THE INFLUENCE OF SOME NUTRITIONAL FACTORS ON EXPERIMENTAL HOG-CHOLERA INFECTION

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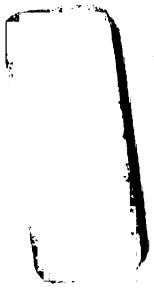

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By

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AN ABSTRACT

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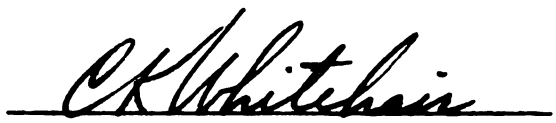
Approved by 

ABSTRACT

A series of experiments was conducted in an effort to determine possible relationships between some nutritional factors and experimental hog-cholera infection in baby pigs.

Ascorbic acid was administered to groups of animals in doses ranging from 25 to 200 mg. per pound body weight per day. This was administered by the intraperitoneal, subcutaneous, or oral routes. In 1 experiment the ascorbic acid appeared to lengthen the survival time of the infected pigs somewhat. Other evidences of beneficial effects were not noted. Of the 19 pigs given ascorbic acid, only 1 survived, and it is probable that its survival was due to immunity obtained from ingestion of colostrum.

The effects of a tryptophan-deficient diet on the symptoms, course, and lesions of experimental hog-cholera infection in baby pigs were also investigated. The deficiency delayed the onset of symptoms and time of death in 1 group of animals, and in 2 groups the deficiency caused a slight decrease in the severity of the gross lesions.

The administration of tryptazan, a tryptophan analogue, to a pig inoculated with hog-cholera virus was accompanied by a recovery from the infection. The results were inconclusive, however, due to the possibility that the animal had obtained some immunity to the hog-cholera virus from having nursed its dam during the first few days of life.

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I. INTRODUCTION

Two factors that play important roles in the life of an animal are nutrition and infection. If the body is to be maintained in a state of good health, it is essential that the dietary intake meet all the nutritional requirements for the species involved, and it is likewise essential that the individual be at least relatively free from infectious diseases. Not only are these two factors important in themselves, but the interactions of one with the other are also of significance. The belief is commonly held that an individual on a high plane of nutrition is more resistant to many of the infectious diseases than an individual whose diet is not adequate in all the essential nutrients. There is some evidence, however, that this relationship does not always hold true and that some nutritional deficiencies actually increase resistance to infection. In this study an attempt has been made to investigate certain aspects of the relationship between nutrition and experimental hog-cholera infection in the baby pig.

A few of the reasons for choosing the pig as an experimental animal follow. The baby pig is readily available at a reasonable cost. Also, the fact that the baby pig is without circulating antibodies at birth and that these antibodies are obtained from the dam only through the colostrum, makes this species desirable. By obtaining colostrum-deprived pigs, one is assured of having animals which have not been influenced by the dam's immunity and are therefore relatively uniformly susceptible to infection with certain agents. The pig is, of course, large enough that gross clinical and pathological observations may be made and tissue samples may be readily obtained.

The hog-cholera virus was used as the infectious agent in this work because of its known pathogenicity for the non-immunized pig. Almost without exception, inoculation of non-immunized pigs with this virus causes death. The incubation period, clinical symptoms, and gross and microscopic lesions have been well described and are fairly uniform.

The objectives of this study were as follows:

1. To determine the effects of administration of large doses of ascorbic acid to pigs experimentally infected with the hog-cholera virus. The role of ascorbic acid (vitamin C) in infection has been investigated by a number of workers. Numerous laboratory investigations have demonstrated the ability of this vitamin to neutralize certain toxins and viruses. Although the results have not been highly consistent, some beneficial effects have been noted from the administration of ascorbic acid to animals experimentally infected with pathogenic viruses. There have also been reports of the successful clinical use of ascorbic acid in human infections. Since the hog-cholera virus produces lesions closely associated with the cardiovascular system in a number of tissues, and since ascorbic acid is considered to be beneficial in maintaining the integrity of the capillary wall, it seemed worth-while to investigate this relationship.

2. To determine the effect of protein and tryptophan deficiency on hog-cholera infection. Experimentally, it has been demonstrated that certain deficiencies tend to increase resistance to infection. This experimental work has been done with animals on low levels of food intake or on diets deficient in protein, certain of the B vitamins, or certain of the amino acids. In most of the experiments, the infectious agent was a virus of known pathogenicity for the species involved. In view of the

results reported in the literature, it seemed advisable to determine if the resistance of the baby pig to experimental hog-cholera infection could be increased by feeding a diet deficient in tryptophan. This amino acid deficiency has been used in a number of the experiments alluded to previously.

3. To determine the effect of administration of a tryptophan analogue to an animal experimentally infected with hog-cholera virus. It has been fairly well established that viruses are high in nucleoproteins. The multiplication and growth of a virus in a host animal would be dependent on the synthesis of these specific proteins. The use of an analogue of one of the essential amino acids might serve to "block" or interfere with the synthesis of these nucleoproteins and thereby interfere with the growth and multiplication of the virus. For this purpose it is essential that the analogue be non-toxic to the host animal and that it be relatively soluble. A small amount of an analogue of tryptophan that met these requirements was made available for use in this study.

II. LITERATURE REVIEW

The interest which has been shown in the possible relationship between nutritional factors and resistance to infection is indicated by the large amount of material which has been published on various aspects of the subject. In preparing this literature review, only those references which seemed pertinent have been cited. Although much has been published on this subject, it was felt that a great deal of the material, for one reason or another, was not in line with the objectives of this study as listed previously. Also it became apparent in reviewing the literature that some of the results, especially those based on clinical observations in the field of human medicine, were of a rather indefinite nature and were not derived by use of well-controlled experiments.

A. The Role of Ascorbic Acid in Infection

It is known that most mammals require no dietary source of ascorbic acid (vitamin C). A series of three enzymes or enzyme systems is involved in the biosynthesis of l-ascorbic acid from d-glucuronic acid in animal tissues. In those mammals requiring ascorbic acid in the diet -- man, monkey, and the guinea pig -- enzymes catalyzing the first two reactions are present, but the last reaction cannot be carried out (18).

Numerous references are made to the possibility of ascorbic acid affecting the resistance of an organism to infection (3). A number of the functions of ascorbic acid have been pointed out in the literature as related to the defense mechanism of the body.

One of the functions of ascorbic acid in the body is related to its high concentration in the adrenal cortex. Although the exact

relationship has not been determined, it is postulated that the vitamin is important in the activity of this gland. It has been shown that the ascorbic acid concentration in the adrenal falls markedly when adrenal corticotrophic hormone is injected into rats (14). At the same time there is an increase in the amount of ascorbic acid in the adrenal vein (48). For this reason ascorbic acid may play a part in stress conditions such as those brought about by some of the infectious diseases.

Madison and Manwaring (32) report that the injection of rabbits with 100 mg. of ascorbic acid along with 0.5 ml. of horse serum causes the production of a precipitin titer in the blood which is several times as high as that found in rabbits injected only with the horse serum. Ecker et al. (12) have shown that a correlation exists between the concentration of ascorbic acid in the serum of guinea pigs and the complementing activity of the serum.

Ames and Nungester (1) have shown that the number of exudative polymorphonuclear cells showing phagocytic activity is increased as the concentration of ascorbic acid in the cells is increased. Massa (34) states that the respiration of leucocytes is increased by the presence of ascorbic acid.

Ascorbic acid is also important in the body's defense mechanism because it is necessary for the formation of intercellular substances such as collagen, osteoid, and dentine (36). Roskin (46) reports that the endothelial cells of capillaries are relatively rich in ascorbic acid. Sokoloff (50) states that one of the most important features of viral inflammation is the capillary syndrome. The endothelial cells are invaded by the viral particles, and this results in damage to the capillaries. Generalization of the viral infection is enhanced and the inflammatory

process intensified. Polozhintseva (40) mentions that the lowered capillary resistance can be demonstrated by means of negative pressure applied to the skin of children during the eruption of measles rash.

In 1935 Jungeblut and Zwemer (26), at Columbia University, reported the in vitro inactivation of diphtheria toxin by ascorbic acid. They also found that the vitamin helped protect guinea pigs against the fatal outcome of diphtheria intoxication to the extent that about 50 per cent of the animals survived when given 2 M.L.D. of toxin and 5 to 200 mg. of ascorbic acid simultaneously. Jungeblut (24) proposed that the inactivation of diphtheria toxin by ascorbic acid occurs as the result of direct interaction by the two substances. He found that this inactivation does not follow the laws of multiple proportions and is limited to relatively small doses of toxin and ascorbic acid. He also suggested that the toxin is inactivated during the auto-oxidation of ascorbic acid and that the substance responsible is probably a peroxide. In further work Jungeblut (23) also observed the inactivation of tetanus toxin in vitro by ascorbic acid. This is not due to an acid effect, since it occurs when the ascorbic acid has been adjusted to a pH at which the potency of the toxin is undiminished. Here again, the inactivation is limited to a rather definite quantitative range of toxin and vitamin.

Campillo (8) found that ascorbic acid is effective in the neutralization of influenza A and influenza B viruses in vitro, but it seems to be lacking in either prophylactic or therapeutic values when used in vivo in mice inoculated with such viruses. He attributed this failure to the presence of catalases in the mouse body which neutralize the normal viricidal powers of ascorbic acid.

