A POSSIBLE CONDITIONING EFFECT OF METHIONINE AND VITAMIN B_{12} IN GROWTH STIMULATION BY ANTIBIOTICS

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CHAPTER I

INTRODUCTION

The discovery that antibiotics stimulate growth of animals when added to the diet happened rather by accident. Research workers at Lederle Laboratories were seeking a practical source of vitamin B_{12} and found that fractions obtained from the fermented mash of Streptomyces aureofaciens gave better growth than vitamin B_{12} itself (46). These investigators subsequently proved that an antibiotic, aureomycin, contained in the fermented mash was the factor responsible for the added growth response.

It is difficult to say when or where the thought first arose that there may be a nutritional quality associated with animal protein which is not shared by vegetable protein and is not dependent on amino acid balance. Mapson (104, 105) in 1932 and 1933 and Byerly, Titus and Ellis (30) in 1933 were the first to put the idea into words and Mapson is generally regarded as the pioneer in this field. Unfortunately, however, Mapson did not continue these studies. Byerly's group carried on and were instrumental in laying the groundwork, both for more rational studies with vegetable proteins and for important advances towards the isolation of the unknown factor. As a period of scarcity of protein from animal sources came on with general war-time shortages, and since increased production of livestock necessitated additional protein supply, soybean meal was employed in poultry feeding on a much larger scale than previously. An intensified study of both the slight amino acid deficiencies of soybeans and the postulated unknown factor in animal proteins was made necessary due to the unavailability of adequate animal protein supplements. For scientific purposes it was necessary, step by step, to differentiate the effects of various vitamins and amino acid deficiencies and for practical purposes to find suitable sources of the unknown factor. It was firmly established that the unknown factor not present in any vegetable feeds was a determinate in the quality of various animal protein supplements. The factor in question became known as the "animal protein factor" or "A.P.F." and later the term "animal protein factor complex" reflected the conviction that more than one chemical entity was involved.

It was shown by Hammond and Titus (58) in 1944 and Rubin and Bird (135) in 1946 that chicks failed to make normal gains when grown on wholly vegetable diets, even when these diets were supplemented with adequate amounts of all the then known vitamins. In 1948 Zucker and Zucker

(182) demonstrated that such diets were inadequate for the proper nutrition of the rat. Previously it had been commonly accepted for a long time that a certain amount of protein of animal origin was necessary for the optimal growth of pigs and poultry.

Very soon after the isolation of vitamin B_{12} by Rickes _et al_ (134) in 1948 in the United States and by Smith and Parker (160) also in 1948 in England, it was shown by Ott, Rickes and Wood (121) that vitamin B_{12} increased the growth of chicks fed an entirely vegetable ration. In 1949 Hogan and Anderson (71) demonstrated that vitamin B_{12} also increased the growth of pigs fed a vegetable ration.

The results of these experiments were misleading in that they suggested that vitamin B_{12} and the "animal protein factor" were identical. Further work, however, showed that vitamin B_{12} could not adequately supplement vegetable proteins to bring them up to a nutritional par with animal proteins. Sunde _et al_ (168) showed that chicks fed an all vegetable ration supplemented with three per cent fish solubles or liver extract made greater weight gains than those receiving the same ration supplemented with vitamin B_{12}. It was demonstrated by others that fish meal (6) and liver meal (170) also were better growth stimulants for chicks than pure vitamin B_{12}.

In 1946, two years before vitamin B_{12} was isolated, Moore et al (108) observed the beneficial effects of dietary antibiotics. They found that the growth of chicks on adequate diets was improved by the addition of streptomycin and sulphasuccidine to the diet.

In 1946 Rubin and Bird (136) demonstrated that cow manure contained the factor to which fish meal owed its distinctive value for hatchability and growth of chicks and were able to concentrate the factor a thousand-fold. Rubin, Bird and Rothchild (137) also found that the factor occurred in hen droppings. A very significant contribution was made by McGinnis, Stevens and Groves (102) who showed that the fresh droppings were practically inactive but on incubation high potency developed. These findings gave assurance that the factor was not necessarily dependent on feeding animal tissues but could be of an independent microbiological origin. Immediately several large chemical companies began a series of important investigations pointing toward the development of fermentation methods of producing A.P.F. concentrates.

In 1949 workers at Lederle Laboratories were seeking a practical source of vitamin B_{12} and found that fractions obtained from the fermented mash of Streptomyces aurofaciens gave better growth than vitamin B_{12} itself (167). These workers attributed their results to a second animal protein factor but since aureomycin was known to be present in such fermented products it was also tried and found to be effective as reported in 1950 (165). These results were soon confirmed by others who showed that mixtures of aureomycin and vitamin B_{12} were as effective as animal protein supplements in promoting the growth of chicks (40, 178), turkeys (163), pigs (32, 53, 79), and young non-ruminating calves (90). Soon after, other antibiotics, namely penicillin, terramycin, streptomycin and bacitracin were found to improve the growth of non-ruminants.

It was very surprising to the majority of workers when Biely et al (13) in 1951 found that the antibiotics aureomycin, penicillin, terramycin and streptomycin would improve the growth of chicks receiving three per cent fish meal, and even more surprising when Coates et al (40) showed antibiotics improved the growth of chicks on a first class diet containing ample animal protein.

It was thought by many workers that vitamin B_{12} together with a suitable antibiotic could replace animal protein in vegetable diets based on soybean or peanut meal. However, evidence is now accumulating to show that, under certain conditions and with more highly purified diets, the combination will not completely replace an equal level of animal protein. It was noted by several workers (43, 118, 124) that the hatchability of eggs of hens fed an all vegetable diet, especially when the protein was supplied by purified soya protein, decreased rapidly within a few weeks. Although hatchability was improved by administering vitamin B_{12}, it ultimately fell below that of eggs from hens fed on similar diets containing added liver powder or those on a good commercial ration. It has also been reported that the growth of chicks is increased for ten weeks with vitamin B_{12} and antibiotics but that liver causes an increase for 12 weeks (170). Thus, it appears that there are unidentified factors of animal origin required for maximal prolonged fertile egg production and perhaps even for growth.

The concept of adding antibiotics to animal feeds for improved growth rates is very new but already they have proven themselves and are widely accepted by the feed trade. It has been estimated (129) that over $65,000,000 worth of antibiotics will be sold as supplements for pig, chicken and turkey feeds during the year 1952-1953. The monetary benefits from feeding antibiotics are enormous. For example, Catron (36) has calculated that the potential saving to Iowa swine producers alone through use of these materials is over $16,000,000 annually. Such being the case, the challenge offered to research workers in the designation of improved methods of using antibiotics so as to reap the greatest benefits from their activity cannot be ignored.

CHAPTER II

REVIEW OF LITERATURE

Effect of Antibiotics on Growth

Since April, 1950, a considerable number of reports have appeared which confirm and extend the observations that when certain antibiotics such as procaine penicillin G, aureomycin, terramycin, streptomycin and bacitracin are added to the diets of non-ruminant farm animals, growth usually but not always is stimulated to a variable but definite extent. It was shown by numerous workers that supplementing chick rations with antibiotics resulted in increased body-weight gains (10, 15, 39, 40, 49, 56, 67, 72, 83, 84, 85, 100, 106, 122, 132).

Stimulation of growth of young turkeys is also seen even to a more marked degree with antibiotic feeding as first reported by Stokstad and Jukes (163) and later by many others (2, 6, 19, 83, 97, 100, 101, 179). Reyniers *et al* (130), however, in 1952 reported that antibiotics gave no positive growth stimulation to germ-free chicks. Also, Coates *et al* (38) in England found that no response was obtained to penicillin with chicks which were placed in new cages in a building which had not been previously used for keeping animals. However, a response appeared in later experiments after the quarters had been used for some time.

In conducting a review of the literature on the effects of feeding antibiotics to animals, it was noted that experiments were conducted under different degrees of sanitation and animal health, and that there were wide variations in the composition of the basal rations. It is possible that these variations may account for at least part of the differences in the results obtained by the above investigators. As will be seen elsewhere in this review, conflicting results were obtained by investigators working with swine, cattle and sheep.

Recently the literature has been filled with reports that antibiotics promote growth in young pigs. In 1949 Cunha et al (46) found that the addition of a commercial A.P.F. (Animal Protein Factor) supplement, a fermentation product of Lederle Laboratories, to a basal ration containing all the vitamins known to be needed by pigs resulted in improved growth. The authors concluded that the A.P.F. supplement supplied an unknown growth factor or factors for the pig, and that it increased the feeding value of peanut meal and soybean meal.

Jukes et al (79) of Lederle Laboratories demonstrated that the major factor contained in their A.P.F. supplement which was responsible for the increased weight-gain in pigs was aureomycin. Such findings have been confirmed and extended by many investigators employing not only aureomycin, but also penicillin, streptomycin and terramycin (9, 18, 21, 23, 24, 35, 48, 57, 65, 86, 93, 94, 150, 158).

In contrast to the beneficial effects reported by the above mentioned investigators, Speer et al (159) found that the addition of antibiotics to the basal ration failed to increase the daily weight gains or to improve the feed efficiency of healthy pigs kept under fairly sanitary conditions. Heidebrecht et al (65) also found that the addition of 0.75 gram of aureomycin per 100 pounds of an adequate ration for healthy pigs did not significantly improve growth or performance from weaning to 125 pounds. This observation led the authors to suggest that the response of pigs to antibiotics depends upon the health of the animal and the adequacy of the ration with respect to various nutrients. Biely, March and Smith (12, p.870) also made the statement that "in order to take full advantage of the growth stimulating properties of antibiotics it is necessary to provide a diet of high energy and optimum protein content, as well as one well balanced in amino acids."

There appears to be a difference of opinion as to whether antibiotics promote growth in young fattening hogs. Hanson et al (59) reported that the addition of antibiotics to a fattening ration resulted in a more rapid growth rate and improved feed efficiency in pigs from 125 to 200 pounds body weight. Hoefer et al (70) stated that almost all the growth response (25 per cent) due to antibiotics was obtained in young pigs from 25 to 100 pounds body weight and only a slight advantage of five per cent in favor of antibiotic-fed pigs from 100 to 200 pounds. Brinegar and Warner (26) found that all increase in body weight due to feeding antibiotics occurred before the pigs reached 125 pounds. Heidebrecht et al (65) have also shown that the addition of an antibiotic to an adequate fattening ration did not significantly improve the rate of gain of healthy pigs from 125 to 225 pounds body weight.

It is commonly accepted that the addition of antibiotics to nutritionally adequate "synthetic" milk rations for baby pigs from two to 56 days of age causes an increase in average daily gain and feed efficiency, as first demonstrated by Nesheim, Krider and Johnson (114) and Schefchik et al (147) in 1950. Other investigators have also obtained beneficial effects from the addition of antibiotics to "synthetic" milk rations for baby pigs (60, 95, 116, 148, 149, 174, 175, 180).

Unlike the effects obtained with non-ruminants, the effect of antibiotics when added to rations of ruminant farm animals appears to be one of little or no benefit. However, beneficial results have been obtained with young calves fed antibiotics before they have reached the stage of active rumination.

The earliest reports on the feeding of antibiotics to dairy calves was made in 1950 by Loosli and Wallace (90) of Cornell University. They reported a significant increase in body weight of calves fed an aureomycin supplement from birth to eight weeks of age over the unsupplemented calves. Since 1950 many experiments have been conducted on the feeding of antibiotics to dairy calves of several different breeds. A favorable effect on growth and health, calf scours, feed utilization and physical appearance was noted in the majority of the experiments (7, 16, 17, 33, 73, 80, 91, 98, 111, 139, 140, 173). It was noted that the beneficial results were obtained in young calves which had not reached the stage of active rumination. Some workers, however, found no beneficial effect from feeding antibiotics to dairy calves. Morrison and Deal (110) observed no significant difference in the incidence of scouring general health, feed consumption and gain in weight to 12 weeks in Holsteins fed an antibiotic supplement from birth to two weeks of age. No significant difference in body weight gains or incidence of scours was found by Logan (89) between groups of dairy calves fed an antibiotic supplement from birth to 12 weeks and the unsupplemented controls.

No beneficial effect has been reported from feeding antibiotics to mature ruminating cows. Stokstad (162) reported no beneficial or adverse effects, except a temporary reduction in appetite, when aureomycin was fed to yearling beef heifers for 150 days. Neumann, Sapp and Gall (115) obtained no benefit when aureomycin was added to a fattening ration fed to yearling heifers for 150 days. Total bacterial counts were about the same in the group receiving the antibiotics as in the unsupplemented controls, but the types of organisms found in the heifers receiving aureomycin were much less diverse, suggesting that the normal bacterial flora had been disturbed. Rusoff et al (141) found no significant difference in the amount, composition and bacterial count of the milks of two groups of dairy cows, one group receiving aureomycin and the other none.

