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Aureomycin as Preservative of Small Animal Bodies

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AUREOMYCIN AS PRESERVATIVE OF SMALL ANIMAL BODIES

By

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SMALL ANIMAL BODIES

This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser

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INTRODUCTION

During the last six years research has been conducted on the use of antibiotics in the preservation of foods for human consumption. The first published statement suggesting the use of antibiotics in the preservation of foods was made by Morris B. Jacobs in 1944. (9) The first commercial use of antibiotics in the processing of perishable food became possible on an interstate basis on November 30, 1955. On that date the Pure Food, Drug and Cosmetic Administration approved Aureomycin chlortetracycline for use "in or on uncooked poultry." (12)

The first antibiotics had narrow-spectra and showed little promise. Later there were developed the broad-spectra antibiotics, so called because such compounds are effective against large numbers of different species of bacteria. These antibiotics were used in the major portion of research dealing with antibiotics for food preservation. Today, using Aureomycin, the keeping period of dressed, refrigerated, poultry has been lengthened from 14 days to 21 days. (12) Deatherage was able to keep beef carcasses from spoiling at room temperature 70-85 degrees Fahrenheit for 72 hours by using 55 parts per million of Aureomycin. (3)

The major problems in using antibiotics for food preservation are the type of material one is preserving and the type of antibiotic to use. Deatherage states: "One of the first problems to solve was to select the best antibiotics for the products you wished to preserve; and to use the antibiotics effectively one must know something of the nature of the spoilage in a particular food and the specific properties of the antibiotics. Whereas the tetracyclines may be effective in preserving meat, penicillin

is worse than nothing at all, for it promotes a more rapid development of the normal spoilage flora. Streptomycin is of no value in preserving the high protein foods, yet is quite effective in bacterial soft rot in certain fresh vegetables. All of these antibacterial agents are useless against fungal spoilage, which is of primary importance in many fruits and vegetables." (3)

In selecting an antibiotic suitable for use as a preservative of meat and working on the assumption that deep spoilage may be the result of organisms found in the lymph nodes, Lepovetsky, Weiser and Deatherage isolated 93 strains of organisms from the lymph nodes. The majority of these organisms were proteolytic in nature. They tested these organisms against Aureomycin, terramycin and chloromycetin and found that Aureomycin inhibited growth in 81 strains, terramycin in 77 strains, and chloromycetin in 74 strains. Nine strains were not affected by any of the three antibiotics. (11)

Because Aureomycin proved effective for inhibiting the growth of organisms found in the lymph nodes, perhaps deep spoilage could be controlled by the injection of Aureomycin into the circulatory system of animals. Weiser, Goldberg, Cahill, Kunkle and Deatherage tried to control deep spoilage in animals by injecting Aureomycin into the circulatory system. They found that by using a solution of 55 parts per million of Aureomycin and injecting a volume equal to 10 per cent of the body weight, the bacteria in the lymph nodes could be controlled and a few dressed animals remained sound for 76 hours at a temperature of 70 to 85 degrees Fahrenheit. (17)

The purpose of this study was to learn the effect of Aureomycin

as a preservative when using a higher dose of Aureomycin on a whole animal. It was desired to have undesirable conditions, therefore; the animals were left at room temperature, 30 degrees centigrade, with the intestines intact.

REVIEW OF LITERATURE

The value of antibiotics for the preservation of food was first published by Morris B. Jacobs. (9) For the preservation of meat, investigation has indicated Aureomycin as being the most beneficial. Niven and Chesbro found that of all the antibiotics tested, only the tetracyclines appeared to have potential value. (13) Lepovetsky and co-workers screened Aureomycin, terramycin and chloramphenicol against 93 strains isolated from meat and found that Aureomycin was the best. (11) The findings of Tarr, Southcott, and Bissett were the same as Lepovetsky and co-workers. (16) Goldberg, Weiser and Deatherage also found Aureomycin to be the best antibiotic for meat preservation. (6) The results of such research were summarized as late as 1954 with the statement that "chlortetracycline was found to be more effective in preserving such foods than any of 14 other antibiotics studied." (12)

A very good definition of an antibiotic has been stated as "A chemical compound derived from or produced by living organisms, which is capable, in small concentration, of inhibiting the life processes of microorganisms." (16) Aureomycin is one of many of the new antibiotics which has been developed in recent years. It is a yellow, crystallizable antibiotic biosynthesized by Dr. B.M. Duggar (1948) during the course of a screening program, in which he investigated hundreds of different species and strains of soil-inhabiting microorganisms--principally actinomycetes. (15) The antibiotic is produced by the fungus Streptomyces aureofaciens. (18)

Aureomycin has a wide antibacterial spectrum which includes a number of Gram-positive and Gram-negative organisms. (15) It is active against certain penicillin resistant organisms as well as against streptomycin resistant and dependent strains. It is stable in the dry powder at room temperature and in high concentration in solution in distilled water at 37 degrees Fahrenheit or 4 degrees centigrade. Loss of activity occurs rapidly when the antibiotic is incubated at 37 degrees centigrade in low concentration in broth, plasma or blood agar. The loss in activity in the same media is less rapid on refrigeration. (14) The antibiotic is most active in the acid pH range. (14) The pH of the medium is very important. For some organisms 64 times as much Aureomycin may be required to cause total inhibition at pH 8.0 as at pH 6.0. (15) Aureomycin is bacteriostatic. (15) "Aureomycin is most effective against actively multiplying organisms, and exerts relatively little effect against fully grown or metabolically relatively inactive cultures. In serial dilution studies, the density of the population is an important factor, more Aureomycin being required for inhibition of dense cultures than for more sparsely populated ones." (15)