Holden and Molloy (20) demonstrated the inactivation of herpes virus by ascorbic acid *in vitro* but not *in vivo*. They found that an excess of ascorbic acid was toxic when injected intracerebrally in rabbits and that 20 mg. caused death in a few hours.

In 1935 Jungeblut (22) reported the inactivation of poliomyelitis virus by ascorbic acid when the two were mixed before being injected into monkeys by the intracerebral route. Using 0.1 ml. of the supernatant of a centrifuged 10 per cent poliomyelitis suspension, he found the effective range of the vitamin to fall between 5 and 10 mg., although there was some protection above and below this point. In 1937 the same author (25) reported a decrease in the incidence of paralysis when doses of ascorbic acid ranging from 5 to 50 mg. per day were given to monkeys infected with the poliomyelitis virus. Prophylactic administration of the vitamin failed to protect against subsequent intracerebral infection.

Numerous reports have appeared in the literature relative to the clinical use of ascorbic acid in human medicine. McCormick (35) states that there is an unusually broad spectrum of antibiotic activity in ascorbic-acid therapy and that this activity includes all bacterial and viral infections. Manwaring (33) noted that doses of 1 g. of ascorbic acid appear to be of marked help in the treatment of the common cold. Brown and associates (7) indicate that ascorbic acid is helpful in checking the further development of colds under certain conditions when given in two 1-g. doses 24 hours apart. Klenner (30) has reported quite favorable clinical results in treating such diseases as diphtheria, poliomyelitis, herpes zoster, herpes simplex, chickenpox, influenza, viral encephalitis, measles, mumps, and viral pneumonia with massive doses of vitamin C. He used 1 to 2 g. of the vitamin intravenously or

intramuscularly every 2 to 4 hours and gradually increased the time interval with the subsiding of the infection as evidenced by return of the body temperature to normal and disappearance of symptoms. In a later report (29) he indicated that the usual dose of vitamin C is 65 mg. per kg. of body weight every 2 to 4 hours but that under certain conditions a satisfactory response was obtained by giving 250 mg. per kg. of body weight as a single intramuscular dose. In some cases it was necessary to repeat with half this amount 8 hours later. The vitamin was given in a concentration of 500 mg. per ml. of solution. Klenner refers to vitamin C as a "super-antibiotic" and attributes some of the reported failures of ascorbic acid therapy to inadequate amounts of the vitamin.

Egan (13) obtained beneficial effects from the use of ascorbic acid in combination with sulfonamides in the treatment of 6 Afghan hounds affected with chorea resulting from distemper. The daily dose was six 100-mg. tablets per animal. Recovery occurred in 2 to 6 months.

A recent report (4) indicates that the citrus fruit flavonoids, commonly found associated with ascorbic acid in natural sources of the vitamin, play an important role in resistance to such infections as the common cold, acute follicular tonsillitis, and influenza. The effectiveness of the flavonoids is thought to be related to their ability to restore to normal the increased permeability and fragility of capillaries. Boines (5) observed abnormal capillary fragility in severe, acute poliomyelitis. The capillary integrity was improved by the daily administration of 600 mg. each of hesperidin (a flavonoid) and ascorbic acid in divided doses. In 80 per cent of the cases improvement occurred in an average of 5 weeks. An improvement in the patient's sense of well-being and an increased appetite were noted within 1 week.

B. The Effect of Protein and Tryptophan Deficiency on Infection

The belief has been expressed that any deficiency in nutrition, whether it be of a quantitative or qualitative nature, is invariably accompanied by increased susceptibility to infection (31). The increased incidence of tuberculosis in the central European countries following World War I has also been attributed, at least in part, to the severe food restrictions imposed upon the population (10). This increased susceptibility may be due to the fact that protein depletion interferes with antibody production (2).

There has, however, been considerable evidence to support the idea that deficiencies do not always cause increased susceptibility, but, on the other hand, sometimes decrease susceptibility to disease. This has been especially true with diseases of viral origin. Rous (47), working with the experimental transmission of an avian tumor by cell-free filtrate, made the observation that the developing tumor nodule showed retrogression when the host became sick. If the health of the experimental animal returned, the tumor frequently reappeared and grew rapidly. Olitsky et al. (37) made similar observations in work with the foot-and-mouth disease virus. They found that guinea pigs suffering from malnutrition were more resistant to infection when inoculated with the virus than were normal animals. Sprunt (51) found that simple undernourishment decreased the susceptibility of the rabbit's skin to infection with vaccinia virus and theorized that poorly nourished cells may be lacking in some of the materials necessary for the formation of new viral particles. He also found (52) that, when well-nourished animals are placed on a poor diet, they become first more susceptible to viral infection, then

less, and terminally more susceptible again. Foster et al. (15) report that the feeding of about 40 per cent of the usual daily consumption definitely extended the time before the onset of paralysis and the time of death in mice infected with the Lansing strain of poliomyelitis virus. However, the difference between the deficient and control mice had disappeared by the end of 28 days.

Several workers (16,17,27,43) report that thiamine-deficient mice are less susceptible to infection with the Lansing strain of poliomyelitis virus, Theiler's virus, and Western-equine-encephalomyelitis virus. Lowering of the incidence of paralysis, lower mortality rate, and lack of the characteristic signs of infection were noted.

Sprunt and Sands (53) found that chickens, kept on an adequate diet for weight gain but moderately reduced in protein for 3 to 6 weeks, developed more tumors when inoculated with the Rous sarcoma virus than did those on a diet adequate in protein. If, however, the chickens were kept on the diet for a longer period of time, they developed statistically fewer tumors than did the controls. Jones et al. (21) reported that protein deficiency delayed the onset of symptoms when mice were inoculated with poliomyelitis virus. Kearney et al. (33), on the other hand, have reported that a low-protein diet has no influence on the infection of mice with Theiler's encephalomyelitis virus.

Jones and co-workers at Pennsylvania (21) inoculated tryptophan-deficient mice with poliomyelitis virus and noted that symptoms were delayed to a greater extent than in the case of protein deficiency. Pond et al. (41) reported that a deficiency of any one of the amino acids, tryptophan, isoleucine, methionine, and valine reduced the incidence of paralysis and other signs of infection in mice inoculated with

Theiler's-GD-VII-encephalomyelitis virus. They also found that tryptophan deficiency caused a slower multiplication of the virus in the brain and spinal cord. Davies and his group (11) found that deficiencies of all the amino acids except lysine affected the course of experimental poliomyelitis in mice in similar fashion. There was an increase in the incubation period, a decrease in the number of animals paralyzed at the end of 28 days, and an increase in the number of animals which died without characteristic signs of disease.

C. The Effect of Tryptophan Analogues on Infection

It has been demonstrated that tryptophan is required for viral multiplication and that the addition of 5-methyl tryptophan inhibits viral growth in the Escherichia coli-T2 bacteriophage system. This analogue apparently interferes with viral utilization of the tryptophan produced by the bacteria. Cohen and Fowler (9) report that this inhibition is overcome by the addition of tryptophan to the system. Rasmussen et al. (42) found that the inclusion of 0.4 per cent 6-methyl tryptophan in the diet of tryptophan-deficient mice increased the time of survival in mice infected with Lansing-poliomyelitis virus. The control animals lived 8.3 days while those treated with 6-methyl tryptophan lived 12.5 days. They attributed this increase to either an immediate requirement for tryptophan by the virus or to a manifestation of a more profound change in protein metabolism. The same group of workers (44) found that the addition of the analogue to a low-tryptophan diet influenced the incidence of paralysis in monkeys infected orally with poliomyelitis virus. In one experiment 7 of 9 monkeys receiving 6-methyl tryptophan became paralyzed, while 9 of 9 controls became paralyzed. Using a smaller amount of virus as the

infecting dose, it was found that 1 of 20 in the treated group showed paralysis while 12 of 20 in the control group became paralyzed.

It is evident from this literature review that certain nutrients and metabolites have an effect on the course, symptoms, and lesions of certain infections. It is also evident that the data on this subject are in conflict. This is probably due, at least in part, to the wide variety of factors involved in experiments of this nature, such as susceptibility of the host to infection, virulence of the pathogen, lesions produced, and the specific nutrient under study.

III. EXPERIMENTAL

The work for this study was conducted in the facilities of the Department of Veterinary Pathology. The animals were kept in semi-isolation, entrance to the room being made through a small anteroom. A pan containing an antiseptic solution was placed on the floor of this small room for disinfecting footwear. The main room was provided with an exhaust fan, and air entered the room only through or around the doors.

The baby pigs, after being delivered by caesarian section or removed from the dam at 1 to 3 days of age were brought to the room and placed in individual metabolism cages (Figure 1). Each cage was provided with a floor of one-half inch wire mesh, and a sheet-metal pan below this floor drained liquid waste material into a glass jar. Additional heat was provided for the baby pigs during the first few days of life by suspending a 250-watt heat lamp over 2 adjacent cages.

