in the bones of Lot 1 were, for the most part, negative, showing only a few evidences of slight abnormalities, not sufficient to warrant the conclusion that Lot 1 had suffered or was suffering from rickets.

### ROENTGENOGRAPHY

The findings, as revealed by X-ray study on Lot 1, agree in general with the macroscopic study. However, Table 28 indicates that the X-ray may be relied upon to bring out more of the less conspicuous bone abnormalities. The chief finding in the tibiae of Lot 1 was bone atrophy, though in every case the change was slight. In two instances a slight spreading of the epiphyseal line was noted. The ribs, like the tibiae, were not seriously affected, since eight revealed negative findings. One rib showed marked beading and spreading of the epiphyseal line and another showed a healed fracture with resulting deformity.

Evidence of defective bone structure was generally more pronounced in the humeri of Lot 1 than in the tibiae or ribs. In only two instances was the tibia or rib more affected than

TABLE 28.—*Summary of X-ray findings (Trial III)*

| Findings | Cases observed | | | | | |
|---|---|---|---|---|---|---|
| | Lot 1 Basal ration +C. L. O. (indoors) | | | Lot 2 Basal ration (indoors) | | |
| | Humerus | Tibia | 6th rib | Humerus | Tibia | 6th rib |
| Widening epiphyseal line | | | | | | |
| a—Proximal | 0 | 2 | 0 | 7 | 3 | 0 |
| b—Distal | 0 | 0 | 1 | 5 | 2 | 5 |
| Separation epiphyseal line | | | | | | |
| a—Proximal | 2 | 2 | 1 | 6 | 2 | 0 |
| b—Distal | 1 | 0 | 2 | 5 | 2 | 3 |
| Increased condensation both sides epiphyseal line | | | | | | |
| a—Proximal | 1 | 0 | 0 | 5 | 3 | 1 |
| b—Distal | 1 | 0 | 0 | 3 | 2 | 2 |
| Erosion articular cartilages | 0 | 0 | 0 | 7 | 3 | 4 |
| Periostitis | 0 | 0 | 0 | 8 | 4 | 6 |
| Bone absorption | 1 | 3 | 0 | 5 | 3 | 5 |
| Bone atrophy, shaft | 3 | 1 | 0 | 6 | 5 | 4 |
| Thickening of cortex | 0 | 0 | 0 | 3 | 2 | 2 |
| Deformity | | | | | | |
| a—Proximal | 2 | 0 | 0 | 7 | 4 | 4 |
| b—Distal | 1 | 0 | 0 | 5 | 1 | 3 |
| c—Shaft | 1 | 0 | 1 | 5 | 4 | 5 |
| Bone fractures | 0 | 0 | 1 | 0 | 1 | 4 |
| Number of bones | 10 | 10 | 10 | 8 | 8 | 8 |

the humerus from the same pig, indicating, as in previous studies, that the humerus is affected sooner and to a greater degree than the tibia. In addition to slight bone atrophy, the humeri of Lot 1 showed a spreading of the epiphyseal line in two cases, and in several instances slight deformity. In Lot 1 there were slight bone lesions, suggestive of a rachitic disturbance, and they probably explain why Lot 1 did not make more satisfactory growth. If rickets causes a susceptibility to pneumonia by lowering the vitality of the animal, as stated by Hess (21), then the prevalence of pneumonic lungs in Lot 1 is not surprising.

The abnormalities found in the bones of Lot 2 were many and characteristic of severe rickets. The ribs from three pigs of Lot 2 were the only bones that appeared normal. The humeri, tibiae, and remaining ribs showed marked rachitic changes. Widening and separation of the epiphyseal line was one of the common findings. Increased condensation of the bone was common, as was bone atrophy, thickening of the cortex, deformity, erosion of the articular cartilages, decalcification, and absorption of lime salts. Thickening of the periosteum was also noted. Rib fractures and beading observations were borne out by X-ray studies.