It is speculated that deep spoilage of meat may begin at the lymph nodes. (17) Lymph nodes serve a phagocytic function in the animal and it would be at these nodes where large numbers of organisms would be found. From inspection of many sound rounds of beef and hams it appeared that deep spoilage might be caused by organisms residing not in the meat but in the other tissues--mainly lymph nodes and bone marrow. (11) The work of Lepovetsky, Weiser and Deatherage on the bacteria found in lymph nodes of cattle gives us an idea of the type of organisms that may be found

in deep spoilage. These workers isolated 93 different species of organisms. In their plating procedures to isolate the organisms they found that counts made from plates incubated anaerobically at 37 degrees centigrade were usually lower than those from plates incubated aerobically at this temperature. Such results were not surprising, since subsequent investigations revealed that 85 of the 93 isolates were facultative anaerobes, three were microaerophiles and the remainder were obligate anaerobes. They isolated 12 genera of bacteria from the various lymph nodes. (11)

In the classification of the 93 organisms isolated, they classified 31 in the genus Streptococcus. Six of these resembled S. faecalis, nine appeared to be strains of S. liquefaciens. A number of strains could not be assigned to these groups. Coliform bacteria appeared frequently with 28 isolates having been recovered. Eighteen of these were strains of Escherichia coli and E. freundii, six were identified as strains of Aerobacter cloacae, and the remaining organisms resembled A. aerogenes. The other Gram-negative rods consisted of the genera Psuedomonas, Alcaligenes, Flavobacterium, Serratia and Proteus. (11)

Lepovetsky, Weiser and Deatherage studied the proteolytic properties of the isolates so that their role as potential spoilage organisms could be tentatively evaluated. This was accomplished by observing the reactions in Litmus Milk, Nutrient Gelatin, cooked meat medium, and Loeffler blood serum medium. They found that many of the isolates were proteolytic as shown: by their ability to liquefy gelatin and blood serum, peptonize milk and digest the meat in tubes of cooked meat medium. They stated that the proteolytic nature of these organisms, together with their ability to grow anaerobically and at a wide range of temperatures, suggest that they

might be capable of producing a deep spoilage in carcasses that are inadequately refrigerated. Furthermore, the large number of organisms in the lymph nodes suggests that these tissues may be the point from which deep spoilage in beef arises. (11)

Deatherage reports the results of work done on antibiotic treatment of meats are: For whole or half carcasses a keeping time of at least 72 hours is obtained even if the internal temperature of the meat remains in the 70 degree-85 degree Fahrenheit range. It will greatly retard the slime formation and surface spoilage in meat in carcass, cut, and comminuted forms. The length of holding again depends on the temperature and original bacterial load. For ground beef and pork sausage originally containing 200,000 organisms per gram, 2 parts per million chlortetracycline will increase the usable life at 10 degrees centigrade by an average of six or more days. The keeping period of dressed poultry has been lengthened from 14 days to 21 days, with refrigeration, by using Aureomycin. (3)

If the meat has a high bacterial load; the Aureomycin will be of no value in keeping the meat. Broquist, Kohler and Miller found that in many laboratory experiments, chlortetracycline, which is primarily bacteriostatic, was less effective in extending the shelf life of poultry with a higher bacterial count than when freshly killed poultry was employed. When poultry was used that smelled strong and had a bacterial load of 514×10^6 microorganisms per gram, dipping it in a solution of 300 parts per million of chlortetracycline had no beneficial effect. Processing such poultry with 300 parts per million of antibiotic subsequently reduced the bacterial load only about tenfold with no organoleptic improvement. (1)

When the bacteria are retarded or inhibited another problem arises in keeping the meat fresh and that is the appearance of molds and yeasts. Yeasts are the predominating organisms in this case. Tarr, Southcott, and Bissett found in one series of experiments yeasts and noticeable bacteria, developed in the antibiotic-treated samples. (16)

Ziegler and Stadelman found when studying poultry meat, using antibiotics, that the microflora of the surface of treated halves differed from that of the control halves. The predominant organisms on the treated halves were yeasts having the shape and budding characteristics of the family Saccharomycetaceae. On the controls the predominant organisms were Gram negative rod shaped bacteria. In many instances on the halves treated with a 10 parts per million Aureomycin solution, a considerable number of the Gram-negative bacteria were present with yeasts predominating. (21)

Whitehill in studying the increase of yeast growth that occured on several occasions when meat samples were treated with antibiotics, or in combination with irradiation; found evidence that suggested the bacterial flora of meat produced antifungals which kept the yeast growth in check on stored normal meat. But when the bacteria are retarded by antibiotics the yeasts can grow unimpeded. (19)

Weiser, Goldberg, Cahill, Kunkle and Deatherage found in their experimental work with Aureomycin in beef that the pH was 5.50 and 5.60. (17) Jensen found that on numerous determinations of pH of beef muscle, neck, chuck and round, a rapid drop occured within 48 hours, 7.2 to 6.0 and after 48 hours, 5.7. The muscle sheaths were always less acid than the muscle fibers. (10)

PROCEDURE

White rats were chosen as suitable test animals for the following reasons: 1. They were available and of adequate size (150-300 grams). 2. Injection trials proved they were easy to inject, and required less time. 3. Only small quantities of Aureomycin solution would be needed per animal. 4. Their small size presented a minute problem in storage and disposal.