A. The Effect of Ascorbic Acid on Experimental Hog-Cholera Infection

1. Experiment I

a. Procedure. This experiment, involving only 2 animals, was conducted as a preliminary investigation to observe the effects of high doses of ascorbic acid on baby pigs. The 2 animals were obtained from a registered Yorkshire herd. One pig was from the fourth litter farrowed by a sow which had been immunized against hog cholera at weaning age. This pig was removed from the dam at 3 days of age and placed in an individual metabolism cage. It was fed a daily ration of 200 ml. of pasteurized, homogenized, whole milk with 1 egg yolk added. The ration was divided into 4 feedings. After 13 days the diet was changed to

Figure 1

Metabolism cages used for experimental pigs. Cage on left has container for feed and water. Front has been removed from cage on right to demonstrate wire mesh floor and removable tray.

ration A shown in Table I. This ration was prepared in liquid form so that it contained 20 per cent solids and was homogenized. The mineral mixture was that formulated by Phillips and Hart (38). To each 100 ml. of the diet, 1 ml. of vitamin solution A was added (Table II). The vitamins were dissolved in 30 per cent ethyl alcohol. This vitamin solution was a modification of that used by Reber et al. (45). Additional amounts of vitamins A and D in cod-liver oil were given in the ration every third day. This supplied an excess of the calculated daily

TABLE I

Composition of Rations Used in

Experiments I and II

Ingredient	Ration A %	Ration B %
Casein	35.0	43.8
Lard	40.0	25.0
Lactose	20.0	25.0
Mineral mixture	5.0	6.2

requirements of the various vitamins. The amount of this ration fed was gradually increased to 300 ml. 4 times a day.

The second pig was from a first-litter gilt and was left with its dam until it was 3 days of age. The dam had been immunized against hog cholera at weaning age. At this time the pig was transferred to the dam of the other pig used in this experiment and was left with her until it was 46 days of age. It was then removed from the sow and placed on ration A.

When the 2 animals were 59 days of age, they were inoculated with a titrated hog-cholera virus furnished by the United States Department of Agriculture Hog Cholera Research Station, Ames, Iowa. This virus (serial no. 312) had been found to kill pigs at dilutions of 1/10,000 and 1/100,000. Two ml. of this agent were diluted with sterile physiological saline to a total volume of 100 ml. Each pig was then given 5 ml. of this solution subcutaneously in the axillary space. Using the 1/10,000 dilution as a practical end-point, each animal received 1000 infective doses.

TABLE II

Composition of Vitamin Solutions Used in Preparing Rations

Vitamin	Vitamin Solution A Mg./ml.	Vitamin Solution B Mg./2 ml.
E	1.5	1.5
K	0.29	0.29
Thiamine hydrochloride	1.1	1.1
Riboflavin	1.8	1.8
Pyridoxine	2.0	2.0
Calcium pantothenate	7.0	7.1
Inositol	10.0	26.8
p-Aminobenzoic acid	2.0	5.0
Biotin	0.025	0.025
Nicotinic acid	10.1	10.1
Choline	50.0	260.0
Folic acid	0.13	0.13
Ascorbic acid	25.0	100.0
B_{12}	0.005	0.005

Twenty-five grams of crystalline ascorbic acid (Merck) were dissolved in sterile saline and diluted to a volume of 125 ml. Each 5-ml. dose, therefore, represented 1 g. of ascorbic acid. The pig removed from the sow at 46 days of age was given 1 g. of ascorbic acid intraperitoneally at the time of inoculation with hog-cholera virus. This dose was repeated

on 2 subsequent days. A necropsy was performed as soon as possible after death of the pig.

The remaining pig was given 1 g. of ascorbic acid intravenously at the time of inoculation. Subsequent daily injections were given intraperitoneally due to excessive swelling in the region of the thoracic inlet where the first injection was given. These daily injections of 1 g. of ascorbic acid were continued for a total of 14 days.

This animal was given 1 ml. of the original undiluted virus (10,000 infective doses) 22 days after being inoculated in an effort to determine if it had acquired an immunity to the hog-cholera virus.

b. Results. The animal in which all injections of ascorbic acid were made intraperitoneally died approximately 48 hours after the original injection. Necropsy revealed a large blood clot in the peritoneal cavity along with much free blood. There was also hemorrhage, edema, and a blood clot in the right flank region of the abdominal wall, indicating that the spermatic vessels had been punctured during the administration of ascorbic acid. This apparently led to hemorrhage, inflammation, and death of the animal.

The second pig received ascorbic acid intravenously on the day of inoculation with hog-cholera virus and intraperitoneally thereafter due to the swelling which developed in the region of the thoracic inlet from the first injection. Food consumption was reduced slightly and intermittently during the first 6 days following inoculation with the virus. After this it was normal. The body temperature reached a peak of 106.7° F. on the day following the initial intravenous injection. From here it fluctuated between 105.5° F. and 103.5° F. for the next 12 days. The leukocyte count was 16,650 per cmm. at the time of inoculation.

From here it varied quite widely, a low of 9,300 per cmm. being obtained on the sixth day and a high of 29,850 per cmm. on the eighth day.

After being challenged with 10,000 infective doses of virus 22 days following the original inoculation, the animal evidenced no changes suggestive of hog cholera.

c. Discussion. The death of 1 of the animals was not thought to be due to the hog-cholera virus but rather to the irritating effect of the ascorbic acid in the tissues of the abdominal wall, along with hemorrhage. Evidence of this irritation was also seen in the other animal when the first injection was given intravenously, but no evidence of peritonitis was observed when the intraperitoneal method of administration was adopted. This does not, however, preclude the possibility that peritonitis existed and might have been detected if a post-mortem examination had been made.

The apparent recovery of the 1 animal from hog-cholera infection may have two possible explanations. First of all, the possibility exists that the ascorbic acid administered to the animal had a direct effect in preventing the virus from exerting its full pathogenic activity. The second possibility is that the pig may have obtained enough antibodies from its dam through the colostrum to provide at least partial protection against the virus. The fact that it nursed the sow for 3 days after birth and that the sow was vaccinated against hog cholera helps substantiate this possibility. Smith and King (49) report that pigs from dams vaccinated at 6 weeks of age with virulent virus and antiserum were able to withstand challenge with hog-cholera virus at 4 and 6 weeks of age, but not at 8 weeks, while pigs from dams vaccinated with a modified live virus vaccine at 6 weeks of age failed to withstand challenge at 4, 6, 8,

or 10 weeks. Brambell et al. (6) state that the period during which the gut of the new-born calf can absorb antibodies is of brief duration and lasts only about 1 day. They further state that a similar situation exists in pigs. This being the case, the animal nursing the dam for 2 or 3 days would probably have as high an antibody titer as the animal left with the dam until weaning age. Pickens et al. (39), however, propose that the milk of immune mothers has some influence in maintaining the immunity of suckling pigs. They demonstrated a sharp division between immunity and susceptibility at weaning age.

In Experiment V a littermate of this animal, which had been left with its dam until it was 46 days of age, survived when injected with the same amount of virus. Also in Experiment V, 2 littermates of these animals, after being fed a tryptophan-deficient ration for 31 days, also survived when injected with hog-cholera virus. The pig given a tryptophan analogue in Experiment VI was also a littermate. The fact that 5 animals from the same litter, on 4 different treatments, survived suggests that the failure of the animal in Experiment I to die of hog cholera was due to immunity received from the dam rather than to the effect of the ascorbic acid.

2. Experiment II

a. Procedure. Thirteen pigs were used to study the effects of the intraperitoneal administration of ascorbic acid on hog-cholera infection. They were from a litter of 15 farrowed by a Yorkshire sow. This was her third litter, and she had not previously been vaccinated against hog cholera. The pigs were removed from the dam at 48 hours of age and were placed on a ration consisting of 50 ml. of pasteurized, homogenized,

whole milk with 1 egg added per quart. This was fed 4 times each day, and the amount was gradually increased. Each pig was given 1 tablet containing iron, copper, and cobalt (FeCuCo, Ft. Dodge) at 5 and 13 days of age.

When the pigs were 18 days of age, they were placed on ration A shown in Table I. Two days later the ration was modified in such a way that the amount of lard was reduced 50 per cent. The modified ration is shown as ration B, Table I. This was done in an effort to lower the incidence of diarrhea which was apparently caused by uneven distribution of fat resulting from inadequate homogenization. The diet was prepared in liquid form so that it contained 16 per cent solids, and it was then homogenized. .The vitamin solution was added as in Experiment I. The amount of the ration fed was gradually increased until each animal was receiving 400 ml. 3 times each day.

At 33 days of age, 1 pig was placed in a room isolated from the area in which the other animals were kept. The remaining 12 animals were inoculated with a titrated hog-cholera virus furnished by the United States Department of Agriculture Hog Cholera Research Station, Ames, Iowa. This virus (serial no. 313) had been found to kill pigs at a dilution of 1/750,000. One ml. of this virus was diluted to 100 ml. with sterile saline. Each pig was then given 2 ml. of this solution subcutaneously in the axillary space. Each pig, therefore, received 15,000 infective doses.

Ten g. of crystalline ascorbic acid (Merck) were dissolved in distilled water and diluted to 100 ml. Each ml. represented 100 mg. ascorbic acid. This solution was made fresh daily and refrigerated. The

remaining 12 pigs were randomly divided into 4 groups of 3 pigs each and treated as indicated in Table III.

