THE EFFECT OF VITAMIN B_{12} AND/OR AUREOMYCIN UPON THE GROWTH, GESTATION, AND LACTATION OF RATS FED ALL-PLANT RATIONS

By

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INTRODUCTION

Every year tremendous mortality at early age is encountered among farm animals. In pigs, 20 per cent die before they reach the age of eight weeks, and 3.5 per cent die after weaning time. Mortality in calves is lower, 9.8 per cent before one month of age and 2.2 per cent thereafter. Chicks have the greatest death rate, about one in every four chicks dying at an early age.

The isolation of vitamin B_{12} from liver led to much research work to ascertain whether this nutrient is essential for reproduction, lactation and growth of simple stomach animals. Attention has also been focused on the role of antibiotics in improving growth of various species. It has been shown that the antibiotics have anti-infectious action and also destroy bacteria and other micro-organisms that cause disease. It has been postulated that antibiotics improve the utilization of various growth factors, including vitamin B_{12}, in the following ways: (1) inhibit the growth of micro-organisms that destroy or inactivate growth factors, (2) encourage growth of micro-organisms that synthesize growth factors, and (3) eliminate bacteria which produce toxins that decrease the growth of the desirable types of bacteria or affect the host directly.

From previous observations, it is evident that maternal nutrition affects the incidence of mortality in the young. Requirements for different nutrients increase during gestation and lactation. The average energy needed daily for reproduction in a sow producing a litter of eight pigs is 115 Calories. On the basis of this figure, the average daily need for reproduction adds only 6 per cent to the maintenance requirement and less than 15 per cent during the last week of gestation when the demand is greatest. Approximately the same relations hold for the requirement in terms of digestible nutrients. The requirement for protein is also increased by 32 per cent over the maintenance requirement. At the close of the gestation period the requirement is twice this average value. Mineral requirements increase too, especially in the last half of the gestation period and during lactation.

Furthermore, the young animal requires readily available and abundant nutrients to meet its daily needs. The young animal does not possess as well developed digestive enzyme system and moreover, its metabolic rate exceeds that of the adult, and consequently its energy requirement for maintenance alone is greater in proportion to body size. Young animals have not fully developed their active resistance to bacterial infections thus have lower resistance to disease than the adult animal.

The series of studies reported in this thesis was designed to investigate the nutritional essentiality of vitamin B_{12} and/or aureomycin, during gestation, lactation and growth of rats fed all-plent rations.

REVIEW OF LITERATURE

The literature on this topic is so voluminous that only references which were especially significant to this study has been included.

Corn-Soybean Meal Rations for Reproduction

In extensive series of experiments made by Ross et al (1942a) with rats and swine, simplified corn-soybean oil meal diets failed to support normal reproduction and lactation. Suckling pigs from sows fed the basal ration were abnormally thin and in many cases died before reaching weaning age. The inclusion of 15 per cent alfalfa meal in the ration adequately fortified the ration to permit normal reproduction and lactation in the sow. In rats the corn--soybean basal diet failed to promote normal function during fetal development and during the suckling period. Intrauterine hemorrhage was observed which often resulted in resorption or death from toxemia if the fetuses were large. If the fetuses were carried to term, lactation was insufficient to support growth and the young usually died before weaning. The supplementation of 15 per cent alfalfa meal promoted normal reproduction and lactation, even in some instances through the second generation. Five per cent alfalfa meal when added to the basal ration, failed to make the ration adequate to support normal lactation.

Ross and coworkers (1942b) continued these studies by increasing the level of corn in the basal ration from 76.35 to 82.0 per cent, and at the same time decreasing the level of soybean meal from 17.50 to 11.50 per cent. Sows fed this ration failed to reproduce normally. The litters were small in size and numbers. The addition of wheat bran plus middlings, fish meal, tankage, brewer's yeast or molasses failed to prevent the occurrences of embryological abnormalities or to stimulate lactation enough to raise pigs until weaning.

Spitzer and Phillips (1946) fed rats a basal ration consisting of ground yellow corn, 75.34 per cent; soybean oil meal, 17.50 per cent; alfalfa meal 5.0 per cent; monophosphate, 1.0 per cent, calcium chloride, 0.65 per cent; iodized sodium chloride, 0.50 per cent; and manganese sulfate, 0.01 per cent. When young received such a ration from birth to breeding complete failure in lactation resulted. Death of young was due to lack of milk secretion. A peculiar type of hemorrhage was noted in the fetal attachment of the pregnant rats. Breakdown of capillaries of the cotyledons in many cases was observed during the early part of the gestation period. Female rats that were fed the basal diet from weaning to reproduction weaned only twenty-five per cent of those of the litter that were alive at birth.

Inadequacy of diets containing whole cereal grains supplemented with soybean oil meal for lactation and growth of rats was investigated by VanLandingham _et al_. (1947). Their basal ration contained principally ground whole wheat, ground whole oats, and soybean oil meal. Weanling rats fed this ration grew normally when fed this ration during lactation, however their young did not survive until weaned. Most of the young died during the first week, and many were eaten by their mothers. These workers suspected that the cause of heavy mortality was due to lack of milk from the mothers. Addition of alfalfa meal, bone meal, choline hydrochloride and riboflavin to the basal diet did not correct the inadequacy of the basal ration for normal lactation. However, normal lactation was attained when 2 per cent dried pig's liver was added to the basal diet.

Growth and reproduction of rats on rations devoid of animal protein was comprehensively studied by Schultze and Briggs (1949). Their basal ration consisted of soybean sodium proteinate, 274.4 grams; sucrose, 598.5 grams; D,L-methionine, 5.6 grams; salt mixture, 40.0 grams; Crisco, 70.0 grams; corn oil (fortified with 100 mg. D,L-alpha-tocopherol, 10,000 I.U. vitamin A, 1,500 A.O.A.C. unit D_3), 10 grams water soluble vitamins (Nelson and Evans, 1947) and folic acid, 0.2 mg. This ration promoted normal reproduction. There were two hundred sixty-three live and only fourteen dead young in the forty-three litters obtained. When ethanol-extracted casein replaced the soybean sodium proteinate and methionine in this ration the young failed to survive beyond the third day after birth. The cause of the early mortality in the ethanol-extracted casein group was not known but in no case was it ascribed to failure of the young to obtain sufficient milk since milk production in all cases was apparently adequate.

In a subsequent study, using almost the same ration, Schultze (1950a) found that further extensive purification of the commercial soybean protein did not cause a decline in growth rate in weanling rats. Supplementation of the ration with 2 per cent liver extract (1:20) or with 3 per cent condensed fish solubles did not improve the growth rate in rats. Daily administration of 0.25 ug. of crystalline vitamin B_{12} to rats also failed to stimulate post-weaning weights or to increase the efficiency of food utilization.

The above ration was further investigated by Schultze (1950b) in regards to its influence on the reproduction performance of rats. All available vitamins except ascorbic acid and vitamin B_{12} were added to the ration. High incidence of mortality of the young during the first four days of life was encountered, and symptoms of acute uremia of the newborn preceded most of these early deaths. The addition of liver extract or condensed fish solubles to the ration of the mother remedied the severe early mortality and greatly increased the survival rate of the young. When the survivors from the unsupplemented diet were continued for two subsequent generations, the same rate of mortality prevailed.

Halverson and Schultze (1950) fed rats a diet consisting primarily of ground yellow corn and expeller processed soybean oil meal and corroborated the findings of Schultze (1950b). Acute uremia was observed in young rats born to mothers receiving this ration. From forty to fifty per cent of the litters from mothers fed the unsupplemented ration incurred symptoms of acute uremia. The addition of a mixture of D,L-methionine, choline, or the B-vitamins except vitamin B_{12} did not prevent the occurrence of the syndrome. However, the condition was mitigated by the supplementation of the diet with 2 per cent Wilson's 1:20 liver extract, condensed fish solubles or by subcutaneous injections of vitamin B_{12} into the young rats shortly after birth.

Watts, _et al_. (1950) conducted an extensive series of reproduction and lactation studies with rats fed a ration consisting of ground yellow corn, expeller-processed soybean oil meal, alcohol-extracted casein, alfalfa leaf meal, minerals and B-vitamins except vitamin B_{12}. Normal gestation and lactation performances were obtained. Neither resorptions nor toxemias were observed as had been previously encountered by Ross, *et al*. (1942a). Supplementation of the basal diet with fish solubles, liver powder, Merck's B_{12} supplement, or Lederle's APF supplement consistently increased the weaning weights of the young rats in the first reproductive cycle and these differences were highly significant. However, when the females were maintained on the same ration for the second reproductive cycle, the differences were less striking. Statistical significance was not attained, although, in general, there was a tendency for the results to be in the same direction.

Vitamin B_{12} and Growth

Hogan and Anderson (1949) performed a series of experiments to study vitamin B_{12} as a growth factor in baby pigs. Six pigs were taken from their mothers at 2 days of age and transferred to individual cages with wide-mesh wire floors. They were fed synthetic milk which included alcohol-extracted casein, sucrose, corn starch, lard, mineral salts, and 13 known vitamins except vitamin B_{12}. Intramuscular injections of crystalline vitamin B_{12} were given to three pigs; one pig received a total of 50; a second, 100; and third, 200 micrograms of the vitamin. One of the unsupplemented pigs died unexpectedly in the sixth week, and one had not gained consistently. The third pig began to decline in body weight, but upon injection of 15 micrograms of vitamin B_{12}, it began to gain at a moderate rate. In the following 4-week period the treated pigs made an average gain of 26.8 pounds as compared to gains of only 15.9 pounds in the untreated pigs. The three pigs that received the vitamin by injection grew at a uniform rate attaining an average of 58.8 pounds during this period.