From the above findings, no doubt exists that the pigs of Lot 2 were suffering from severe rickets. It is apparent that some of the pigs in Lot 1 were close to the border line of rickets if not actually suffering from a mild form of the disease, or else that recovery was taking place. A study of the clinical symptoms of Lot 1, together with the fact that the radiographic plates revealed bone deformity in the presence of normal calcification, would lead to the conclusion that healing was occurring.

### CHEMICAL ANALYSES OF THE BONES

Trials I and II showed that the bones from rachitic animals are significantly lower in ash content than similar bones from normal animals. The ash determinations in those trials indicated that the severity of the rachitic condition was the principal factor in lowering the ash in the bones of the affected animals.

It is interesting to note that the results of this trial are not in agreement with those of Trials I and II. The previous studies in this experiment leave little doubt that the pigs in Lot 2 were suffering from severe rickets, while the pigs in Lot 1 were, at the most, suffering from a very mild form of rickets. If the conclusion drawn from previous trials was correct, then Lot 2, the rachitic pigs, should have shown a lower bone ash than Lot 1. Contrary to this expectation, the ash content of the humerus in Lot 2 was 4.03 per cent and of

the tibia 6.23 per cent higher than in Lot 1. The ash of the ribs seemed not to have been affected to any appreciable extent, since Lot 1 averaged only 0.41 per cent higher than Lot 2.

A possible explanation of these findings has been advanced by Hart and by Hess (21), who state that starvation due to severe rickets and decreased appetite would cause a redeposition of lime salts in the bones and ash determinations would, therefore, be of little value. The results obtained in this trial would tend to bear out these statements. A study of the feed records shows that the food intake of Lot 2 was very small, since these pigs were consuming at the close of the trial only one pound of feed per pig daily. It should also be remembered that post-mortem examination revealed that three pigs had suffered severely from emaciation, death having resulted in one case. Loss of appetite due to severity of rickets is probably the cause of what seemed to be starvation. Any feed other than that absolutely necessary for the maintenance of life was consistently refused. It is quite possible that these conditions were responsible for the fact that ash determinations showed negative results.

Besides the ash content of the bones, the percentage of moisture was calculated. Although moisture determinations may or may not be of any significance, the difference in average percentage between Lots 1 and 2 is interesting. The moisture content of the humerus in Lot 1 was 12.83 per cent, of the tibia 8.38 per cent, and of the rib 2.90 per cent higher than in Lot 2. The fat content, though accurate determinations were impossible due to the loss in drying, seemed noticeably higher in Lot 1. The results obtained from ash determinations are presented in Table 29.

### BREAKING STRENGTH OF THE TIBIAE

Both breaking strength per square inch of bone area and the stress required to break the tibiae are reported in Table

TABLE 29.—*Percentage of ash and moisture in the humerus, tibia, and sixth rib (Trial III)*

| | Humerus | | Tibia | | 6th rib | |
|---|---|---|---|---|---|---|
| | Ash[1] | Moisture | Ash[1] | Moisture | Ash[1] | Moisture |
| Lot 1—Basal ration +cod-liver oil (indoors) | | | | | | |
| Minimum | 41.21 | 47.68 | 39.47 | 55.98 | 45.05 | 50.23 |
| Maximum | 48.67 | 64.45 | 46.92 | 68.19 | 53.81 | 67.26 |
| Average | 45.48 | 57.59 | 44.03 | 61.68 | 50.48 | 58.57 |
| Lot 2—Basal ration (indoors) | | | | | | |
| Minimum | 43.94 | 38.45 | 46.83 | 46.35 | 51.21 | 47.03 |
| Maximum | 53.18 | 50.32 | 54.25 | 63.08 | 56.58 | 62.50 |
| Average | 48.51 | 44.76 | 49.49 | 53.30 | 53.61 | 55.67 |

[1]Ash based on fat and water free sample.

