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Growth Rate of Calves and In Vitro Metabolism of Liver and Thymus Tissue as Affected by Antibiotics

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This bulletin reports on Department of Dairy Husbandry Research Project 55, Diet and Growth

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J. M. Buckalew and C. P. Merilan

INTRODUCTION

The use of antibiotics in commercial animal feeds is quite widespread despite the lack of adequate information concerning their varied effects on different animals and under different conditions. Among the questions which have arisen concerning the use of antibiotics in feeding is the means by which antibiotics produce accelerated growth in young animals. In general, the theories which have been advanced concerning the mode of action may be grouped under two headings:

1. *Antibiotics act in modifying the bacterial flora of the alimentary tract thereby affecting the over-all physiological processes of the animal.*
2. *Antibiotics exert a direct effect on the metabolism of certain body organs or tissues, thus influencing the animal's growth and development.*

Although little is actually known about the mode of action of antibiotics in producing the growth response, evidence available seems to indicate that changes in the alimentary bacterial flora and direct effects on body tissues may both occur.

Aureomycin* (chlorotetracycline) has been the antibiotic most commonly used in feeding trials with calves and this antibiotic is known to be effective against a wide range of bacteria. It has also been indicated that aureomycin may affect directly the metabolism of certain body tissues. Since this antibiotic is already widely available in commercial calf rations, it is imperative that its effects on the animal be carefully studied.

This study was undertaken in an effort to provide additional information on the physiological effects of aureomycin by observing, *in vitro,* its effects on the metabolic activity of certain body tissues.

*Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y., supplied the aureomycin used in these investigations.

REVIEW OF LITERATURE

Effects of Aureomycin on Growth and Development of Dairy Calves

Numerous papers published since 1950 have demonstrated that aureomycin produces a noticeable effect on the growth and development of dairy calves. Bartley, *et al.* (1950), described the response of young calves to an A.P.F. concentrate containing aureomycin. Eight calves receiving aureomycin gained an average of 30.8 pounds compared to an average gain of 18.0 pounds for four control calves. The incidence of scours was much less in the experimental group, indicating to the authors that the aureomycin enhanced growth by preventing scours in young animals.

Loosli and Wallace (1950) and Loosli, Wasserman and Gall (1951) reported that crystalline aureomycin significantly increased the rates of growth and reduced the incidence and severity of scours in young calves. Rusoff and co-workers (1951) reported that calves receiving aureomycin gained an average of 20 percent more than their controls over a 16-week experimental period starting when the calves were four days old. No differences were noted in the efficiency of feed utilization between the experimental and control groups; however, the antibiotic appeared to stimulate the appetite of calves on this experiment. The authors state that "a bacteriological study of the effect of aureomycin on the rumen flora of calves failed to reveal any changes in the usual microscopic appearance of the flora".

In observations by Rusoff, *et al.* (1954), a larger yield of meat and larger skeletons were produced by aureomycin supplemented calves than by control animals. Analysis of the rib sections indicated that the meat from the experimental calves contained 9 percent more fat than that from the controls. It is of interest to note that the intramuscular injection of 400 mg. of aureomycin weekly as an aluminum chloride complex in sesame oil, produced a growth response equal to or better than that obtained by the oral administration of 50 mg. of aureomycin daily. In a recent report, Pritchard, *et al.* (1955), stated that aureomycin increased the growth rate and improved the efficiency of feed utilization of young calves over an eight-week experimental period; however, a seven-day collection period at the end of the eight-week growth period showed practically no differences in the digestibility of dry matter, ash, protein, crude fibre, nitrogen free extract or fat when aureomycin was included in the ration. Identical twin calves were used as the experimental animals in this study. Over the past six years, many other papers have been presented describing the growth response of calves to aureomycin and this work has been reviewed by Braude, Kon and Porter (1953) and by Reid, Warner, and Loosli (1954).

Effects of Terramycin on Growth and Development of Dairy Calves

Terramycin, an antibiotic similar to aureomycin in chemical structure, has produced growth responses in some animals. The first study reported was by Cason and Voelker (1951) in which calves were fed terramycin at the rate of 15 and 30 mg. per 100 pounds body weight. At the end of eight weeks there was no growth response to these two amounts of terramycin.

In further studies by Voelker and Cason (1951), a slight growth response was obtained by feeding 30 mg. of terramycin per 100 pounds body weight. In a separate experiment, the amount of terramycin was increased to 100 mg. per 100 pounds body weight and a 28 percent increase in the rate of growth was observed. No differences in efficiency of feed utilization were noted. In a third experiment, terramycin also increased the rate of gain in older calves. Further evidence for the growth promoting effects of terramycin has been reported by Kesler and Knodt (1952), MacKay, *et al.* (1953), and Kesler (1954).

Although terramycin has been used in comparatively few studies with dairy calves, the evidence available indicates that it may be just as effective as aureomycin in producing a growth response.

Possible Modes of Action of Antibiotics in Producing Growth Response

The actual mechanism of antibiotics in producing a growth response in animals is still unknown. Many theories have been advanced to explain the mode of action but as yet, none of these theories have been confirmed. The general topic of mode of action has been reviewed in detail by Stokstad (1954). He states that the action of antibiotics in increasing growth is probably confined to its effect on bacteria within the intestinal tract of the animal. This belief is based on the following observations: 1. Antibiotics of widely varying chemical structure are effective. This seemingly precludes their being incorporated into any compound essential for the animal's growth. 2. The ineffectiveness of antibiotics in increasing growth in the germ-free animal as observed by Luckey (1952). 3. The ineffectiveness of aureomycin in increasing growth of the developing chick embryo reported by Jukes, *et al.* (1952). 4. The effect of sanitation on the magnitude of the growth response.

Coates, *et al.* (1951), reported that chicks kept in previously unused quarters showed no response to aureomycin supplementation while chicks of similar breeding kept in old "infected" quarters gave a growth response. The total gain in weight of chicks housed in the new quarters and of those housed in the old quarters which were given aureomycin was about the same, indicating that the action of the antibiotic consisted in preventing the inhibition of growth caused by some agent present in the old quarters. Many

other workers have since reported that keeping animals under highly sanitary conditions tends to lower the response to antibiotic supplementation; however, it is of interest to note that a recent report by Landagora, *et al.* (1955), states that calves housed in new, "uncontaminated" quarters showed a response to aureomycin feeding. Jersey and Holstein calves showed an increase in weight over the controls of 25 percent and 15 percent, respectively.

If the antibiotic acts directly upon bacteria in the intestinal tract, a number of possible modes of action may be listed: 1. Inhibition of bacteria which compete with the host for essential nutrients. 2. Inhibition of microorganisms which are deleterious because they produce toxic compounds or damage the intestinal tissues. 3. Increased bacterial synthesis of essential or stimulatory growth factors.

It is entirely possible that one if not all of these pathways may be utilized in producing the growth response in young animals. Although much evidence may be present to support the various theories as to the way in which antibiotics promote growth by modifying bacterial action, none of these theories have as yet been proven and the specific mode of action remains unknown.

Effects of Aureomycin on Liver Function and Metabolism

It has long been known that aureomycin, administered either orally or parenterally, is soon concentrated in the liver and the portal circulation. Herril and Heilman (1949) reported the presence of high concentrations of aureomycin in the hepatic system and bile following oral administration of the drug. Small amounts of aureomycin were also found in a number of tissues including the liver, kidney, spleen and lungs. Zaslow, Hewlett and Lorry (1950a) studied the concentration of aureomycin in the gall bladder contents of 25 patients given the antibiotic before cholecystectomy, and showed significant levels when the cystic duct was not obstructed. Zaslow and co-workers (1950b) also measured aureomycin excretion in bile drained by a T tube in eight patients immediately after cholecystectomy, and noted levels as high at one hour after injection as at three. Two patients had transient absence of aureomycin in the bile; one of these was jaundiced and the other had evidence of mild parenchymal liver damage. Jacob, *et al.* (1951), observed changes in the fecal flora of man and dogs during intravenous administration of aureomycin and postulated that the drug must be excreted into the intestinal tract via the bile and salivary glands.

In studies by Wright and Prigot (1951), bile levels of aureomycin following oral and parenteral administration were found to be 10 times higher than that of blood serum. According to these workers, the high concentration of aureomycin in the bile shows that the antibiotic is extracted by the liver from the blood and is excreted by the liver into the bile. With the bile,

it may then be reabsorbed from the intestinal tract. This may explain why the blood level of aureomycin is maintained relatively longer than most antibiotics.