TABLE III

Treatments Applied to Pigs in Experiment II

Group	No. of Pigs	Treatment
25 mg.	3	Virus plus 25 mg. ascorbic acid *
50 mg.	3	Virus plus 50 mg. ascorbic acid *
100 mg.	3	Virus plus 100 mg. ascorbic acid *
Control	3	Virus only
Uninoculated	1	Isolated, no treatment

* Ascorbic acid values are mg. per pound body weight per day. The dose was divided into 3 equal parts and was administered intraperitoneally every 8 hours.

Records of body weight, body temperature, food consumption, and white blood cell counts were made at selected intervals. Ascorbic acid determinations were made on the plasma every third day using a modification of the method described by Stotz (54). The blood was centrifuged immediately after collection, and 3 ml. of the plasma were added to 3 ml. of carbon dioxide-free distilled water in a centrifuge tube. Six ml. of 6 per cent metaphosphoric acid were then added, and the tube was gently agitated for 30 seconds. The sample was then allowed to stand for 30 minutes, after which it was centrifuged. Eight ml. of the supernatant were placed in a glass-stoppered Erlenmeyer flask, and a blank was

prepared containing 4 ml. of carbon dioxide-free distilled water and 4 ml. of 6 per cent metaphosphoric acid. From this point both the sample and the blank were treated in the same manner. Three drops of bromcresol-green indicator were added to the liquid, and 0.8 N sodium hydroxide was added in sufficient quantity to just produce a green color. One ml. of phosphate-citrate buffer was added, and the solution was mixed. Then 2 ml. of 2,6-dichlorobenzenone solution were added rapidly with mixing. Within 30 seconds 12 ml. of xylene were added to each flask, and the flask was shaken for 30 seconds. After the layers had separated, the pink xylene layer was filtered. The readings were made with a Bausch and Lomb Spectronic 20 colorimeter. The wave length was set at 500 mμ. and the transmittance at 50 per cent with the blank in the machine. The sample was then placed in the machine, and the transmittance was read.

An ascorbic acid curve was determined by analyzing a series of known standards. These were prepared by dissolving a known amount of crystalline ascorbic acid in carbon dioxide-free distilled water. A chart was then prepared so that the transmittance reading of the unknown could be changed to mg. of ascorbic acid per 100 ml. of plasma.

Post-mortem examinations were performed soon after death on those animals not surviving. The uninoculated control pig was given 50 ml. of anti-hog-cholera serum and returned to the room with the other animals 10 days after they were inoculated. This was done because the animal had developed a high temperature and leukopenia.

b. Results. The food consumption of the animals in this experiment remained normal until the second day following inoculation. At this time there was a decrease in the amount of the ration consumed by all groups except the 50 mg. pigs and the uninoculated animal. During

succeeding days there was a decline in consumption in all groups injected with the virus. This decline was most marked in the 100 mg. group. The amount consumed in all groups was quite variable, but on the average the animals consumed approximately one-third of the amount eaten before inoculation. There were no marked differences between the groups, but the control pigs receiving only the virus consumed slightly more than those receiving the virus plus ascorbic acid.

The leukocyte counts, body temperatures, and plasma ascorbic acid values are presented in Tables IV, V, and VI, respectively. The values in the tables are averages of the values obtained for the number of animals in that particular group on any given day. Examination of Tables IV and V reveals the type reaction normally expected following the injection of hog-cholera virus into susceptible pigs. Total leukocyte counts were quite low on the fourth day after inoculation in all pigs injected with the virus, and body temperature rose quite markedly on the second and third days. The ascorbic acid determinations revealed that rather high plasma levels were produced by the intraperitoneal injections.

The pigs receiving virus began to show evidence of infection on the third day following inoculation. They became lethargic, some developed weakness, and diarrhea was present in all inoculated animals on the fourth day. The symptoms developed quite rapidly, and the animals receiving 100 mg. ascorbic acid per pound body weight died on the fifth, sixth, and seventh days. The others gradually became weaker so that it was necessary to support them while they stood to drink. They were killed on the eleventh day following inoculation when most of them were in a very weak condition, and post-mortem examinations were made.

TABLE IV

Total Leukocyte Counts of Pigs in Experiment II

(Leukocytes per cmm. blood)

	Days after Inoculation			
Group	0	4	7	10
25 mg.	9,917	2,767	2,633	3,500
50 mg.	9,167	2,817	3,267	5,633
100 mg.	10,883	2,550	---	---
Control	9,700	1,883	1,317	3,783
Uninoculated	9,400	8,950	8,900	4,450

The uninoculated pig developed a temperature of 105.1° F. and a leukocyte count of 4,450 on the tenth day after the other animals were inoculated. The administration of anti-hog-cholera serum was followed by a rapid return to normal temperature (Table V), and other signs of hog-cholera infection failed to develop.

Necropsy of the 3 animals in the 100 mg. group revealed that their deaths were primarily due to severe fibrinous peritonitis, apparently caused by the irritating effect of the ascorbic acid injected intraperitoneally. An abscess was noted in the peritoneal cavity of 1 animal. Some edema in lymph nodes along with numerous petechiae on the surface of the kidneys of 1 animal were the only gross lesions suggestive of hog cholera.

Necropsy of the remaining pigs revealed that fibrinous peritonitis and abscesses within the peritoneal cavity were present in those

TABLE V

Body Temperatures of Pigs in Experiment II

(^{o}F.)

Group	Days after Inoculation										
	0	1	2	3	4	5	6	8	9	10	11
25 mg.	102.8	102.2	104.1	105.8	105.1	104.6	105.1	105.5	104.5	104.2	102.2
50 mg.	102.8	102.4	104.3	106.1	105.1	104.8	105.5	104.9	104.3	103.8	102.1
100 mg.	103.1	102.8	104.5	105.6	105.2	104.2	103.2	---	---	---	---
Control	102.7	102.5	104.9	106.4	105.7	105.2	105.7	105.9	105.6	104.8	103.8
Uninoculated	103.3	103.0	103.6	103.3	103.0	103.0	102.3	102.3	103.2	105.1	103.1

TABLE VI

Plasma Ascorbic Acid Values of Pigs in Experiment II

(Mg. per 100 ml. plasma)

Group	Days after Inoculation			
	0	4	7	10
25 mg.	0.66	2.69	3.17	3.31
50 mg.	0.09	>5.54*	4.92	3.83
100 mg.	0.82	>5.54*	—	—
Control	1.24	1.28	0.80	0.46
Uninoculated	1.15	1.79	1.02	1.15

* 5.54 represents the upper limits of this determination. These samples contained more than 5.54 mg. per 100 ml.

animals receiving ascorbic acid but not in those inoculated only with the virus. The lesions of peritonitis appeared to be more severe in the animals receiving 50 mg. ascorbic acid per pound body weight than in the 25-mg. group.

Gross lesions of hog cholera were seen during necropsy of the animals receiving only virus and in the groups receiving 25 mg. and 50 mg. of ascorbic acid. The changes observed in the lymph nodes included edema, congestion, and peripheral hemorrhage. These lesions were seen most frequently in the internal iliac and the prefemoral lymph nodes. Petechial hemorrhages were seen on the surface of the kidneys of all pigs. The spleens of 6 of the 9 pigs contained lesions varying from slight enlargement to numerous infarcts. Lesions of enteritis or gastroenteritis were seen in all but 1 pig. Ulcers around the ileo-cecal valve were found

quite consistently. Grossly, the lungs of some pigs were normal while others exhibited a moderate degree of pneumonia.

There was an indication that the lesions of hog cholera were slightly more severe in the pigs receiving only virus than in those receiving ascorbic acid, but the differences were not great and were confined to the lymph nodes and kidneys.

c. Discussion. The slightly decreased food consumption of the animals receiving virus and ascorbic acid as compared to those receiving only the virus was probably due to the developing peritonitis. The leukocyte counts and body temperatures did not differ greatly in the various inoculated groups.

The plasma ascorbic acid values were quite high in the animals receiving injections of the vitamin. According to Grummer et al. (19) the level of plasma ascorbic acid for young pigs up to weaning age is about 0.8 mg. per 100 ml. The control pigs and the uninoculated animal had ascorbic acid levels above this figure before the onset of hog-cholera symptoms. These differences were not great when one considers the fact that a variation of one point on the transmittance scale of the colorimeter used in determining ascorbic acid represents a difference of more than 0.1 mg. per 100 ml. and that the needle sometimes fluctuates 1 or 2 points as readings are being made. The fact that plasma ascorbic acid levels of more than 7 times the normal values were obtained in both the 50- and 100-mg. level pigs with no significant differences being produced in either the symptoms or lesions of hog cholera indicates that the ascorbic acid was of no value in altering the outcome of the infection. However, the complications produced by the peritonitis in all animals receiving

ascorbic acid cannot be disregarded. The deaths of the 100-mg. pigs were apparently due primarily to peritonitis rather than to hog-cholera infection.