Newmann *et al*. (1951) produced vitamin B_{12} deficiency in baby pigs fed an alpha-protein (soybean isolate) diet. The symptoms were extreme irritability, posterior incoordination, voice failure and pain in the rear quarters. Supplementation of the basal diet with vitamin B_{12} concentrate at levels which gave a vitamin B_{12} potency of 51 and 68 ug.. per kilogram of dry matter of the diet overcome the vitamin-deficiency symptoms. There was also a marked and steady increase in the average weight gains as the level of vitamin B_{12} potency per kilogram of dry matter in the diet rose from 0.0 to 50 ug... The addition of vitamin B_{12} concentrate to supply more than 50 ug. per kilogram of dry matter did not stimulate further growth response. This indicates that about 50 mcgs. of vitamin B_{12} per kilogram of dry matter in the diet is the optimal level of the vitamin necessary for maximum growth of baby pigs.

Catron and associates (1952) using 18 per cent plant protein ration investigated the growth effect of vitamin B_{12} when fed to 75 or 100 pound pigs. Twenty-four pigs were divided into four lots and received 0.0, 2.0, 4.0, and 6.0 ug. . of crystalline vitamin B_{12} per pound of total ration respectively for lots 1, 2, 3, and 4. The addition of vitamin B_{12} to the basal diet promoted significantly greater gains in both the 75 and 100 pound-pigs. Vitamin B_{12} not only stimulated growth but also increased feed efficiency. No differences in the average daily gains of the pigs receiving 4.0 and 6.0 ug. . of vitamin B_{12} were observed.

Emerson, Wurtz, and Zannetti (1949) performed an extensive series of experiments to investigate the influence of vitamin B_{12} upon the growth of rats. The young rats were maintained during gestation and lactation on a diet containing 60 per cent soybean meal. From the time of impregnation, one group received 0.5 ug. of vitamin B_{12} daily while the other group was maintained as the control. At weaning time, one-half of the offsprings from each group were given 0.5 ug. of vitamin B_{12} daily for ninety days and the other half of the rats were unsupplemented. Treated young rats from mothers receiving no supplement gained one hundred grams more than those rats that received no vitamin B_{12}. Supplementation of young with B_{12} had no effect when the mothers received vitamin B_{12} in their rations during gestation and lactation. Sure (1951) studied the stimulatory effect of vitamin B_{12} on weanling albino rats during a 10-week growth period. When 0.1 ug. daily of crystalline vitamin B_{12} was added to rations containing low-fat soybean flour, alpha-soybean protein, and low-fat cotton seed flour increased growth of 3.6, 38.8 and 38.0 per cent, respectively was obtained. Baker (1951), using a purified basal diet containing all known nutrients except vitamin B_{12} and with sodium soybean proteinate as a source of protein, found that the addition of vitamin B_{12}, aureomycin, liver residue, vitamin B_{12} plus aureomycin, vitamin B_{12} plus liver residue plus aureomycin, did not improve the growth rate of weanling rats during a four-week growth period.

In an earlier experiment, Schultze (1950a) found that the daily administration of 0.25 ug. of crystalline vitamin B_{12} to weanling rats receiving a highly purified soybean protein diet did not improve growth.

Downing (1950) found that intramuscular injections of vitamin B_{12} stimulated growth of premature infants. Forty-eight infants with birth weights between 1,245 grams and 2,326 grams were divided into two groups.

Twenty-five received a dosage of 10 ug. vitamin B_{12} administered from once daily to once every three days. The other twenty-three infants served as controls. At the termination of the study there was no appreciable difference between those receiving vitamin B_{12} and those serving as controls in respect to average total gain, average daily gain, and average time to reach a weight of 2.5 kilograms. Since the infants were assumed to be clinically healthy, Downing stated that vitamin B_{12} is effective only in promoting weight gain in children who have slow growth due to a deficiency of the vitamin.

Chow (1950) found that the growth rate of both normal and chronically ill children was improved by the administration of vitamin B_{12}. Ninety-six ill children in a convalescent home were divided into two groups, without regard to sex, age or disease. All of the children received the regular home diet throughout the experimental period. The children in the control group received no supplement of vitamin B_{12}. One tablet containing 25 ug. of vitamin B_{12} was given daily to children (6 years old or older) who were able to swallow it. The younger children in the experimental group, who could not be depended upon to swallow the tablet, received daily an equivalent amount of this vitamin in solution. Infants below two years of age received 10 ug. of crystalline vitamin B_{12}. After three months of vitamin B_{12} therapy the mean gain in body weight in the experimental group was practically twice that in the control group. The difference between the means, 53 ounces, was highly significant at the one per cent level. Chow investigated further the effect of vitamin B_{12} on clinically healthy children. Twenty-four children in a foundling house were divided into two groups. One group received a daily supplement of 25 ug. of vitamin B_{12} by addition to milk of 0.5 ml. of a solution of 25 mg. of the crystalline vitamin in 500 milliliters of water. The control group received a placebo in the form of 45 milligram of Vinta certified dye in 2,000 ml. of water. At the end of 24 weeks, the supplemented group showed a weight gain of 58 ounces compared to the control, 40 ounces. The differences of 18 ounces was statistically significant at the five per cent level.

Vitamin B_{12} and Reproduction

Anderson and Hogan (1950a) conducted two experiments to investigate the adequacy of purified diets for reproduction of swine. Two gilts that had formerly received injections of crystalline vitamin B_{12} at 3-day intervals, beginning when they were 3 and ending when they were 38 days of age, were bred. One gilt, Number 113, received a total of 50 ug. and the other, Number 171, 200 ug. of crystalline vitamin B_{12} during the 35-day period. The gilts were bred at 195 and 238 days of age. A solution of iron, copper, and manganese was supplied daily during the first three weeks of lactation to udders of the sows to prevent anemia in their litters. When the sows were 231 days old the vitamin injections were resumed and continued at intervals until well into the lactation period. The total amounts injected during this time were 442 ug. for sow Number 113 and 383 ug. for sow Number 171. There was some slight evidence that the ration of the sow was inadequate. A few of the pigs were born with kinked tails. Mild diarrhea was observed in both litters. Sow Number 171 was observed to vomit prior to feeding. Two pigs in the litter of sow Number 171 had died by the 10th day. However, the injection of 21.5 ug. of crystallin vitamin B_{12} into each pig on the 11th day reduced mortality. An injection of 10 ug. vitamin B_{12} immediately after birth to each pig of sow Number 113 prevented early death. One pig died at the age of 19 days but the cause was attributed to an intestinal obstruction. In the second trial, the same sows were used. They were fed with the same ration except that sow Number 113 from the 59th day of gestation received 2.5 per cent of water extract of liver at the expense of the vitamin free-casein in the diet. The liver extract was increased to 5 per cent when her litter was six weeks old. Sow Number 171 was given 1.54 mg. of vitamin B_{12} during gestation and 0.84 mg. during lactation. Each pig from sow Number 171 received an injection of 50 ug. of vitamin B_{12} shortly after birth. The pigs from sow Number 171 were normal at all times. The pigs from sow Number 113 had recurrent mild attacks of diarrhea. The vitamin B_{12}-injected pigs were uniform in size and attained an average weight of 47.1 pounds at 8 weeks of age, while the pigs reared by the liver extract-treated sow were not uniform in size and were 12 pounds lighter at 8 weeks of age.

In a subsequent study by Anderson and Hogan (1950b), the value of vitamin B_{12} concentrate for brood sows was thoroughly investigated. Eighteen 200-pound gilts were divided equally into three groups and fed a ration consisting primarily of; 70 per cent corn, 5 per cent tankage, 20 per cent soybean oil meal, 2.5 per cent alfalfa meal, 0.5 per cent Cod liver oil, and 2 per cent complex mineral mixture. Lot 1 received the control diet. Lot 2 received the basal diet supplemented with enough Merck's APF No. 3 to supply 10 ug. of vitamin B_{12} per pound of feed. Lot 3 received the basal diet supplemented with 15 per cent of fish meal and 2 per cent of fish solubles combined. The pigs in Lot 2 attained an average weaning weight of 36.2 pounds which 8.2 and 3.7

pounds heavier than the control and the fish meal-solubles-supplemented groups, respectively. Lots 2 and 3 weaned all their pigs that were alive at birth as compared to the control lot which had 13.3 per cent mortality. As a continuation of this experiment, these investigators divided fifteen gilts which were raised on the basal lot of the first trial into three lots. They were fed rations which had the same components as the original basal ration except that the tankage was replaced with soybean oil meal, riboflavin, calcium pantothenate, and nicotinic acid were also added to this ration. Lot 1 received the control diet. Lot 2 was supplemented with fresh cut green rye, while Lot 3 was supplemented with 10 ug. of vitamin B_{12} per pound of feed. The sows of the basal lot weaned 91.9 per cent of the pigs they littered, at an average weight of 35.8 pounds. Those in Lot 2 weaned 100 per cent of their pigs at an average weight of 37.7 pounds; and those of Lot 3 weaned 97.5 per cent of their pigs at an average weight of 37.7 pounds.

Heidebrecht, _et al._ (1950) investigated the adequacy of a corn--soybean meal ration for reproduction and lactation of swine. Twenty--four gilts and eight sows that had been confined since weaning on concrete floors were alloted to four groups at the time of breeding. They were fed a corn-soybean meal ration fortified with alfalfa leaf meal and steamed bone meal and received the following supplements: Lot 1, none; Lot 2, riboflavin, nicotinic acid, and calcium pantothenate; Lot 3, same as Lot 2 plus an APF concentrate containing 12.5 ug. vitamin B_{12} per pound; Lot 4, same as Lot 2 plus and APF containing aureomycin. The workers observed diarrhea in all lots, but Lot 4 had the condition to a lesser degree than the other groups. The highest percentage of weaned pigs was in Lot 2, which was followed by Lots 4, 3, and 1, respectively. However, the pigs receiving aureomycin achieved the highest weaning weights, and was followed by the vitamin B_{12}-treated pigs, the B-vitamins-treated pigs, and the control, consecutively. Nevertheless, many pigs died in the lot of pigs receiving B-complex vitamin shortly after weaning time as compared to only a few in the other groups.