In 1953, Cole made observations on the recovery of aureomycin from the intestinal tract following intravenous administration. These observations demonstrated that aureomycin rapidly appeared in the contents of the gastrointestinal tract following the injections. Levels obtained in the stomach and in the duodenum of one patient with complete common bile duct obstruction, did not indicate active concentration of the aureomycin in these secretions. The duodenal contents, on the other hand, exhibited aureomycin in high concentration even when partial biliary obstruction was present. The bile thus provided the most concentrated source of aureomycin for excretion into the intestinal tract when the antibiotic was given intravenously. Three patients who had parenchymal liver damage showed a lag in the development of peak levels of aureomycin in the duodenum. An interesting factor in this study was the observation that aureomycin appeared in the colon one and one-half to two hours after injection. To the author, this suggested an active secretion along the lower portion of the intestinal tract.

Considering the importance of the liver to the physiological processes of the body, it seems possible that some of the effects produced by aureomycin on the growth and development of animals might be due to its affecting the functions or metabolism of the liver. At present, aureomycin is commonly used in the treatment of liver diseases. This use stems from numerous reports of the effectiveness of the antibiotic in combating such hepatic diseases as cirrhosis and hepatitis.

Shaffer, *et al.* (1950a), reported beneficial effects from aureomycin therapy in cases of acute viral hepatitis in humans. These workers also reported in a later paper (1950b) the successful treatment of 13 cases of chronic hepatitis. Farquhar and co-workers (1950) studied the effect of aureomycin therapy in cases of hepatic coma. Four cases were treated with aureomycin given both orally and parenterally. In every case, recovery was rapid following treatment with aureomycin. In this report, the authors state that "the action of aureomycin in relieving hepatic coma could have occurred in the intestinal tract or by antibacterial action within the liver itself or possibly, within the circulatory system." They also suggest the possibility that aureomycin may have some direct effect upon the liver.

Rumball, *et al.* (1950), published a case report on the treatment of hepatic cirrhosis with aureomycin. Beneficial results were obtained and it was suggested that aureomycin might have some non-specific anti-viral properties enabling it to be effective in treating such cases.

The nutritional importance of aureomycin in preventing liver malfunctions was indicated by Gyorgy and co-workers (1950) who described the prevention of experimental hepatic necrosis in rats by aureomycin. The

disease was produced by a diet consisting principally of corn starch and high amounts of a special yeast and could be prevented by cystine, methionine or vitamin E. Twenty-five mg. of aureomycin daily (4000 ppm. in the diet) greatly increased survival time and rate of gain. Other antibiotics including streptomycin, polymyxin, chloromycetin, penicillin and terramycin were only partially as effective as aureomycin.

In further studies by Gyorgy, *et al.* (1951), it was found that a delay in, rather than the prevention of hepatic necrosis produced by the starch yeast diet was obtained with aureomycin and, to a lesser extent, with terramycin and streptomycin. The authors postulated that necrosis eventually appeared in the rats receiving aureomycin because organisms later reappeared in the intestinal tract which were resistant to aureomycin. Additional support for such a view comes from the observation that liver necrosis occurs mainly in the left lobe of the rat liver which derives its portal circulation from the large intestine and the stomach. The right lobe which is relatively free of necrosis derives its portal circulation largely from the small intestine. The temporary effect of aureomycin on the prevention of hepatic necrosis may therefore be contrasted with its effects on growth which are commonly maintained throughout the growing period of animals.

Goldbloom and Steigmann (1951) have also demonstrated the ability of aureomycin to delay hepatic necrosis. These workers have advanced the theory that aureomycin acts on the intestinal flora when given orally and thus prevents the production of toxic substances which under normal conditions, are destroyed by the liver but which in liver disease accumulate and finally produce the toxic changes characteristic of hepatic insufficiency. In this experiment, response of hepatic insufficiency to aureomycin therapy did not occur until several days after the start of treatment indicating to the workers that the production of toxins in the intestinal tract must be lessened before recovery could begin.

However, Luckey, *et al.* (1954), reported that rats on a necrogenic diet grown under germ-free conditions developed liver necrosis when their feed consumption was restricted to that of the controls raised under ordinary conditions. The necrosis did not develop when the germ-free animals were fed ad libitum. The observation of the development of necrosis in germ-free animals does not support the theory that the intestinal bacteria are the sole cause of necrosis. The indications are that even if the toxin production of intestinal bacteria is a major cause of necrosis, other mechanisms must be involved.

In addition to its effects in delaying necrosis of the liver, aureomycin and some other antibiotics are believed to have a lipotropic effect when present in certain diets. Gyorgy (1952) demonstrated that aureomycin exerted a lipotropic effect, decreased cirrhosis, and increased the growth rate of rats on a high fat, low protein, choline-free diet. Methionine exerted a similar lipotropic effect.

A possible mechanism for the lipotropic effects of aureomycin was suggested by the observations of de la Huerga and Popper (1951, 1952) who found that in dogs the antibiotic reduced the urinary excretion of trimethylamine which followed oral dosage with large amounts of choline. The theory presented was that choline was to a considerable extent broken down to trimethylamine by bacteria in the intestine and that this action was prevented by aureomycin. The lipotropic effect of aureomycin observed by Gyorgy (1952) cannot be accounted for by a decreased choline destruction since choline-free diets were being fed. However, a reduction of the bacterial destruction of other lipotropic agents may be involved. It is of interest to note that Baxter and Campbell (1952) found that aureomycin fed at the rate of 5 gm. per kg. of diet had a protective effect against the renal lesions produced by choline deficiency in rats on high fat, low choline diets.

Further evidence of a lipotropic effect of aureomycin was provided by Kaplan, *et al.* (1953), who studied the effects of the antibiotic on dogs with ligated pancreatic ducts. These animals retain the endocrine secretion of the pancrease while losing the "anti-fatty liver" factor which is present in pancreatic juice and which may be replaced by free choline or methionine. Aureomycin was given in doses of 0.75 or 1.0 gm. daily and its administration was accompanied by a restoration of the normal blood patterns as shown by the serum total and ester cholesterol levels; this change was paralleled by an increase in phospholipids while the alkaline phosphatase activity decreased. These changes are characteristically produced in animals by lipotropic substances such as choline. The changes with aureomycin reached a peak in three or four weeks and then retrogressed to a plateau. This effect was similar to the effect on necrosis observed by Gyorgy (1952). In one animal, vitamin B_{12} and folic acid were administered after the plateau had been reached and lipotropic response was obtained; however, no such response was obtained when these vitamins were given to dogs not receiving aureomycin. In contrast to the results of de la Huerga and Popper (1951, 1952), Kaplan, *et al.* (1953) noted that aureomycin produced no consistent depression of choline destruction as measured by trimethylamine.

More recent evidence of the lipotropic effect of aureomycin has been presented by Seto and Lepper (1954) who demonstrated the effect while studying the action of various antibiotics on hepatic fat content.

At present, little evidence is available to indicate whether or not aureomycin has an effect on the metabolism of liver tissue. Loomis and Lipmann (1948, 1949) observed that low concentrations of aureomycin inhibited phosphorylation in kidney homogenates. Respiration was not affected by aureomycin in these concentrations. In this action, aureomycin resembles dinitrophenol, atabrine, gramicidin, methylene blue and certain other compounds. Penicillin, chloromycetin and sulfadiazine were inactive when tested in a similar fashion. Loomis (1950) also observed that aureomycin inhibited

aerobic phosphorylation in mitochondria. While phosphorylation was severely inhibited, oxygen uptake was not influenced by the aureomycin in this experiment.

In 1950, Van Meter and Oleson reported on the effect of aureomycin on respiration of normal rat liver homogenates. They observed a definite inhibition of respiration in the homogenates and the rate of this inhibition was influenced by the amount of citrate in the medium. In the absence of citrate, aureomycin produced a rapid decline in respiratory activity. These workers suggest that aureomycin may act by blocking some part of the Krebs Cycle.

Brody and Bain (1951) observed in rat liver and brain tissue that in low concentrations, aureomycin uncoupled phosphorylation from oxidation while in higher concentrations it inhibited both oxidation and phosphorylation. Terramycin did not give this effect under the conditions of this study.

Effects of Aureomycin on the Thymus

A large number of workers have studied the relationship between aureomycin and liver function but little information is available on the relationship between aureomycin and thymus function or metabolism. Meites and Ogle (1951) and Meites (1951) observed that aureomycin or vitamin B_{12} partially protected the thymus against atrophy caused by cortisone injections in rats. A combination of aureomycin and vitamin B_{12} was more effective in preventing thymus atrophy than either one used separately. The corresponding atrophy of the adrenal gland induced by cortisone injections was not affected by the aureomycin, indicating that the action on the thymus was a direct one. The loss of hair and inhibition of growth caused by the cortisone were also prevented by aureomycin.