3. Experiment III

a. Procedure. Twelve pigs were used in this experiment to continue the study of the effects of administration of ascorbic acid on hog-cholera infection. Six of the animals were purchased at about 10 days of age and 6 at 7 days of age. They were from 2 Hampshire gilts bred to a Yorkshire boar. Neither of the dams had previously been immunized against hog cholera. After they were removed from the sows, these pigs were placed in individual cages and were fed pasteurized, homogenized, whole milk with 1 egg added per quart. The amount fed was gradually increased from 50 ml. 4 times a day to 200 ml. 3 times a day. One iron-copper-cobalt tablet (FeCuCo, Ft. Dodge) was given each pig at approximately 2 weeks of age.

When the pigs were 18 and 20 days of age, they were divided into 5 lots, with animals of both age groups being represented in each lot. Two of the animals were removed from the group and placed in a large stall in another part of the building where they were cared for by a separate attendant.

The remaining 10 animals were inoculated with the titrated virus used in Experiment II (serial no. 313). Each animal was given 0.8 ml. of the virus subcutaneously in the axillary space. Using the original titration values, each animal received 600,000 infective doses. The actual dosage, however, may have been somewhat less than this, considering the fact that the virus had been thawed for use in Experiment II and then refrozen. The animals were treated as indicated in Table VII.

TABLE VII

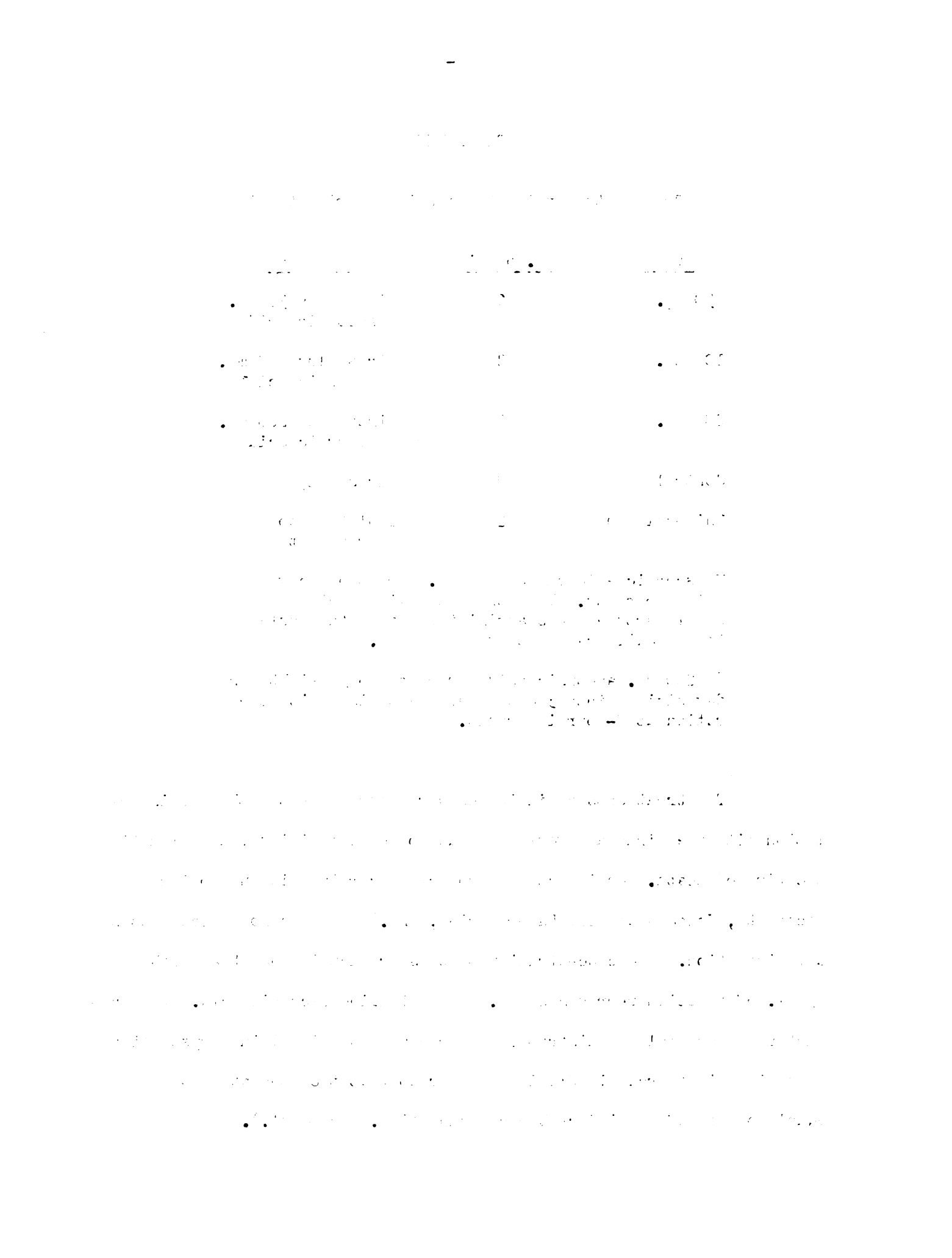
Treatments Applied to Pigs in Experiment III

Group	No. of Pigs	Treatment
50 mg.	2	Virus plus 50 mg. ascorbic acid*
100 mg.	3	Virus plus 100 mg. ascorbic acid*
200 mg.	3	Virus plus 200 mg. ascorbic acid**
Control	2	Virus only
Uninoculated	2	Isolated, no treatment

* Ascorbic acid values are mg. per pound body weight per day. The dose was divided into 3 equal parts and was administered subcutaneously in the axillary space every 8 hours.

** 200 mg. ascorbic acid per pound body weight per day divided into 3 equal parts and mixed with the ration at 8-hour intervals.

The treatments as indicated were started at the time of inoculation with the virus and were continued on each individual animal until the time of death. An injectable product (Ascorbic Acid and Sodium Ascorbate, Jensen-Salsbery Laboratories, Inc.) was used for subcutaneous administration. The concentration of this product is such that each 25 ml. of solution represents 2 g. of crystalline ascorbic acid. The product used for oral administration was prepared by dissolving crystalline ascorbic acid (Merck) in distilled water so that the concentration was equal to that of the injectable compound (2 g. per 25 ml.).



Records of body weight, body temperature, food consumption, and white blood cell counts were made at selected intervals. Ascorbic acid determinations were made on the plasma every 2 to 4 days. Necropsies were performed on the animals at the time of death.

Blocks of tissues were fixed in Zenker's fluid, except for the brain which was fixed in 10 per cent neutral formalin. These materials were imbedded in paraffin, sectioned, and stained with Harris's alum hematoxylin and eosin.

b. Results. The food consumption began to drop in the younger group of animals on the second day following inoculation, but in the older group the decrease was not enough to be considered significant until the fourth day. The amount consumed was quite variable, but most animals were taking 50 per cent or less of the normal amount by the seventh day.

Symptoms suggestive of infection were first noticed on the third day in the younger group. These included diarrhea, lethargy, weakness, and some trembling. Similar symptoms in the older animals were observed 1 or 2 days later. Convulsions were seen in 5 of the animals at some time during the course of the infection. This condition was seen in all the groups inoculated with the virus. The animals gradually became weaker but did not show the extreme weakness observed in those of Experiment II.

The leukocyte counts, body temperatures, and plasma ascorbic acid values are presented in Tables VIII, IX, and X, respectively. The values in the tables are averages of the values obtained for the number of animals in that particular group on any given day. As in Experiment II the total leukocyte counts and body temperatures are similar to what might be expected when susceptible pigs are inoculated with hog-cholera virus. The total leukocyte counts became quite low between the third and

TABLE VIII

Total Leukocyte Counts of Pigs in Experiment III

(Leukocytes per cmm. blood)

Group	Days after Inoculation											
	-1	3	6	8	11	14	18	21	25	28	31	34
50 mg.	11,275	5,825	4,600	3,275	4,750	4,900	—	—	—	—	—	—
100 mg.	10,317	7,200	5,650	5,483	4,800	4,150	—	—	—	—	—	—
200 mg.	15,600	5,567	3,667	4,533	4,600	1,950	2,500	2,050	5,250	6,300	8,900	—
Control	11,650	6,400	12,725	4,375	5,200	350	—	—	—	—	—	—
Uninoculated	19,550	16,275	6,100	9,975	13,000	12,550	12,700	7,550	12,725	11,400	9,775	12,300

TABLE IX

Body Temperatures of Pigs in Experiment III

(°F.)