Unlike the results obtained with calves, the majority of the investigators report no beneficial effects from feeding antibiotics to either suckling lambs or to fattening lambs. In fact, several have reported harmful effects from their use. Colby, Rau and Miller (42) found that the administration of 100 mg. of aureomycin daily by capsule to fattening lambs resulted in a loss of appetite and a subsequent loss of body weight during the first week on experiment, but the controls continued to gain more rapidly throughout the experimental period. It should be noted, however, that Colby, Rau and Miller administered exceedingly high levels of aureomycin to the lambs in their experiments. Stokstad (162) has reported that aureomycin supplementation at levels of five to 15 mg. per day produced growth increases in lambs of 10 to 20 per cent, while larger doses of 100 mg. per day produced an adverse effect.

Kinsman and Riddell (81) reported no significant difference in rate of gain of five groups of creep-fed suckling lambs, four of which received 15 mg. of antibiotic per pound of ration.

Hardie (61, p.68) found that the administration of five mg. aureomycin per lamb per day had no significant effect on the rate of gain of fattening lambs.

In contrast to the results obtained by the above mentioned workers, Jordan and Bell (77) reported an increase in body weight gains of suck-lambs which were drenched with five mg. of aureomycin daily for six weeks. The treated lambs averaged 0.64 pounds gain per day as compared to 0.54 pounds per day for the controls. The above investigators also obtained a favorable growth response from the addition of from six to 12 mg. of aureomycin daily to a fattening ration for weanling lambs.

With the widespread and rather indiscriminate use of antibiotics in feed for livestock, there arose several questions which had to be answered. These questions were, according to Bird (14):

1. In what feeds are antibiotics useful?
2. Is there danger of toxicity?
3. Will feeding antibiotics result in their accumulation in the tissues?
4. By what mechanism do they stimulate growth?

The first three questions have been fairly satisfactorily answered. It has been established that antibiotics are useful in feeds for growing non-ruminant animals. The answer to the second question is an unqualified "No." The antibiotics that are being used in commercial feeds have been fed at levels 20 to 30 times as great as are being used commercially, with no apparent harmful effects. Information on the accumulation of antibiotics in the tissues of animals is still rather meager, but the results reported thus far indicate that there is no significant accumulation in the flesh. The fourth question, concerning the mechanism by which antibiotics promote growth, has not as yet been adequately answered. The literature has been filled with contradictory and confusing results on the mode of action of antibiotics. In view of the confusing situation existing on the mode of action of antibiotics, this thesis was undertaken in an effort to clarify a part of the picture regarding their growth-promoting effect, namely that of essential nutrient sparing action.

Mode of Action of Antibiotics

The exact reason for the growth-promoting action of antibiotics has not been determined as yet. There have been many reports concerning the effects of antibiotics when fed at the low "nutritional" levels. Most workers in the field agree that the following possibilities as to the mechanism of action of antibiotics exist:

1. Antibiotics may have a direct "vitamin effect" on animals.
2. Antibiotics may act indirectly through an effect on the micro-organism of the intestines.
    a. Toxin-producing bacteria are eliminated.
    b. Micro-organisms competing with the host for nutrients are eliminated.
    c. Vitamin-producing micro-organisms are favored, thus "sparing" certain vitamins.
    d. Beneficial changes occur in the chemistry of the micro-organisms.
    e. Antibiotics spare the protein requirement of animals.

f. Antibiotics spare certain essential amino acids.

The first of these possibilities does not seem likely since such a wide variety of compounds: penicillin, aureomycin, terramycin, streptomycin and bacitracin, with dissimilar structures, are effective. Their only known point of resemblance is their ability to inhibit the growth of micro-organisms. It would seem, therefore, most obvious, since all of the compounds reported to have growth activity are also effective germicides, that the intestinal flora is altered in such a manner as to benefit the host, as has been suggested by a majority of workers in this field (6, 38, 51, 56, 108, 178). In fact, significant changes in the intestinal flora, which were correlated with increased growth, have been found in chickens fed antibiotics (4, 54, 85, 108, 125, 133, 154, 181).

The second theory is also supported by the observation that the growth-promoting effects of antibiotics are most marked when the animals are kept under unsanitary conditions and are suffering from various intestinal diseases. The exact changes which are brought about in the intestinal flora are not understood, and there is no direct experimental proof as to just how changes in the flora could bring about increased growth.

Little evidence has been offered that "toxic substances" are being produced by the flora in the absence of antibiotics, although this remains a distinct possibility. Sieburth *et al* (154, p.18) presented evidence that there was a marked change in the intestinal content of *Clostridium perfringens* of turkeys fed penicillin and terramycin and suggested that "these antibiotics promote growth by preventing enterotoxemia." However, it has been suggested more recently by Williams *et al* (181) that although pronounced changes in *Clostridium* counts do occur they apparently do not account for the changes in growth.

It has been postulated by some workers that organisms detrimental to the host, by competing with the host for nutrients in some manner, are decreased by the antibiotics. Lih and Baumann (88) in studies with the rat, and Coates *et al* (37) and Biely and March (11) in studies with the chick, have offered some evidence that the flora may compete with the host for certain vitamins. Lih and Baumann concluded from their data that "the mechanism by which the antibiotics stimulate growth is by preventing micro-organisms from depriving the host of limiting nutrients." The competition between host and micro-organisms is likely for some "minor" nutrient which is required by the host in very small or "catalytic" amounts, since it would be highly improbable that the requirement for calories by the microbial population in the digestive tract of non-ruminant animals would be such that they could seriously affect the carbohydrate, fat and protein available to the host. This theory, regardless of the support it has received, does not quite explain how antibiotics increase growth when they are added to diets apparently adequately fortified with all the essential nutrients.

Another theory proposed by some investigators as to the mode of action of antibiotics is that they favor certain vitamin-producing organisms, thus sparing certain B vitamins. Sauberlich (143) found the addition of penicillin to the diet at a level of 0.01 per cent caused a marked stimulation in the growth of rats fed diets free of or low in thiamine, pyridoxine, pantothenic acid and, to some extent, riboflavin. Aureomycin at the 0.01 per cent level had a similar effect in the case of pantothenic acid and thiamine deficiencies. The addition of penicillin or aureomycin to the completely vitamin-supplemented basal diet had no effect upon the growth of the animals. Biely and March (11, p.331) also concluded that "dietary levels of nicotinic acid, folic acid or riboflavin that were sub-optimal for maximum growth rate of the chick, may be adequate when aureomycin is fed." However, Stokstad, Jukes and Broquist (166) reported that differences in vitamin requirements were not seen with antibiotic feeding. These workers reported that the requirement of chicks on purified diets for niacin, pantothenic acid, pyridoxine and folic acid was not changed by adding aureomycin to the diet. Sunde _et al_ (169) fed chicks a grain ration containing peanut meal as the protein supplement and found that 30 micrograms of vitamin B_{12} plus 500 mg. of streptomycin per kg. of ration stimulated growth to the same extent as 200 micrograms of vitamin B_{12} per kg. without antibiotic supplement. No additional growth was observed when streptomycin was added to the ration containing the high level of vitamin B_{12}. The authors concluded that one function of the antibiotics is the sparing of vitamin B_{12}.

Oleson, Hutchings and Whitehill (163) found that no growth stimulation was obtained in chicks when aureomycin was added to a ration free of vitamin B_{12}, but when the ration contained suboptimal levels of vitamin B_{12} a marked weight increase was obtained by the addition of aureomycin.

Stokstad and Jukes (164) reported that in some experiments with chicks aureomycin seemed to have a sparing effect on vitamin B_{12}, but in others no effect was observed. Some evidence exists that organisms may be competing with the host for an unknown growth factor (66, 75, 106), but this does not seem to explain the entire growth-promoting action since antibiotics are still effective when added to rations containing a wide variety of animal products, such as fish meal, meat scraps, liver meal, tankage and whey as demonstrated by numerous investigators (18, 25, 26, 27, 172).

As another possible explanation of the mode of action it is conceivable that growth of organisms beneficial to the host is favored by the antibiotics, due possibly to the suppression of other organisms. Further, such organisms could be beneficial by producing nutrients, known or unidentified, or by protecting the host in some other manner. For example, Kratzer et al (85) showed that a five to tenfold increase in yeast organisms resulted from the feeding of streptomycin. If this situation is true, growth should be equally improved by the addition of various concentrates such as yeasts, fermentation solubles, etc. to the diet without antibiotics, although such has not been observed in every case in common practice. Furthermore, chicks grown in the complete absence of intestinal organisms are not adversely affected and may even grow faster than chicks grown under normal conditions as shown by Reyniers et al (131) of Notre Dame. In another experiment with germ-free chicks, antibiotics were added to the ration at a level of 50 mg. per kg. of diet (130). Steam sterilization was used for the chloromycetin; filtration was used to sterilize streptomycin and cathode ray sterilization was used for penicillin, terramycin and bacitracin. The potency of the antibiotics was not drastically affected by the sterilization.

The growth of the germ-free chicks indicated no response to the antibiotics, but antibiotics sterilized in the same manner as those fed to the germ-free chicks did produce growth stimulation in conventional birds. The germ-free chicks grew at a somewhat faster rate than conventional birds which did not receive the antibiotic supplement. However, the weight of germ-free birds at four weeks of age was never greater than the conventional birds receiving penicillin. Schwarz (151) suggested, as the result of data obtained with rats, that micro-organisms produce a known or an unidentified vitamin which is inactivated or destroyed by the pH of the gut or by other means. However, the above-mentioned work with germ-free chicks presents evidence that this cannot be the case.

It has been reported that the injections of small amounts of antibiotics from 1.2 mg. to 3.0 mg. weekly has increased the growth of chickens (50, 54) and of pigs (8, 119), and that the growth of baby pigs was improved by the subcutaneous implantation of bacitracin pellets (117). The pigs that received one pellet containing 1000 units of bacitracin were heavier than those given either two or four pellets of the same potency. In both studies with the chick, changes were observed in the microflora of the tract after injection. Also, the findings of Larson and Carpenter (87) that aureomycin when given either orally or injected was excreted in the feces may explain these results without discrediting the mode of action theories involving changes of intestinal flora. Larson and Carpenter suggested that injected aureomycin enters the intestinal tract of the pig through the bile. No real evidence has yet been presented that the action of antibiotics is within the tissues of the animal. Furthermore, many of the effective promotants are very poorly absorbed from the intestinal tract (22).

A few suggestions have appeared in the literature which may give a clue to the growth-promoting action of antibiotics. For instance, it has been reported by Machlin et al (103 and McGinnis (99) that antibiotics may spare the protein requirement of chickens and turkeys. Several investigators have presented evidence which indicates that antibiotics may spare the protein requirement of pigs (29, 34, 47, 70). Machlin et al (103) fed growing chickens diets containing 15, 17, 19, and 21 per cent protein (all-plant protein ration) alone or supplemented with 20 mg. of crystalline aureomycin per kg. of feed. With every protein level in each experiment, average weight was greater with aureomycin than without aureomycin. McGinnis (99) found that equally good growth of young turkeys was obtained at lower levels of protein when penicillin was added to the ration. A diet containing approximately 24 per cent protein and penicillin gave growth at four weeks of age comparable to that obtained with protein levels as high as 28 per cent without antibiotic supplement.

Cunha _et al_ (47) working with young growing pigs fed rations varying in protein content from 12.2 per cent to 19.6 per cent with and without an A.P.F. concentrate containing aureomycin. In each case the addition of A.P.F. increased the rate of gain, and the 17.9 per cent protein ration with A.P.F. was equal to the 19.6 per cent protein ration without A.P.F. Work at the Iowa station by Catron, Jensen and Maddock (34) indicates that aureomycin has a sparing effect on the protein requirements of pigs. Sixteen lots of eight pigs each were fed an all-plant ration adequately supplemented with vitamins and minerals. Initial protein levels were 20, 18, 16 and 14 per cent. Two lots of pigs on each level of protein received 10 mg. of aureomycin per pound of ration. There were no significant differences in gains of the pigs on the various levels of protein receiving aureomycin. In the absence of the antibiotic, the per cent protein in the rations significantly affected the average daily gains.