In further studies by Meites (1952a), it was again demonstrated that aureomycin could prevent cortisone induced atrophy of the thymus without affecting the accompanying atrophy of the adrenals. The author states that "it is of interest that increased food intake, testosterone and growth hormone have all been observed to protect the body against the catabolic actions of A.C.T.H. and cortisone in a manner similar to that reported here for B_{12} and aureomycin. It seems possible that the factor which all of these have in common is their ability to enhance protein retention in the body."

Meites (1952b) also reported that antibiotics did not appear to influence the normal effects of diethylstilbestrol, thyroprotein, thiouracil or cortisone in the body. However, the protection of the thymus from cortisone was an exception.

Rusoff, Landagora, and Hester (1954) observed that oral or intramuscular administration of aureomycin to calves resulted in an increase in thymus weight out of proportion to the accompanying increase in body weight.

MATERIALS AND METHODS

Preliminary Growth Study

This study was designed to show the effect of aureomycin and terramycin on the growth of calves from four days to eight weeks of age. Eighteen heifer calves were divided into 3 groups of 6 calves each. Birth weight of the calves served as the basis for grouping. The calves were fed whole milk at the rate of 1 pound milk per 10 pounds of body weight until they were four weeks old, at which time they were changed over to skim milk fed at the rate of 1 pound per 10 pounds body weight to a maximum of 14 pounds daily. An 18 percent protein calf starter was fed ad libitum and green leafy alfalfa or lespedeza hay was always available. In addition to the basal ration, the calves were supplemented as follows: Group No. I, control; No. II, 80 mg. aureomycin given daily by capsule; and No. III, 80 mg. of terramycin given daily by capsule. Daily observations were made on incidence of scours and weight and height at the withers were recorded weekly.

Manometric Studies

The effect of aureomycin on tissue metabolic activity was studied with liver and thymus tissue from rats and cattle. Rats served as the source of liver tissue for the preliminary manometric determinations; whereas procedures for thymus study were developed on tissue from young dogs, since in this species the thymus is easily removed and is large enough to provide adequate amounts of tissue. Final determinations were made on tissue from one mature cow and three calves of different ages.

In some cases it was necessary to store tissue slices before they could be used. These slices were stored in a solution consisting of 10 percent bovine blood serum and 90 percent modified Tyrode's solution containing 0.5 percent glucose. Each slice of tissue to be stored was placed in a sterile 125 ml. Erhlenmeyer flask containing 3 ml. of the storage solution. Then the flasks were tightly sealed with a rubber stopper and stored at 4-5° C. until the tissue was used.

Modified Tyrode's Solution

Na Cl	8.00 g.
K Cl	0.20 g.
$Ca\ Cl_2$ (anhyrous)	0.20 g.
$Mg\ Cl_2$	0.10 g.
$Na\ H_2PO_4$	0.05 g.
Glucose	5.00 g.
$NaH\ CO_3$	0.70 g.

In the order listed, each of the first six compounds are weighed and added separately (stirring until each is dissolved before adding the next one)

to 950 ml. of redistilled water in a 2-liter Erhlenmeyer flask. The sodium bicarbonate is prepared separately in a 250 ml. Erhlenmeyer flask by adding 0.7 g. of sodium bicarbonate to 150 ml. of distilled water. Both solutions are then autoclaved at 15 pounds pressure for 30 minutes and allowed to cool in the autoclave. After cooling, enough of the sodium bicarbonate solution is added to the salt solution to give a pH of 7.4. The flask containing the final solution is sealed with a sterile rubber stopper and paper cap and stored at 0-5° C., as described by Cameron (1950). The bovine blood serum was added just prior to use of the storage solution.

The manometric determinations were made using a rectangular Warburg apparatus accommodating 14 flasks per experimental run and adjusted for a shaking speed of approximately 110 strokes per minute. All determinations were made on tissue slices prepared by removing the tissue from the animal immediately after slaughter and then slicing, by means of a Stadie-Riggs tissue slicer, into sections about 0.5 mm. thick and weighing approximately 100 mg. The tissue slices were then placed immediately either in the modified Tyrode storage solution or in Warburg reaction flasks for the manometric determinations.

The respiratory rate of the tissues was measured by using the direct method for determining oxygen uptake in Krebs-Ringer phosphate buffer plus 0.5 percent glucose with air as the gas phase (Umbreit, *et al.,* 1949).

The rate of anaerobic glycolysis was determined by measuring the release of carbon dioxide from Krebs-Ringer bicarbonate buffer plus 0.5 percent glucose (Umbreit, *et al.,* 1949). Prior to the anaerobic determinations, all manometers and reaction flasks were gassed with a 95 percent nitrogen and 5 percent carbon dioxide gas mixture, using a water vacuum pump and manifold gassing arrangement. The flasks and manometers were alternately evacuated and filled with the gas mixture a minimum of 10 times to insure replacement of the original atmosphere by the nitrogen-carbon dioxide mixture.

The various levels of aureomycin to be studied were dissolved in the appropriate buffer (phosphate or bicarbonate) and placed in the reaction vessel sidearms before placing the tissue slices in the flask. Control flask sidearms contained equivalent amounts of buffer without aureomycin.

After placing the flasks in the 38° C.bath, they were allowed to equilibrate for 15 minutes. Following equilibration, four manometer readings were taken at five-minute intervals to determine a base metabolic rate for each flask. The sidearms were then tipped and readings taken at 15 minute intervals for the duration of the experimental run.

Tissue slices from all reaction flasks were removed at the end of the run, extracted for 24 hours with acetone and then dried for 24 hours at 100° C. The slices were then weighed and tissue activity calculated on the basis of dry weight.

OBSERVATIONS AND RESULTS

Results of the preliminary growth study, summarized in Table 1 and Figure 1, indicate that aureomycin significantly increased the growth rate of calves from birth to eight weeks of age. Calves receiving 80 mg. of crystalline aureomycin daily gained an average of 24 percent more than the control animals during the experimental period. Both control and experimental animals were healthy and no differences were noted in general appearance between the two groups.

Although the average gain in weight for the calves receiving terramycin was 9 percent higher than for the controls, this difference was not significant at p.05.

While supplementation of the diet with antibiotics resulted in an increased gain in weight during the experiment, no differences could be noted in height at the withers between the supplemented and the unsupplemented groups. According to Reid, *et al.* (1954), this is not an uncommon occurrence in antibiotic feeding studies.

The incidence of scours was extremely low in both control and experimental animals during the study. Observed cases of scours were all of short duration and did not appear to affect the growth and well being of the animals.

TABLE 1 -- EFFECT OF ANTIBIOTIC SUPPLEMENTATION ON HOLSTEIN HEIFER CALVES FROM BIRTH TO EIGHT WEEKS OF AGE

Treatment	Avg. Total Increase in Weight Lb.	Avg. Total Increase In Height at Withers cm.	Avg. Incidence of Scouring Days
Control	54.1	8.2	2.0
Aureomycin (80 mg. daily)	67.1	8.1	1.5
Terramycin (80 mg. daily)	59.0	8.4	1.8

Effect of Aureomycin on Metabolism of Rat Liver

Under the conditions of this experiment, aureomycin did not appear to affect the uptake of oxygen by rat liver slices over a two and one-half hour experimental period. Considerable variation was noted among the individual slices studied but the average oxygen uptake of the liver tissue exposed to aureomycin tended to coincide closely with the average uptake of the control tissue. Varying the levels of aureomycin from 3.5 to 10.5 micrograms per microliter of basal activity did not appear to produce change in respiratory activity of the tissue used in this study.