METHOD OF INJECTION:

The animals were killed by using ether, and the animal was opened by the following incisions. First, longitudinally just below the diaphragm exposing the liver. Next, along the left side of the sternum to expose the heart. The heart was used as the site of injection, because the arteries and veins were small and difficult to inject. Injection was made by using a short ($\frac{1}{2}$ inch) 24 gauge needle. A longer needle might go into the other ventrical, the auricle or all the way through the heart, while applying pressure to the syringe. After swabbing the heart with alcohol the needle was placed halfway into the left ventrical, and a slight amount of pressure was applied to observe if the ventrical enlarged. This was used as a method to check on the correct placement of the needle. When the ventrical enlarged, the inferior vena cava was used for drainage because of its large size and its location near the heart giving complete circulation. By cutting the vena cava in this location the diaphragm could be used to retain the blood in the area of the lungs instead of allowing it to flow into the abdominal cavity; therefore, providing an easy and quick removal of blood with cotton swabs.

AUREOMYCIN SOLUTION

The Aureomycin used for injection was supplied by the American Cyanamid Company. Trade name for the Aureomycin was Acronize PD Chlortetracycline. The composition is 10 per cent chlortetracycline, blended with a mixutre of edible ingredients, salt and citric acid. The Acronize was added to distilled water until the desired concentration of Aureomycin was obtained. Each new solution used was plated out for sterility. Growth appeared only once and that was a single colony. The solutions used contained 100 parts per million or 200 parts per million.

QUALITY OF INJECTION

Pre-trial experiments gave the following methods of measuring a thorough injection to all parts of the animal; 1. The amount of material injected (10 per cent of body weight). 2. The removal of blood from the liver giving the liver a white appearance. 3. Watching visible veins and arteries to see if the blood was removed. 4. The flowing of liquid from the incisions. 5. The lungs filling and the purging of liquid from the nose. A rapid purging at the onset of injection indicated the needle was in the right ventrical instead of the left ventrical or a blockage had occured in the circulatory system. 6. Another observation, but not proved, was the violent twitching of the body, especially the tail and hind legs. Assay tests for Aureomycin showed a large amount of the antibiotic in the muscle of the hind legs when this occured. If the above steps (1-5) were not observed the animal was not used for the experiment.

MOLD INHIBITOR

When the animal had been injected the opening was covered with cotton dipped in "Sorbistat". Sorbistat (Sorbic acid, Pfizer) is a

selective growth inhibitor for many molds and yeasts and certain bacteria which cause spoilage in food products. Sorbistat appears to inhibit mold growth owing to its unsaturated fatty acid structure. This was used because pre-trial experiments had disclosed that mold growth would appear around the incisions and spread throughout the body cavities. Only in a few cases did mold growth appear on the outer edges of the incisions when Sorbistat was used.

Because of the low aqueous solubility of Sorbistat, it is difficult to prepare stock solutions. Chas. Pfizer and Co., Inc. prescribe this method: To make 200 milliliters sodium bicarbonate solution equivalent to 10 per cent Sorbistat (weight/volume); (1) Dissolve 5 grams sodium bicarbonate and 5 grams sodium hydroxide in 150 milliliters of water; (2) Add the alkaline solution slowly to 20 grams Sorbistat with stirring and make to 200 milliliter with water.

REFRIGERATION OF TEST ANIMAL

After the incision was closed the animal was placed in a refrigerator for 18 hours giving the antibiotic a chance to penetrate into the muscular tissue. After removal from the refrigerator, four hours were allowed to reach room temperature, before timing or sampling was started. Hounie observed that by placing meat in a refrigerator for five days, he was producing conditions that favored penetration of the antibiotic. The meat was better preserved and the low temperatures reduced the growth of bacteria. (8)

OBTAINING SAMPLES FOR ANTIBIOTIC ASSAY AND BACTERIA COUNT

The hind leg muscle was used for the microbiological assay and the determination of the number of organisms present in the tissues. Reasons

for selecting the hind leg muscle are as follows: 1. At least three to four grams of meat were available. 2. It was easy to dissect and aseptic technique could be used. 3. It was the largest quantity of muscle farthest from the heart. If one is able to detect a large amount of Aureomycin compared to the amount injected after 24 hours, it would serve as an indication of complete injection. The liver and kidney could not be used because Deatherage showed that those organs trapped a large amount of the Aureomycin. (3) 4. The meat was covered by the skin and not exposed to the air, making outside contamination neglible and possible contamination would occur only during injection or while the muscle was being removed to be weighed.

METHOD OF ASSAY

The method of testing for Aureomycin was a microbiological assay, which was introduced by Dornbush and Pelcak. Dornbush and Pelcak demonstrated that the plate method of assay for Aureomycin showed that difficulty would be encountered in reading and evaluating the zone of inhibition. Favorable points for using this test are the short length of test, 4 hours, use of standard laboratory equipment, simple media and non-sterile glassware. In a period of less than 45 minutes, three assays may be started. (5) The microorganism used for the assay was Bacillus cereus #5, American Type Culture Collection 10702, which was obtained from Mr. Dornbush of the American Cyanamid Company at Pearl River, New York.