It should be noted that the effect of antibiotics on protein utilization may be very indirect and related more to feed consumption. Slinger _et al_ (156) have shown a negative relationship between antibiotics and proteins. These workers obtained a greater growth response in broilers from penicillin at a level of 10 p.p.m. and from aureomycin at a level of 20 p.p.m. added to a ration containing 26 per cent protein than when added to rations containing 17, 20 or 23 per cent protein. In the absence of antibiotics, the highest level of protein tended to depress growth and feed efficiency as compared to the 23 per cent protein. The authors concluded that the protein requirement for maximum fleshing was 23 per cent in the presence or absence of antibiotics. Biely, March and Smith (12) reported that antibiotics did not lower the protein requirement of growing chicks; in fact, they might even enhance the dietary protein requirements. This observation is in agreement with Slinger _et al_ (157) who found an increased requirement of turkeys for protein in the presence of antibiotics. These investigators found the response to antibiotics much greater with a 28 per cent than with a 20 per cent protein diet. Hoefer, Luecke and Thorp (69) found that terramycin at five mg. per pound of total ration had a highly significant effect on the rate of gain of weanling pigs, but it did not seem to affect the requirement of pigs for protein.

It has been claimed by some investigators that antibiotics spare certain essential amino acids. Jones and Combs (75) reported that aureomycin, added to diets suboptimal in tryptophan and lysine, appeared to spare the dietary requirement of chicks for tryptophan, but not for lysine. In another experiment, using a corn-soybean meal type ration, it was found that dietary penicillin did not spare the requirement for methionine. In fact, the growth response resulting from the addition of 0.1 per cent DL-methionine to the ration containing 10 p.p.m. of penicillin was much greater than that obtained from the addition of the same amount of methionine to the basal ration. Nelson and Scott (113) concluded that neither aureomycin nor penicillin, both added at 20 mg. per kg. of diet, spared the niacin requirement of chicks reared on a semi-purified diet containing either dextrose or sucrose as the sole carbohydrate. Biely, March and Smith (12) added penicillin at 15 mg. per pound of ration and an aureomycin supplement at one per cent to rations deficient in lysine and tryptophane and found that the antibiotics had little or no stimulating effect on growth rate of chicks fed rations in which the limiting factor was either of these amino acids. The response to penicillin with the lysine-adequate rations was highly significant, an increase in average weight of 41 grams at four weeks of age, but there was no response with the lysine-deficient ration. These workers noted that with a better balance of amino acids a greater response to antibiotics was possible.

Cunha et al (47) obtained an increase in growth rate of pigs fed a corn-peanut meal ration supplemented with minerals, vitamins and an A.P.F. supplement containing aureomycin. Methionine added to this ration did not stimulate growth; however, when it was added to a similar ration without A.P.F., growth was increased. This observation led the authors to conclude that A.P.F. spared methionine. Since the A.P.F. supplement used by Cunha et al was a crude aureomycin fermentation product, it is likely that other nutrients, and possibly methionine itself, was responsible for the methionine sparing action rather than the aureomycin it contained.

Levels

It is very difficult to arrive at the most effective level of antibiotics, when added to the diets of animals, from data which have appeared in the literature, due to differences in diets and condition of the animals employed by the many investigators. Braude, Wallace and Cunha (20, p.275) have listed the growth index of pigs fed antibiotics at varying levels as outlined in Table 1.

The most common practice recently has been to follow the recommendations of the manufacturer of the antibiotic to be used. These recommended levels are given in Table 2.

TABLE 1

The Effect of the Level of Antibiotics in the Diet on Growth Stimulation of Swine (Growth Index) (20, p.275).

Level of Antibiotics Gm. per Ton of Ration	Aureomycin *Growth Index	Penicillin *Growth Index	Streptomycin *Growth Index	Terramycin *Growth Index
Less than 5	114.7	108.4	108.0	109.0
6-9	108.3	104.0	111.0	114.0
9	115.3			
10	115.5	110.0	105.0	122.1
11-15	116.1	110.4		108.0
16-20	113.8	109.0	114.6	107.5
21-25	118.1	115.8	121.7	116.0
26-50	115.8	109.0	104.5	117.0
51-75	123.5			
76-100	129.0			
Above 100		104.0	118.0	

*Mean growth index of pigs receiving antibiotics related to the daily weight gain of the control.

TABLE 2

Manufacturers Recommendations for the Amounts of the Various Antibiotics to be Added to Rations of Farm Animals.

Species	Antibiotics			
	Aureomycin Gms. per Ton of Feed	Bacitracin Gms. per Ton of Feed	Penicillin Gms. per Ton of Feed	Terramycin Gms. per Ton of Feed
Calves	35-40	--	--	--
Chickens	8-10	8-10	3-5	8-10
Pigs	18-20	8-10	3-5	8-10
Turkeys	18-20	8-10	3-5	8-10

CHAPTER III

EXPERIMENTAL INVESTIGATIONS

Experimental: Rats

In recent years, great emphasis has been placed upon the use of vitamin B_{12} and of certain antibiotic substances in the rations of domestic animals and poultry. The value of vitamin B_{12} supplementation was found to be due mainly to its ability to improve the feed value of certain proteins, especially those of vegetable origin, in livestock feeding, and to improve the overall efficiency of protein utilization by exerting a "sparing" action upon the metabolism of certain essential amino acids. Hartman, Dryden and Cary (62) obtained data with young rats which suggested that vitamin B_{12} helps to metabolize protein when it is present in the diet in very large amounts. It has been found that both vitamin B_{12} and folic acid play some part in the synthesis and metabolism of labile methyl groups and possibly of other one-carbon fragments. Jukes, Stokstad and Broquist (78) found that the methionine requirement of chicks could be met by homocystine in conjunction with vitamin B_{12}, thus sparing the essential amino acid methionine. Similarly, Stekol and Weiss (161) demonstrated that rats 30 days old, but not younger, could use diets free from labile methyl compounds if they were supplemented with vitamin B_{12} as well as homocystine. It was shown by Salmon (142), and Schaefer, Salmon and Strength (146) that in rats and chicks vitamin B_{12} and folic acid were concerned in transmethylation reactions.

The mode of action of antibiotics in causing growth stimulation is not entirely clear, as was pointed out in the review of literature. It has been noted by many investigators that the maximal effect of antibiotics is noticeable when the experimental animals are in an unthrifty condition, or are on a suboptimal diet. Many of the suboptimal diets used in antibiotic studies included soybean oil meal or peanut oil meal as the major source of protein. Each of these protein supplements, and especially the latter, is known to be somewhat deficient in methionine as compared to animal proteins such as those of milk, eggs and meat.

This study was undertaken in an effort to determine whether there was some metabolic interrelationship between the activity of antibiotics and of vitamin B_{12} and the level of methionine in the ration. Cunha _et al_ (47) reported that a crude A.P.F. supplement containing aureomycin spared the methionine requirement of pigs. Since the crude A.P.F. supplement used by Cunha _et al_ possibly contained other growth factors in addition to aureomycin, it was doubted that aureomycin was the substance responsible for the methionine sparing action of the supplement. It was thought that the preliminary work on this problem should be done with rats due to advantages in preparation of a more purified diet. The results of the investigations with rats were also useful in setting up further experiments with pigs.

Experiment I

The Effect of Penicillin on Growth and Feed Efficiency of Young Rats Fed Diets Deficient in and Adequately Fortified with Methionine and Vitamin B_{12}.

According to Rose (107, p.481), the minimal requirement of methionine for normal growth of the rat is 0.6 per cent of the diet. The methionine content of the diets employed in this study was approximately 0.12 per cent as determined by microbiological assay. The purpose of this experiment was:

1. To determine whether the low level of methionine in such feedstuffs as peanut oil meal was detrimental to the rate and efficiency of growth of animals.
2. Whether vitamin B_{12} or antibiotic supplementation may allow more efficient utilization of low methionine feeds.

Materials and Methods: Weanling rats of the Oregon State Agricultural Chemistry Department strain, weighing from 40 to 60 grams, were placed in individual cages and randomly distributed, 10 animals to each group. Feed and water were supplied *ad libitum*. Individual food consumption records were kept throughout the experimental period of six weeks duration. All animals were weighed weekly.

The diets, freshly prepared at about two-week intervals, were stored in tightly covered jars in the refrigerator at four degrees centigrade. The composition of the diets used for the four groups is given in Table 4. Since plant protein is almost completely devoid of vitamin B_{12} (62), it was added to the diets of some groups of rats at the rate of 0.0315 mg. per kg. Penicillin was added at 5.0 mg. per kg. of diet as recommended by Merck and Company.

The crude protein content of the peanut meal and of the complete rations was determined by the Kjeldahl method as outlined by Hawk, Oser and Summerson (63, pp.814-816) and Association of Official Agricultural Chemists: Methods of Analysis (5, pp.26-27) with the following modifications:

1. The distillate from a 2.0 gm. sample was collected in a four per cent boric acid solution and titrated directly with 0.2284N sulfuric acid.
2. A mixed indicator, consisting of five parts of a 0.1 per cent bromcresol green and one part of a 0.1 per cent methyl red solution in 95 per cent ethyl alcohol, was routinely used.

The methionine content of the peanut meal was determined microbiologically using the basal medium of Henderson and Snell (64, p.17) with slight modification as shown in Table 3. The peanut meal was prepared for analysis by hydrolyzing one-half gram with 10 ml. of 1.2 N. HCl for 10 hours in an autoclave at 15 pounds pressure, according to the method of Klosterman et al (82, p.440). The organisms used for this determination were Leuconostoc mesenteroides P-60 N.R.R.L. No. 8042 and Streptococcus fecalis R., N.R.R.L. No. 8043. A standard curve, run with L-methionine, is included for reference purposes as Figure I.

TABLE 3

Composition of Medium for Microbiological Determination of Amino Acids (64, p.17).

Component	Quantity per Liter	Component	Quantity per Liter L	DL
Glucose	20 gm.	Histidine	100 mg.	200 mg.
Sodium Citrate	20 gm.	Isoleucine	"	"
Sodium Acetate (Anhydrous)	1 gm.	Leucine	"	"
Ammonium Cl.	3 gm.	Methionine	"	"
K_2HPO_4	5 gm.	Phenylalanine	"	"
Salts C	20 ml.	Proline	"	"
Adenine Sulfate	10 mg.	Threonine	"	"
Guanine HCl	10 mg.	Tyrosine	"	"
Uracil	10 mg.	Valine	"	"
Xanthine	10 mg.	Tryptophan	"	"
Thiamine	1 mg.	Cystine	"	"
Riboflavin	1 mg.	Serine	"	"
Pyridoxal	200 gamma	Glycine	100	
Ca. Pantothenate	1 mg.			
Niacin	1 mg.			
P-Amino Benzoic Acid	200 gamma			
Biotin	10 gamma			
Folic Acid	10 gamma			
DL-Alanine	1 gm.			
DL-Aspartic Acid	1 gm.			
L-Glutamic Acid	1 gm.			
L-Arginine-HCl	200 mg.			
L-Lysine-HCl-H_2O	200 mg.			

Adenine, guanine, and uracil solutions were prepared at concentrations of 1 mg. per ml. with sufficient HCl to keep them in solution. The xanthine at the same concentration was dissolved with the aid of dilute ammonia.

Salts C solution was prepared by dissolving

10 gm. $MgSO_4.7H_2O$
0.5 gm. $FeSO_4.7H_2O$
0.5 gm. NaCl
2.0 gm. $MnSO_4.7H_2O$

Made up to 250 ml. with distilled water.

Amino Acids:

These were prepared in a solution at such concentrations that 250 ml. contain the amounts indicated. Cystine and tyrosine were dissolved first in the minimum quantity of 3N HCl, then diluted with water and the other amino acids added. The mixture was heated until complete solution was effected. All solutions were kept in the cold room under toluene, and replaced at intervals of one month. The medium was adjusted to pH 6.8-7.0 before use.

The procedure outlined in Table 3 was followed in the determination of the methionine content of peanut meal with the following modifications:

1. All amino acids used were of the DL configuration except for L-histidine and L-lysine. L-methionine was used as the standard.
2. The medium was autoclaved for 10 minutes at 15 pounds steam pressure.
3. The assays were read 16 hours after inoculation in a Coleman Universal Model 14 spectrophotometer at 540 mu.