Group	Days after Inoculation									
	-1	0	1	2	3	4	5	6	7	8
50 mg.	102.3	103.8	102.0	103.5	104.4	104.4	106.9	105.9	105.6	105.8
100 mg.	103.1	103.7	101.7	104.7	105.1	103.9	105.5	105.3	105.2	101.0
200 mg.	103.8	104.1	102.2	104.2	104.4	104.3	106.0	106.0	106.5	105.6
Control	104.1	104.1	102.1	104.9	106.4	105.2	106.5	106.0	105.9	104.7
Uninoculated	102.9	104.2	102.1	100.5	102.0	101.8	102.3	102.1	102.1	101.2
	9	10	11	12	13	14	15	16	17	18
50 mg.	104.1	101.2	105.6	104.1	103.4	101.6	103.8	102.3	--	--
100 mg.	105.3	104.5	103.1	103.4	100.9	102.3	101.6	103.5	103.5	--
200 mg.	105.2	104.0	105.0	101.8	104.7	105.3	105.3	104.0	103.7	102.5
Control	104.4	103.7	104.8	103.5	99.3	--	--	--	--	--
Uninoculated	101.2	101.8	101.8	--	102.2	103.2	102.5	101.1	102.1	100.4

TABLE X

Plasma Ascorbic Acid Values of Pigs in Experiment III

(Mg. per 100 ml. plasma)

Group	Days after Inoculation										
	-1	3	6	8	11	14	18	21	25	28	31
50 mg.	0.39	1.41	2.08	2.57	2.02	1.90	—	—	—	—	—
100 mg.	0.60	1.73	2.61	3.07	2.59	2.48	—	—	—	—	—
200 mg.	0.70	1.62	2.45	2.21	2.75	1.66	2.37	1.79	1.15	2.25	1.54
Control	0.46	0.61	0.75	0.61	0.61	0.75	—	—	—	—	—
Uninoculated	0.39	0.61	0.75	0.75	0.31	0.46	0.31	0.68	0.75	0.82	1.09

sixth days after injection of the virus. One apparent discrepancy was noted in the results obtained on the sixth day following inoculation. At this time the average leukocyte count of the uninoculated animals was well below that of the control group receiving only the virus. It is difficult to account for this discrepancy other than by assuming that the counts were in error. Of the 4 animals involved in these 2 groups, only 1 had a relatively high count (19,000) at this time. It is possible that the accidental shifting of this count from the uninoculated group to the control group may have caused the error. Body temperatures rose between the second and fourth days and in most cases remained above normal until the death of the animal. Table X reveals that the plasma ascorbic acid values in this experiment were not as high as those obtained in Experiment II.

Gross lesions suggestive of hog cholera were seen upon necropsy of all the animals inoculated with the hog-cholera virus. These lesions included edema, congestion, and hemorrhage of the lymph nodes; congestion, petechial hemorrhage, and ecchymotic hemorrhage on the surface of the kidney; infarcts involving the edge of the spleen; enteritis or gastro-enteritis of varying extent and degree; and varying degrees of congestion and pneumonia in the lungs. Severe submeningeal hemorrhage was present in the area of the cerebellum of 1 animal in the 50-mg. group.

The lesions were quite variable, but there appeared to be no correlation between the extent and severity of the lesions and the level of ascorbic acid administered to the individual animals. There was more variation within groups than between groups.

Microscopic examination of sections of lymph nodes, kidneys, and four regions of the brain (medulla oblongata, brain stem at the level

of the cerebral peduncles, cerebellum, and cerebrum) revealed numerous lesions suggestive of hog cholera. In the lymph nodes the changes were predominantly those of edema, congestion, and hemmorrhage, most of the lesions being confined to the periphery of the nodes. However, in a few cases, the lesions were spread rather uniformly throughout the nodes. The kidney lesions were most prominent in the area of the cortex adjacent to the capsule. Congestion, varying from slight to marked, was seen both in the glomeruli and in the vessels supplying the tubules. Hemorrhages were also found in this area, some of them being just under the capsule, and in others the blood had escaped into the area between the tubules. Microscopically, congestion was a more prominent feature than hemorrhage, and apparently many of the lesions appearing grossly to be petechial hemorrhages were actually areas of congestion.

The microscopic lesions found in the brain included both perivascular cuffing and swelling and proliferation of the endothelial cells. These changes were most commonly found in the sections of the medulla and the sections of the anterior brain stem taken at the level of the cerebral peduncles. However, in a few animals, the lesions were also seen in sections of the cerebrum and cerebellum. The perivascular cuffing consisted of the collection of lymphocytes within the Virchow-Robin spaces. This cuffing varied from a condition in which only a few lymphocytes were seen to the presence in 1 animal of great numbers of lymphocytes. The animal in which extensive cuffing was found was from the 100-mg. group. A more consistent lesion was that involving the endothelial cells themselves. These cells exhibited swelling, and in some cases there appeared to be an increase in the numbers of the endothelial cells. Other microscopic lesions such as varying degrees of vacuolation, apparent degeneration of

Purkinje fibers in the cerebellum, and extensive hemorrhage in the cerebellum of one animal in the 50-mg. group were also found. These changes were not, however, consistent enough to be considered important.

Gross lesions in the uninoculated animals were negligible. Microscopically, however, there were areas of congestion in the kidneys, and slight congestion and hemorrhage in the internal iliac lymph nodes of both pigs.

The level of ascorbic acid administered appeared to have some influence on the length of survival after inoculation with the virus. As the amount of the vitamin was increased, the survival time in both litters was increased, with the exception of one animal in the younger litter (Table XI).

TABLE XI

Survival Time of Pigs in
Experiment III after Inoculation

	Survival Time (Days)	
Group	Younger Litter	Older Litter
Virus	8	14
50 mg.	10	17
100 mg.	8, 12	19
200 mg.	12	21, 34

c. Discussion. The differences in time of onset of symptoms and survival time between the two litters may have been due to the small age difference present or possibly to a slight immunity obtained from

the dam of the older litter, although neither had been vaccinated against hog cholera.

The plasma ascorbic acid levels were not as high using the injectable product subcutaneously as those obtained when a solution made from crystalline ascorbic acid was administered intraperitoneally in Experiment II. It is not known whether this was due to less complete absorption of the subcutaneously administered product, a more rapid elimination, or some other factor.

The apparent correlation between level of ascorbic acid administered and time of survival after inoculation with the virus suggests that a partial or transient protection was offered by the vitamin. If one attributes the survival of the pig in Experiment I to a carry-over of immunity acquired from the dam, this constitutes the only evidence of ascorbic acid exerting an effect on the hog-cholera virus in these experiments.

The lack of significant beneficial effects from ascorbic acid in this experiment is in contrast to some of the results cited in the review of literature. One possible explanation is that the vitamin used in at least part of the reported work may have been from natural sources and may have contained some of the citrus fruit flavonoids. It is quite possible that the beneficial results were due to a combination of the vitamin and the flavonoids. It would be desirable at some future time to investigate the influence of a combination of ascorbic acid and citrus fruit flavonoids on hog-cholera infection.

B. The Effect of Protein and Tryptophan Deficiency on Hog-Cholera Infection

1. Experiment IV

a. Procedure. This experiment was conducted in an attempt to determine if the feeding of a ration deficient in tryptophan to baby pigs would have any influence on resistance of the animals to hog-cholera infection. Pigs for this experiment were removed from the dam by caesarian section at the time of the appearance of milk in the udder. Twelve pigs were procured from 1 Yorkshire gilt and 9 from another. The second litter was delivered 15 days after the first. The pigs were kept either in individual cages or 2 pigs to each cage with heat lamps being placed above the cages. These lamps were used for the first several days. The ration for the first week consisted of pasteurized, homogenized, whole milk with 1 egg yolk added to each 200 ml. of milk. A 50-ml. portion of this mixture was offered to each pig 5 times a day for the first few days and then 4 times a day.

After the first week the diet was gradually changed to the control ration listed in Table XII. This diet was prepared in liquid form so that it contained 20 per cent solids, and it was then homogenized. The mineral mixture was that formulated by Phillips and Hart (38). To each 100 ml. of the control diet, 2 ml. of vitamin solution B (Table II) were added. The vitamins were dissolved in 30 per cent ethyl alcohol. Additional amounts of vitamins A and D in cod-liver oil were given in the milk every third day.

The tryptophan-deficient diet was prepared from the ingredients listed in Table XII. Since the gelatin caused solidification when the ration was cooled, it was found necessary to dissolve the ingredients in

TABLE XII

Composition of Rations Used in Experiments IV and V

Ingredient	Control Ration %	Deficient Ration %
Casein	35.0	—
Zein	—	17.5
Gelatin	—	17.5
Lard	40.0	40.0
Lactose	20.0	19.3
Mineral mixture	5.0	5.0
Lysine	—	0.6
Methionine	—	0.1

enough hot water to make half of the required volume. The vitamin solution was added in the amount of 4 ml. per 100 ml. of the concentrate. This was then homogenized and divided into the desired volume for individual feedings. At the time of feeding an equal volume of hot water was added and the ration was mixed in a Waring Blendor. Twenty mg. of ferric citrate were given in the ration of each pig approximately every week.

Nine animals from the first litter and 3 from the second litter were used in this experiment. Eight of the older pigs were placed on the tryptophan-deficient diet when they were 17 days of age. The remaining animal from the first litter and 3 from the second litter were left on the control ration. One of the animals from the control group died 5 days after it was placed on the control ration. The amount of ration was gradually increased until each animal received 85 ml. 4 times a day.

After they had been on the deficient ration for 4 weeks, these pigs along with the 4 control animals were injected with 2 ml. of commercial hog-cholera virus (Pitmore Moore, serial no. 2654). The treatments are listed in Table XIII.

TABLE XIII

Treatments Applied to Pigs in Experiment IV

Group	No. of Pigs	Treatment
Deficient	8	Deficient ration plus hog-cholera virus
Control	4	Control ration plus hog-cholera virus

Records were kept of food consumption, body weight, and body temperatures. Blood counts were also made, and post-mortem examinations were conducted soon after death of the pigs.

b. Results. The pigs on the tryptophan-deficient ration began to consume less food after about 5 days. They began to eat rather slowly and developed diarrhea with some vomiting. After 19 days on the ration, the pigs were consuming only about 70 per cent of the amount offered to them and were becoming quite weak. Additional food in the form of 10 to 20 ml. of homogenized, whole milk was given at each feeding to those animals severely weakened. This was done only when it was thought necessary to prevent the pigs from dying of the deficiency. The food consumption of the pigs on the control ration remained quite good, and they consumed all the ration offered them.