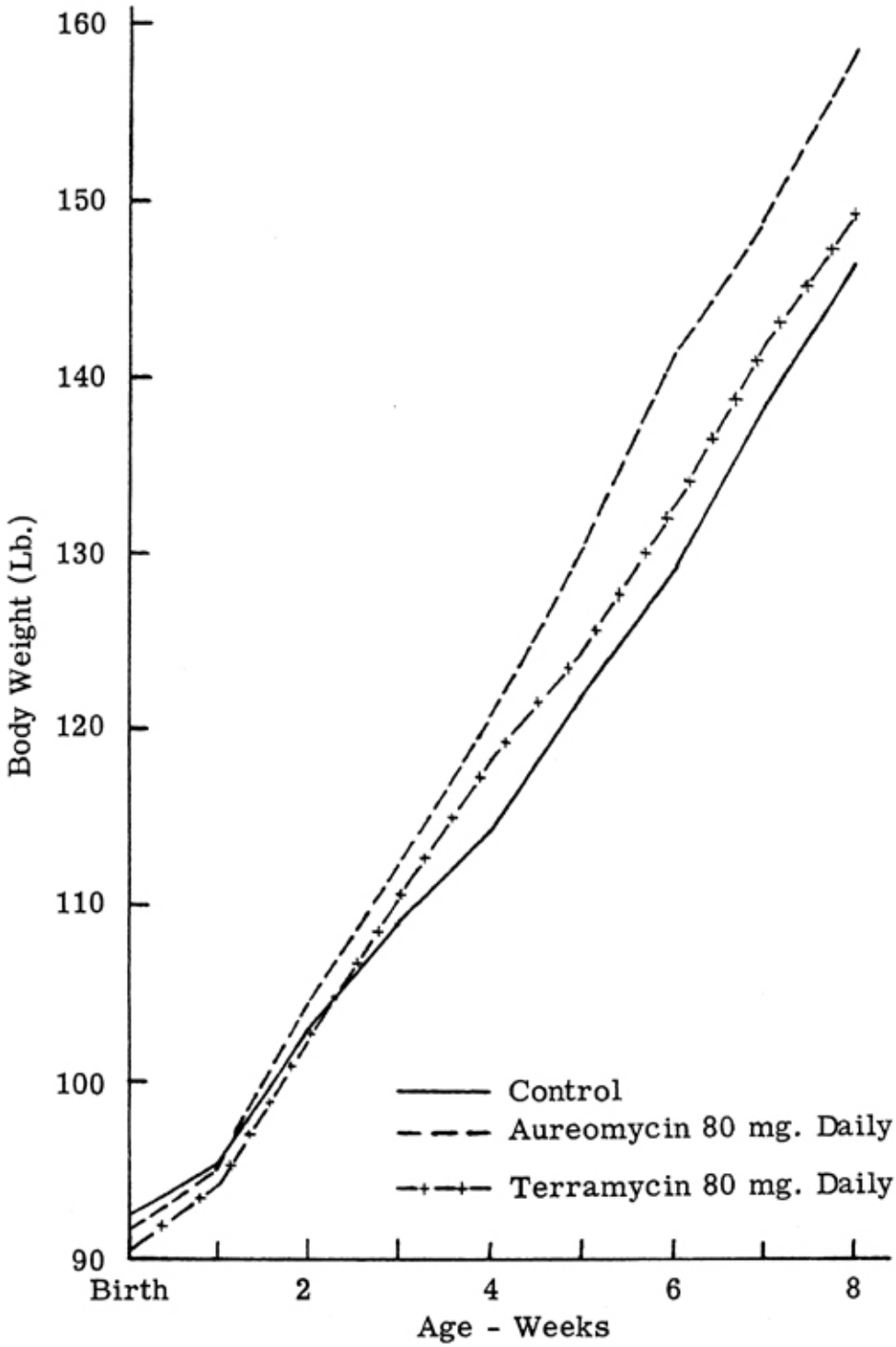


Fig. 1—Effect of Aureomycin and Terramycin Supplementation on Body Weight of Holstein Heifer Calves.

Under anaerobic conditions, concentrations of aureomycin ranging from 1.5 to 2.5 micrograms per microliter of basal activity appeared to have an inhibitory effect on the metabolic rate of liver tissue. When levels above 2.5 micrograms were used, the metabolic rate closely approached that of the controls as shown in Figure 2. No apparent inhibitory effect could be noted when levels of from 2.5 to 5.5 micrograms of aureomycin were used.

Effect of Aureomycin on Metabolism of Bovine Liver

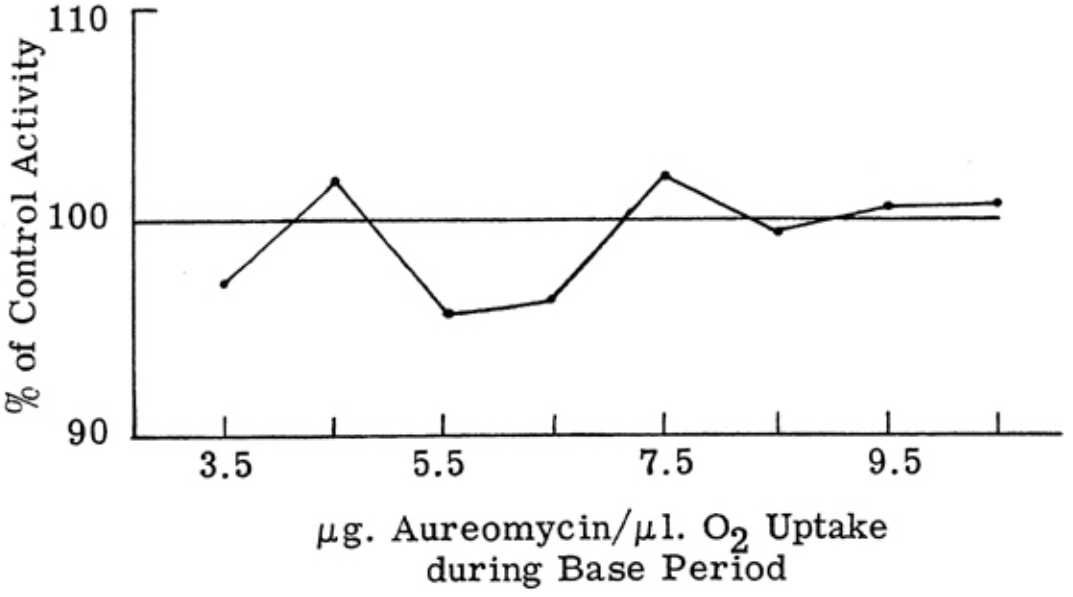
Concentrations of aureomycin ranging from 2.5 to 6.0 micrograms per microliter of basal activity did not appear to affect the oxygen uptake of bovine liver slices as shown in Figure 3. Average metabolic activity of the tissue exposed to aureomycin ranged from 93.4 percent to 103.6 percent of the average control activity and showed no relation to the experimental levels of aureomycin. Insufficient data were available to determine the effects of concentrations of aureomycin below 2.5 micrograms per microliter basal activity.

Aureomycin concentrations below 6.0 micrograms per microliter of basal activity appeared to depress anaerobic glycolysis (Figure 3) but concentrations ranging from 6.0 to 12.0 micrograms appeared to have a stimulatory effect on apparent carbon dioxide production by the tissue. The control tissue slices produced an average of 3.81 microliters of CO_2 per mg. of dry tissue per hour, while tissue subjected to concentrations of aureomycin averaging 3.4 and 4.5 micrograms produced 3.47 and 3.43 microliters of CO_2 per mg. of dry tissue per hour, respectively, and concentrations of aureomycin averaging 7.4, 9.5 and 11.8 micrograms resulted in the production of 4.33, 4.58 and 4.97 microliters of CO_2 per mg. of dry tissue per hour.

It would appear from these results that low concentrations of aureomycin inhibit anaerobic metabolism whereas higher concentrations seem to stimulate the anaerobic activity of bovine liver slices as measured by the amount of CO_2 released under anaerobic conditions.

Effect of Aureomycin on Metabolism of Bovine Thymus

As indicated in Figure 4, concentrations of aureomycin used in this experiment appeared to have neither a stimulatory nor an inhibitory effect on the metabolism of the thymus tissue. Although reports by Meites, *et al.* (1951, 1952a, 1952b), have indicated an *in vivo* effect of aureomycin on the thymus and have suggested the possibility of a direct effect on thymus metabolism, no *in vitro* effect could be demonstrated in this experiment.