A two gram sample of muscle was removed from the hind leg of the animal and ground with a mortar and pestle using sand and distilled water. Distilled water was added to bring the solution to a one to ten dilution. This solution was filtered through three thick nesses of Whatman number

three filter paper to remove meat and other particles. A nearly clear solution could be used, providing a large amount of Aureomycin was in the solution, because the serial dilutions would give you a clear solution at the end-point.

Dornbush and Pelczar give this method for conducting the assay test: 1. One half milliliter of a 0.2 microgram per milliliter solution of an Aureomycin reference solution is placed in a ½ x 4½ inch Wasserman tube. 2. A second one half milliliter portion of the same solution is diluted serially two-fold through an additional five tubes with sterile Nutrient Broth. 3. Samples submitted for assay are similarly diluted for as many tubes as may be required to give an end-point. 4. When all of the samples and the standard have been placed in tubes, 1½ milliliters of a 1:100 broth dilution of an overnight broth culture of the test organism is added and the tubes are incubated at 37 degrees centigrade in a water bath. 5. After four hours, the tubes are examined visually and the growth is recorded as either negative or positive. The end-point is usually quite distinguishable and even faint growth is recorded as positive. Quite consistently, an end-point in the third tube of the standard series was obtained; thus the smallest detectable amount of Aureomycin is 0.05 microgram per milliliter of sample. (5) The end-point of the sample was considered the same as the end-point of the standard solution. If the end-point of the standard solution fell at tube three, which contained 0.05 microgram per milliliter of Aureomycin solution. You then count back to the first tube of the sample series to determine the amount of Aureomycin present in the sample.

BACTERIAL COUNTS OF THE MUSCLE

When removing the meat for the bacterial count, the hind leg was completely covered with alcohol. The scissors were dipped in alcohol or kept in alcohol when not in use. The skin was cut and then ripped back exposing the flesh. A one or two gram sample was removed and weighed on a sterile folded piece of paper. This was quickly transfered to 99 milliliters or 198 milliliters of sterile water buffered with Nutrient Broth, giving a 1:100 dilution and placed into a sterile metal Waring Blender and blended for two minutes. Serial dilutions were made to receive an end-point of 30-300 colonies per plate. All dilutions were made in duplicate. The contents were plated out on TYG agar (7) and Endo Agar. Each dilution was also placed in a tube of Fluid Thioglycollate Medium. All plates and end tubes were placed in the incubator at 30 degrees centigrade for 72 hours.

A RAPID METHOD FOR DETERMINING SPOILAGE

The procedure used was a modification of the rapid method of Ziegler, Spencer and Stadelman for the determination of spoilage in fresh poultry. This test was conducted in the following manner: 1. Removal of the skin covering the leg muscle using asceptic means. 2. A smear was taken by means of a sterile wire loop and spread uniformly on a glass slide. 3. The smear was subsequently fixed, stained with Gram's stain, and examined under a microscope with oil immersion objective. 4. The smears were classified as negative when relatively few organisms were present, and as positive when a great number of organisms was present. (20)

MEDIA USED

The medium used for the plate counts was recommended by W. R. Chesbro (2) of the American Meat Institute Foundation, which was formulated by Haynes, Wickerhan and Hesseltine. (7) The formula is stated as such:

Tryptone	5.0 g.
Yeast extract	5.0 g.
Glucose	1.0 g.
K_2HPO_4	1.0 g.
Agar	20.0 g.
Tap H_2O	1000.0 g.

The pH was adjusted to 7.0 and sterilized in the autoclave at 15 pounds pressure.

In this work a modification of the above formula was that the pH was adjusted to pH 6.6 instead of pH 7.0. Fluid Thioglycollate Medium was used for anaerobic growth.(4) Endo agar was used to detect the presence of lactose fermenters. (4) Litmus Milk was used to test representative organisms for their ability to peptonize milk and Nutrient Gelatin was used to test for their ability to liquify gelatin.

The representative colonies from the plate counts were removed, purified, and then placed in Litmus Milk and Nutrient Gelatin for observation. These isolated bacteria were also tested for their sensitivity toward Aureomycin, terramycin and chloromycetin by using Difco Bacto-Sensitivity Disks for antibiotics 10 microgram per disk. (4)

CRITERIA FOR SPOILAGE

The criteria for spoilage was a combination of observations: 1. Odor. 2. Plate count over 200,000 bacteria per gram of meat. 3. The color of the areas around the nose and anus, a blue or dark color

indicated spoilage. 4. The color of the tail, a pink color indicated good condition, a brown color indicated spoilage. 5. The condition of the eyes. 6. The ease of removing the fur from the skin. 7. The condition of the leg muscle. All of these points were observed for each rat at the time of assay and bacterial plate count of the leg muscle, but the presence of odor was the primary factor.

RESULTS AND DISCUSSION

Ninety-seven animals were used in the course of this study on the effect Aureomycin has on the keeping quality of whole small animals at room temperature. The animals were used in the following experiments: Twenty-four were injected using a 100 parts per million Aureomycin solution, 72 were injected using a 200 parts per million Aureomycin solution. Eighteen of the last group had their intestines removed following the completion of injection.