After consuming the deficient ration for 4 weeks, the pigs weighed an average of 32 g. each less than they did when the deficient ration was started. The control pigs, on the other hand, gained an average of more than 1 kg. each during the same interval.

Body temperatures of the deficient pigs became quite low, and at the time of inoculation with the virus, the deficient animals had average temperatures of 98.8° F. as compared to 102.0° F. for the control animals.

Average hematologic values for the 2 groups of animals at the time of inoculation with the virus are shown in Table XIV.

TABLE XIV

Hematologic Values of Pigs in Experiment IV at Time of Inoculation with Virus

	Control Animals	Deficient Animals
Erythrocyte counts	7,530,000 per cmm.	7,384,000 per cmm.
Hemoglobin	11.8 g. per 100 ml.	9.28 g. per 100 ml.
Leukocyte counts	17,812 per cmm.	23,106 per cmm.

Symptoms of hog cholera began developing in the control pigs in 4 days and included profuse, fetid diarrhea, respiratory difficulty, mild conjunctivitis, and nervous manifestations in 1 animal. Symptoms in the deficient animals were much less marked and consisted primarily of a gradually increasing weakness. Food consumption in the control animals remained nearly normal until about 1 day before death. There was a gradual decline in food consumption in the deficient group. Three days after the

virus was injected, it was thought advisable to change the deficient pigs to a mixture of the control ration and homogenized, whole milk. This was done because 1 pig died the day following inoculation and another on the third day. There was a temporary increase in the amount consumed. The average body temperatures of the control pigs increased to 105.5° F. on the third day after inoculation, while it rose to 105.3° F. on the fourth day in the deficient pigs. Leukopenia developed in both groups, but the values were not consistent, due in part to the fact that 1 pig in the control group developed a transient leukocytosis. Leukocyte counts are shown in Table XV.

TABLE XV

Total Leukocyte Counts of Pigs in Experiment IV

(Leukocytes per cmm. blood)

	Days after Inoculation						
Group	0	1	2	3	4	5	6
Control	20,250	12,488*	6,638*	8,262*	12,725*	4,150	---
Deficient	21,606	22,686*	13,879*	8,371*	10,058*	11,050	4,410

* Blood samples drawn from ear vein. Other samples drawn from anterior vena cava.

One of the control pigs died on the fifth day following inoculation with the virus, 2 on the sixth day, and 1 on the seventh day. In the deficient group, 1 pig died on the day following inoculation, 1 on the third day, and 1 on the fifth day (Table XVI). The other 5 animals were killed on the seventh day when they were in a moribund condition, and post-mortem examinations were made.

TABLE XVI

Survival Time of Pigs in Experiment IV after Inoculation

Group	Survival Time (Days)
Deficient	1, 3, 5*
Control	5, 6, 6, 7

* Remaining 5 animals killed on seventh day.

Necropsy of the pigs revealed lesions suggestive of hog cholera in all except the deficient animal which died on the day following inoculation. Lesions included edema, congestion, and hemorrhage of the lymph nodes; petechial hemorrhages on the surface of the kidney; infarcts in the spleen; pneumonia; and enteritis. These lesions, especially those in the kidneys, were slightly more severe in the control than in the deficient animals. Edema was present in all but 1 animal. In the deficient pigs this edema was quite extensive in the subcutaneous tissues of the ventral part of the body, often extending from the mandible posteriorly to the flank region. Edema was also found in the mesentery of the coiled part of the colon. The control pigs were not so severely affected but 3 of the 4 exhibited some edema.

c. Discussion. Although the ration fed to the deficient pigs in Experiments IV and V was theoretically lacking only in tryptophan, it is highly probable that the deficiency state produced was actually one of lack of protein as well as of tryptophan. This ration seemed to be absorbed rather poorly and was evidently not digested as thoroughly as the control ration in which protein was supplied in the form of casein. The

extensive edema observed in the deficient group of pigs is also evidence of a protein deficiency. The fact that some edema was also observed in the control group of pigs suggests that the control ration may have been lacking in some way or perhaps was not fed in sufficient quantities.

The time of death was delayed in the deficient animals in this experiment, if one assumes that the deficient animals dying on the first and third days died of the deficiency rather than from effects of the hog-cholera virus. This is in agreement with the findings of Kearney et al. (28), Davies et al. (11), and Pond et al. (41) in that the deficiency was effective in prolonging the incubation period and lengthening the survival time.

2. Experiment V

a. Procedure. This experiment was a continuation of the study of the tryptophan-deficient diet and its effects on hog-cholera infection. Ten pigs were purchased at 3 days of age from a Yorkshire herd. These pigs were litter mates of those used in Experiment I and were from a gilt and third-litter sow, both of which had been immunized against hog cholera at weaning age. These animals were fed egg yolk and whole milk as were those in Experiment IV for the first few days and then changed to the control ration. Vitamin solution A (Table II) in the amount of 1 ml. per liter was added to the ration. Two of these animals were lost before the deficient diet was started. Six pigs were placed on the tryptophan-deficient ration when they were 4 weeks of age. This ration was formulated as in Experiment IV, except that 2 ml. of vitamin solution A per liter were substituted for the vitamin solution B. The amount of ration fed was gradually increased until the animals on the control ration were consuming

300 ml. 4 times a day while those on the deficient ration were consuming about 125 ml. 4 times a day.

When these pigs were 46 days of age, a litter mate which had been left with its dam was removed from the sow and added to the experimental group. It was given the control ration.

At the age of 59 days all the pigs were inoculated with a titrated hog-cholera virus furnished by the United States Department of Agriculture Hog Cholera Research Station, Ames, Iowa. This virus (serial no. 312) had been found to kill pigs at dilutions of 1/10,000 and 1/100,000. Two ml. of this agent were diluted with sterile physiological saline to a total volume of 100 ml. Each pig was then given 5 ml. of this solution subcutaneously in the axillary space. Using the 1/10,000 dilution as a practical end point, each animal received 1000 infective doses. Two pigs of the deficient group were also given 20 ml. each of commercial anti-hog-cholera serum (Fort Dodge, serial no. 459). The treatments are listed in Table XVII.

TABLE XVII

Treatments Applied to Pigs in Experiment V

Group	No. of Pigs	Treatment
Deficient	4	Deficient ration plus hog-cholera virus
Deficient plus serum	2	Deficient ration plus hog-cholera virus and anti-hog-cholera serum
Control	2	Control ration plus hog-cholera virus
Control	1*	Control ration plus hog-cholera virus

* Removed from sow at 46 days of age.

Records were kept of food consumption, body weight, and body temperatures. Blood counts were made, and post-mortem examinations were carried out at the time of death of the animals.

The animals surviving were given 1 ml. of the original undiluted virus (10,000 infective doses) 22 days after being inoculated in an effort to determine if they had acquired an immunity to the hog-cholera virus.

b. Results. The food consumption began to decrease in the deficient pigs about 11 days after they were changed to the deficient ration. The animals became lethargic and ate quite slowly, but they did not become as weak as did those in Experiment IV. At the time they were inoculated with the virus, they were consuming approximately 75 per cent of the 125 ml. offered at each feeding. The control animals were at this time consuming 300 ml. of the control ration at each feeding.

After consuming the deficient ration for 32 days, the pigs weighed an average of 422 g. each less than they did when the deficient ration was started. The control pigs, on the other hand, gained an average of 6422 g. each during the same interval.

The body temperatures of the deficient pigs did not become as low as did those in Experiment IV, and at the time of inoculation with the virus, the temperature of both groups of animals averaged 102.7° F.

Average hematologic values for the 2 groups of animals at the time of inoculation with the virus are shown in Table XVIII.

Symptoms of hog cholera began developing in the control pigs on the third day following inoculation with the virus. At this time 1 pig developed anorexia, had a black, pasty stool, exhibited respiratory difficulty, and became lethargic. Some convulsions were also noted in this animal. The deficient pigs became rather lethargic at this time and developed

diarrhea, although the stool was not as dark nor as fetid as that of the control animals. The remaining control pig began to exhibit diarrhea and became lethargic on the fourth day. It developed convulsions on the eighth day, and conjunctivitis was noted at this time.

TABLE XVIII

Hematologic Values at Time of Inoculation

	Control Animals	Deficient Animals
Erythrocyte counts	7,865,000 per cmm.	7,555,000 per cmm.
Hemoglobin	11.2 g. per 100 ml.	9.7 g. per 100 ml.
Leukocyte counts	14,838 per cmm.	13,933 per cmm.

Average food consumption of the deficient pigs receiving only the virus followed about the same pattern as that of the 2 animals receiving both the virus and serum. There was a drop of approximately 20 ml. per pig per feeding the day following inoculation. Following this there was a fluctuation in the amount consumed for several days until, at the end of 8 days, the consumption was slightly above the original value. Food consumption of the control group gradually declined after the second day. The pig left with the sow until it was 46 days of age exhibited a drop in consumption on the fourth day but returned to normal by the eleventh day.