It was felt desirable to divide the rat experiments among several feeding trials. These trials are referred to by number in the results and discussion which follow. Trial 1 was an investigation of the effects of methionine and penicillin supplementation of rations low in vitamin B_{12}. Trial 2 involved the effectiveness of penicillin when added to rations low and adequate in both methionine and vitamin B_{12}. Trial 3 continued with the investigation of the growth-stimulatory effects of methionine, penicillin and vitamin B_{12} alone or in combination in the basal ration described in Table 4. The results of these three trials are shown in tabular form in Tables 5, 6 and 7, respectively.

TABLE 4

Composition of Diets Used in the Rat Experiments.

Component	Quantity	Jones and Foster[1]		
Basal	Gms. per Kg. Diet	Mineral Mix		
Peanut meal	250	NaCl	292.5	gms.
Sucrose	100	KH_2PO_4	816.6	"
Corn starch	590	$MgSO_4$	120.3	"
Minerals (Jones and Foster[1])	40	$CaCO_3$	800.8	"
Cod liver oil[2]	20	$FeSO_4.7H_2O$	56.6	"
		$MnSO_4.2H_2O$	9.35	"
		KI	1.66	"
Vitamin supplement	Mg. per kg. diet	$CuSO_4.5H_2O$	0.9988	"
Thiamine	2	$ZnCl_2$	0.5452	"
Riboflavin	4	$CoCl_2.6H_2O$	0.0476	"
Pyridoxine	2			
Ca. pantothenate	10			
Niacin	20			
Inositol	200			
Choline	425			
Folic acid	2			

Diet 414. Basal + 0.525% DL-methionine.
Diet 415. Basal + 5.0 mg. procaine penicillin G per kg. diet.
Diet 416. Basal + 5.0 mg. procaine penicillin G + 0.0315 mg. vitamin B_{12} per kg. diet.
Diet 417. Basal less choline chloride.
Diet 422. Basal + 0.525% DL-methionine + 5.0 mg. procaine penicillin G per kg. diet.
Diet 423. Basal + 0.0315 mg. vitamin B_{12} per kg. diet.
Diet 424. Basal + 0.525% DL-methionine + 0.0315 mg. vitamin B_{12} per kg. diet.
Diet 425. Basal + 0.525% DL-methionine + 0.0315 mg. vitamin B_{12} + 5.0 mg. procaine penicillin G per kg. diet.

[1]Jones and Foster mineral mix was included in all diets at a level of four per cent (76, p.254).

[2]The cod liver oil contained 1800 U.S.P. units of vitamin A and 180 U.S.P. units of vitamin D per gram.

FIGURE I

METHIONINE STANDARD CURVE

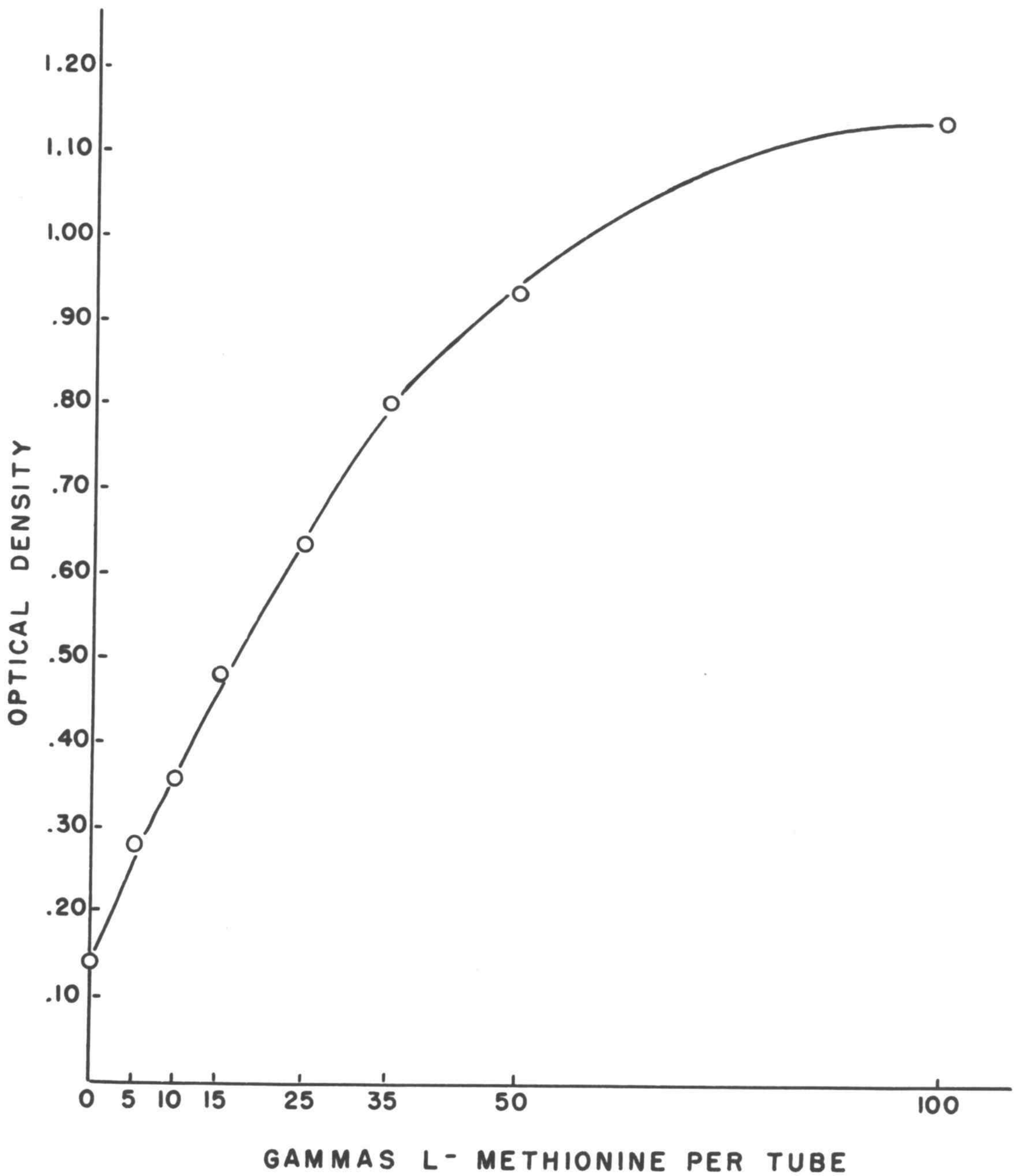

TABLE 5

Comparison of Weight Gains and Feed Efficiency of Weanling Rats Fed Vitamin B_{12} Deficient Diets With and Without Methionine and Penicillin Supplements.

Treatment	Average Initial Weight	Average Final Weight	Average Weight Gain 6 Wks.	Feed Req. per Gm. Gain
	Gms.	Gms.	Gms.	Gms.
Basal	51.4	145.4	94.0	5.9
Basal + 0.525% DL-Methionine	54.2	173.5	119.3	5.6
Basal + 5 Mg. Penicillin per Kg. Diet	50.4	151.7	101.3	5.7

TABLE 6

Effect of Penicillin on Growth and Feed Efficiency of Young Rats Fed Diets Low and Adequate in Methionine and Vitamin B_{12}.

Treatment	Average Initial Weight	Average Final Weight	Average Weight Gain 6 Wks.	Feed Req. per Gm. Gain
	Gms.	Gms.	Gms.	Gms.
Basal	56.7	116.0	59.3	7.2
Basal + 0.525% DL-Methionine	55.3	149.1	93.8	5.6
Basal + 0.525% DL-Methionine + 5 Mg. Penicillin per Kg. Diet	54.2	148.1	93.9	5.8
Basal + 0.0315 Mg. Vitamin B_{12} per Kg. Diet	52.5	136.2	83.7	5.6
Basal + 5 Mg. Penicillin + 0.0315 Mg. Vitamin B_{12} per Kg. Diet	55.4	142.4	87.0	5.6
Basal + 0.525% DL-Methionine + 5 Mg. Penicillin + 0.0315 Mg. Vitamin B_{12} per Kg. Diet	54.2	144.1	89.9	5.5

TABLE 7

Effect of Methionine, Vitamin B_{12} and Penicillin Alone or in Combination on Growth and Feed Efficiency of Young Rats.

Treatment	Average Initial Weight	Average Final Weight	Average Weight Gain 6 Wks.	Feed Req. per Gm. Gain
	Gms.	Gms.	Gms.	Gms.
Basal	67.0	156.4	89.4	5.7
Basal + 0.525% DL-Methionine	67.1	156.2	89.1	5.6
Basal + 0.0315 Mg. Vitamin B_{12} per Kg. Diet	66.6	158.7	92.1	5.6
Basal + 0.525% DL-Methionine + 0.0315 Mg. Vitamin B_{12} per Kg. Diet	65.4	164.6	99.2	5.4
Basal + 5 Mg. Penicillin per Kg. Diet	66.8	151.6	84.8	5.9
Basal + 5 Mg. Penicillin + 0.525% DL-Methionine	66.0	160.9	94.9	5.4
Basal + 5 Mg. Penicillin + 0.0315 Mg. Vitamin B_{12} per Kg. Diet	67.0	167.7	100.7	5.5
Basal + 5 Mg. Penicillin + 0.525% DL-Methionine + 0.0315 Mg. Vitamin B_{12} per Kg. Diet	65.9	173.6	107.7	5.2

<u>Results</u>: The addition of 0.525 per cent DL-methionine to a diet deficient in both methionine and vitamin B_{12} resulted in a highly significant increase in body weight and feed efficiency in the younger rats employed in trials 1 and 2 as shown in Tables 5 and 6. The addition of 5.0 mg. penicillin to the basal diet deficient in both methionine and vitamin B_{12} also exerted a significant effect on body weight gains and feed efficiency of the younger rats (Table 5). However, when penicillin or methionine was added to the methionine-vitamin B_{12} - deficient diet of the older rats in trial 3 (Table 7), no significant effect on rate of growth was obtained. Supplementing the diet with 0.525 per cent DL-methionine did reduce the amount of feed required per gram of gain to a slight extent in this trial. The addition of penicillin to the diet fortified with methionine had no beneficial effect on either rate of growth or feed efficiency of rats in trial 2; however, when added to the methionine low diet containing adequate vitamin B_{12}, growth of rats was stimulated to a slight extent, but no beneficial effect on feed efficiency occurred. The group of rats in trial 2 receiving the basal mixture supplemented with methionine, penicillin and vitamin B_{12} required slightly less feed per gram gain than did any of the others. They required approximately 1.7 grams less feed per gram gain than did the controls

fed the basal mixture. The addition of vitamin B_{12} to the methionine-vitamin B_{12} - deficient diet of the rats in trial 3 resulted in a slight increase in growth and feed efficiency over the control animals fed the basal diet, but when the ration contained both methionine and vitamin B_{12} a highly significant effect was obtained on both growth and feed efficiency. The rats receiving supplements of both vitamin B_{12} and methionine made an average weight-gain of 9.8 grams more and required 0.3 grams less feed per gram gain than did the control over a six-week period. When penicillin was added to diets of the rats supplemented with either methionine or vitamin B_{12} a beneficial effect on growth and feed efficiency, over the rats fed the same diets less the antibiotic, was obtained. The animals which made the largest weight gains and required less feed per gram of gain were those receiving the diet fully supplemented with methionine, vitamin B_{12} and penicillin.

Discussion: It appears that the diets employed in this experiment contained an inadequate amount of methionine for the young rats in trials 1 and 2, and that neither penicillin nor vitamin B_{12} was able to compensate entirely for this deficiency. When both of the latter factors were added to the basal mixture, however, slightly larger increases in body weight were obtained, which would possibly indicate that a slight methionine "sparing" action was exerted by both penicillin and vitamin B_{12} alone and to a greater extent in combination. However, when penicillin and vitamin B_{12} were both added to the diet adequately fortified with methionine, a slightly greater growth response was obtained, which is in agreement with the observation of Biely, March and Smith (11) that antibiotics exert their greatest effect on growth of chicks when the ration contains an adequate supply of essential nutrients. Statistical analysis revealed that, in trials 1 and 2, the addition of methionine produced a significant increase in weight-gain and feed efficiency. This was to be expected since the peanut meal contained only 1.13 per cent methionine as determined microbiologically. This level of methionine is far less than the 0.6 per cent required by the rat (107, p.481).