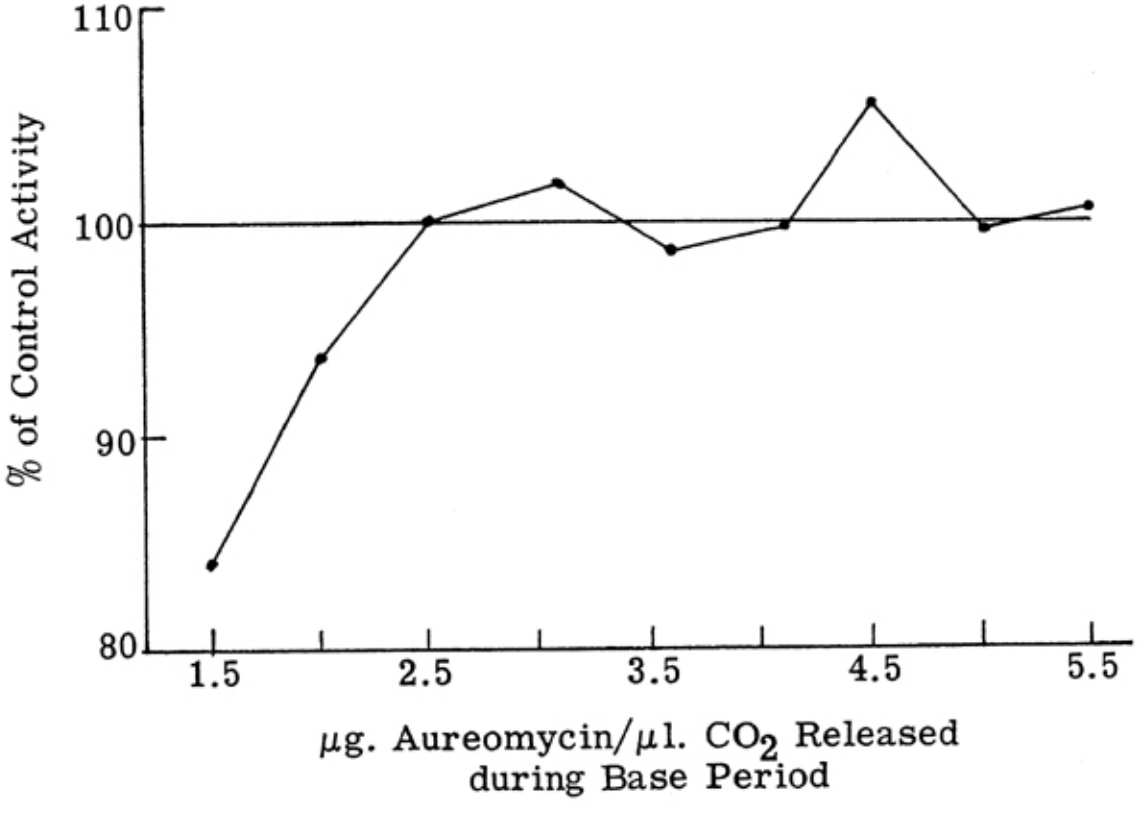


Fig. 2—Effect of aureomycin on the *in vitro* metabolism of rat liver.

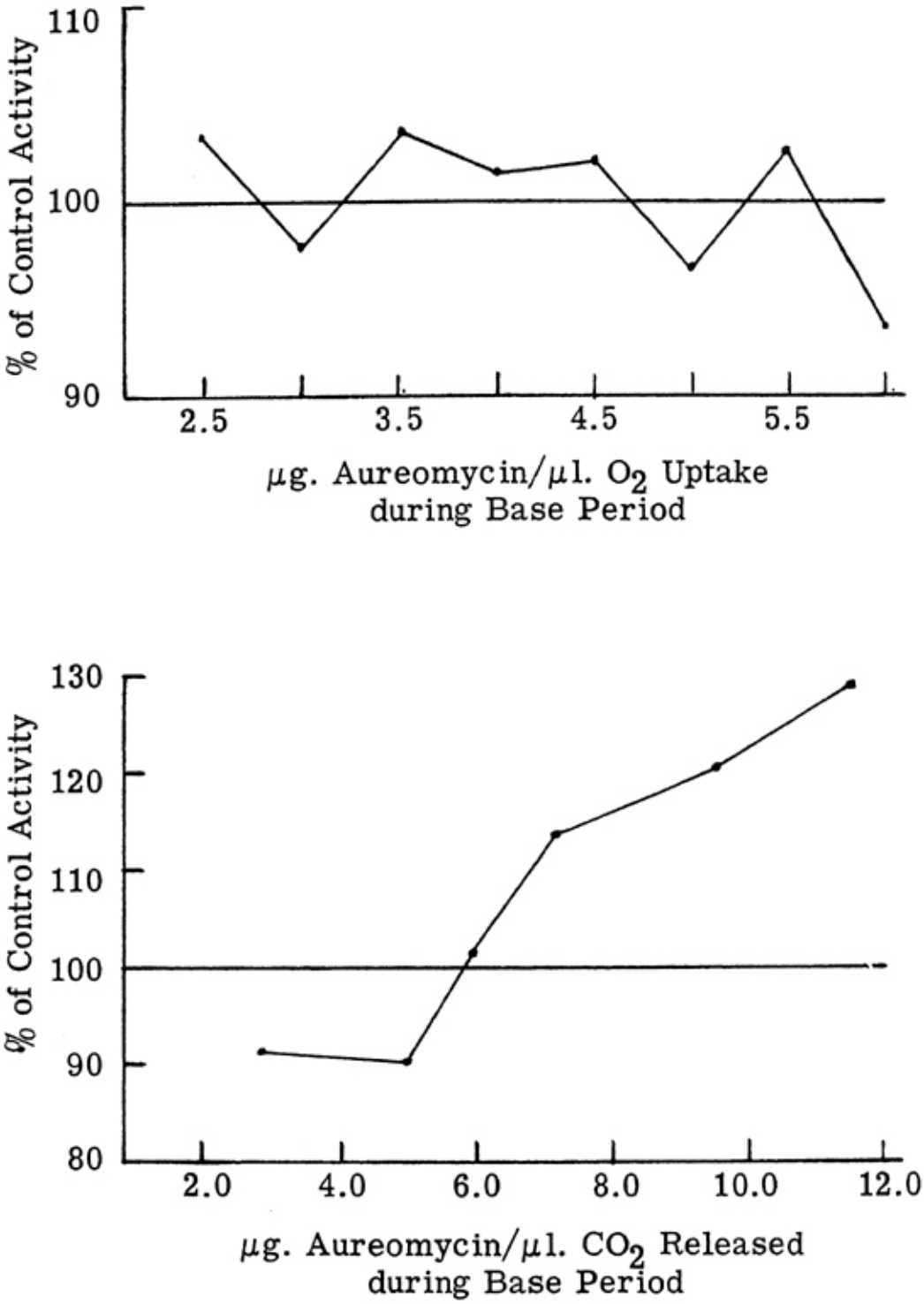


Fig. 3—Effect of aureomycin on the *in vitro* metabolism of bovine liver.

