Absorption and Metabolism of Antimycin Administered with a Rat Diet

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Absorption and metabolism of ^{3}H-antimycin was investigated by feeding it with the diet to female rats. At least 80% of each of two dose levels—20 and 106 μg. per rat per day—was absorbed, since only 20% appeared in the feces. About 27% of the ingested radioactivity appeared in the urine; 20% of this was neutral, volatile material, presumably ^{3}H-water, while less than 15% was intact antimycin or any of several known hydrolysis products. Radioactivity was distributed throughout the carcass with higher levels in heart, liver, and kidney than in muscle. One third to one half of the ingested tritium could not be accounted for and was probably lost as ^{3}H-water. Less than 10% of the total dose remained in the bodies of animals killed 7 days after dosing was discontinued. Antimycin, therefore, is rapidly degraded and eliminated by rats and is not a cumulative poison.

Antimycin (Strong, 1956), a potent fish poison (Derse and Strong, 1963) has been extensively tested for use as a fish eradicant (Vezina, 1967; Walker *et al.*, 1964). The residues in tissues of fish killed by antimycin are low. Maximum whole body antimycin levels in trout and carp killed at 5 and 10 p.p.b. water concentrations averaged 203 μg. per kg. (Ritter and Strong, 1966). Since fish killed in this manner might be used for human or animal consumption, the present studies were undertaken to provide information on the intestinal absorption, body distribution, excretion, and metabolism of this antibiotic in rats.

EXPERIMENTAL

Materials and Methods. Determination of Radioactivity. All counting was done in a Packard Tri-Carb liquid scintillation spectrometer, Model 3003, equipped with Packard automatic standardization, Model 3951. Unless stated otherwise, all samples were counted until the standard deviation of the observed counts, when corrected for background radiation, was less than 10%. In most cases, the standard deviation was less than 5%.

A 2-ethoxyethanol–toluene scintillation solution (EE-2) (8 grams of PPO, 0.2 gram of POPOP, 850 ml. of 2-ethoxyethanol, and 1400 ml. of toluene) (Hall and Cocking, 1965) and an ethanol–toluene scintillation solution (Ritter and Strong, 1966) were used. If not stated otherwise, the ethanol–toluene solution was used. Two external standardization curves were required, one for each of the scintillation solutions.

Randomly Tritiated Antimycin. Approximately 1 gram of blastmycin (antimycin A_3 + a trace of antimycin A_4) was tritiated by the Nuclear Chicago Corp. by mixing with an equal weight of a catalyst consisting of 10% palladium on charcoal and exposing to 7 to 9 curies of tritium gas for 14 days at room temperature. The catalyst-antimycin mixture, still containing labile tritium, was then returned for purification.

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The crude antimycin was separated from the catalyst by filtration through a Celite 606 column, and the labile tritium was removed by repeatedly dissolving the antibiotic in methanol and removing the methanol in a vacuum rotary evaporator (Ritter, 1967). The antimycin was partially purified through silicic acid chromatography, then recrystallized from ethyl acetate–Skellysolve B to a constant specific activity of 0.44 mc. per mmole (1.87×10^8 d.p.m. per mg.) (Ritter, 1967). The recrystallized material (m.p. 168.5–169.5° C.; m.p. of blastmycin 164–166° C.; mixed m.p. 165–167° C.) was spotted along with an authentic sample of nonlabeled antimycin on each of three silica gel G thin-layer plates. R_f values of 0.51, 0.55, and 0.68 were determined for antimycin when 20% ethanol in benzene, 4% methanol in chloroform, and butanol–acetic acid–water (3:1:1) (all percentages and proportions by volume), respectively, were used for the development of these plates. In every case, over 95% of the total radioactivity on the developed plate was in the resultant ^{3}H-antimycin spot which was located by its R_f value and its fluorescence when exposed to long wavelength ultraviolet light.

The radioactivity in most samples was determined by the oxygen flask combustion technique (Ritter and Strong, 1966; Kelly *et al.*, 1961; Oliverio *et al.*, 1962). All samples which were combusted were predried under vacuum at or below room temperature.