The keeping period of the controls at room temperature, 30 degrees centigrade, was two days, as seen in plate 1 page 18. At the end of two days a foul odor was present and the tails were turning from pink to a dark brown. When using a 100 parts per million Aureomycin solution for injection into the circulatory system, the maximum keeping period was five days. After the fifth day an odor was present, but the appearance of the outside of the animals was excellent until approximately the seventh day. At this time, the tail was beginning to turn brown, the eyes had started to decompose, the fur could be pulled out with ease and the odor was becoming stronger. When the amount of Aureomycin in the solution was increased to 200 parts per million the keeping period was lengthened to seven days, plate 1 page 18. A few animals appeared excellent at eight days, but it was questionable whether a slight odor was present.

When the animals spoil or decompose, spoilage appeared to begin in the area of the intestine. In many instances a slight amount of spoilage was first observed in the area around the nose. It was indicated by a blue appearance. This may have been the result of an incomplete injection or a blockage of the blood vessels may have occurred in this area. Rapid

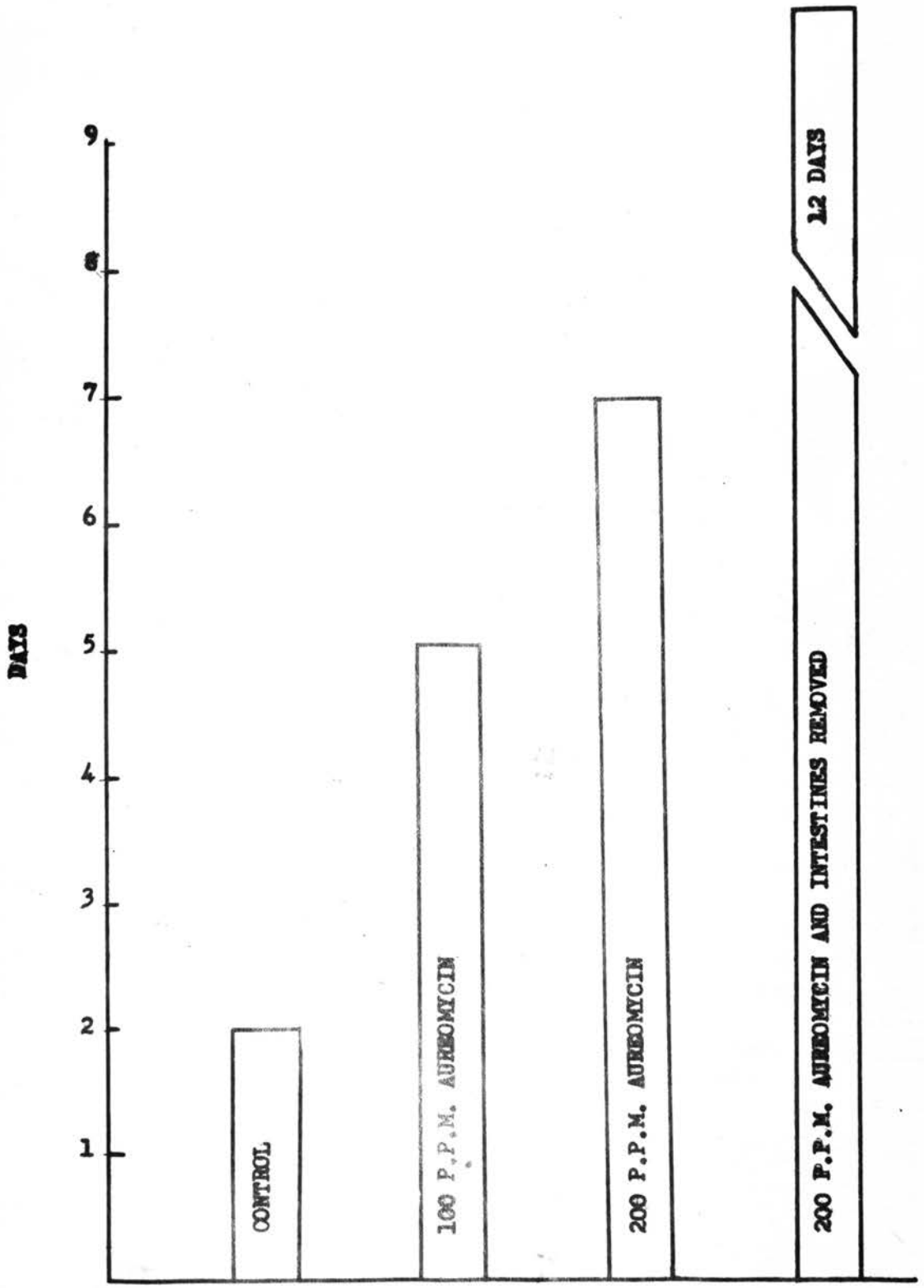

Plate 1. **MAXIMUM KEEPING PERIOD OF ANIMALS**

spoilage also occurred many times around the anus, unless it was properly cleaned. Since spoilage seemed to begin in the area of the intestines, the intestines were removed from 18 rats of the last group. Using a 200 parts per million Aureomycin solution to flush out the peritoneal cavity after the removal of the intestines, the keeping period was increased to twelve days. However, by removing the intestines, the results were not always favorable because a large percentage of the animals spoiled before seven days of storage. This may have been the result of poor technique in the removal of the intestines, because a small amount of the fecal contents may have been spread around the body cavity. The factor of excessive handling must also be taken into account. In referring to the fact that the spoilage appeared to begin in the area of the intestines, the intestines were removed from the controls and the keeping period was not lengthened. The only change was that the odor was not as strong at two days as when the intestines had been left in.