In trial 3, the rats were larger when put on experiment than they were in trials 1 and 2. This was necessitated in order to make use of all the rats available at the time the experiment was begun. One of the most striking observations of this trial was that the addition of methionine to the basal diet did not produce a marked effect on either growth or feed efficiency. This may have been due to the size of the rats when placed on experiment. However, this seems unlikely since significant increases in body weight and feed efficiency were obtained on the fully supplemented basal in this same trial. Likewise, the addition of either penicillin or vitamin B_{12} did not exert any significant effect on rate of growth or feed efficiency of rats in this trial which is in contrast to the results obtained with the younger rats in trials 1 and 2, but did not exert the same effect on the rats in trial 3 which were approximately one week older. When either penicillin or vitamin B_{12} was added to the diets fortified with methionine and penicillin, growth and feed efficiency were increased with the combination of vitamin B_{12} and methionine being better in this respect than methionine and penicillin. This may indicate that in the presence of adequate methionine, penicillin may "spare" the vitamin B_{12} requirements of rats. In trial 3 the rats fed the fully supplemented diet made the greatest weight gains and, as in trial 2, were the most efficient in terms of feed consumed per unit gain.

Summary: The addition of either methionine, vitamin B_{12} or penicillin to diets low in methionine and vitamin B_{12} stimulated growth and decreased the amount of feed required per gram gain in young rats, while the addition of either methionine or penicillin to similar diets did not result in increased growth of slightly older rats.

When penicillin was added to diets fortified with either vitamin B_{12} or methionine, a significant increase in growth was obtained in the older rats.

Penicillin exerted a greater effect on growth and feed efficiency in both the younger and older rats when the diet was supplemented with adequate amounts of both methionine and vitamin B_{12}.

Experimental: Pigs

Since 1949 when the first report appeared (46) that small amounts of "animal protein factor" obtained from the fermented mash of Streptomyces aureofaciens stimulated growth of young pigs fed an all-plant ration, and the subsequent finding that similar growth responses could be obtained with an antibiotic aureomycin (79), many workers have been engaged in this field of research. As a rule, the response to antibiotics was found to be greater in rations containing peanut oil meal or soybean oil meal as the sole source of protein. However, it must be admitted that the majority of these experiments have been carried out in localities where these protein supplements are commonly used. It has seemed possible from these studies that the stimulatory effect of vitamin B_{12} and certain antibiotic substances for the pig may be conditioned to some extent by the quality of protein in the ration.

Experiment II

The Effect of Penicillin and Methionine on Growth and Feed Efficiency When Added to a Methionine-low Ration for Young Pigs.

Cunha et al (47) reported that a crude aureomycin fermentation product containing aureomycin spared the methionine requirements of pigs fed an all-plant ration with peanut oil meal as the major source of protein. Results obtained with rats at Oregon State College, discussed in detail elsewhere in this thesis, indicated that penicillin may exert a slight "sparing" action on the methionine requirement of young rats. The purpose of this experiment was to determine whether the growth-stimulatory activity of an antibiotic, penicillin, would be influenced by the level of methionine in the ration of young pigs.

Methods and Materials: Pigs used in this experiment were young purebred Berkshires obtained from the Oregon State College swine herd. Thirty-two pigs, 11 to 12 weeks of age averaging approximately 66 pounds each, were randomly divided into four groups of eight pigs. Feed and water were supplied ad libitum and daily food consumption was recorded for each group throughout the experimental period of six weeks duration. All pigs were kept in concrete-floored pens and weighed at weekly intervals. The composition of the diets used in this experiment is listed in Table 8. The diets, freshly prepared at about 10-day intervals, were stored in a cool room, away from direct light.

TABLE 8

Composition of Rations for Pigs in Experiment II.

1. Basal (O.S.C. Ration No. 3)

Component	Quantity
Ground barley	160.5
Peanut meal	120.0
Ground alfalfa hay	15.0
Ground limestone	3.0
Iodized salt	1.5
Vitamin Supplement*	Gms. per 300 Pounds Ration
Riboflavin	0.240
Calcium pantothenate	1.350
Niacin	1.500
Choline chloride	136.200
Vitamin B_{12}	0.0045

2. Basal + 0.3 per cent DL-methionine (O.S.C. Ration No. 4).

3. Basal + 5.0 mg. procaine penicillin per pound (O.S.C. Ration No. 5).

4. Basal + 0.3 per cent DL-methionine + 5.0 mg. procaine penicillin per pound (O.S.C. Ration No. 6).

*Recommendations of the National Research Council were followed in determining quantity of vitamins added.

FIGURE II

COMPARISON OF WEIGHT GAINS OF PIGS IN EXPERIMENT I

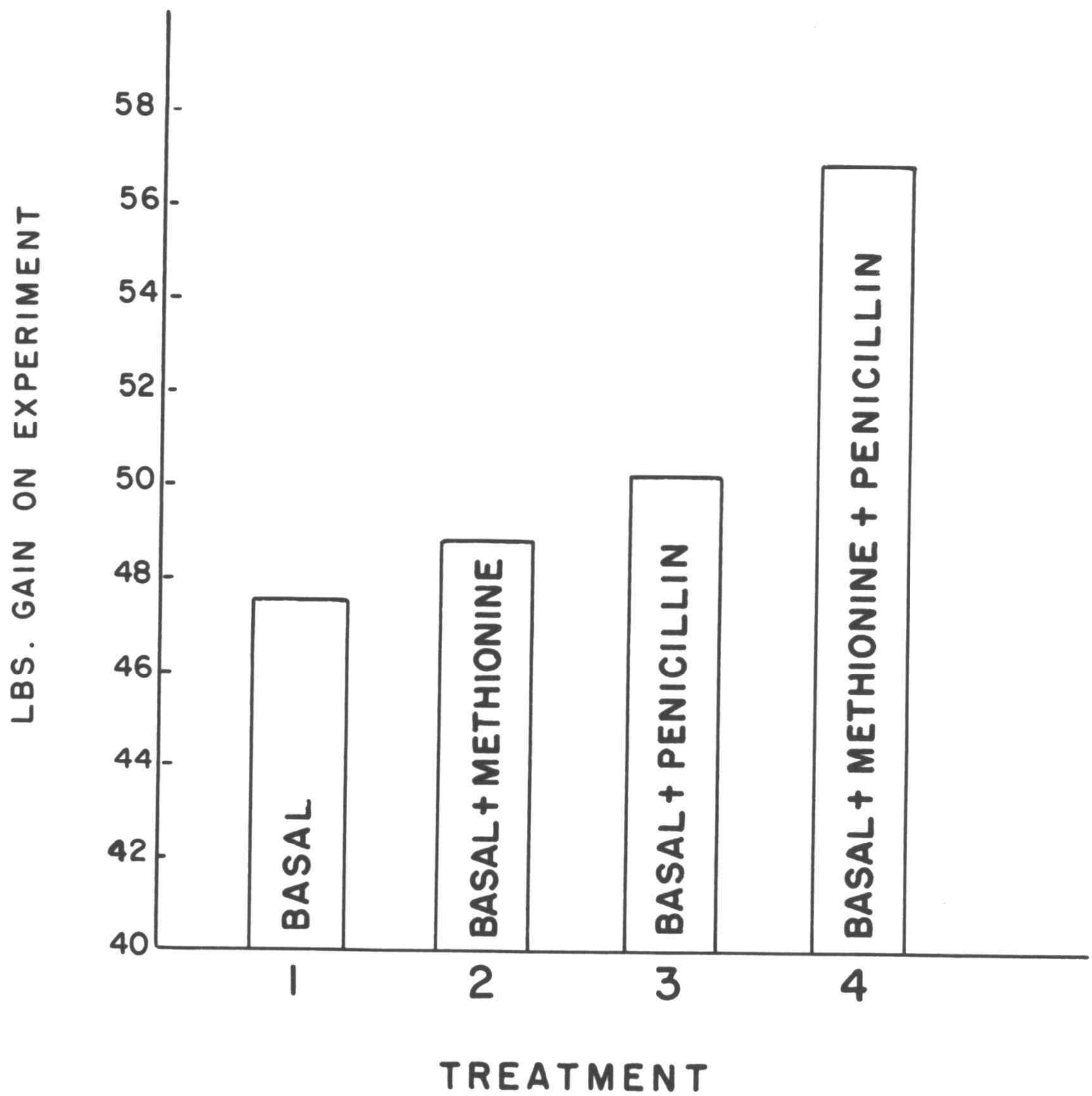

TABLE 9

Effect of Penicillin and Methionine Alone and in Combination on Growth and Feed Efficiency of Young Pigs.

Treatment	Average Initial Weight	Average Final Weight	Average Weight Gain 6 Wks.	Feed Req. per Pound Gain
	Lbs.	Lbs.	Lbs.	Lbs.
Basal	65.9	113.5	47.6	3.3
Basal + 0.3% DL-Methionine	66.3	115.2	48.9	3.3
Basal + 5 Mg. Penicillin per Lb. Feed	64.8	115.1	50.3	3.2
Basal + 0.3% DL-Methionine + 5 Mg. Penicillin per Lb. Feed	68.1	125.1	57.0	3.2

<u>Results</u>: The effects of supplementing an all-plant ration with penicillin and methionine alone and in combination on growth and feed efficiency of young pigs are shown in Table 9. It may be seen that under the conditions of this experiment, growth of young pigs was not stimulated markedly by 5.0 mg. procaine penicillin per pound ration unless the ration was also supplemented with 0.3 per cent DL-methionine. Penicillin and methionine when added separately to the ration stimulated growth only to a very slight extent. The average growth stimulation due to the penicillin was 2.6 pounds over a six-week period while that due to the added methionine was 1.3 pounds over the same length of time. However, when DL-methionine and penicillin were both added to the basal ration a pronounced growth response was produced. Animals fed the ration containing both methionine and penicillin made an average weight gain of 57.0 pounds while those fed the unsupplemented basal ration made an average weight-gain of 47.6 pounds. These gains are illustrated graphically in Figure 2. The pigs receiving 0.3 per cent DL-methionine supplement required the same amount of feed per pound of gain as did the pigs fed the basal ration. However, the pigs receiving either penicillin or penicillin and methionine supplements required slightly less feed (0.1 pound) per pound of gain than did those fed the basal ration or

the basal ration supplemented with only methionine. There was practically no difference in the amount of feed required per pound of gain between the group fed the basal ration plus penicillin and the group receiving the basal ration fortified with both methionine and penicillin. It will be noticed that there was a slight difference in the average starting weight of pigs in the four groups; however, this is practically unavoidable when choosing experimental animals from a small herd.

Discussion: On the basis of the results reported above, it was apparent that the addition of both methionine and penicillin to an all-plant ration containing peanut meal as the major source of protein did stimulate growth of young pigs to a marked extent. When both methionine and penicillin were added to the basal mixture, a synergistic effect on growth occurred. This finding is in agreement with that reported by Biely, March and Smith (11) who found that penicillin had a greater stimulatory action on the growth of chicks with rations which were well balanced in amino acids than with rations deficient in some amino acid. The fact that penicillin alone did not stimulate growth to any larger extent is in direct contrast to results obtained by other investigators. Penicillin has been reported by Luecke et al (93), Heidebrecht et al (65), Briggs, Elrod and Beeson (25) and

Brinegar and Warner (26) to stimulate significantly growth of pigs fed a corn-soybean meal ration. Under the conditions of this experiment, penicillin did not appear to exert a "sparing" action on the methionine requirement of the pigs.

One of the most striking findings was that the addition of methionine to the methionine-deficient ration did not markedly stimulate growth. Since the ration contained a high level of peanut meal protein (20.52 per cent), the pigs may have received enough methionine for growth under these conditions, and thus it may not have been the limiting growth factor. However, Cunha et al (47) reported that added methionine did stimulate growth of young pigs fed a similar type ration in which peanut meal composed 41.5 per cent of the total ration. The different responses to antibiotics obtained by other investigators may have been due to varying "disease levels" since Heidebrecht et al (65) found that the response of pigs to antibiotics depended upon the health of the animal as well as to the adequacy of the ration with respect to various nutrients.

Methionine and penicillin supplementation either alone or in combination had very little effect on feed efficiency of pigs in this experiment. Almost without exception, the pigs which consumed the most feed made the largest gains. The pigs in the group receiving both penicillin and methionine consumed a total of 1452 pounds of feed and made 456 pounds total gain in weight while those fed the basal ration consumed 1272 pounds of feed and gained 361 pounds body weight. It was observed that the pigs fed the ration containing both penicillin and methionine never "went off feed" while the pigs fed the other three rations did refuse to consume all their feed at times. The fact that the antibiotic, penicillin, did not exert a greater effect on feed efficiency is not in agreement with the majority of other investigators in this field. Heidebrecht et al (65) found that penicillin lowered the feed required per pound of gain in young pigs fed a corn-soybean meal ration from 3.58 to 3.06 pounds. Brinegar and Warner (26) reported that penicillin increased feed efficiency in young pigs fed a similar ration by 11 per cent. However, it may be noted from Table 9 that the pigs in this experiment made better than average daily weight-gains and required less feed per pound gain than is considered average for pigs of this weight range as calculated by Morrison (109, p.937). Morrison lists the average daily weight gains and feed required per pound gain of pigs from 60 to 125 pounds as approximately 1.1 and 3.65 pounds, respectively. Such being the case, it might be assumed that the so-called "disease-level" in the animals used on this experiment was lower than that encountered by other investigators.