Baker (1950) studied the reproduction and lactation performance of rats fed all-vegetable protein rations supplemented with all known minerals and vitamins except vitamin B_{12}. Eighty-four 200-gram female rats, which were previously used in a growth experiment, were bred. These rats were fed a basal diet (Lot 1), the basal plus vitamin B_{12} (Lot 2), the basal plus aureomycin (Lot 3), the basal plus liver residue (Lot 4), the basal plus vitamin B_{12} and aureomycin (Lot 5), the basal plus liver residue and vitamin B_{12} (Lot 6), and the basal plus liver residue and aureomycin (Lot 7). The study was conducted for two lactation periods. Satisfactory reproduction was obtained in the rats fed the all-plant ration and the addition of any supplement produced unsignificant differences. Lactation performances of these rats were consistently improved by supplementing the all-plant ration with vitamin B_{12}, aureomycin, liver residue, or combinations of these substances as indicated by greater weaning weight of young.

Meyer, <u>et al</u>. (1951) found that the oral administration of crystalline vitamin B_{12} failed to overcome lactation failures. In his experiments seven groups of fifteen rats each were bred at 100 days of age. Three different protein sources in the diet, with and without vitamin B_{12}, were fed to test their effects upon reproduction performances in rats. Lots 1, 3, and 5 were fed a diet in which roast beef, roast pork, and casein were the main sources of protein, respectively. Lots 2, 4, and 6 were fed the same rations, except each rat received 0.10 ug. vitamin B_{12} per day. The seventh lot was fed casein plus liver residue. All rations were compounded isocallocically and contain 24 per cent protein. Their results indicated that oral administration of crystalline vitamin B_{12} did not improve lactation performances of the rats. Pork diets not only impaired lactation but also hindered conception. The casein group receiving the methanol extract of liver had 100 per cent reproduction and lactation performance.

Dryden, Hartman, and Cary (1951) found that vitamin B_{12} supplementation did not influence uterine mortality in rats. No real dissimilarity to the number of conceptions and resorptions was attributable to the presence or absence of vitamin B_{12} in the ration. These data indicated that there was no effect of vitamin B_{12} deficiency upon the conception or maintenance of pregnancy in rats. However, birth weights were improved by either liver extract or crystalline vitamin B_{12}. Dryden, et al. (1951) in a later study investigated the effect of vitamin B_{12} upon the survival of young from mother rats fed purified casein rations. In this study a number of B_{12}-deficient rations containing yeast instead of B-vitamins were also studied. In the first experiment the mothers were started on the vitamin B_{12}-deficient rations at mating time and were given an oral supplement 0.10 ml. per day of an APF liver extract daily, and in the second experiment 1 ug. per day of crystalline vitamin B_{12} was given. In the third and fifth experiments primiparous stock colony mothers were used. They were put on the vitamin B_{12}-deficient diet at parturition and received 1 ug. and 2 ug. per day of crystalline vitamin B_{12}, respectively for experiments 3 and 5. In the fourth experiment, colony mothers at parturition were put on a vitamin B_{12}-deficient diet. This study was conducted for three lactation periods. For the first mating, vitamin B_{12}-deficient mothers raised as large a proportion of their young to weaning as did the vitamin B_{12} supplemented mothers; however, when the mothers were continued on the experimental rations through additional matings, quite different results were obtained. Few young survived on the yeast-free vitamin B_{12}-deficient rations in two experiments. In none of the three experiments was there better than 39 per cent survival of the young. Out of the 48 mothers on the yeast--free vitamin B_{12}-deficient rations, only 10 carried any young to weaning. The dosage of 2 ug. per day crystalline vitamin B_{12} at parturition to yeast-free B_{12}-deficient mother did not correct the mortality of the young rats. Nevertheless, the supplementation of crystalline vitamin B_{12} to yeast-containg rations prevented early mortality in the young.

Schultze and associates (1952) confirmed the work of Dryden, et al. They found that the survival of young rats whose mothers received vitamin B_{12}-low diets containing soybean oil meal was improved by the addition of vitamin B_{12} to the maternal ration. Incidence of uremia in newly born rats, which was common to the control, to the alfalfa leaf meal--supplemented, and to the corn-soybean-alfalfa leaf meal groups; was reduced to minimum by the supplementation of 40 ug. of vitamin B_{12} per kilogram of the maternal diet. The incidence of uremia was zero when the diet was low in vitamin B_{12}. The inclusion of 15 percent of alalfa leaf meal into a 22 percent protein diet increased the incidence of uremia to 3 per cent. The vitamin B_{12}-deficient diet, with or without methionine, gave no cases of uremia. The feeding of the soybean oil meal-alfalfa leaf meal-yellow corn diet containing 21.8 or 15.6 per cent protein induced symptoms of acute uremia in 40 to 60 per cent of the litters. Increasing the crude protein content of the ration fed to the young rats after weaning time, from 15.6 to 33 per cent, incurred a high incidence of mortality during the first two weeks after weaning. Post-weaning mortality was prevented by the addition of vitamin B_{12} to the diet.

Antibiotics and Growth

Edwards, et al. (1950) fed a ground yellow corn-peanut meal ration to pigs and found that either aureomycin or an APF supplement, containing vitamin B_{12} and aureomycin stimulated growth. Sixteen purebred Duroc pigs were divided into four lots. Lot 1 was supplemented with 44 ug. vitamin B_{12}, alone; Lots 2 and 3, 50 mg. and 200 mg. of aureomycin, respectively, in addition to the crystalline vitamin B_{12}; and Lot 4 received 10 grams crude APF supplement per kilogram of basal diet. The pigs were kept on concrete floors which were washed once daily. Aureomycin supplementation increased the growth rate of the pigs. The higher level of aureomycin was more effective in stimulating growth than the lower. The pigs receiving the APF supplement gained faster than those receiving only vitamin B_{12}, but in all cases slower than those receiving aureomycin.

Catron and co-workers (1950) observed that pigs fed an all-plant basal ration with different levels of aureomycin and vitamin B_{12}, sepa- ately, made faster gains than the controls. Less incidence of scouring was observed in the vitamin B_{12} and aureomycin supplemented lots. These materials were further investigated with runt pigs (unthrifty pigs weighing less than 20 pounds at 56 days of age). The addition of vitamin B_{12} failed to improve the daily gains. However, the slow-growing pigs were responsive to aureomycin therapy. They were more uniform in size, more thrifty in appearance and exhibited less scouring than the pigs on the other treatments.

In a recent work by Catron and colleagues (1952), they found that protein level in the rations of growing pigs can be reduced by the addition of aureomycin to their diets. Four levels of protein, 20, 18, 16, and 14 per cent, were tested. The various protein levels were obtained by adjusting the primary components of the basal ration, ground corn and soybean oil meal. The protein level of each ration was decreased 3 percentage points when the pigs reached an average weight of 75 pounds and again at 150 pounds. These data showed that the 16, 13, and 10 per cent protein levels supplied the pigs' needs for protein from weaning to market, when they received the basal ration, whereas in the presence of aureomycin the 14-11-8 per cent level combination produced gains equivalent to higher levels of protein.

Beker, Terrill, Meade, and Edwards (1952) compared the efficacy of various antibacterial agents for stimulating the rate of gain in pigs. Results obtained implied that in all cases the feeding of these agents promoted a highly significant growth response over the basal diet. However, there was a pronounced variation in the magnitude of this response. The gains were highest in the aureomycin and terramycin treated lots. Rough haircoats, dermatitis, and a brownish exudate over the entire body were observed in the basal group. The pigs in this lot were also extremely emaciated and unthrifty. On the other hand, the supplemented groups had smooth skin and haircoats. The prevalence of diarrhea, which was rampant in the control Lots, was reduced to a minimum when antibiotics were fed. As a continuation of this study, the basal-fed pigs were divided into 2 subgroups. One group continued to receive the basal ration, and the other group received 5 mg. of aureomycin in addition. The aureomycin-supplemented pigs attained 1.10 pounds average daily gain more than the control group during the succeeding 3 weeks period.

In rats, Swick, Lih, and Baumann (1951) observed increased growth rates of rats receiving limiting amounts of thiamin, riboflavin, and pantothenic acid supplemented with penicillin, aureomycin, and streptomycin. They observed that penicillin was most effective in thiamin deficiency. In pantothenic acid deficiency, aureomycin, and streptomycin were superior to penicillin. Linkswiller, et al. (1951) prevented acrodynia in rats by supplementing a pyridoxine-deficient diet with aureomycin, but failed to receive any stimulation of growth. In the pyridoxine-adequate diet (.41 ug. per gram of diet), the presence of aureomycin increased the growth of rats sufficient enough to cause over-all errors of 10 to 100 per cent in a bioassay for vitamin B_6. Nevertheless, the increase in growth would disappear when high levels of pyridoxine were fed.

Sauberlich (1952) in a series of experiments studied the effect of aureomycin and penicillin upon the vitamin requirements of the rat. Weanling rats were placed individually in wire-bottomed cages and fed a basal diet which contained 20 per cent methanol-extracted casein, 70.5 per cent carbohydrate, 4 per cent salt mixture, 0.3 per cent L-cystine and adequate water-soluble vitamins to support maximum growth. The presence of aureomycin or penicillin in the fully supplemented basal diet had no effect upon the growth of rats, while the addition of sulfamerizine had an inhibitory effect upon growth. In diets free of, or low in, thiamin, pyridoxine, pantothenic acid or riboflavin, the addition of penicillin to the diet caused a marked stimulation in the growth of rats. Aureomycin was ineffective in diets low in pantothenic acid or riboflavin but gave a stimulatory effect where added to diets low in thiamin or pyridoxine. The type of carbohydrate used (dextrin or starch) was without effect in these studies.