When using a 100 parts per million Aureomycin solution, the maximum amount of the antibiotic that could be detected in the muscle after twenty-four hours was 67.5 micrograms per gram of sample. At forty-eight hours 33.75 micrograms per gram of sample was detectable. At seventy-two hours 16.87 micrograms per gram of sample was detectable and at ninety-six hours no Aureomycin could be detected, plate 2 page 20. When using a 200 parts per million Aureomycin solution, the maximum amount of antibiotic that could be detected at twenty-four hours was 135 micrograms per gram of sample. At forty-eight hours 65 micrograms of Aureomycin was detected in the muscle sample. At seventy-two hours 33.75 micrograms of the Aureomycin was found in the sample and at ninety-six hours 8.4 micrograms of the

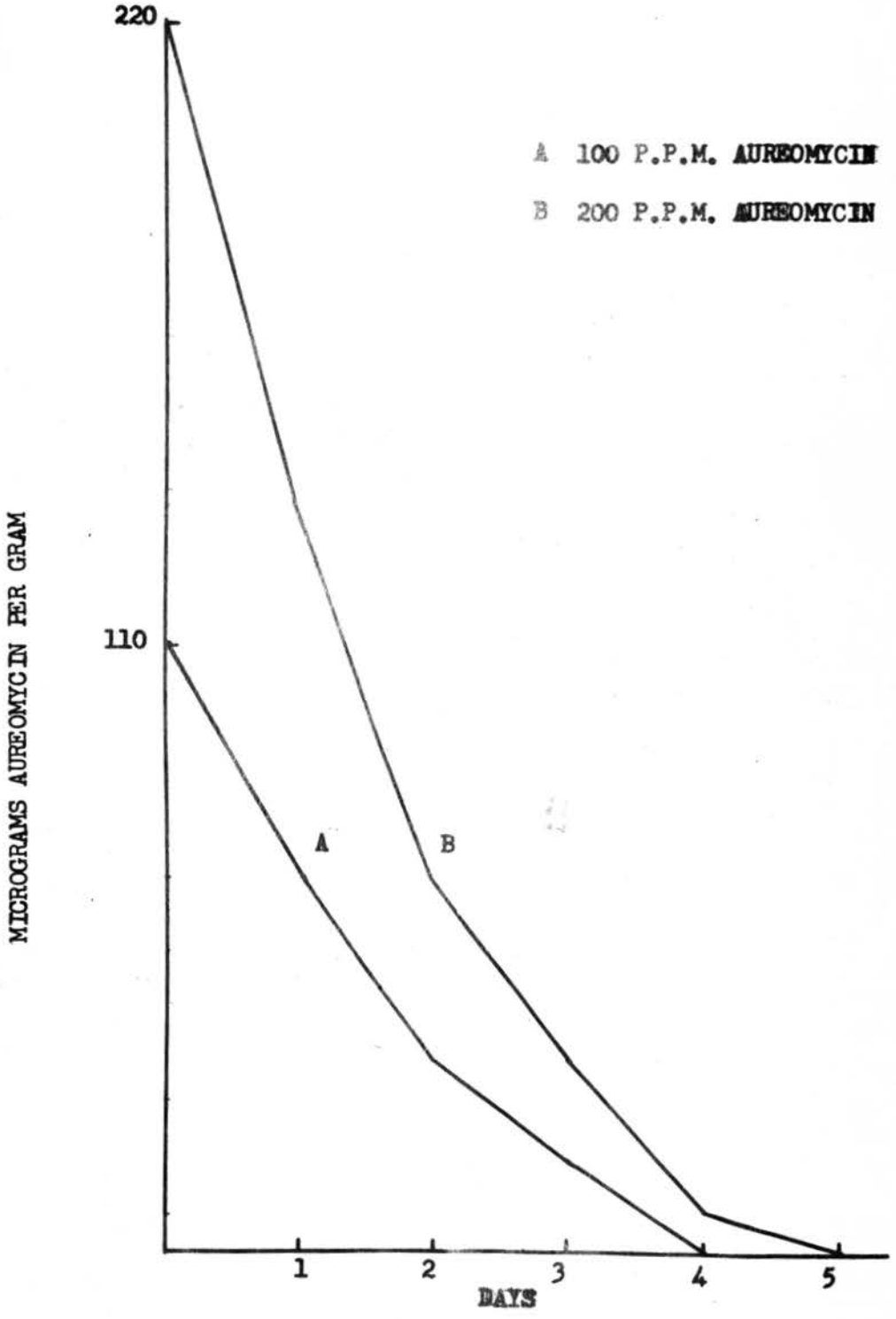

Plate 2. AUREOMYCIN CONTENT OF MUSCLE

antibiotic was detected in one gram of sample. At one hundred and twenty hours, no Aureomycin was detectable, plate 2 page 20.

As stated in the procedure the agar used for the plate counts was TYG agar. In pre-trial experiments, using a pH of 7.0, the growth was poor and at a slow rate. In many cases, spoiled meat showed only a few colonies on the agar plate whereas a smear showed many bacteria on a stained slide. It was found that by lowering the pH of the TYG agar to a pH of 6.5 rapid growth appeared.