Summary: The feeding of 5.0 mg. penicillin per pound ration to young pigs fed an all-plant ration with peanut meal as the main source of protein did not stimulate growth to any significant extent. However, when 0.3 per cent methionine and 5.0 mg. penicillin per pound ration were both added to the ration, growth was stimulated to a marked extent. Thus, penicillin apparently exerted a greater effect on growth when the ration was more nearly balanced with respect to essential amino acids.

Experiment III

The Effect of High Levels of Penicillin in the Diet on Growth and Feed Efficiency of Young Fattening Hogs.

The greatest growth response to feeding antibiotics to pigs has been obtained with young pigs before they reach 125 pounds body weight. Luther and Brown (96) obtained a 20 to 45 per cent increase in growth of young pigs from weaning to 125 pounds and eight to 15 per cent increase in growth from 125 pounds to market weight. Hanson *et al* (59) found that the withdrawal of antibiotics from the ration of pigs when they reached 125 pounds body weight had an adverse effect on feed consumption and rate of growth. However, Brinegar and Warner (26) reported that all increases in weight gains of pigs receiving terramycin occurred before the pigs reached 125 pounds, and no additional benefits were obtained by antibiotics beyond this weight.

The purpose of this experiment was to determine whether penicillin added at a high level to a ration low in methionine would stimulate growth in young fattening hogs over 125 pounds in weight.

Materials and Methods: Twenty-four Berkshire shoats averaging approximately 135 pounds body weight were randomly divided into four lots of six pigs each. The treatment of the animals was essentially the same as that in the previous pig experiment, with the exception of the composition of the rations which were modified as shown in Table 10. A lower protein level was used in this experiment since the later stage of growth of the animals was such as to lower the protein requirement. Moreover, it was thought that the level of protein in these rations might be an important conditioning factor in the response to antibiotics under different conditions of amino acid balance.

TABLE 10

Composition of Rations for Young Fattening Hogs in Experiment III.

1. Basal (O.S.C. Ration No. 8)

Component	Pounds per 100 Pounds Ration
Ground barley	73.5
Peanut meal	20.0
Ground alfalfa hay	5.0
Limestone	1.0
Iodized salt	0.5

Vitamin Supplement*	Gms. per 100 Pounds Ration
Riboflavin	0.080
Calcium pantothenate	0.450
Niacin	0.500
Choline chloride	181.600
Vitamin B_{12}	0.0015

2. Basal + 50 mg. procaine penicillin G per pound of ration (O.S.C. Ration No. 9).

3. Basal + 0.3% DL-methionine (O.S.C. Ration No. 10).

4. Basal + 0.3% DL-methionine + 50 mg. procaine penicillin G per pound of ration (O.S.C. Ration No. 11).

*Recommendations of the National Research Council were followed in determining the quantity of vitamins added.

FIGURE III

COMPARISON OF WEIGHT GAINS OF PIGS IN EXPERIMENT II

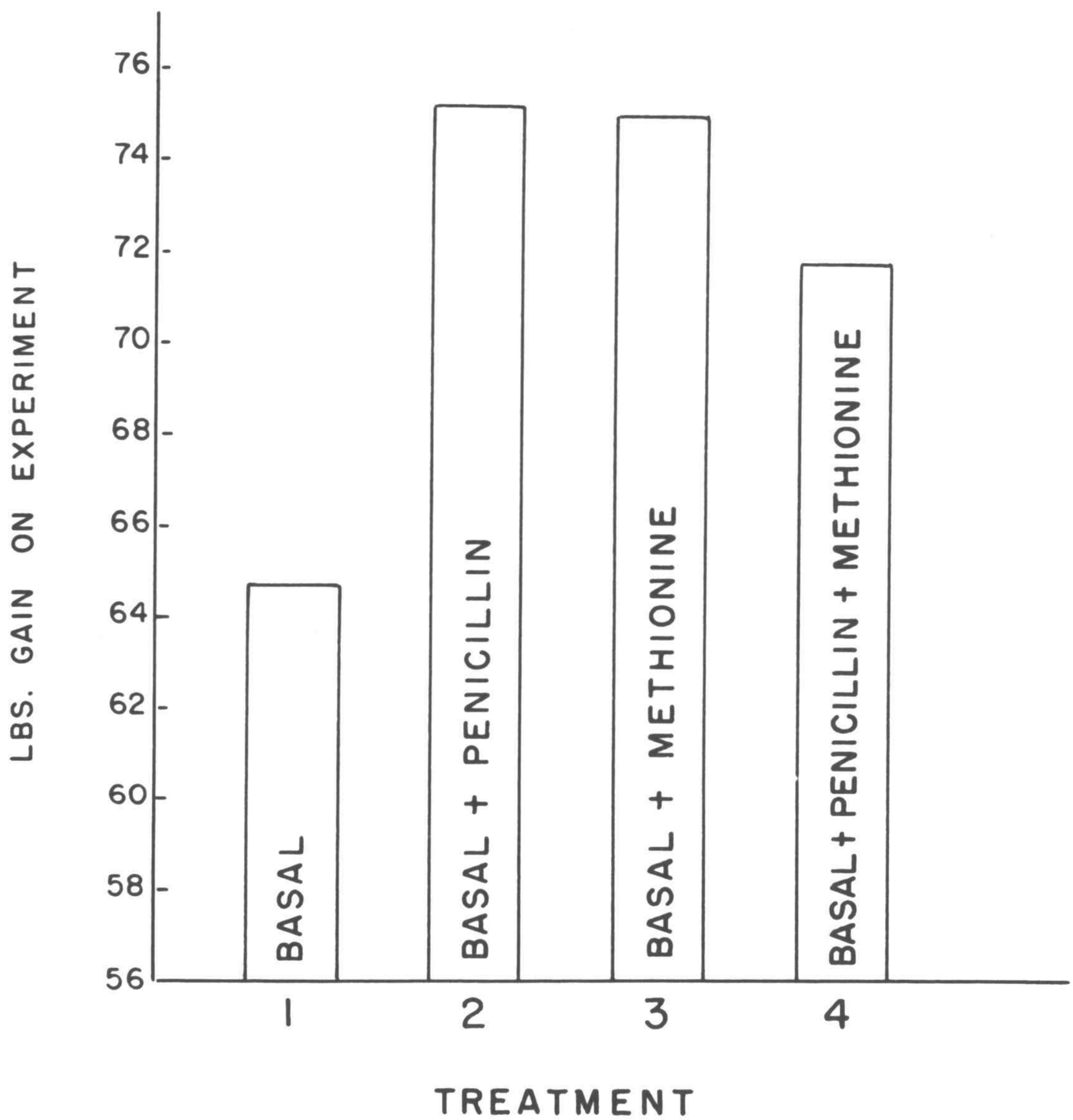

TABLE 11

The Effect of High Levels of Penicillin
on Growth and Feed Efficiency of Young Fattening Hogs.

Treatment	Average Initial Weight	Average Final Weight	Average Weight Gain 6 Wks.	Feed Req. per Pound Gain
	Lbs.	Lbs.	Lbs.	Lbs.
Basal	135.7	200.4	64.7	4.7
Basal + 50 Mg. Penicillin per Lb. Feed	137.5	212.7	75.2	4.1
Basal + 0.3% DL-Methionine	133.3	208.3	75.0	4.1
Basal + 0.3% DL-Methionine + 50 Mg. Penicillin per Lb. Feed	137.3	209.1	71.8	4.4

Results: The effect of high levels of dietary penicillin on growth and feed efficiency of young fattening hogs fed rations low in and adequately fortified with methionine is shown in Table 11. When the basal ration was supplemented with 50.0 mg. of procaine penicillin per pound, growth was stimulated to a marked extent. The pigs fed the basal mixture gained on the average 64.7 pounds during the experiment while those receiving penicillin in the ration gained an average of 75.2 pounds, or an average increase of 10.5 pounds each. Feed required per pound of gain was also much less for the pigs receiving penicillin in the ration. The pigs fed the basal ration required 4.7 pounds of feed per pound gain as compared to 4.1 pounds feed required by those fed the basal ration containing penicillin.

Pigs fed the basal ration supplemented with 0.3 per cent DL-methionine also made larger body weight gains and required less feed per pound gain than did those fed the basal mixture. Pigs receiving methionine supplementation gained on the average 10.3 pounds more and required 0.6 pounds less feed per pound gain than did pigs fed the basal ration without added methionine. Total gains of the animals on the various treatments are illustrated in Figure 3.

There was essentially no difference in body weight gains and feed required per pound of gain between the two groups receiving either penicillin or methionine supplements. Both groups made approximately the same body weight gains and required the same amount of feed per pound of gain.

When both penicillin and methionine were added to the basal ration, growth was not stimulated to as great an extent as when either was added alone. Also, there was an increase of 0.3 pound feed required per pound of gain in the group receiving both penicillin and methionine as compared to either the group receiving only penicillin or methionine. However, the pigs receiving both methionine and penicillin still made better gains and required less feed per pound gain than did those fed the basal ration without either methionine or penicillin supplements.

Discussion: Unlike the previous experiment with young pigs, penicillin under the conditions of this experiment employing older pigs and a lower level of dietary protein, stimulated growth to a marked extent and exerted a favorable effect on feed efficiency. This finding is more in agreement with those of other investigators who have found that penicillin stimulated growth and lowered the feed required per pound of gain when added to an all-plant ration for young pigs (25, 26, 65, 94). Penicillin appeared to exert a methionine "sparing" action in the older pigs in this experiment, since the addition of either penicillin or methionine to the ration resulted in approximately equal growth stimulation. This observation is in agreement with that of Cunha et al (47) who reported that a crude A.P.F. supplement containing an antibiotic, aureomycin, spared the methionine requirement of young pigs fed a corn-peanut meal ration. The fact that penicillin appeared to exert a methionine "sparing" action in this experiment, as opposed to no apparent effect on the methionine requirement of the younger pigs in Experiment II, may be explained on the basis of the different peanut meal content of the two rations. Sauberlich (144) found that penicillin did exert a "sparing" action on the methionine requirement of weanling rats fed a low protein diet. The ration fed to pigs in Experiment II contained 40 per cent peanut meal as compared to 20 per cent peanut meal in this experiment. Analysis of the peanut meal indicated a methionine content of 1.13 per cent of the crude protein, which is in close agreement with analyses of Murphy and Dunn (112) who reported that the protein of peanut meal contained 1.1 per cent methionine, as determined microbiologically. This means that the methionine content of the ration fed the pigs in Experiment II, supplied by the peanut meal, was 0.232 per cent, while the amount of methionine supplied by the peanut meal in Experiment III was 0.116 per cent of the total ration. It may have been that the high level of peanut meal employed in Experiment II furnished enough methionine for growth of the young pigs, whereas the lower level of peanut meal fed to the older pigs in this experiment did not contain enough methionine to meet their needs for growth. However, in both experiments the amount of methionine supplied by the peanut meal is far less than the estimated optimal level of 0.6 per cent of the ration, reported by Shelton, Beeson and Mertz (153).

In contrast to results obtained with the younger pigs in Experiment II, the addition of both penicillin and methionine to the basal ration did not stimulate growth to even the same extent as did either penicillin or methionine alone. However, the addition of both methionine and penicillin resulted in an increase in average body weight gains of 7.1 pounds during the experiment and lowered the amount of feed required per pound gain by 0.3 pound over those fed the basal mixture.

The fact that penicillin lowered the feed required per pound gain by 0.3 pound is in agreement with results obtained by Heidebrecht _et al_ (65) who showed that the additions of penicillin to a corn-soybean meal ration lowered the feed required per pound gain of weanling pigs by 0.52 pound. The addition of 0.3 per cent DL-methionine to the ration also had the same effect on feed efficiency as did 50.0 mg. procaine penicillin.