Antibiotics and Reproduction

Carpenter (1951) studied gestation performances of sows fed a ground yellow corn-oats-barley ration as compared to those receiving the same ration plus an APF concentrate containing aureomycin. He observed that the feeding of the antibiotic supplement had effected neither litter size nor number of pigs born dead. However, the incidence of diarrhea was reduced to minimum in the treated groups, and the weaning weights of pigs farrowed by the supplemented sows were much higher than the pigs from the control sows. Carpenter (1951) further investigated the effects of feeding an APF supplement during lactation. Three groups of sows with litters were placed on a corn--alfalfa leaf meal diet immediately after parturition. The pigs were creep fed 10 to 14 days after birth. Lot 1 was the control, and the dams and pigs received no supplement. In Lot 2 the dams received 0.5 per cent APF supplement while the pigs received APF supplement at levels of 0.5 per cent and 1 per cent, respectively. The pigs nursing dams fed a diet containing 0.5 per cent APF did not grow any faster than pigs nursing the dams receiving the control ration. The pigs which received the APF in their diets attained a weaning weight which was 6 pounds heavier than the other two lots, implying that the growth stimulatory effect of APF concentrate was attributable to its presence in the creep diet rather than its transmission into the milk.

Heidebrecht, et al. (1951) found that the addition of aureomycin to the ration of sows and gilts provided some protection against an infectious type of disturbances to pigs. Sure (1951) observed that the supplementation of 1 per cent APF from a fermentation product of aureomycin to a "Cerevim" ration produced very favorable effects on reproduction and lactation performances of rats. Baker (1951) corroborated the work of Sure. Rats fed with all-plant protein ration, supplemented with aureomycin, singly or with vitamin B_{12} and liver residue, had higher average weaning weights than the untreated groups.

Robinson (1952) stated that aureomycin stimulates growth rate in premature infants by preventing infections. In his studies he used eleven sets of twins and two sets of triplets which were dosed daily with 50 mg. of aureomycin per kilogram of body weight. At first the weaker in the twins and the weakest in the triplets were administered with the drug and the stronger and strongest of the twins and triplets, respectively, served as controls, but when one of these could not tolerate the antibiotics, the treatment was reversed with the weaker and the weakest being used as controls while the stronger and the strongest received the drug. At the termination of the investigation all the babies who received aureomycin gained more weights than the controls. There was also 33 per cent death rate in the controls from intercurrent infections; whereas all the babies who received a full course of aureomycin survived.

Other Nutritional Factors

Recent reports have indicated that there are still unknown nutritional factors in various feedstuffs and feed by-products which are essential for life, maintenance and growth.

Sunde, *et al.* (1952) found that the growth rate of chicks receiving an all-plant diet containing vitamin B_{12} and an antibiotic was stimulated by fish solubles, torula yeast, meat scraps, and condensed homogenized fish. Kratzer (1952) observed that growth stimulation resulting from liver concentrate was independent from that provided by aureomycin. In 5 out of 6 tests aureomycin and liver concentrate had additive growth stimulatory effects. Fuller and associates (1952) compared vitamin B_{12}, fish solubles and whey in the diets of chicks fed corn-soya diet. He found that the growth response of chicks depleted of vitamin B_{12} for 2 weeks was greater when 3 per cent condensed fish solubles supplemented the basal corn-soya ration, than when vitamin B_{12} was added. Four per cent dried whey was about one-half as effective as vitamin B_{12}. This finding suggested that there are other unknown factors needed for growth besides vitamin B_{12}.

OBJECTIVE

The experiment which was initiated April 5, 1952, and discontinued December 24, 1952, was designed to test the nutritional adequacy for gestation, lactation and growth of rats of an all-plant ration, alone and supplemented with vitamin B_{12} and/or aureomycin.

Experimental Procedure

In order to simplify the discussion of the experimental procedure of this study, it has been divided into three major parts: (A) Gestation and lactation performance of first generation female rats, (B) Gestation and lactation performance of second generation female rats and (C) Growth performance of second generation weanling rats.

A. Gestation and lactation performance of first generation female rats.

Eighty-six weanling female rats of Sprague-Dawley strain were divided into four equal lots and fed the rations shown in Table I. These animals were housed in wire cages, four rats per cage, and given food and water *ad libitum*. In addition each rat received two drops of cod-liver oil alpha-tocopherol mixture by dropper once each week. Fresh water was given daily. Feed and water containers were cleaned frequently, and the sugar cane baggase used for litter was changed weekly.

During a three-week breeding period, the males were rotated daily among the various pens. The females were weighed weekly and those that gained 20 grams or more during the preceding week were removed to an individual maternity cage. At the end of the three-week mating period all females that failed to show signs of pregnancy were further observed for ten days. At this time all open females were continued on their respective experimental diets for use in the following gestation and lactation period.

One or two days before parturition, the cages were lowered and placed in contact with clean litter. Frequent observations were made during this period in order to account for the number of young born either alive or dead. Since cannibalistic characteristics among the mothers were observed, this procedure was necessary. Birth weights and numbers of rats born were recorded soon after parturition. The young rats were weighed again at three days of age and then the number of young was adjusted to six per nursing female. Thereafter, weekly weights were taken until they were weaned at the age of 21 days. In this trial the mothers were weighed weekly during the lactation period.

After the young were weaned, the mothers were given a short rest and then rebred for a second gestation and lactation period. This same procedure was also followed in preparing the females for the second and final gestation and lactation period.

B. Gestation and lactation performance of second generation female rats.

In order to further test the nutritional adequacy of the purified all-plant protein rations, thirty weanling females from mothers receiving each ration were selected at the end of the first trial. These were fed the same diets that their respective mothers had received. When they were three months old, twenty females from each group were selected on basis of weight and appearance and were bred. Thereafter, the same management procedure followed in the case of their mothers was followed during the one gestation and lactation period with the exception wheat germ oil was used in lieu of alpha-tocopherol.

C. Growth performance of second generation weanling rats.

Twenty uniform weanling rats of mixed sex from each of the four lots were selected at the end of the third reproductive cycle of the first generation rats. The rats were put in wire cages, three in a cage. They were ear-notched for identification and fed the same experimental diets that their respective mothers had previously received during the gestation and lactation period. Food and water were given ad libitum during the entire experiment. The water was changed daily and the feeding bottles were cleaned frequently. The rats were given two drops of cod-liver oil once each week throughout the growth trial. Weights were taken weekly for 3 weeks following weaning.

RESULTS

A. Gestation and lactation performance of first generation female rats.

The results of the first gestation and lactation period are shown in Table III. These results, which involved 86 females and their litters, indicated no improvement of the basal diet by vitamin B_{12} and/or aureomycin when the per cent of young born alive, per cent young alive at the third day and the average weaning weights of litters were used as the criteria. Statistical analysis of the data (weaning weights) indicated that the small differences existing between treatments were not significant.

The results of the second gestation and lactation period are shown in Table V. Using the per cent of young alive until the third

TABLE I

Experimental Rations

Rations	I	II	III	IV
Supplements	None	Vitamin B_{12}	Aureomycin	Vit. B_{12} + Aureomycin
Ingredients				
Sodium Proteinate*	20.0	20.0	20.0	20.0
Corn Oil	5.0	5.0	5.0	5.0
Hegsted Salt Mixture**	4.0	4.0	4.0	4.0
Corn Starch	70.6	70.6	70.6	70.6
D,L-Methionine	0.2	0.2	0.2	0.2
Vitamin Mixture**	2g/kg diet	2g/kg diet	2g/kg diet	2g/kg diet
Vitamin B_{12}***	-	50 ug/kg	-	50 ug/kg
Aureomycin HCl****	-	-	25 mg/kg	25 mg/kg

* Manufactured by Archer-Daniels-Midland Co.

** Hegsted Salt Mixture (J. Biol. Chem. 138:459. 1941)
Calcium carbonate 1200 grams; Potassium phosphate (dibasis) 1290 grams; Di-calcium phosphate 300 grams; Ferric citrate 110 grams; Potassium iodide 3.2 grams; Manganese sulfate 20 grams; Zinc chloride 1 gram; Copper sulfate 1.2 grams.

Vitamin Mixture
Thiamin 4 grams; Riboflavin 6 grams; Pyridoxine HCl 3 grams; Niacin 20 grams; Choline chloride 100 grams; Calcium pantothenate 20 grams; Inositol 20 grams; Para-aminobenzoic acid 20 grams; Folic acid 0.5 gram.

*** Manufactured by Merck and Company, Inc.

**** Manufactured by Lederle Laboratories Division, American Cyanide Co.

TABLE II

Calculated Amino Acids Content of the Ration (20 per cent Sodium-proteinate plus 0.2 per cent D,L-Methionine.)

Amino Acids[1]	Per cent in the diet	Required by rats[3] (Rose Science 86:298, 1937)
Arginine	1.35	0.20
Histidine	0.38	0.40
Isoleucine	0.91	0.50
Leucine	1.23	0.90
Lysine	1.00	1.00
Methionine[2]	0.40	0.60
Phenylalanine	0.85	0.70
Threonine	0.65	0.60
Tryptophan	0.19	0.20
Valine	0.87	0.70

[1] The protein content of the ration was 16.34 per cent.

[2] D-Methionine included at 80% biological activity.