The number of organisms in leg muscle of the controls varied from less than 3,000 to 18,000 per gram at twenty-four hours. At 48 hours the count was 125,000 bacteria per gram of muscle. When the period of 72 hours was reached, the counts were approximately 610 million per gram of muscle and at 96 hours they had reached 950 million to 3.5 billion organisms per gram of muscle, plate 3 page 22.

In the animals injected with a 100 parts per million Aureomycin solution the number of organisms would remain less than 3,000 to 3,500 organisms per gram of muscle through the fourth day. On the fifth day the counts increased to approximately 160,000 organisms per gram of muscle. After the fifth day the counts increased at the same rate as the controls did after three days, plate 2 page 20.

With the injection of 200 parts per million Aureomycin solution, the counts ranged from less than 3,000 to 10,000 organisms per gram of muscle until the sixth day. The seventh day the counts started to rise as on the fourth day for the 100 parts per million Aureomycin injection, plate 3 page 22.

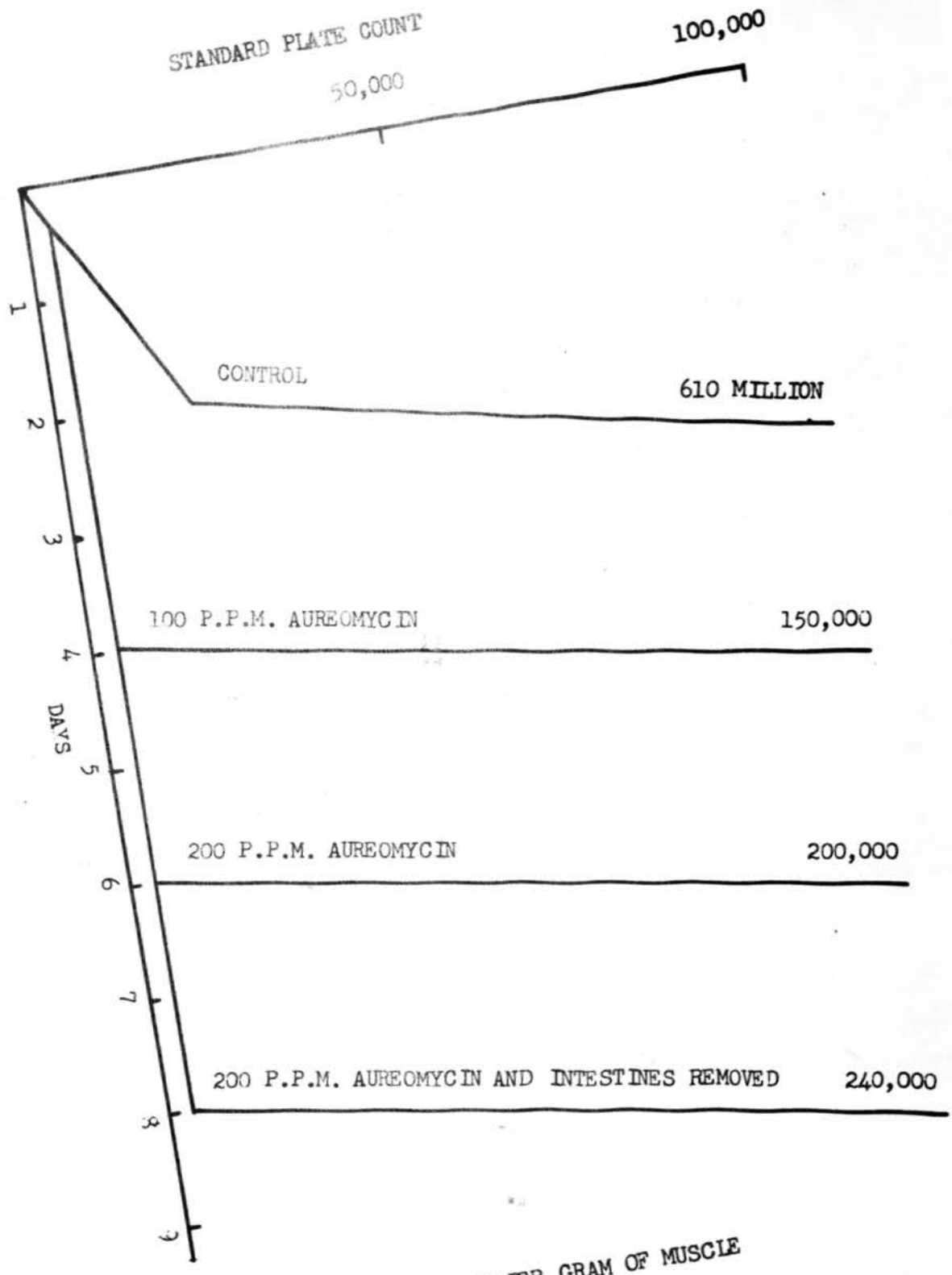
STANDARD PLATE COUNT
50,000
100,000
1
2
3
4
5
6
7
8
9
DAYS
CONTROL
610 MILLION
100 P.P.M. AUREOMYCIN
150,000
200 P.P.M. AUREOMYCIN
200,000
200 P.P.M. AUREOMYCIN AND INTESTINES REMOVED
240,000
NUMBER OF ORGANISMS PER GRAM OF MUSCLE

Plate 3.

The animals with the intestines removed were compared with whole animals to which 200 parts per million Aureomycin had been injected. After the ninth and tenth days the bacterial counts averaged 240,000 bacteria per gram of muscle without demonstrating any odor. After the 12 days an odor was detected, plate 3 page 22.