Summary: The addition of 50.0 mg. of procaine penicillin to a ration composed of 20 per cent peanut meal as the main source of protein appeared to exert a "sparing" action on the methionine requirement of young growing-fattening hogs.

The addition of either 50.0 mg. per pound of penicillin or 0.3 per cent methionine to the ration resulted in equally greater feed efficiency of the pigs in this experiment.

Experiment IV

A Comparison of Methods of Administration of Penicillin to Suckling Pigs.

Experiences of numerous investigators using antibiotics in rations for weanling pigs, and more recently the market release of a synthetic sow's milk containing an antibiotic, have focused attention on the importance of providing such materials to pigs at an early age for optimum results. Becker et al (8) reported that the injection of procaine penicillin G suspended in sesame oil containing two per cent aluminum monostearate on alternate days gave a statistically significant increment in rate of gain of pigs from weaning to 100 pounds. Many investigators have reported large increases in body weight-gain of baby pigs raised on synthetic milks fortified with an antibiotic, as compared to the controls receiving no antibiotic supplement. Williams et al (180) obtained an average increase of 0.31 pounds per day in baby pigs raised on synthetic milk containing aureomycin over the unsupplemented controls. Similarly, Schendel and Johnson (149) reported a growth stimulatory effect in baby pigs due to aureomycin and terramycin added to the synthetic milk. Therefore, there exists the practical problem of getting antibiotics into young pigs while still nursing the sow, and before they begin taking dry feed, in order to take fullest advantage of the growth stimulation due to antibiotics.

The purpose of this experiment was to investigate the activity of an antibiotic (procaine penicillin G, Merck) when administered to very young suckling pigs by several different methods.

<u>Materials and Methods</u>: Nine litters of purebred Berkshire pigs, seven to 10 days old, were used, and pigs from each litter were randomly assigned to four groups. Pigs in group one served as controls and received no supplemental treatment. The pigs in group two received 280 mg. of procaine penicillin G in sesame oil containing two per cent aluminum monostearate (weight by volume) intramuscularly seven days after birth. Pigs in group three received the same dosage of penicillin orally by capsule, while those in group four were given the same total amount of penicillin divided among four oral doses at weekly intervals. The amount of procaine penicillin G administered were calculated on the basis of 5.0 mg. per pig daily from birth to weaning, which was thought to be a reasonable level of feeding. In addition to regular weekly weighings, the pigs in this experiment were also weighed at weaning at eight weeks of age. A 22-gauge stainless surgical needle and a 1.0 cc hypodermic syringe were used to inject the pigs in group two. The procaine

penicillin G in sesame oil was injected intramuscularly into the ham.

TABLE 12

The Effect of Methods of Administration of Penicillin on Growth of Young Suckling Pigs.

Treatment	Average Initial Weight	Average Weight After 4 Wks.	Average Weight at 8 Wks.	Average Weight Gain at 8 Wks.
	Lbs.	Lbs.	Lbs.	Lbs.
Control	8.37	19.82	28.56	20.19
One Injection of 280 Mg. Penicillin in Sesame Oil	8.11	20.71	31.39	23.29
One Dose of 280 Mg. Penicillin by Capsule	7.56	19.31	29.12	21.55
Four Weekly Doses of 70 Mg. Penicillin by Capsule	8.02	19.44	28.17	20.14

<u>Results</u>: The effect of methods of administration of penicillin on growth of young suckling pigs is listed in Table 12. The average body weight-gain per pig in each of the three groups receiving penicillin was slightly superior to that of the control animals. One intramuscular injection of 280 mg. procaine penicillin G in sesame oil stimulated growth to a slightly greater extent than did either one large dose of 280 mg. procaine penicillin or four smaller doses of 70 mg. each administered orally by capsule. Although the pigs in the group receiving penicillin by injection were slightly smaller than the controls at the time the experiment was begun, their average weight gain at eight weeks of age was 3.1 pounds more than was the average weight gain of the controls which received no penicillin. The pigs in the group receiving one large dose of 280 mg. procaine penicillin orally by capsule, although initially weighing considerably less than the controls, also made larger body weight-gains at eight weeks than did the control animals. However, at no time during the experiment were the pigs in the group fed the four weekly doses of 70 mg. procaine penicillin G as heavy as were the controls.

<u>Discussion</u>: The observation that the intramuscular procaine penicillin G in sesame oil containing two per

cent aluminum monostearate stimulated growth of young pigs is in agreement with that of Becker et al (8) who found that the injection of 200,000 units of the same type penicillin on alternate days gave a statistically significant increase in rate of gain. It may be noticed that the increase in rate of gain was obtained with the higher concentrations of penicillin, that is, in both the groups receiving the one large dose of 280 mg. penicillin either by intramuscular injection or by capsule. Further, both types of administration of relatively large doses of penicillin appeared to produce a lasting stimulation throughout the experimental period. This would appear to indicate that perhaps the administration of a single large dose of penicillin might produce as lasting a growth effect as continued administration of lesser quantities of the material, since the four smaller doses of 70 mg. each given at weekly intervals did not stimulate the rate of growth. It is not possible, of course, from the results of this experiment, to preduct whether the action of the antibiotic took place in the blood stream or the digestive tract, since both sites would be available to each method of administration. Larsen and Carpenter (87) found that an antibiotic, aureomycin, when given either orally or injected was excreted in the feces and suggested that injected aureomycin enters the intestinal tract of the pig through the bile. The author, as will be shown elsewhere, has detected measurable quantities of penicillin in the blood of hogs fed large amounts of the antibiotic in the feed. Since the smaller animals in the treated groups made considerably superior gains when compared to the smaller animals in the control group, it might be supposed that the method of growth promotion was through antibacterial action rather than any effect on the normal metabolism of nutrients.

Summary: Under the conditions of this experiment, the administration of a single large dose of procaine penicillin G (280 mg.) either orally by capsule or by deep intramuscular injection (in a sesame oil vehicle with two per cent aluminum monostearate) resulted in slight increases in growth rate of suckling pigs over the untreated animals.

The administration of four smaller doses of procaine penicillin G (70 mg.) at weekly intervals did not result in an increase in growth rate over the untreated animals.

Experiment V

Blood Plasma Levels of Penicillin in Hogs Given Oral Procaine Penicillin.

The absorption and excretion of penicillin in man was first studied by Abraham et al (1) who found that from 50 to 68 per cent of the dose injected intravenously could be detected in the urine. Rammelkamp and Keefer (127) studied the fate of penicillin when given by various routes to the human subject. They found that oral doses of penicillin were poorly absorbed and little penicillin was excreted in the urine after oral administration. By no means all of the penicillin given orally or its metabolites are recovered from the urine. Studies in which penicillin has been "tagged" with radioactive sulphur demonstrate that the limited excretion of penicillin or its metabolites is due to incomplete absorption from the gastro-intestinal tract. This means that a considerable amount of penicillin may be excreted in the feces. This observation, coupled with those which show that penicillin is destroyed by gastric juice of the stomach and by penicillinase produced by many strains of *Escherichia coli* in the large intestine (3, pp.111-112), made it clear that the administration of penicillin by the oral route is an uncertain therapeutic procedure.

The purpose of this experiment was to determine the amount of penicillin absorbed into the blood stream of hogs fed varying levels of the antibiotic in the feed.

Materials and Methods: Eight young Berkshire hogs weighing from 185 to 195 pounds each were used in this experiment. Three hogs were allotted to each of the two groups fed rations supplemented with 50 and 250 mg. procaine penicillin per pound, while two hogs were allotted to the group fed the ration containing 500 mg. procaine penicillin per pound. The Oregon State College (O.S.C.) stock ration, whose composition is listed in Table 13, was used as the basal ration. The hogs were placed on the feed containing penicillin at 8:00 A.M. and blood samples were taken at exactly 24 and 48 hours later. A final sample of blood was drawn at the time of slaughter about 12 hours after the hogs had been removed from feed.

Blood samples were obtained from the vessels of the ear. The animals were restrained by passing a noose around the upper jaw and then tied to a post. A tourniquet of one-half inch o.d. soft rubber tubing was applied around the base of the ear, which caused the vessels to dilate markedly. Bleeding was then accomplished using a 14-gauge hyperchrome stainless steel bleeding needle. Approximately 10 ml. of blood was drawn into test tubes containing three drops of saturated sodium citrate solution. After collection of the desired quantity of blood, the tourniquet was quickly removed and bleeding soon ceased.

Plasma penicillin levels were determined by the cup plate diffusion method obtained from Dr. A. C. Cuckler (45) of Merck and Company. This method was used in preference to several others investigated because it was a consistently reproducible, reliable and efficient method for assay of plasma concentrations. The media consisted of a base layer of 10 ml. of sugarless agar, prepared by dissolving 25.5 gms. Bacto Penassay Base Agar (Difco) in 1000 ml. of distilled water, and a seed layer of four ml., prepared by dissolving 30.5 gms. of Bacto Penassay Seed Agar (Difco) in 1000 ml. distilled water. The media was adjusted to a final pH of 6.6. The organism used was Sarcina lutea obtained from Dr. A. C. Cuckler of Merck and Company. The organism was transferred daily onto slants prepared from seed layer agar and was incubated at 30°C. In the preparation of the saline seeding suspension of Sarcina lutea, the material on one agar slant was washed off with 0.9 per cent sterile saline into a flask, and the total volume was brought up to 50 ml. The suspension was then adjusted to a light transmittancy of 80 per cent using 610 mu in a Coleman Universal Model 14

Spectrophotometer. The plates were prepared for the assay by adding 10 ml. of sterile base layer agar to pyrex petri dishes (90 mm. diameter of bottom). This layer was allowed to harden, and while the dishes were still warm, four ml. of seed agar to which had previously been added two ml. of <u>Sarcina lutea</u> suspension per 100 ml. of seed agar were added. The melted seed agar was cooled to 48-50°C. before adding the inoculum. The plates were stored at 4°C. as soon as they had hardened; and to insure good setting of the agar, they were not used until they had cooled for at least one-half an hour. In the preparation of the assay, "penicillinders" (stainless steel cylinders eight mm. in diameter) were placed on the plates (3 per plate). The cups were warmed slightly by passing through a flame to make a seal with the agar. The plasma to be tested was placed in the cups, 0.3 ml. per cup, and three replicates of the unknown were run. The plates were then incubated at 30°C. for a minimum of 15 hours. The diameters of the zones of inhibition were measured to the closest 0.1 mm. with a vernier caliper. The three replicates were averaged and the potency of the unknown was determined by comparing the zone sizes obtained with those of the penicillin standard. The plasma standard was prepared by weighing out 30 mg. of crystalline penicillin G sodium and placing in a sterile 50 ml. volumetric flask and pH 6.0

phosphate buffer. Using a serum pipette, 0.2 ml. of this stock was diluted with 9.8 ml. of normal plasma to give a penicillin concentration of 20 units per ml; 0.50 ml. of this solution was diluted with 4.5 ml. of normal plasma to give 2.0 units of penicillin per ml. Serial dilutions (2 ml. plus 2 ml.), using normal plasma, were then made to produce concentrations of 1.0, 0.5, 0.25, 0.125, 0.062 and 0.031 units per ml. The standard solutions were placed in penicylinders, 0.3 ml. per cylinder, using at least three replicates for each standard solution. The standard curve was prepared by plotting the zone diameters in millimeters (abscissae) against the plasma concentrations (ordinates) on three-cycle semi-logarithmic graph paper. A line was fitted to these points by inspection. The standard curve used is shown in Figure IV, and a photograph of the assay is pictured in Figure V.

TABLE 13

Composition of Oregon State College (O.S.C.) Ration for Growing Shoats in Experiment V.