TABLE 30.—*Breaking strength of the tibia (Trial III)*

| | Pounds per square inch of bone area | Pounds required to break bone |
|---|---|---|
| Lot 1—Basal ration + cod-liver oil (indoors) | | |
| Minimum | 12,442 | 280 |
| Maximum | 23,839 | 660 |
| Average | 19,843 | 433 |
| Lot 2—Basal ration (indoors) | | |
| Minimum | 5,027 | 100 |
| Maximum | 13,234 | 240 |
| Average | 9,753 | 179 |

30. The tibiae from Lot 1 showed an average breaking strength of 19,843 pounds per square inch of bone area, or 2.02 times that of Lot 2. The average stress required to break the tibia in Lot 1 was 433 pounds, or 2.43 times that of Lot 2. Thickness of the bone wall is responsible for the difference in strength relation between lots when the two methods of calculation are used. The lowest breaking strength in Lot 1 was 12,442 pounds and the highest 23,839 pounds per square inch of bone area. Only two pigs in this lot showed a breaking strength that was less than 19,448 pounds. A similar variation in Lot 2 occurred; however, only one tibia in Lot 2 was stronger than the weakest in Lot 1. This fact seems significant and indicates that the slight disturbance apparently present in Lot 1 was not sufficient to materially affect the strength of the bones. It is also apparent that rickets had a profound effect on the strength of the bones in Lot 2, showing a direct positive correlation between breaking strength and severity of rickets.

### DISCUSSION—TRIAL III

In this trial a positive correlation was shown between the severity of rachitic condition and the rate of growth. Those animals most affected grew the least, and in several instances barely maintained their weight during several weeks. A comparison between Lots 1 and 2 showed that the growth of the pigs in Lot 1 was three times as great as that of Lot 2, although the pigs in Lot 1 did not make satisfactory gains. It is possible that a mild rachitic disturbance retarded the gains of the pigs in Lot 1.

Studies made from the ante-mortem and post-mortem examinations and the macroscopic examination of the bones were in close agreement, showing that the pigs in Lot 2 were suffering from severe rickets, while those in Lot 1 were normal or only slightly affected. However, the findings in

Lot 1 indicated that the pigs were in or near the danger zone of rickets. Ante-mortem study was not as valuable in detecting these border-line cases of rickets in Lot 1 as were post-mortem studies or macroscopic examination of the bones.

A study of breaking strength of the tibiae showed that vitamin D, as obtained from cod-liver oil, caused the production of stronger bones in Lot 1 than in Lot 2. The difference in strength between lots was quite marked, the tibiae in Lot 2 being less than half as strong as those in Lot 1. Ash analyses of the bones in this trial were contrary to the results secured in Trials I and II.

X-ray studies were in accord with other studies made in this experiment. Radiographs strengthened the conclusion that the humerus is more affected than the tibia and rib and also that the proximal ends of the humerus and tibia were more affected than the distal ends, while in the case of the rib the opposite was true. When the bones of Lot 1 were examined radiographically some evidence of mild rickets was found. Evidence pointed toward the fact that healing was taking place. If this were true, then Lot 1 was beginning to suffer from rickets about March 25, when it was found necessary to increase the allowance of cod-liver oil. This would indicate that cod-liver oil such as was used in this test must make up at least one per cent of the ration to prevent rickets in pigs fed indoors on a rickets-producing ration.

In this trial rachitic symptoms among the pigs of Lot 2 began to develop about the fifth or sixth week of the experiment. This was also true of the two previous trials. By the tenth week all the pigs in Lot 2 were showing very distinct symptoms and some were suffering from severe rickets.

That rickets lowers the vitality of the animal and makes him susceptible to other diseases, especially pneumonia, is evident from this trial. Practically every pig in Lot 2 and several in Lot 1 showed evidences of pneumonia at autopsy. Several attacks occurred during the course of the experiment, but fortunately most of the pigs recovered. At no time during the entire trial, however, were all the pigs in Lot 2 free from this disease.

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