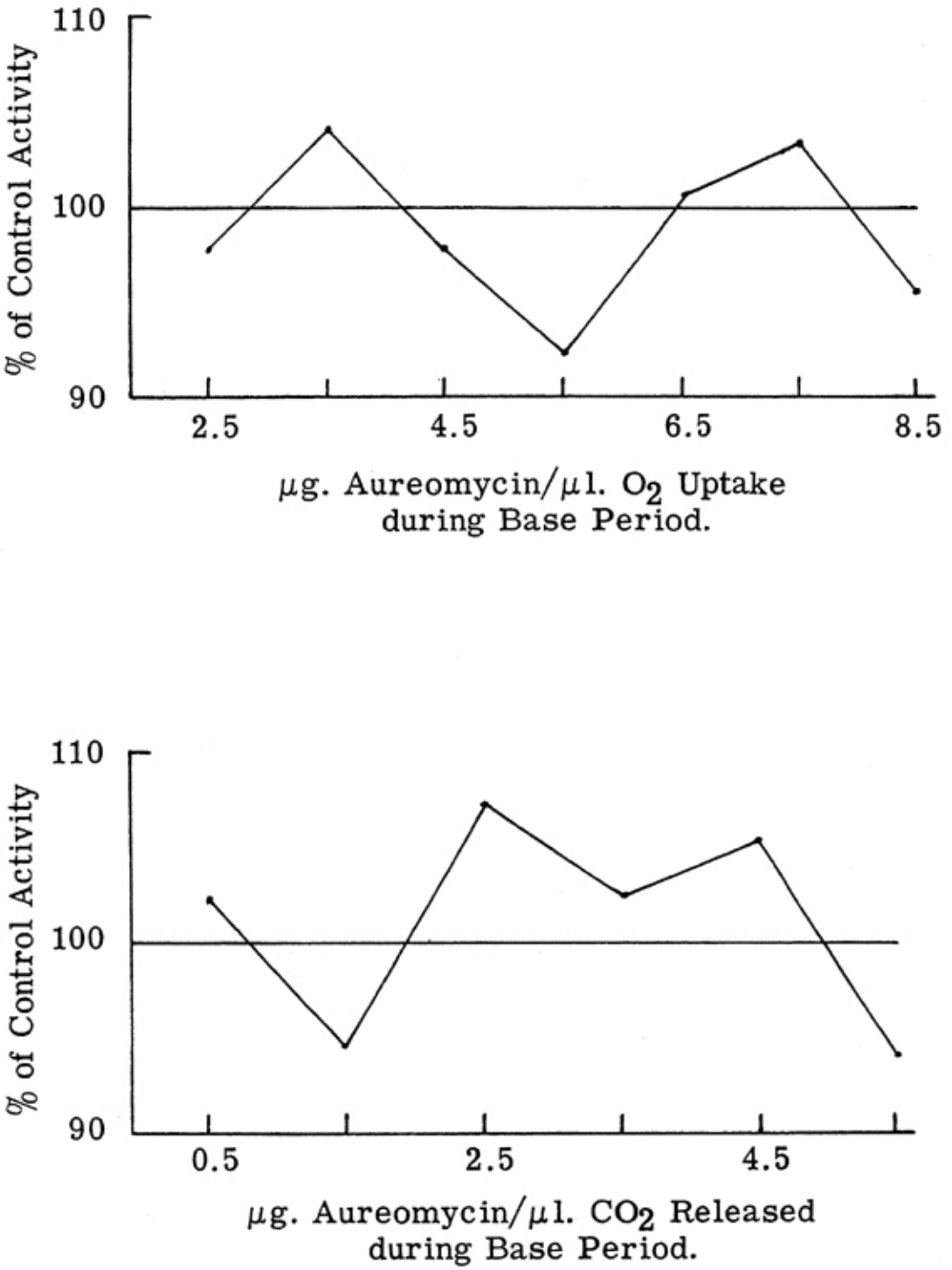


Fig. 4—Effect of aureomycin on the *in vitro* metabolism of bovine thymus.

Oxygen uptake by thymus tissue subjected to aureomycin showed no great average variation from the uptake of the controls and appeared to be independent of the concentration of aureomycin employed.

Anaerobic metabolic rate of the aureomycin-treated thymus tissue also tended to coincide closely with the metabolic rate of the controls and, like the aerobic metabolism, appeared to be unaffected by varying concentrations of the antibiotic.

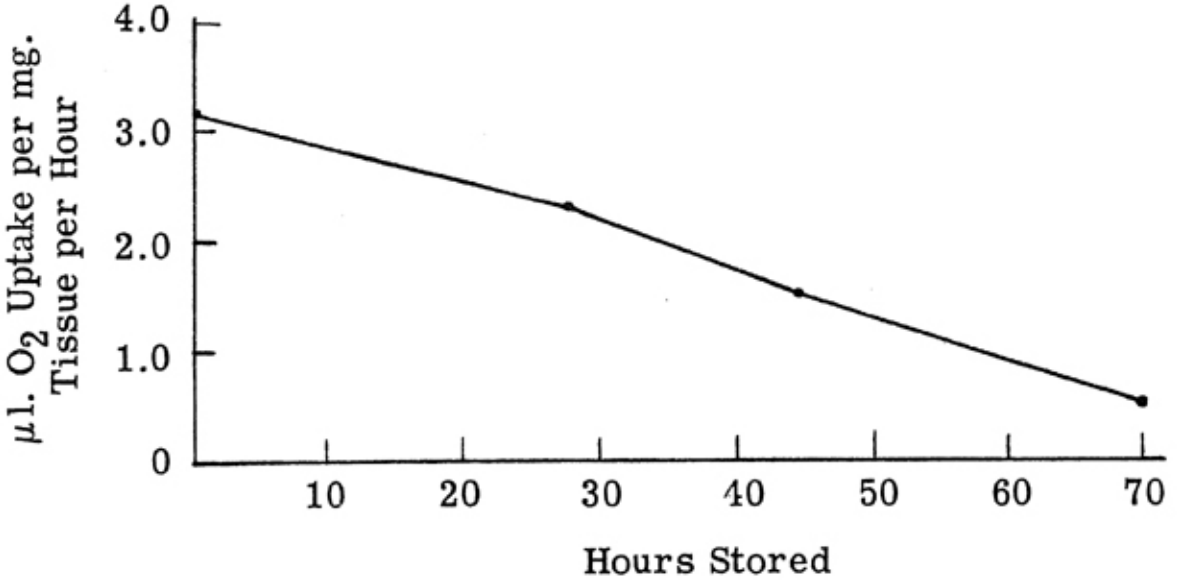
The difficulty in handling thymus tissue would seem to be a possible source of error in this experiment. The tissue was difficult to slice and exhibited a considerable amount of fragmentation when subjected to shaking in the experimental flasks. Consequently, it was difficult to obtain the correct dry weight of the tissue used and impossible to determine the effect of varying amounts of fragmentation on the subsequent metabolic rate of the tissue being studied.

Effect of Storage on Tissue Metabolism

In order to obtain sufficient data on the *in vitro* metabolic activity of tissue obtained from dairy animals, a considerable amount of this tissue was stored prior to the manometric determinations. The effect of storage on metabolic activity of bovine liver is shown in Figure 5. In slices of tissue stored for periods ranging up to 70 hours, the oxygen uptake varied from an average of 0.317 microliters per hour per mg. of tissue, dry weight, in fresh tissue to an average of 0.048 microliters per mg. of tissue, dry weight, per hour in slices stored for a period of 70 hours. Metabolic activity declined slowly during the first 30 hours of storage, after which the decline was more rapid.

In this experiment, storage for periods ranging up to 40 hours appeared to have only a small effect on the anaerobic metabolic rate of liver slices. Average activity, as measured by microliters of carbon dioxide produced per mg. of tissue dry weight per hour, varied from 0.214 in fresh tissue to 0.187 in tissue stored for a period of 40 hours. During the first 24 hours of storage there appeared to be very little decline in the anaerobic metabolic rate of the liver slices.

Thymus tissue slices were not stored in sufficient numbers to adequately study the effect of storage on metabolism. However, from the limited data available it would seem that the aerobic metabolic activity of the thymus tissue declined in a manner similar to the decline of aerobic activity in bovine liver slices on storage. Anaerobic metabolic activity of the thymus tissue was high even after long periods of storage. Six slices of thymus tissue stored for a period of 50 hours produced an average of 11.8 microliters of carbon dioxide per mg. of dry tissue per hour at the end of this storage period. However, six slices stored for a period of 80 hours averaged only 3.0 microliters of carbon dioxide per mg. of dry tissue per hour. No relationship was observed between length of storage time and response to aureomycin in this experiment.



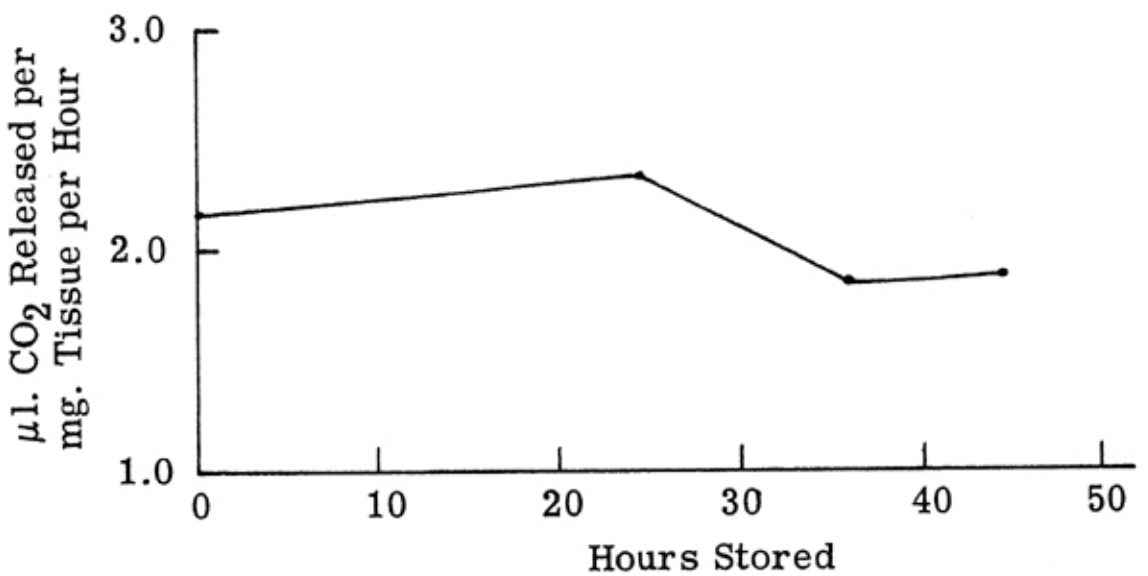


Fig. 5—The effect of storage on the metabolic activity of bovine liver slices.