Component	Quantity Pounds per 100 Pounds Ration
Ground barley	50.0
Ground oats	20.0
Meat meal 55%	10.0
Soybean meal	7.0
Linseed meal	5.0
Ground alfalfa hay	5.0
Steamed bone meal	1.0
Ground limestone	1.5
Iodized salt	0.5

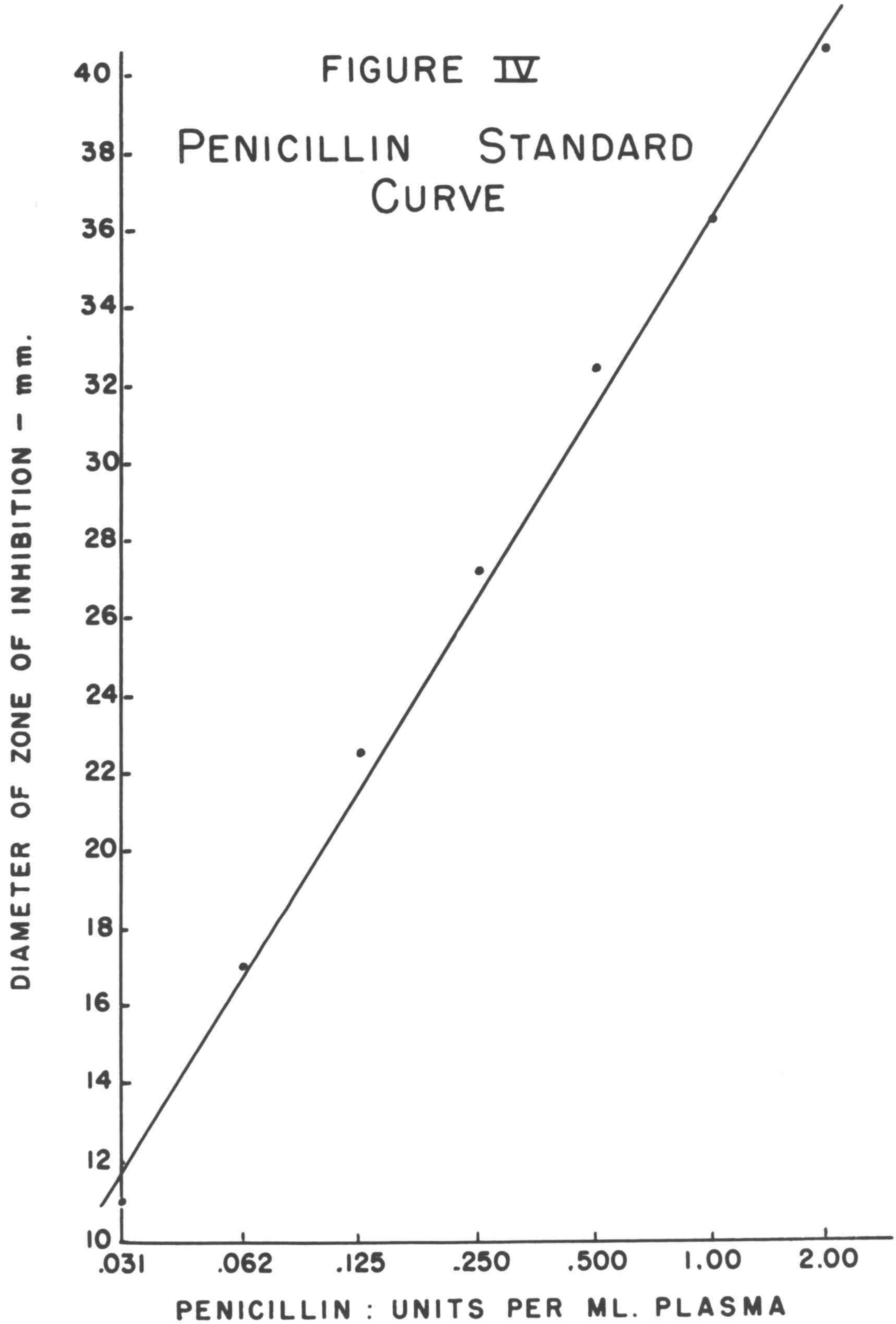
FIGURE IV
PENICILLIN STANDARD CURVE
DIAMETER OF ZONE OF INHIBITION - mm.
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
.031
.062
.125
.250
.500
1.00
2.00
PENICILLIN : UNITS PER ML. PLASMA

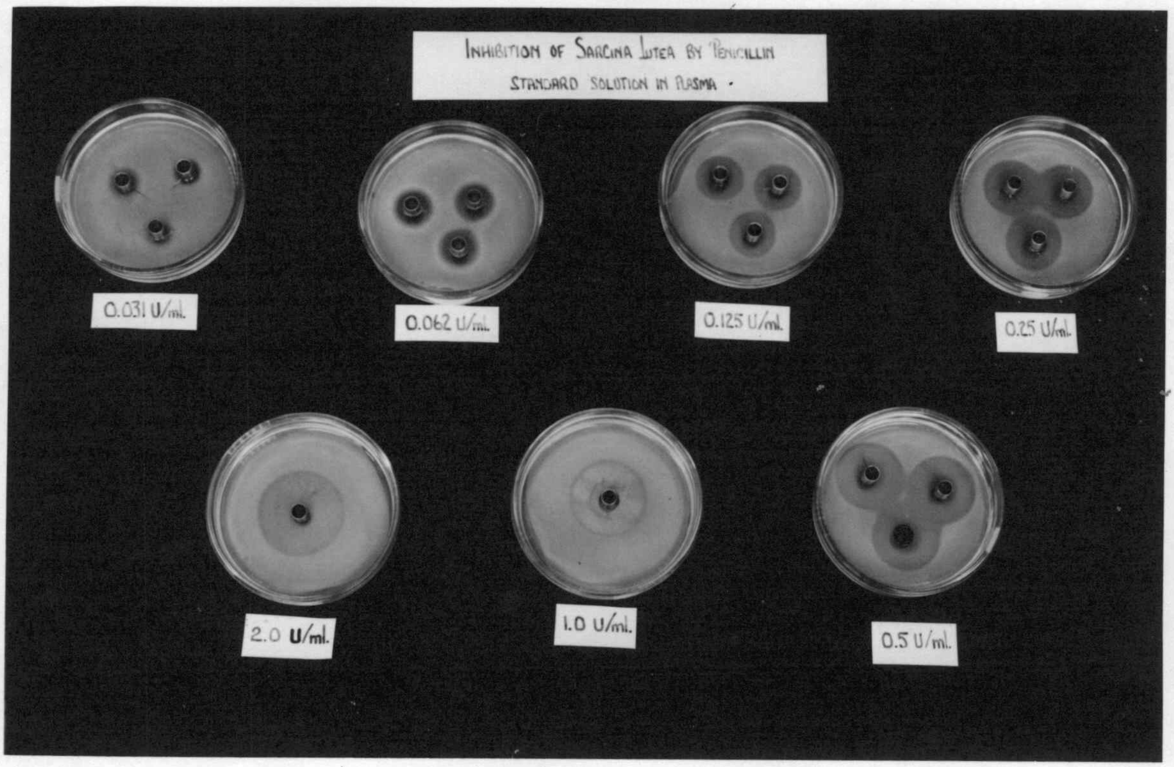

FIGURE V

Photograph of Plates Used in Preparation of a Standard Curve for Penicillin in Plasma, Showing the Diameters of Inhibition Zones at Varying Levels of the Antibiotic.

TABLE 14

The Penicillin Blood Plasma Concentrations of Hogs Fed High Levels of Procaine Penicillin.

Treatment	Average Plasma Penicillin Levels 24 Hours	48 Hours	After 12 Hours Fast
	Units[1] Penicillin per Ml. Plasma		
Basal + 50.0 Mg. Procaine Penicillin per Lb. Ration	Not detectable	Not detectable	Not detectable
Basal + 250 Mg. Procaine Penicillin per Lb. Ration	0.115	0.060	"
Basal + 500 Mg. Procaine Penicillin per Lb. Ration	0.088	0.600	"

[1]One unit is equivalent to 0.6 micrograms of benzylpenicillin sodium.

Results: The analytical results of this experiment are presented in Table 14. The most striking observation in this table is that no traces of penicillin could be detected in the blood of hogs fed the ration containing 50 mg. procaine penicillin per pound. Likewise, no detectable traces of penicillin remained in the blood stream of the hogs in any of the groups 12 hours after they had been removed from feed. The blood of the hogs fed the rations containing 250 mg. procaine penicillin per pound for 24 hours contained more penicillin than did that of the hogs fed a ration containing twice as much procaine penicillin over a 24-hour period. However, at the end of 48 hours the hogs fed the ration supplemented with 500 mg. procaine penicillin per pound had considerably more penicillin per unit volume of blood than did the hogs fed the ration containing 250 mg. procaine penicillin. The greatest concentration of penicillin in the blood was found in the hogs that had been fed the ration containing 500 mg. of procaine penicillin per pound for 48 hours.

Discussion: Only a very small fraction of the penicillin consumed by the hogs was detected in the blood stream. Since levels as low as 0.031 units per ml. of plasma could be detected employing the cup-plate diffusion method of analysis (see Figure IV), the blood of the hogs fed the ration containing 50 mg. procaine penicillin per pound must have contained less than this amount per ml. It should be noticed that the amounts of penicillin fed in this experiment were from 25 to 250 times greater than the recommended amounts for growth stimulation made by the manufacturer, and noted previously in this thesis (Tables 8, 10). Speer _et al_ (158) reported that 2.0 mg. of procaine penicillin per pound of ration stimulated growth of young pigs to the same extent as did 20.0 mg. per pound of ration. Briggs, Elrod and Beeson (25) also found that 2.5 mg. of procaine penicillin per pound of total ration was as effective in stimulating growth of pigs as were higher levels. Since the hogs in this experiment each consumed approximately seven pounds of feed per day, those fed the ration supplemented with 50.0 mg. procaine penicillin per pound were receiving about 350 mg. penicillin per day, while those fed the ration supplemented with 500 mg. procaine penicillin were receiving about 3500 mg. per day. The fact that larger amounts of penicillin were not detected in the blood stream of the hogs on this experiment would seem to indicate that very little of the penicillin was absorbed into the blood stream. Rammelkamp and Keefer (127) found that penicillin when given orally to humans was poorly absorbed and very little was excreted in the urine. Due to the fact that very little penicillin was found in the blood of hogs fed even many times the recommended amounts for maximum growth stimulation, it would appear to indicate that the growth-promoting action of penicillin is probably due to some action involving the micro-organisms in the digestive tract.

One hog in the group receiving the 250 mg. procaine penicillin supplement had an exceedingly high concentration of penicillin in the blood at the 24-hour sampling period. This may explain the fact that the average penicillin content of the blood of hogs fed a ration containing 250 mg. procaine penicillin per pound was somewhat greater at the 24-hour sampling period than was that of hogs receiving 500 mg. procaine penicillin per pound of ration. After the hogs had been consuming the feed containing penicillin for 48 hours, those fed the highest level of penicillin had the greatest penicillin concentration in the blood.

The fact that no penicillin could be detected in the blood of the hogs 12 hours after they had been removed from feed is probably due to the rapid excretion of penicillin by the kidneys.

Summary: From the results of the experiment reported herein, it may be concluded that very minute fractions of penicillin, when administered orally to hogs, are absorbed

into the blood stream. Thus, it would appear that the site of action of procaine penicillin in stimulating growth of hogs is in the intestinal tract itself.

CHAPTER IV

SUMMARY OF EXPERIMENTAL INVESTIGATIONS

The addition of either 0.3 per cent DL-methionine or 5.0 mg. procaine penicillin per kg. of diet stimulated growth and increased feed efficiency of young rats fed a low protein diet deficient in vitamin B_{12} and methionine, but had little or no beneficial effect on slightly older rats fed diets of similar composition. The most pronounced effect on growth and feed efficiency was obtained in both age groups of rats when penicillin was added to diets more completely fortified with essential nutrients.

Young pigs fed a barley-peanut meal ration containing 40 per cent peanut meal failed to respond favorably to the addition of either 0.3 per cent DL-methionine or 5.0 mg. procaine penicillin per pound ration, while older pigs fed a barley-peanut meal ration containing 20 per cent peanut meal did respond favorably to the addition of either 0.3 per cent DL-methionine or 50.0 mg. procaine penicillin per pound ration. The young pigs, like the rats, made larger body weight-gains and required less feed per unit gain when the ration was more completely fortified with essential nutrients.

The administration of a single large dose of procaine penicillin (280 mg.) either orally by capsule or by deep intramuscular injection (in oil) resulted in slight increases in growth of suckling pigs over both the untreated animals and those given four smaller doses of procaine penicillin (70 mg.) at weekly intervals.

Employing the cup-plate method of assay for penicillin in plasma, no penicillin was found in the blood stream of hogs fed rations containing 50.0 mg. procaine penicillin per pound ration, while only very small amounts of penicillin were detected in the blood stream of hogs fed rations containing as much as 500.0 mg. procaine penicillin per pound ration.

From the results obtained in these experiments, it would appear that the antibiotic, penicillin, was more effective in promoting growth of animals when added to diets containing adequate amounts of methionine and vitamin B_{12}. The disparity between this conclusion and those of other investigators may be explained on the basis of differences in the "disease level," as reflected in variations in the intestinal microbial populations of the experimental animals.

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