[3] Minimum amount of each natural essential amino acid necessary to support normal growth when the non-essentials are included in the food.

day as the criteria for gestation performance, the addition of vitamin B_{12} and/or aureomycin to the basal ration improved the basal ration. The rats receiving aureomycin alone had the lowest mortality rate. Little difference was noted between vitamin B_{12} supplementation alone and B_{12} and aureomycin. The addition of the supplements to the basal ration improved lactation performance. The average weaning weights per rat of Lots I, II, III, IV were 44.8, 44.4, 48.1 and 46.1 grams, respectively. Statistical analysis of these data indicate that the difference existing between the basal lot and the supplemented lots was highly significant. This difference was due to aureomycin supplementation.

In the third gestation and lactation period only the rats receiving vitamin B_{12} alone maintained a gestation performance above 80 per cent. The rats that received aureomycin alone had the highest mortality rate to the third day, 38.6 per cent. The rats receiving the basal diet also had a high mortality rate (24.5 per cent). The addition of the supplements to the basal ration improved the lactation performance. The difference in weanling weights between the basal lot and the supplemented lots was significant ($P = 0.05$). In contrary to the result obtained in the second gestation and lactation period, the differences in weaning weight between the basal lot and the supplemented lots in this gestation and lactation period was due to the supplementation of vitamin B_{12}.

The gestation-lactation performance of the first generation females is summarized in Table IX.

B. Gestation and lactation performance of second generation rats.

The results of the gestation and lactation period are summarized in Table X. The lactation performance of all lots was normal. However, it was not as good as the performance of their respective mothers. The rats that received vitamin B_{12} and aureomycin had the highest average weaning weight per rat, 42.51 grams, and was followed by the rats that received aureomycin, 41.22 grams. The vitamin B_{12}-fed rats attained the lowest average weaning weight, 36.99 grams, even lower than the control, 38.08 grams. The peculiar feature observed in this trial was that: of the eighty females bred only 52.50 per cent conceived; 40.0, 70.0, 55.0, and 45.0 per cent of Lots I, II, III, IV, respectively.

The slight difference in weaning weights between treatments was not significant. The summary of the analysis of variance was given in Table XI.

C. Growth performance of second generation rats.

The average weekly gains of the growth trial were summarized in Table XII. There were no deaths during the three week growth period. Rats that received aureomycin supplementation made the greatest gain and was followed by the vitamin B_{12}-aureomycin treated rats, the control, and the vitamin B_{12}-treated rats, respectively. The difference in body weight at the end of the three week-growth period was not significant when the data were analyzed by the method of the analysis of variance. This analysis was summarized in Table XIII.

TABLE III

The Results of the First Gestation and Lactation Period

Lots	I	II	III	IV
Supplements	None	Vit. B_{12}	Aureo.	Vit. B_{12} ≁ Aureo.
No. females bred	21	21	22	22
No. litters born	17	14	18	13
No. litters weaned	16	14	17	13
No. young born per litter	9.84	10.36	10.11	10.54
No. live young born/litter	9.30	9.53	9.78	10.39
No. live young third day/litter	9.12	9.29	9.61	9.93
Per cent young born alive	94.61	95.86	96.70	98.54
Per cent young alive third day	93.41	89.66	95.06	94.16
Per cent assigned young weaned	84.15	92.50	91.59	97.43
Ave. birth wt. gram per rat	6.32	6.07	6.13	5.96
Ave. third day wt. gram per rat	7.62	7.25	7.35	7.22
Ave. third day litter wt. gram per rat*	7.55	7.25	7.52	7.28
Ave. 1st. week wt. gram per rat	12.99	12.57	13.02	12.67
Ave. 2nd. week wt. gram per rat	24.66	24.00	25.92	24.40
Ave. weaning wt. gram per rat	40.80	37.92	42.43	40.95
Ave. gain wt. gram per rat**	33.25	30.67	34.91	33.67

* No. of rats in a litter was adjusted to six in number at the third day.

** Average weaning weight minus average third day litter weight.

TABLE IV.

Analysis of Variance of the Weaning Weights of the Young of the First Gestation and Lactation Period.

Sources	d.f.	Sum of squares	Mean of squares	F-value
Total	59	1,854.023	31.424	
Treatment	3	107.039	35.68	1.144
Error	56	1,746.984	31.196	

Not significant

Correlation between the weights of the mothers (X) and of the young (Y) at a weekly period. (Average weight in grams, gain or loss)

Lots	I		II		III		IV	
Treatments	None		Vit. B_{12}		Aureo.		Vit. B_{12} ≠ Aureo.	
	X	Y	X	Y	X	Y	X	Y
No. Weeks								
0	239	6.32 :	233	6.07 :	239	6.13 :	236	5.96
1	224	12.99 :	218	12.57 :	226	13.02 :	229	12.67
2	220	24.66 :	221	24.00 :	229	25.92 :	229	24.40
3	223	40.80 :	220	37.92 :	232	42.43 :	225	40.95
Total	906	84.77 :	892	80.56 :	926	87.50 :	919	83.98
Sx^2	217		138		93		63	
Sy^2		684.95		586.30		765.19		705.15
Sxy	-256.56		-163.91		-69.22		-184.07	
r	- 0.66		- 0.56		- 0.26		- 0.87	

No correlation

TABLE V

The Results of the Second Gestation and Lactation Period

Lots	I	II	III	IV
Supplements	None	Vit. B_{12}	Aureo.	Vit. B_{12} + Aureo.
No. females bred	20	20	22	22
No. litters born	19	19	20	19
No. litters weaned	16	18	20	18
No. young born per litter	9.32	8.89	8.50	9.05
No. live young born per litter	7.32	7.84	8.45	8.53
No. live young at third day per litter	6.84	7.68	8.10	8.05
Per cent young born alive	78.53	88.69	99.41	94.19
Per cent alive young at third day	73.45	86.90	95.29	88.95
Per cent of assigned young weaned	89.11	96.15	97.39	93.52
Ave. birth wt. gram per rat	6.28	6.27	6.41	6.35
Ave. third day wt. gram per rat	8.03	7.90	6.49	7.86
Ave. third day litter wt. gram per rat*	8.01	8.15	7.91	8.00
Ave. first week wt. gram per rat	15.10	15.10	16.89	13.83
Ave. second week wt. gram per rat	27.60	27.52	29.29	27.70
Ave. weaning wt. gram per rat	44.77	44.44	48.07	46.11
Ave. gain wt. gram per rat**	36.76	36.29	40.16	38.11

* No. of rats in a litter was adjusted to six in number at the third day.

** Average weaning weight minus average third day litter weight.

TABLE VI.

Analysis of Variance of the Weaning Weights of the Young of the Second Gestation and Lactation Period.

Sources	d.f.	Sum of squares	Mean of squares	F-value
Total	402	11,263.10		
Treatment	3	1,058.81	352.94	8.72**
Litters in treatment	64	2,591.08	40.49	
Between sex in litters	64	3,306.80	51.67	
Between rats within litters	269	4,207.14	15.64	
Vit. B_{12} vs. W/out	1	125.75	125.75	3.11
Aureo. vs. W/out	1	631.59	631.59	15.60**
Interaction	1	70.74	70.74	1.75

** $P = 0.01$

TABLE VII.

The Results of the Third Gestation and Lactation Period

Lots	I	II	III	IV
Supplements	None	Vit. B_{12}	Aureo.	Vit. B_{12} ≁ Aureo.
No. of females bred	18	19	21	21
No. litters born	18	13	17	17
No. litters weaned	14	11	13	13
No. young born per litter	8.61	5.42	7.52	7.14
No. live young born per litter	6.94	4.80	6.67	6.00
No. live young at third day	6.50	4.47	4.62	5.52
Per cent young born alive	80.64	88.35	88.61	84.00
Per cent alive third day	75.48	82.52	61.39	77.33
Per cent of assigned young weaned	98.78	100.00	82.76	100.00
Ave. birth wt. gram per rat	7.02	6.46	6.61	6.18
Ave. third day wt. gram per rat	7.83	7.95	9.08	7.64
Ave. third day litter wt. gram per rat*	7.85	8.13	7.61	7.49
Ave. first week wt. gram per rat	14.89	15.77	13.78	15.51
Ave. second week wt. gram per rat	26.86	26.03	27.17	28.30
Ave. weaning wt. gram per rat	42.72	46.32	43.22	45.90
Ave. gain wt. gram per rat**	34.87	38.19	35.61	38.41

* No. of rats in a litter was adjusted to six in number at the third day.

** Average weaning weight minus average third day litter weight.

TABLE VIII.

Analysis of Variance of the Weaning Weights of the Young of the Third Gestation and Lactation Period.

Sources	d.f.	Sum of squares	Mean of squares	F-value
Total	285	7,860.47		
Treatment	3	914.72	304.91	2.96*
Litters in treatment	43	4,434.62	103.13	
Between sex in litters	43	601.29	13.98	
Between rats within sex in litters	184	1,909.80	10.38	
Vit. B_{12} vs. W/out	1	550.25	550.25	5.36*
Aureo. vs. W/out	1	6.06	6.06	0.059
Interaction	1	43.49	43.49	0.422

* P = 0.05

TABLE IX.

Summary of the Gestation-Lactation Performance of the First Generation Females

Lots	I	II	III	IV
Supplements	None	Vit. B_{12}	Aureo.	Vit. B_{12} ≠ Aureo.
First Period				
Per cent young alive 3rd. day	93.41	89.66	95.06	94.16
Per cent of assigned young weaned	84.15	92.50	91.59	97.43
Ave. weaning weight gram per rat	40.80	37.92	42.43	40.95
Second Period [1]				
Per cent young alive 3rd. day	73.45	86.90	95.29	88.95
Per cent of assigned young weaned	89.11	96.15	97.39	93.52
Ave. weaning weight gram per rat	44.77	44.44	48.07	46.11
Third Period [2]				
Per cent young alive 3rd day	75.48	82.52	61.39	77.33
Per cent of assigned young weaned	98.78	100.00	82.76	100.00
Ave. weaning weight gram per rat	42.72	46.32	43.22	45.90

[1] The difference in weaning weights between the basal lot and the supplemented lots was significant ($P = 0.01$)

[2] The difference in weaning weights between the basal lot and the supplemented lots was significant ($P = 0.05$).