DISCUSSION

In preliminary growth studies, 80 mg. of aureomycin given daily by capsule resulted in a 24 percent increase over controls in gain in body weight from birth to eight weeks of age. No significant differences were noted in height at the withers, indicating that the increased weight gain of the aureomycin supplemented calves was not accompanied by an increased rate of skeletal development. The incidence of scours was extremely low in both experimental and control animals and thus it appears that the means by which aureomycin produced an accelerated growth rate was something other than an effect on the incidence and severity of scours.

Although calves supplemented with 80 mg. of terramycin gained 9 percent more weight than their controls over the experimental period, this difference was not statistically significant. Kesler (1954) has reported that 20 mg. of terramycin per 100 pounds of body weight daily resulted in a significant increase in body weight gains in young calves. The difference in response to terramycin is in agreement with reports cited by Reid, *et al.* (1954) in which low levels of antibiotics have produced greater responses than high levels.

Kesler (1954) has also demonstrated that terramycin, particularly when given by capsule, decreases cellulose digestion in calves one to two months of age. However, despite the possible effect of the antibiotic on cellulose digestion, the terramycin supplemented calves continued to grow at a faster rate than the controls. It would seem from the information available, that a possible reason for the differences in growth response produced by high and low levels of terramycin is that the detrimental effects of high levels of the antibiotic on rumen function may tend to offset beneficial effects produced elsewhere in the body.

A considerable amount of evidence has indicated that aureomycin may directly affect metabolism of liver tissue. In a report by Rusoff, *et al.* (1954), supplementation of the diet with aureomycin resulted in an increase of fat deposition in experimental calves. It was suggested by these workers that a direct effect on liver metabolism might be the mechanism involved in this increased fat deposition. In demonstrations of a lipotropic effect of aureomycin by Gyorgy (1952) and de la Huerga and Popper (1951, 1952) the suggestion was made that the lipotropic effects of aureomycin were the result of the prevention of destruction of lipotropic agents such as choline and methionine by the intestinal flora. However, the possibility of a more direct effect of the antibiotic was also mentioned by these workers. Further evidence that aureomycin may act directly on the liver was presented by Luckey, *et al.* (1954), in demonstrating that liver necrosis could be developed by germ-free rats. This suggests that the delay in necrosis produced by aureomycin in studies by Gyorgy, *et al.* (1950), may have been due to a direct

effect on the liver, rather than to an action in modifying the bacterial flora of the intestine as has been suggested.

From the results of the manometric studies performed on liver tissue in this experiment, it appears that aureomycin does exert a direct effect on the anaerobic metabolism of the liver. In rat liver slices studied, concentrations of aureomycin below 3.0 micrograms per microliter of basal activity depressed anaerobic metabolism, while levels above 3.0 micrograms appeared to have no effect on total anaerobic activity.

In studies with bovine liver slices, it was found that levels of aureomycin below 6.0 micrograms per microliter of basal activity caused approximately a 10 percent decrease in anaerobic metabolism. However, concentrations above 6.0 micrograms appeared to have a stimulating effect on the anaerobic metabolic rate. This difference in response between high and low levels of aureomycin is interesting in view of the reports cited by Reid, *et al.* (1954), which indicate that low concentrations of an antibiotic often produce a greater growth response than high concentrations.

The reasons for the apparent stimulatory effect of higher concentrations of aureomycin on anaerobic metabolism of bovine liver slices are not clear and indicate the need for further study on this phase of liver metabolism.

The exact mechanism by which aureomycin, in low concentrations, depresses anaerobic metabolism is not known. However, in view of work reported by Van Meter and Oleson (1950) it would appear that the antibiotic may affect phosphorylation reactions in the body and thus affect tissue metabolism.

The data available from this experiment indicated that aureomycin, in the concentrations employed, has no effect on the oxygen uptake by liver slices. This agrees with the reports of Loomis and Lipmann (1948, 1949) who found no effect on oxygen uptake of kidney homogenates. However, Van Meter and Oleson (1950) did observe decreased oxygen uptake with rat liver homogenates exposed to aureomycin. The discrepancy between Van Meter and Oleson's results and those presented in this study may be due to the type of buffer used, since they reported that the most marked inhibition of oxygen uptake occurred in the absence of citrate.

The possibility of a direct effect of aureomycin on the metabolism of the thymus has been suggested in reports by Meites (1951, 1952a, 1952b) and by Rusoff, Landagora, and Hester (1954). These workers demonstrated that orally administered aureomycin tended to prevent the atrophy of the thymus produced by cortisone injections in rats and further that thymus weight in calves receiving aureomycin increased out of proportion to the accompanying increase in body weight.

The results of manometric studies of thymus tissue in this experiment indicate that aureomycin has no direct effect on the *in vitro* metabolism of thymus tissue. As indicated in Figure 4, concentrations of aureomycin

ranging from 2.5 to 8.5 micrograms per microliter of basal activity had no effect on the oxygen uptake of thymus slices. Average oxygen uptake of the experimental tissue slices varied from 94.2 percent to 107.4 percent of the control uptake and showed no effect which could be attributed to varying the concentrations of aureomycin. Similar results were obtained in anaerobic metabolic studies where the concentrations of aureomycin used appeared to have no effect on anaerobic metabolism of the thymus.

Although the possibility exists that the concentrations of aureomycin used were not optimal to produce a metabolic effect, the results of this experiment would seem to indicate that the *in vivo* effects of aureomycin on the thymus demonstrated by Meites (1951, 1952a, 1952b) and Rusoff, Landagora and Hester (1954) were not due to a direct effect of aureomycin on the thymus metabolism but, instead, were produced by some indirect action of aureomycin.

While the results of this experiment indicate that aureomycin has no direct effect on thymus metabolism, the possibility of an indirect effect on the thymus cannot be overlooked. Since steroid hormones produced by various endocrine glands of the body are known to affect the thymus, the po sibility of aureomycin affecting the production of these hormones should be considered. Also, aureomycin may affect the thymus, *in vivo,* by modifying intestinal flora, thereby indirectly affecting thymus metabolism.

A modified Tyrode's solution proved to be of value for storing bovine tissues slices during the course of this experiment. Since only a limited number of tissue slices can be used in one manometric run with the Warburg apparatus, the successful storage of tissue plays an important role in reducing the number of experimental animals needed. From the data available in this experiment, it appears that liver and thymus tissue can be stored for at least three days without materially impairing its value for certain types of *in vitro* metabolism studies.

SUMMARY

Including 80 mg. of aureomycin daily in the diet of Holstein heifers from birth to eight weeks of age resulted in a 24 percent increase in gain in weight over the controls. This increase was statistically significant at p.05. No differences were noted in height at the withers or incidence of scours between the control and experimental animals.

Supplementation of the diet with 80 mg. of terramycin daily produced no significant differences between control and experimental animals in weight, height at the withers or incidence of scouring.

Manometric determinations on rat liver slices indicated that, in the concentrations studied, aureomycin had no effect on oxygen uptake by the liver slices. Low concentrations of aureomycin appeared to inhibit the anaerobic metabolic rate of rat liver tissue.

Aureomycin in concentrations ranging from 2.5 to 6.0 micrograms per microliter of basal activity had no significant effect on the oxygen uptake of bovine liver slices.

Concentrations of aureomycin below 6.0 micrograms per microliter of basal activity appeared to have an inhibitory effect on the anaerobic metabolic rate of bovine liver tissue, while concentrations above this level appeared to be stimulatory.

Under the conditions of this experiment, aureomycin failed to have any significant effect on either the aerobic or the anaerobic metabolism of bovine thymus tissue.

Storage of liver and thymus tissue in a modified Tyrode's solution appeared to preserve this tissue adequately for manometric studies. Tissue could be stored for at least two days without producing a major change in its metabolic activity. No relation was observed between storage of tissue and its reaction to aureomycin.

BIBLIOGRAPHY

Bartley, E. E., Fountaine, F. C., and Atkeson, F. W. 1950. The effects of an A.P.F. concentrate containing aureomycin on the growth and well being of young dairy calves. *J. Animal Sci.* 9:646.

Baxter, J. H. and Campbell, H. 1952. Effects of aureomycin on renal lesions, liver lipid, and tissue choline in choline deficiency. *Proc. Soc. Exper. Biol. & Med.* 80:415.

Braude, R., Kon, S. K., and Porter, J. W. G. 1953. Antibiotics in nutrition. *Nutrition Abstracts and Reviews* 23:473.

Brody, T. M. and Bain, J. A. 1951. The effect of aureomycin and terramycin on oxidative phosphorylation. *J. Pharmacol. Exper. Therap.* 103:338.

Cameron, Gladys 1950. *Tissue culture technique.* 2nd ed. rev. New York, Academic Press, Inc.