Aureomycin was stated to be bacteriostatic in the review of literature. Therefore, Aureomycin does not kill the microorganisms, but inhibits them from growing and increasing in numbers. When the deterioration of Aureomycin takes place, growth commenses at a rapid rate. By using the figures from plate 2, page 20, and assuming that after the first 24 hours the amount of Aureomycin was decreased one-half of the total amount of the preceding day, we would have the following figures:

Days	Aureomycin	
0	110*	220*
2	67.5	135
3	33.75	67.5
4	16.87	33.75
5	8.43	16.87
6	4.26	8.43
7	1.06	4.26
8	0.53	1.06

*Microgram per milliliter

We could say that the Aureomycin is present in the muscle longer than the assay was able to detect and the growth of organisms would not begin until the Aureomycin had reached a level that had no bacteriostatic effect on the microorganisms present. This may explain why there is no growth until a few days after the assay detects no antibiotic.. In order for this to be true, each gram of muscle must contain the same

quantity of the antibiotic and the temperature and the pH must remain constant throughout. When the Aureomycin loses its ability to inhibit growth, there is a wave of rapid growth.

When growth appeared in the tubes of Fluid Thioglycolate Medium it also appeared on the aerobic plates indicating that the organisms were facultative anaerobes. This was used as a method of checking to see that large numbers of anaerobes were not missed. A few plates were placed in a Brewer's Jar from time to time and no difference was obtained in bacterial counts.

The number of organisms found on the Endo Agar plates varied so greatly from time to time that no consistant results were obtained. It was hoped that some corollation would be obtained between spoilage and the lactose fermenters from the intestines. This corollation was not fonnd.

The Sorbistat did inhibit the yeast and mold growth. In only a few cases did a small amount of yeast and mold did appear.

Of the twenty-three organisms picked at random, 16 liquified Nutrient Gelatin and 17 peptonized Litmus Milk. These organisms were then checked for their sensitivity toward Aureomycin, terramycin and chloromycetin. It was found that 19 of the organisms were sensitive toward Aureomycin, 18 toward terramycin and 18 toward chloromycetin. Two of the organisms tested were not inhibited by the antibiotics.

In those instances in which spoilage occurred in a few days, it was found that these organisms were resistant to Aureomycin. It must be remembered that the Aureomycin may have inhibited other organisms, but when the plate counts for the total numbers were made these were the

predominant organisms present. Proteus vulgaris was encountered frequently. This organism is found in the intestine and is not inhibited by Aureomycin.

It appears that Aureomycin does increase the keeping period of small animals with their organs intact. In the majority of cases good results were obtained by using 200 parts per million Aureomycin solution. The only difficulty is that there are a few organisms that are not inhibited by Aureomycin.

SUMMARY AND CONCLUSION

In the course of this study 97 animals were used.

When using 100 parts per million Aureomycin solution, the maximum keeping period was five days. By increasing the amount of Aureomycin in the solution to a 200 parts per million the keeping period was lengthened to seven days. A few animals did appear excellent at eight days, but a slight odor was present. The keeping period of the controls was two days.

The removal of the intestines increased the keeping period to twelve days using a 200 parts per million Aureomycin solution for injection. At nine days the plate counts average 240,000 organisms per gram of muscle, but no odor was present. In removing the intestines the results were not as favorable in that a large percentage of the animals spoiled before seven days.

The Sorbistat did inhibit the yeast and mold growth. In a few cases a small amount of yeast and mold did appear.

In using a 100 parts per million Aureomycin solution; the Aureomycin was present until the third day at 16.7 microgram per gram of sample and at the end of four days no Aureomycin was detectable by the assay method used. In using a 200 parts per million Aureomycin solution the Aureomycin was present until the fourth day at 8.4 microgram per gram of sample, and at the end of the fifth day no Aureomycin could be detected.

Of the 23 organisms picked at random, 16 liquified Nutrient Gelatin and 17 peptonized Litmus Milk. Nineteen of the organisms were sensitive to Aureomycin, eighteen to terramycin and chloromycetin. Two organisms were not inhibited by any of the three antibiotics.

The number of organisms per gram of leg muscle in the controls varied from less than 3,000 to 3,600 at 24 hours and at 48 hours the counts average 18,000 organisms per gram. At 72 hours the count had risen to 610 million organisms per gram of muscle.

The number of organisms in the muscle injected with 100 parts per million remained less than 3,000 to 3,500 until the fifth day. At the fifth day the number of organisms was approximately 160,000 organisms per gram. After this period the count rose as in the controls at three days.

With the injection of 200 parts per million Aureomycin solution the count ranged from less than 1,000 to 10,000 organisms per gram of muscle until the seventh day. After the seventh day the count rose to approximately 200,000 organisms per gram. On the removal of the intestines the count remained low, less than 1,000 to 10,000 until the ninth day when it then rose to 240,000 organisms per gram, but no odor was present until the twelfth day.

No correlation between the lactose fermenters and spoilage was obtained.

The injection of a 200 parts per million Aureomycin solution through the circulatory system preserved whole small animals with intestines intact at 30 degrees centigrade or 87 degrees Fahrenheit for a period of seven days. After seven days the animals began to decompose rapidly, because the bacteriostatic effect of the Aureomycin was lost. If spoilage began before seven days, it was the caused by poor injection or by growth of organisms not inhibited by Aureomycin.

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