TABLE X.

The Results of the First Gestation and Lactation Period of the Second Generation Rats

Lots	I	II	III	IV
Supplements	None	Vit. B_{12}	Aureo.	Vit. B_{12} + Aureo.
No. females bred	20	20	20	20
No. litters born	8	14	11	9
No. litters weaned	6	9	9	7
No. young born per litter	6.00	6.50	5.71	6.89
No. live young born per litter	4.86	5.00	4.86	5.56
No. live young at 3rd. day per litter	4.75	4.36	4.57	5.11
Per cent young born alive	81.25	76.92	85.00	80.65
Per cent young alive at 3rd. day	79.17	67.03	80.00	74.19
Per cent of assigned young weaned	100.00	96.67	96.23	85.37
Ave. birth wt. gram per rat	6.21	6.26	6.15	6.24
Ave. 3rd day wt. gram per rat	7.42	7.48	7.50	7.61
Ave. 3rd day adj. wt. gram per rat*	7.35	7.60	7.47	7.37
Ave. first week wt. gram per rat	14.12	14.07	14.15	13.77
Ave. second week wt. gram per rat	24.41	23.00	24.50	24.74
Ave. weaning wt. gram per rat	38.06	36.99	41.22	42.51
Ave. gain weight gram per rat**	30.71	29.39	33.75	35.14

* No. of rats in a litter was adjusted to six in number at the third day

** Average weaning weight minus average third day litter weight.

TABLE XI

Analysis of Variance of the Weaning Weights of the Young of the Second Generation rats.

Sources	d.f.	Sum of squares	Mean of squares	F-value
Total	177	6,859.96	38.76	
Treatment	3	927.48	309.16	2.83
Litters in treatment	28	3,058.27	109.22	
Bet. sex in litters	28	941.73	33.63	
Bet. rats within litters	144	1,590.14	13.95	

Not significant

TABLE XII.

The Results of the Growth Experiment with Rats from Mothers Maintained on All Plant Protein Rations (average weekly gains in grams, adjusted values)

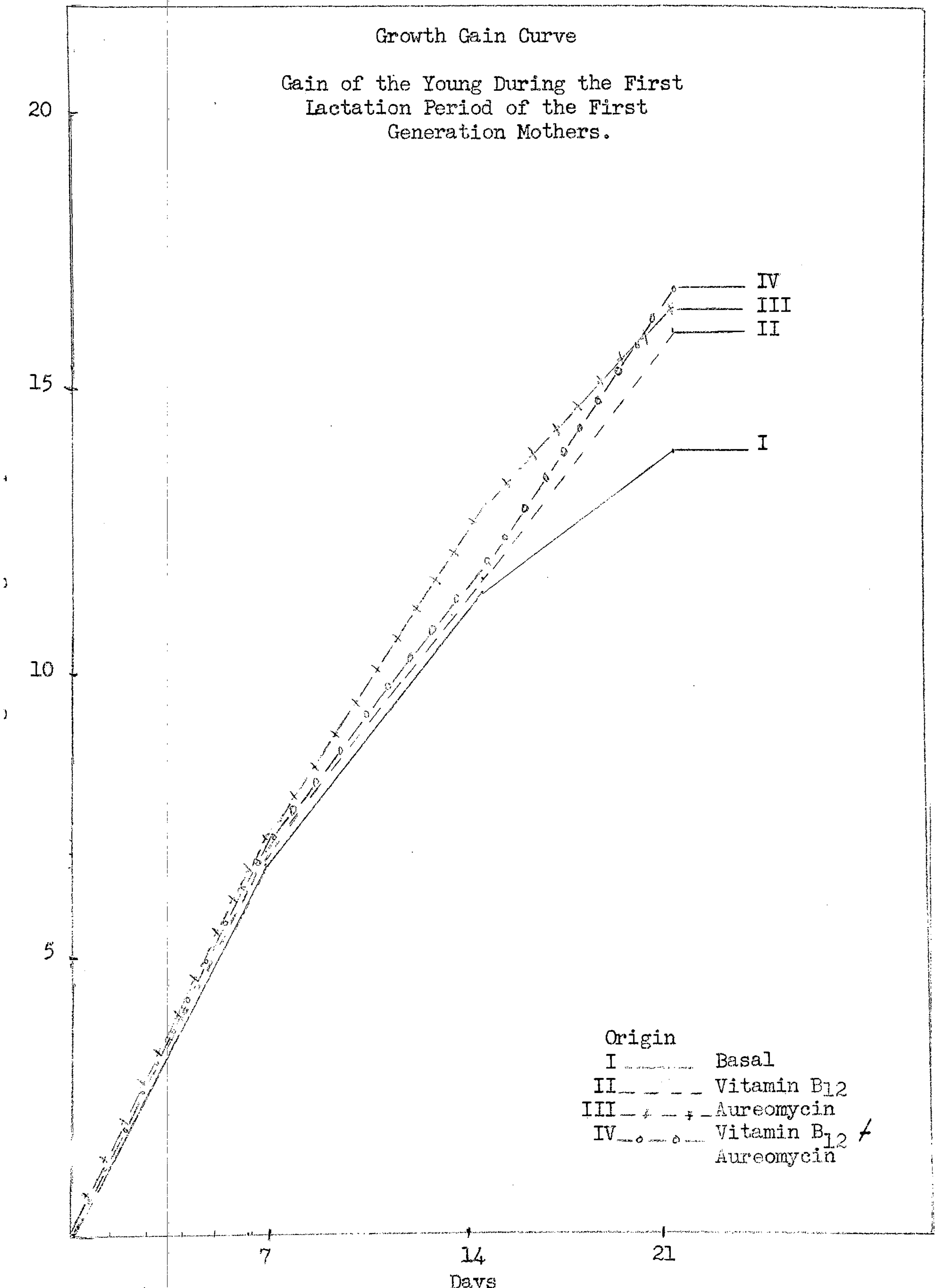
Lots	I	II	III	IV
Supplements	None	Vit. B_{12}	Aureo.	Vit. B_{12} + Aureo.
First week	19.65	19.37	20.71	21.79
Second week	30.02	31.24	35.71	35.69
Third week	32.36	28.94	34.00	28.35
Total Gain	82.03	79.55	90.42	85.83
Ave. daily gain per rat (grams)	3.91	3.79	4.31	4.09

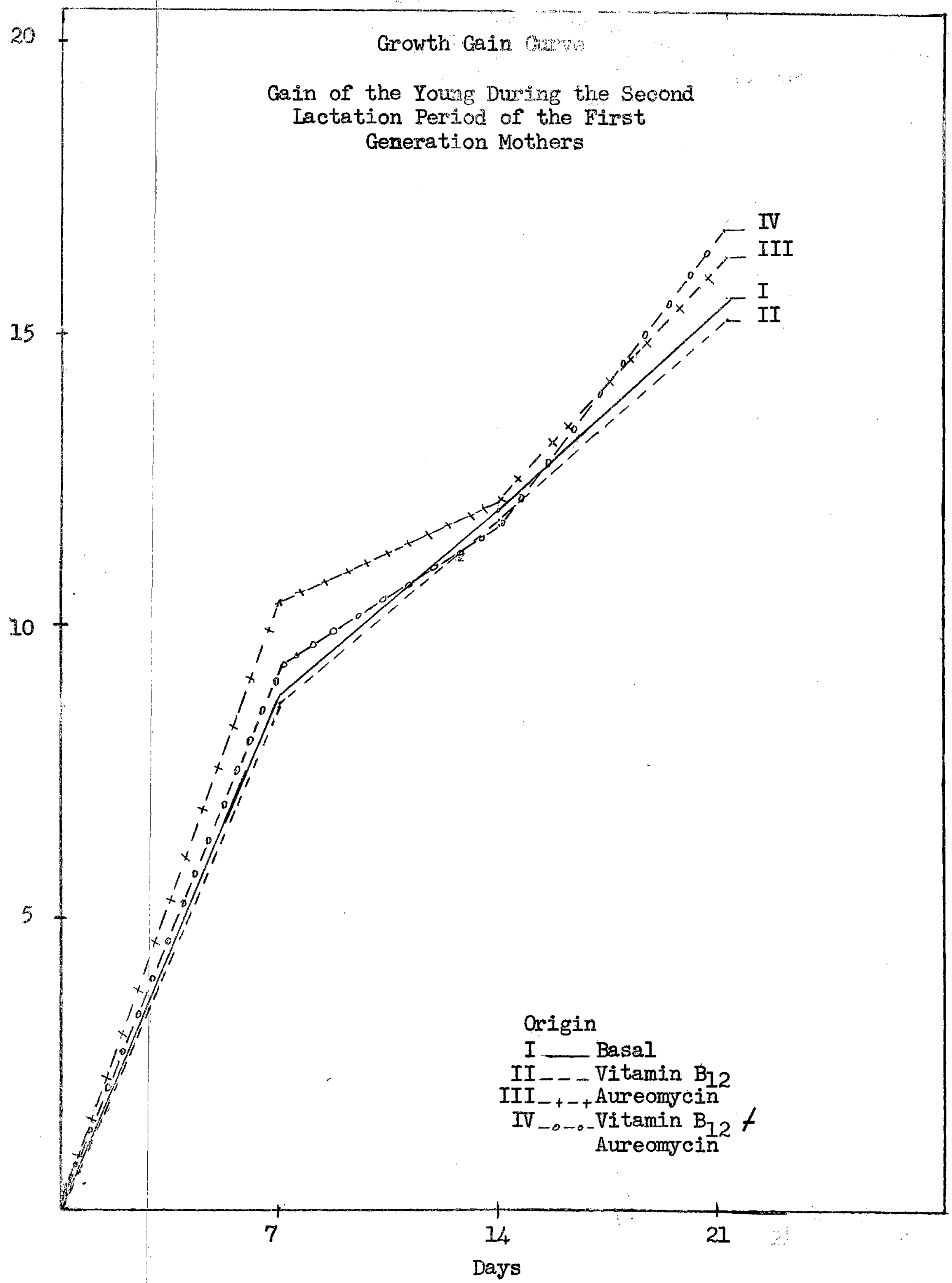
TABLE XIII.