Average body temperatures and leukocyte counts for the pigs are shown in Tables XIX and XX, respectively. These results indicate that the temperature reaction was not so severe in the deficient animals as in those receiving the control diet. The body temperatures of the pigs

TABLE XIX

Body Temperatures of Pigs in Experiment V

(° F.)

Group	Days after Inoculation												
	0	1	2	3	4	5	6	7	8	9	10	11	12
Deficient	102.6	103.8	104.1	103.4	103.4	103.6	103.8	102.7	103.0	102.7	103.2	102.7	103.2
Deficient + serum	102.6	104.3	103.3	102.6	103.3	102.7	103.1	102.3	102.8	102.7	102.7	103.3	103.7
Control	102.7	104.2	105.9	103.8	106.9	107.0	105.6	105.6	108.0	---	---	---	---
Control*	102.8	102.8	103.7	104.0	104.6	105.7	105.5	104.5	106.9	104.5	104.3	104.5	103.1

* Removed from sow at 46 days of age.

TABLE XX

Total Leukocyte Counts of Pigs in Experiment V

(Leukocytes per cmm. blood)

Group	Days after Inoculation							
	0	2	4	6	8	11	15	25
Deficient	12,650	18,178	14,400	6,125	12,500	11,775	14,225	13,800
Deficient + serum	16,500	19,650	14,675	11,825	18,225	15,775	21,375	10,025
Control	14,625	13,925	14,300	7,850	13,150	—	—	—
Control*	11,450	7,350	10,150	6,750	3,900	6,150	13,500	10,050

* Removed from sow at 46 days of age.

receiving the deficient diet and anti-hog-cholera serum remained normal. All pigs except those receiving the anti-hog-cholera serum developed leukopenia. The transient nature of this leukopenia in the group of 3 control pigs which died between the eighth and eleventh day may possibly have resulted from hemoconcentration before death of the animals.

One of the control pigs died on the fourth day after inoculation and the other on the ninth day. One deficient pig died on the fourth day, 1 died on the fifth day, and 2 survived. Both of the deficient pigs receiving antiserum survived as did the animal left with the sow until it was 46 days of age (Table XXI).

TABLE XXI

Survival Time of Pigs in Experiment V after Inoculation

Group	Survival Time (Days)
Deficient	4, 5*
Deficient + serum	Survived
Control	4, 9
Control**	Survived

* Remaining 2 survived.
**Removed from sow at 46 days of age.

Necropsy of the 2 control animals revealed hemorrhage involving the lymph nodes of both animals, petechial hemorrhage on the surface of the kidney of 1 animal, pneumonia in 1 animal, enlargement of the spleen with several infarcts, and enteritis. The hemorrhage of the lymph nodes found upon necropsy of the 2 deficient pigs was not as severe as in the

control animals. Enteritis was seen in both deficient animals, but other gross lesions suggestive of hog cholera were not present.

Inoculation of the 5 surviving pigs with 10,000 infective doses of hog-cholera virus 22 days after the original inoculation produced no significant temperature elevation or other suggestions of hog cholera in any of the animals.

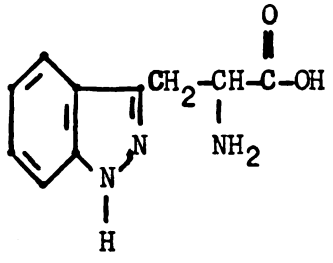
c. Discussion. The survival of the 2 deficient pigs which were given anti-hog-cholera serum at the time of inoculation with the virus indicates that the 2 deficient pigs which failed to survive died either from the effects of the hog-cholera virus or from a combination of the effects of the virus and the deficiency rather than from the deficiency alone. The fact that 2 deficient pigs in this experiment, along with the animal left with the sow until it was 46 days of age, the animal given ascorbic acid in Experiment I, and the animal given a tryptophan analogue in Experiment VI, were all littermates and all survived suggests that a high degree of immunity against hog cholera was probably carried over from the dam.

The results of these 2 experiments indicate that the tryptophan deficiency had a slight effect on the outcome of hog-cholera infection. The longer incubation period and survival time in Experiment IV were not demonstrated in Experiment V. On the other hand, the slightly less severe gross lesions in the deficient pigs were seen in both experiments.

C. The Effect of a Tryptophan Analogue on Hog-Cholera Infection

1. Experiment VI

a. Procedure. This experiment was a preliminary study of the possible inhibiting effects of a tryptophan analogue on hog-cholera virus. Only a small amount of the analogue was available. The 1 animal used in this experiment was a litter mate of part of those used in Experiments I and V. It was removed from the sow at 3 days of age, started on homogenized milk and egg yolk, and later changed to the control diet in Table XII. When the animal was 59 days of age, it was inoculated subcutaneously with 1000 infective doses of the titrated hog-cholera virus (serial no. 312) described in Experiment V. At the same time it was given 100 mg. of the tryptophan analogue tryptazan (dl-α-amino-β-(3-indazole) — propionic acid. This product has the following chemical formula:



One g. of the analogue was dissolved in 50 ml. of sterile physiological saline. Five ml. of the solution were given intravenously once a day to this pig for a total of 10 days. Food consumption, body weight, body temperature, and blood counts were recorded at selected intervals. Twenty-two days following inoculation the animal was given 1 ml. of the original undiluted virus in an effort to determine if it had acquired an immunity to hog-cholera virus.

b. Results. The food consumption of the pig receiving the tryptaphan analogue (tryptozan) began to decline approximately 72 hours after inoculation with the virus. On the fifth day it reached a low of approximately one-sixth of the amount normally consumed. During the next 10 days there was a gradual return to normal.

The body temperature of this pig reached a high of 105.1° F. on the fourth day following inoculation. This was followed by a gradual decline and a return to normal by the twelfth day.

The leukocyte count was 13,450 per cmm. at the time of inoculation, and it remained normal until the sixth day when it dropped to 7,300 per cmm. The counts on the eighth and eleventh days were 9,950 and 9,900 per cmm., respectively. The subsequent counts were normal or above normal.

A dark, fetid diarrhea developed in the animal receiving tryptazan on the fourth day after inoculation. The pig also became somewhat lethargic for a number of days, but it gradually became more active as the food consumption increased and the body temperature became normal.

c. Discussion. While it appears that administration of tryptazan in this experiment modified the course of the hog-cholera infection and the final outcome, it must again be pointed out that this animal was from a litter in which several other animals under various treatments survived inoculation with the hog-cholera virus. Any further conclusions will have to await the results of future experiments.

IV. GENERAL DISCUSSION

The techniques used in maintaining baby pigs on artificial rations for this series of experiments were fairly successful. Some pigs, especially those receiving no colostrum, were found to be quite susceptible to bacterial infections. Some difficulty was encountered with these infections in Experiment IV, and several pigs from the original litters were lost. A number of different micro-organisms were isolated from these animals. Less difficulty was encountered in Experiment V, probably because of the fact that the animals had received colostrum. These results point out the desirability of working with animals in a pathogen-free environment or using animals which have acquired at least some degree of immunity by receiving colostrum for the first few days of life. Work is being continued to determine the effect on colostrum-deprived pigs of some species of bacteria not commonly thought to be pathogenic.

The growth rates obtained in these studies were less than desirable. This is partially explained by the fact that the consumption was limited in some of the experiments in an attempt to reduce the incidence of diarrhea. This diarrhea apparently was caused in some cases by bacterial infection and in other cases by improper homogenization of the prepared ration. For the most part, the ration prepared from homogenized, whole milk and egg caused less difficulty than the one prepared from casein, lard, lactose, minerals, and vitamins. The whole milk ration was also much more convenient to prepare.

Work is being continued to improve the methods of raising baby pigs in artificial surroundings, and at the present time it appears that

the incorporation of some of the techniques used by the Nebraska workers (55,56,57,58) would be desirable.

Statistical analyses were not carried out in this series of experiments due to the small numbers of animals involved and due to the fact that the various groups changed in size as the experiments progressed and animals died.

V. SUMMARY AND CONCLUSIONS

A series of experiments was conducted in an effort to determine possible relationships between some nutritional factors and experimental hog-cholera infection in baby pigs.

Ascorbic acid was administered to groups of animals in doses ranging from 25 to 200 mg. per pound body weight per day. This was administered by the intraperitoneal, subcutaneous, or oral routes. In 1 experiment the ascorbic acid appeared to lengthen the survival time of the infected pigs somewhat. Other evidences of beneficial effects were not noted. Of the 19 pigs given ascorbic acid, only 1 survived, and it is probable that its survival was due to immunity obtained from ingestion of colostrum.

The effects of a tryptophan-deficient diet on the symptoms, course, and lesions of experimental hog-cholera infection in baby pigs were also investigated. The deficiency delayed the onset of symptoms and time of death in 1 group of animals, and in 2 groups the deficiency caused a slight decrease in the severity of the gross lesions.

The administration of tryptazan, a tryptophan analogue, to a pig inoculated with hog-cholera virus was accompanied by a recovery from the infection. The results were inconclusive, however, due to the possibility that the animal had obtained some immunity to the hog-cholera virus from having nursed its dam during the first few days of life.

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