Cason, J. L. and Voelker, H. H. 1951. The effect of a terramycin supplement on the growth and well being of dairy calves. *J. Dairy Sci.* 34:501.

Coates, M. E., Dickinson, C. D., Harrison, G. F., Kon, S. K., Cummins, S. H. and Cuthbertson, W. F. 1951. Mode of action of antibiotics in stimulating growth of chicks. *Nature* 168:332.

Cole, L. R. 1953. Recovery of aureomycin from the gastrointestinal tract following intravenous administration. *J. Lab. and Clin. Med.* 41:670.

Farquhar, J. D., Stokes, J., Whitlock, C. M., Bluemle, L. W. and Gambescia, J. M. 1950. Studies on the use of aureomycin in hepatic disease. III. A note on aureomycin therapy in hepatic coma. *Am. J. Med. Sci.* 220:166.

Goldbloom, R. S. and Steigmann, F. 1951. Aureomycin therapy in hepatic insufficiency. Gastroenterology 18:93.

Gyorgy, P. 1952. Experimental hepatic injury. *Am. J. Digest. Dis.* 19:392.

Gyorgy, P., Stokes, J., Goldblatt, H. and Popper, H. 1951. Antimicrobial agents in the prevention of dietary hepatic injury (necrosis, cirrhosis) in rats. *J. Exper. Med.* 93:513.

Gyorgy, P., Stokes, J., Smith, W. H. and Goldblatt, H. 1950. Studies on the use of aureomycin in hepatic diseases. II. The effect of aureomycin on experimental dietary hepatic necrosis. *Am. J. Med. Sci.* 220:6.

Herril, W. E. and Heilman, F. R. 1949. Aureomycin: Studies on absorption, diffusion and excretion. *Proc. of Mayo Clinic Staff meetings* 24:157.

Huerga, J. de la, and Popper, H. 1951. Urinary excretion of choline metabolites following choline administration in normals and patients with hepatobiliary diseases. *J. Clin. Investigation* 30:463.

Huerga, J. de la, and Popper, H. 1952. Factors influencing choline absorption in the intestinal tract. *J. Clin. Investigation* 31:598.

Jacob, S., Schiweinburg, F. B. and Rutenburg, A. M. 1951. Effect of intravenous aureomycin on the intestinal flora of dog and man. *Proc. Soc. Exper. Biol. & Med.* 78:121.

Jukes, T. H., Williams, W. L., Stokstad, E. L. R., Wong, S. C. and Cox, H. R. 1952. Lack of growth response to antibiotics by sterile chick embryos. Paper presented at informal poultry nutrition conference, Federation Meetings, New York.

Kaplan, A., Cohn, C., Narahara, A., and Jones, K. 1953. The effect of aureomycin administration upon the blood lipids of dogs with ligated pancreatic ducts. Paper presented at New York Acad. Sci. meeting, February 14, 1953.

Kesler, E. M. 1954. Effect of terramycin fed to Holstein calves on growth, in vitro cellulose digestion and B vitamin synthesis. *J. Animal Sci.* 13:10.

Kesler, E. M. and Knodt, C. B. 1952. Effect of terramycin on growth and rumen function in Holstein calves. *J. Animal Sci.* 11:768.

Landagora, F. J., Rusoff, L. L. and Crown, R. M. 1955. Effect of chlorotetracycline (aureomycin) on young dairy calves in a new environment. *J. Dairy Sci.* 38:621.

Loomis, W. F. 1950. On the mechanism of action of aureomycin. *Science* 111:474.

Loomis, W. F. and Lipmann, F. 1948. Reversible inhibition of the coupling between phosphorylation and oxidation. *J. Biol. Chem.* 173:807.

Loomis, W. F. and Lipmann, F. 1949. Inhibition of phosphorylation by azide in kidney homogenate. *J. Biol. Chem.* 179:503.

Loosli, J. K. and Wallace, H. D. 1950. Influence of A.P.F. and aureomycin on the growth of dairy calves. *Proc. Soc. Exper. Biol. & Med.* 75:531.

Loosli, J. K., Wasserman, R. H. and Gall, L. S. 1951. Antibiotic studies with dairy calves. *J. Dairy Sci.* 34:500.

Luckey, T. D. 1952. Effect of feeding antibiotics upon the growth rate of germ free birds. In a colloquim "Studies on the growth effect of antibiotics in germ free animals" held June 4, 1952 at the Lobund Institute, University of Notre Dame.

Luckey, T. D., Reyniers, J. A., Gyorgy, P., and Forbes, M. 1954. Germ free animals and liver necrosis. *Annals. of the New York Academy of Sci.* 57:932.

MacKay, A. M., Riddell, W. H. and Fitzsimmons, R. 1953. Terramycin supplement for dairy calves. *J. Animal Sci.* 12:19.

Meites, J. 1951. Counteraction of cortisone inhibition of body hair and thymus growth by vitamin B_{12} and aureomycin. *Proc. Soc. Exper. Biol. & Med.* 78:692.

Meites, J. 1952a. Changes in nutritional requirements accompanying marked changes in hormone levels. *Metabolism* 1:58.

Meites, J. 1952b. Beneficial effects of vitamin B_{12} and aureomycin in rats given large doses of cortisone. *Proc. Soc. Exper. Biol. & Med.* 81:307.

Meites, J. and Ogle, R. C. 1951. Antithyrotoxic effects of antibiotics in rats. *Proc. Soc. Exper. Biol. & Med.* 77:758.

Pritchard, G. I., Newlander, J. A. and Riddell, W. H. 1955. Aureomycin effects: Growth and digestibility studies with identical twin calves. *J. Animal Sci.* 14:336.

Reid, J. T., Warner, R. G. and Loosli, J. K. 1954. Antibiotics in the nutrition of ruminants. *Agriculture and Food Chem.* 2:186.

Rumball, J. M., Marion, D. F. and Sanders, M. 1950. Post hepatic cirrhosis treated with aureomycin: A case report. *Gastroenterology* 14:432.

Rusoff, L. L., Davis, A. V. and Alford, J. A. 1951. Growth promoting effect of aureo-

mycin on young calves weaned from milk at an early age. *J. Nutrition* 45:289.

Rusoff, L. L., Fussell, J. M., Hyde, C. E. and Crown, R. M. 1954. Parenteral administration of aureomycin to young calves with a note on the mode of action. *J. Dairy Sci.* 37:488.

Rusoff, L. L., Landagora, F. T. and Hester, H. H. 1954. Effect of aureomycin on certain blood constituents, body temperatures, weights of organs and tissues, and thickness of small intestine. Paper presented at annual meeting of the American Dairy Sci. Assoc., State College, Pa. June, 1954.

Seto, J. T. and Lepper, M. H. 1954. The effect of chlorotetracycline, oxytetracycline, and tetracycline administered intravenously on hepatic fat content. *Antibiotics and Chemotherapy* 4:666.

Shaffer, J. M., Bluemle, L. W., Sbrov, V. M. and Neefe, J. R. 1950. Studies on the use of aureomycin in hepatic disease. IV. Aureomycin therapy in chronic liver disease. *Am. J. Med. Sci.* 220:173.

Shaffer, J. M., Farquhar, J. D., Stokes, J. and Sbrov, V. M. 1950. Studies on the use of aureomycin in hepatic disease. I. Aureomycin therapy in acute viral hepatitis. *Am. J. Med. Sci.* 220:1.

Stokstad, E. L. R., 1954. Antibiotics in animal nutrition. *Physiological Reviews* 34:25.

Umbreit, W. W., Burris, R. H. and Stauffer, J. F. 1949. *Manometric Techniques and Tissue Metabolism* second ed., Minneapolis, Minn. Burgess Publishing Co.

Van Meter, J. C. and Oleson, J. J. 1950. Effect of aureomycin on the respiration of normal rat liver homogenates. *Science* 113:273.

Voelker, H. H. and Cason, J. L. 1951. Antibiotic studies with dairy calves. *J. Animal Sci.* 10:1065.

Wright, L. T. and Prigot, A. 1951. Aureomycin as an aid in abdominal surgery. *Ann. Surg.* 133:364.

Zaslow, J., Hewlett, T. H. and Lorry, R. W. 1950a. The excretion and concentration of aureomycin in the abnormal human biliary tract. Part I. Gall bladder. *Gastroenterology* 16:475.

Zaslow, J., Hewlett, T. H. and Lorry, R. W. 1950b. The excretion and concentration of aureomycin in the abnormal human biliary tract. Part II. Hepatic bile. *Gastroenterology* 16:479.