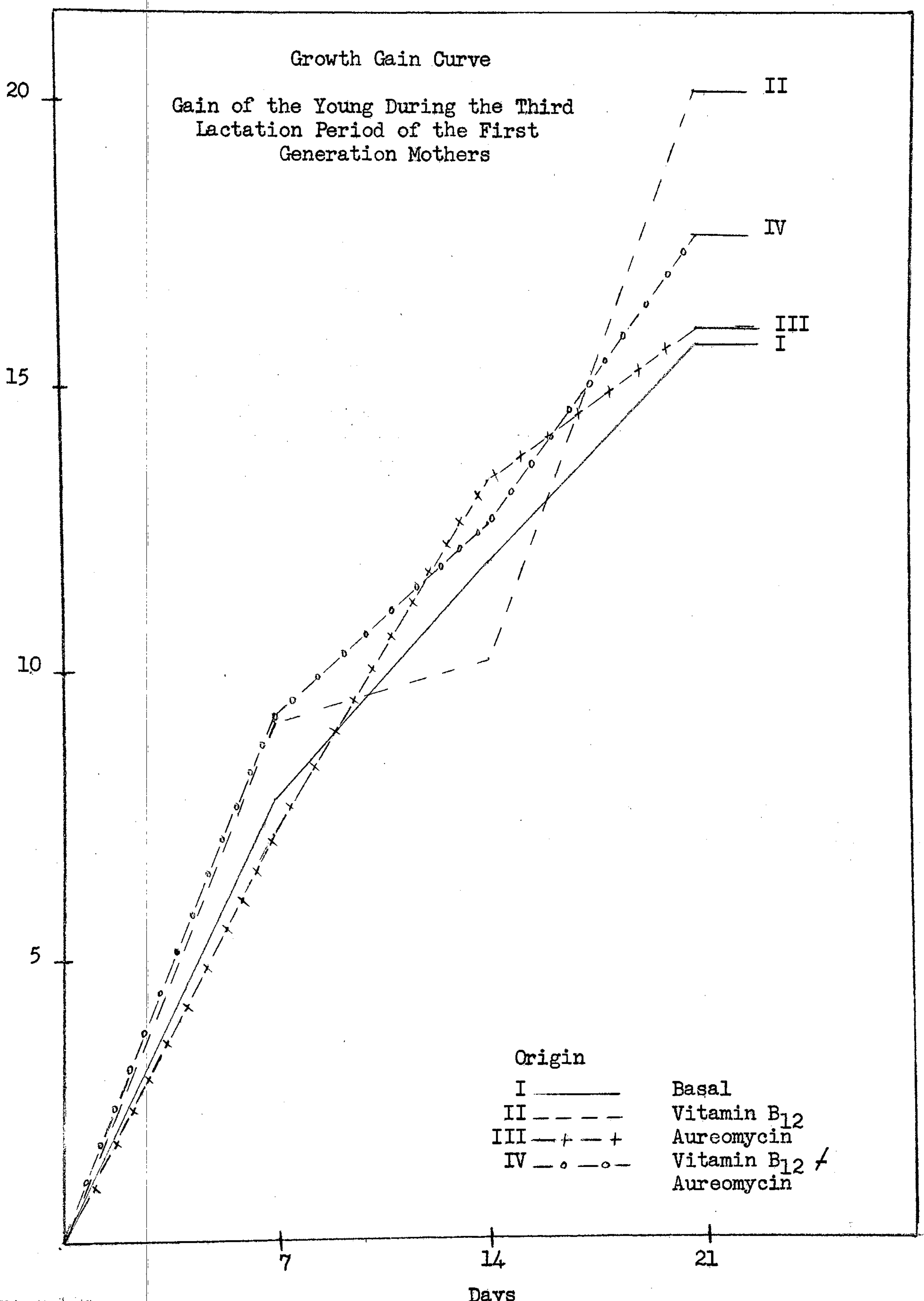
Analysis of Variance of the Weight Gains of the Rats in the Growth Trial

Sources	d.f.	Sum of squares	Mean of squares	F-value
Total	79	11,071.99	140.15	
Treatment	3	809.24	269.75	2.00
Error	76	10,262.75	135.04	

Not significant


Growth Gain Curve
Gain of the Young During the First Lactation Period of the First Generation Mothers.
20
15
10
5
7
14
21
Days
IV
III
II
I
Origin
I Basal
II Vitamin B12
III Aureomycin
IV Vitamin B12 + Aureomycin


Growth Gain Curve
Gain of the Young During the Second Lactation Period of the First Generation Mothers
20
15
10
5
IV
III
I
II
Origin
I Basal
II Vitamin B12
III Aureomycin
IV Vitamin B12 + Aureomycin
7
14
21
Days


Growth Gain Curve
Gain of the Young During the Third Lactation Period of the First Generation Mothers
20
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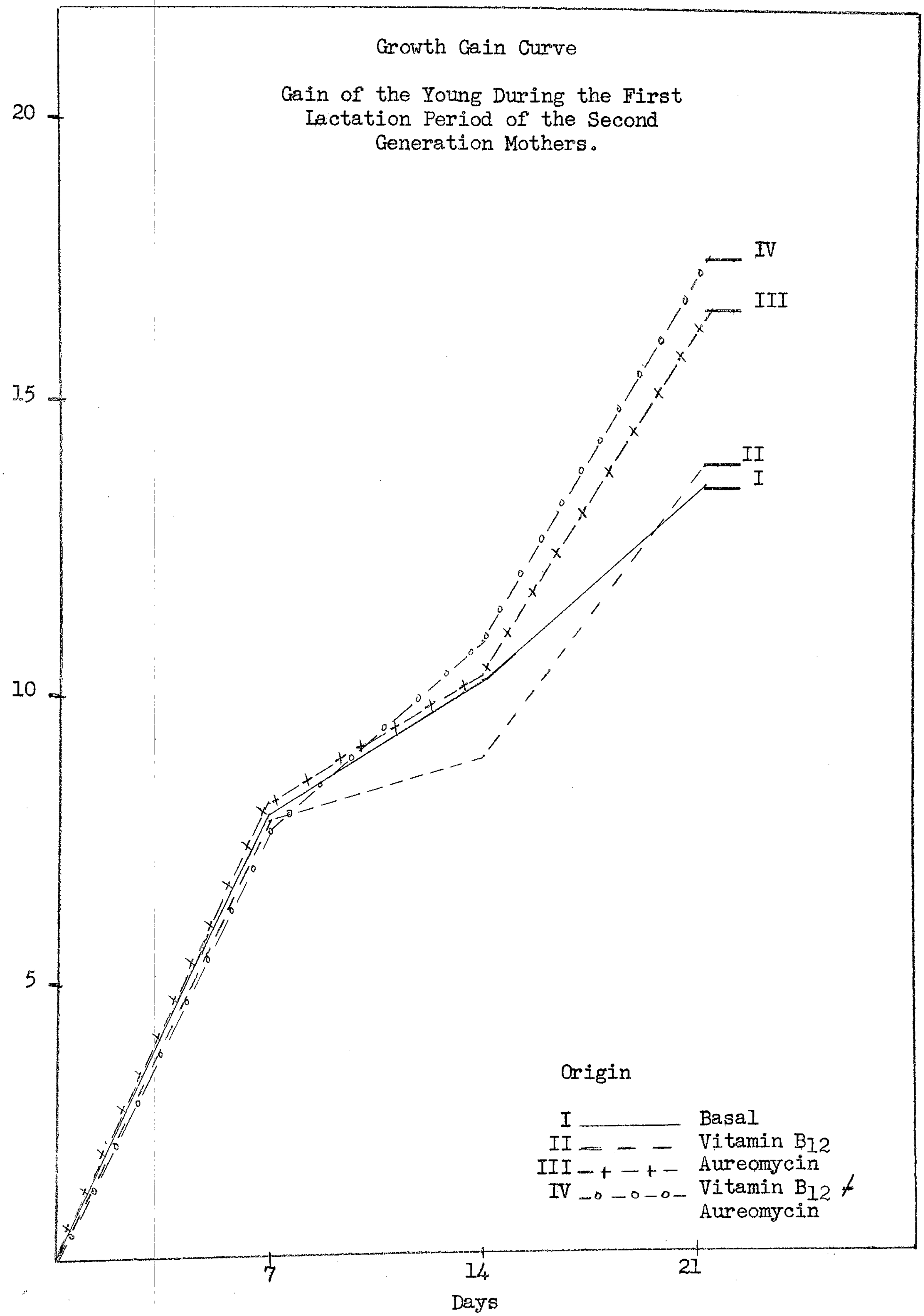
Origin
I Basal
II Vitamin B_{12}
III Aureomycin
IV Vitamin B_{12} + Aureomycin
7
14
21
Days


Growth Gain Curve
Gain of the Young During the First Lactation Period of the Second Generation Mothers.
20
15
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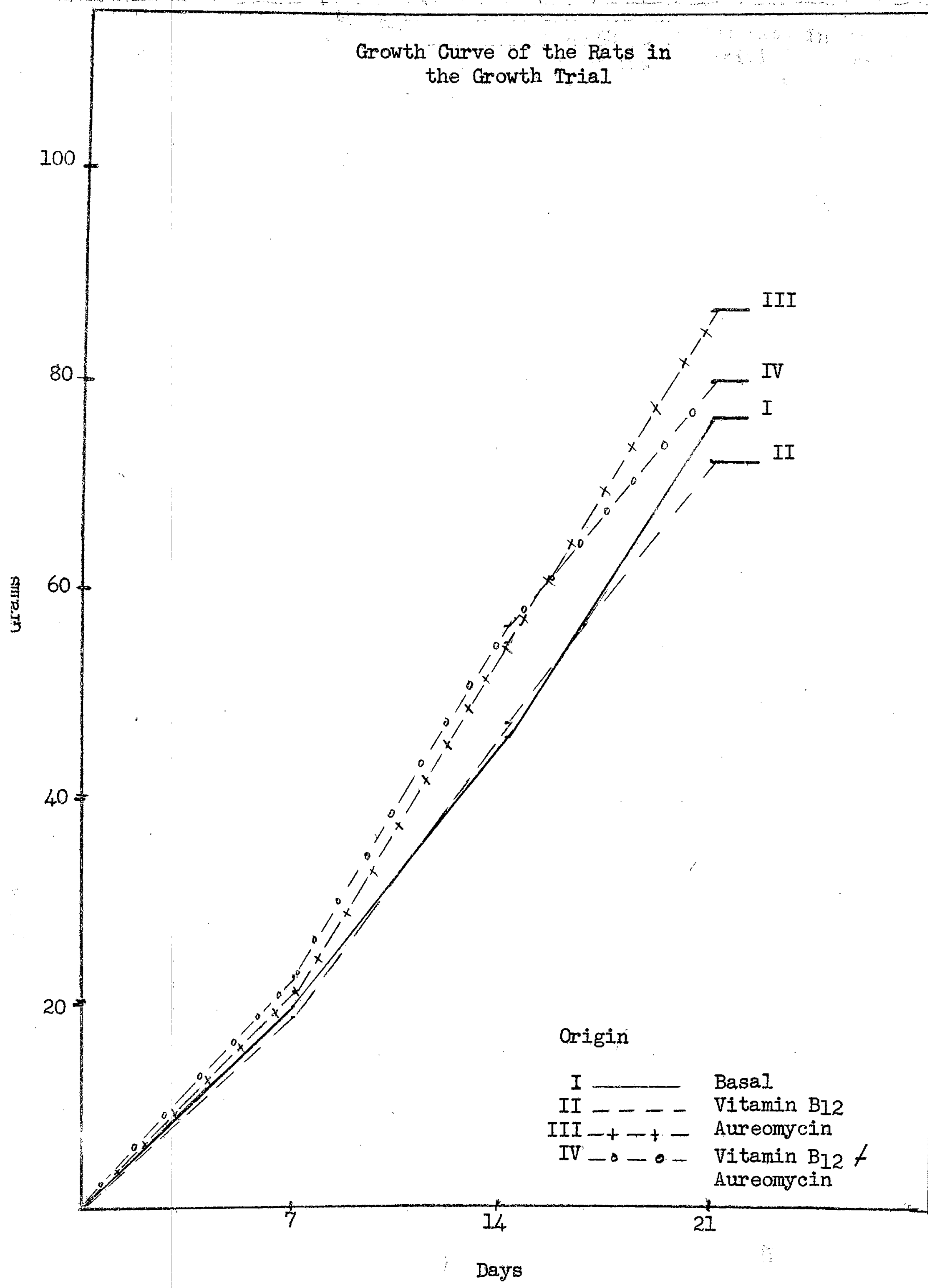
IV
III
II
I
7
14
21
Days
Origin
I Basal
II Vitamin B_{12}
III Aureomycin
IV Vitamin B_{12} + Aureomycin


Growth Curve of the Rats in the Growth Trial
100
80
60
40
20
Grams
7
14
21
Days
III
IV
I
II
Origin
I Basal
II Vitamin B12
III Aureomycin
IV Vitamin B12 + Aureomycin

DISCUSSION

The results of the gestation studies are in agreement with the work of Schultze and Briggs (1949), Watts, et al. (1950), and Baker (1951) in rats which showed that an all-plant ration will support normal gestation performance. This finding is in contradiction to the work of Ross, et al. (1942a) and VanLandingham and coworkers (1947). These workers failed to obtain normal gestation in rats fed all-plant rations. Resorptions or death from toxemia which was previously observed by Ross, et al. (1942a) was not encountered in this study. Expulsion of dead fetus and occurrences of hemorrhage (Spitzer and Phillips, 1946) and symptoms of uremia (Schultze, 1950b and Halverson, et al., 1950) were never observed in this study. If the viability of the young until the third day from birth is taken as a criteria for gestation performance, (80 per cent alive at the third day from birth), the basal ration supported normal gestation performance only in the first pregnancy period. Thereafter, through the third pregnancy period it was subnormal. Upon this basis the data indicate that the basal ration was deficient in vitamin B_{12}. The gestation performance in the vitamin B_{12}-lot was normal and successful throughout the three gestation-lactation periods, viability of the young up to the third day is more than 80 per cent. Or it can be ascribed to some unknown factor(s) essential for gestation since the vitamin B_{12} lot did not continue to have a normal gestation performance through the second generation rats. In no cases can it be attributed to failure of the young to obtain sufficient milk since lactation performance in all trials was normal.

The addition of vitamin B_{12} to the basal diet did not improve lactation performance except in the third gestation-lactation period. In this trial the differences in weaning weights between those rats whose mothers received the vitamin and those rats whose mother which did not receive the vitamin was highly significant ($P = 0.01$). The inconsistent lactation results obtained in this study corroborated mostly that the findings of other workers reported in the literature. In rats (Ross, et al., 1942b; Meyer, et al., 1951; Schultze, 1950a; and Dryden and associates, 1951) the addition of crystalline vitamin B_{12} and other supplements rich with vitamin B_{12} failed to stimulate lactation. Some investigators, Watts, et al. (1950), Baker (1951), Schultze and associates (1952) found that vitamin B_{12} added to an all-plant basal diet donsistently improved gestation and lactation performance of rats.

Aureomycin stimulated gestation performance through the second gestation and lactation period only, but its effect upon the lactation performance was very consistent throughout the entire experimental period. However, the differences in weaning weights between those rats whose mothers received aureomycin and those rats whose mothers did not receive the antibiotic was only significant in the second gestation--lactation period. The consistent improvement of lactation performance substantiated the work of Sure (1951) and Baker (1951) in rats and Heidebrecht, et al. (1951) and Carpenter (1951) in pigs. These workers obtained higher weanling weights from the addition of crystalline aureomycin or from the addition of APF supplements containing aureomycin and B_{12} to basal diets composed of material of plant origin.

Supplementing the basal diet with vitamin B_{12} and aureomycin combined stimulated higher weaning weights. Nevertheless, the increase in weaning weights was not significant as shown by the analysis of variance.

In the growth phase of the study, differences in body gains due to the influence of vitamin B_{12} and aureomycin in the basal diet were not statistically significant. This result corroborated the work of Schultze (1950a), Emerson, et al. (1949) in rats wherein they failed to attain greater growth stimulation by the administration of vitamin B_{12}, orally or by intramuscular injection. In premature infants, Downing (1950) was unable to obtain appreciable growth effect from intramuscular injection of vitamin B_{12} to babies who are clinically healthy. His finding is in contraditcion to the results obtained by Chow (1950). The latter stimulated growth of normal children, and children who are chronically ill by the administration of vitamin B_{12}. Sauberlich (1952) found that aureomycin or penicillin added to a basal ration which was fully fortified with known essential minerals and B-complex vitamins failed to stimulate further growth rate in rats. Linkswiller, et al. (1951) demonstrated that the increase in growth due to aureomycin supplementation to a pyridoxine-deficient diet will disappear when high level of pyridoxine is fed, more than 0.41 ug. per gram of diet. Luckey (1952) reported that workers at Lobund Institute of the University of Notre Dame obtained no growth response in germ-free chicks and poults by the addition of the basal diet with sulfasuxidine, streptomycin, bacitracin and chloromycetin. This finding was substantiated by Coates, et al. (1950) in England. They found no beneficial effect of penicillin upon the growth of chicks in place where no poultry has ever been kept before.

At present it is difficult to evaluate results obtained from the supplementation of vitamin B_{12} and/or aureomycin to all-plant rations as well as from the unsupplemented basal ration. Results obtained by other workers were variable, similar to the results attained in this study. Literature reviewed has shown that results vary with the health of the animal in question, balance of amino acids, level of protein, amounts of the B-vitamins, and the management employed. In view of these facts, further works are necessary to strengthen the information and knowledge in regards to the essentiality of vitamin B_{12} and aureomycin, and other antibiotics to animal nutrition.

SUMMARY

Reproduction and growth studies were conducted with rats to determine the effect of vitamin B_{12} and/or aureomycin when added to an all--plant ration. In the reproduction phase, young females were fed the diet during three reproductive cycles. Also second generation females from the first reproductive cycle were raised to maturity on the same rations, bred, and allowed to produce a single litter. The growth of second-generation young obtained from the third reproductive cycle was studied using the same rations.

The all-plant basal ration supported normal gestation only in the first reproductive cycle. The addition of the supplements to the basal ration supported normal gestation through the second reproductive cycle. Only vitamin B_{12} maintained normal gestation through the third reproductive cycle. None of the supplements supported normal gestation in the second generation of rats.

Satisfactory lactation performances were obtained in all lots during the first generation. Only the aureomycin and vitamin B_{12}-aureomycin fed lots gave normal lactation performances during the second generation. The addition of the supplements to the basal diet increased the weaning weights significantly during the second and third reproductive cycles.

In the growth phase of the experiment the addition of vitamin B_{12} and/or aureomycin did not stimulate further daily gains.

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