The distribution of the tritium in the labeled antibiotic was examined by hydrolyzing portions of the material, diluted with nonlabeled antimycin, then extracting and counting the antimycin lactone formed. Only 1 to 4% of the radioactivity appeared in the extracts. The hydrolyses were carried out as described by Liu *et al.* (1960), and an infrared spectrum of the *n*-pentane-extracted material was indistinguishable from that of antimycin lactone (Van Tamelen *et al.*, 1959). Roughly 55% of the theoretical amount of the lactone was obtained. These results indicate that over 90% of the tritium in the labeled antimycin was bound to the aromatic portion of the molecule which on alkaline hydrolysis yields antimycic and blastmycic acids (Liu *et al.*, 1960).

Replicate portions of the labeled antimycin (217 to 221 × 10^3 d.p.m.) were dissolved in 1-ml. volumes of 95% ethanol. The resultant solutions were allowed to stand in the dark at room temperature for various periods of time, ranging

from 5 min. to 68 hr., then evaporated to dryness, and the residues counted. There was no change in radioactivity which indicates that the tritium on the antibiotic was not exchangeable under these conditions.

Crystalline ^{3}H-antimycin and ethanol solutions of it were found to be stable for at least 6 months when stored in the dark at $-4°$ C. Its potency against goldfish corresponded within limits of experimental error (Ritter and Strong, 1966) to that of untritiated antimycin.

Rats Used and Dosing Technique. Female animals from the Holtzman strain were used. Body weights at the start and during the course of the experiments are given in Table I. For the first two feeding trials the animals were fed a purified diet (18% casein, 0.3% L-methionine, 0.5% vitamin mix, 5% salt mix, 35.5% cerelose, 35.5% starch, 0.2% choline chloride, and 5% corn oil) which was made into gel form by mixing the dry diet with 3% agar in water (Rogers and Harper, 1965). The individual, daily dose of ^{3}H-antimycin was mixed with 2.5 grams (dry weight) of the gelled diet. To accomplish this, the dry diet was placed in a 10-ml. disposable, plastic syringe which had been cut off at the end towards the needle to give a hollow cylinder of uniform diameter, and 2.5 ml. of hot (95° C.). 3% agar solution was added (Harper and Benevenga, 1967). The resultant mixture was stirred with a small glass rod, then 10 μl. of a 100% ethanol solution of the ^{3}H-antimycin was injected with a No. 701, 10-μl. Hamilton syringe. The mixture was restirred, then allowed to cool and gel. The stirring rod was not removed until the mixture had gelled. A cylindrical food pellet approximately 3 cm. long and 1.5 cm. in diameter was obtained when the gelled diet was pushed from the syringe with the plunger.

The reliability of this dosing procedure was verified by dividing a food pellet, prepared as just described, into 10 approximately equal portions, and determining the tritium content of each portion by the oxygen flask combustion technique. It was possible to account for 95% of the tritium supposedly added to the food pellet, and the antimycin level in the various portions ranged from 6.37 to 10.3 μg. per gram dry diet. Thus, the injection procedure was reasonably quantitative, and the antimycin was fairly evenly distributed throughout the pellet, since perfect distribution would have given 8 μg. per gram.

The rats used in the third feeding trial were dosed by squirting a ^{3}H-antimycin–corn oil solution into the back of their mouths with a 0.25-ml. syringe equipped with a blunted 18-gage needle. A small portion of the oil solution leaked out of the rat's mouth each time it was dosed, but the animal immediately started washing its face and presumably consumed essentially all of the oil. These rats were fed a nonpurified stock diet.

First Feeding Trial. A total of 10 weanling rats was used in this experiment. Six of the animals (Nos. 1 through 6 inclusive) were kept in separate metabolism cages which were placed over screens and glass funnels for the collection of feces and urine. The remaining four animals (Nos. 7 through 10 inclusive) were kept in separate suspended galvanized cages with wire mesh bottoms (not metabolism cages). Each rat had a constant supply of water and was weighed each morning during the experimental period. All rats were given an unlimited amount of untreated stock diet for three days prior to the initial antibiotic treatment (start of experiment).

Each of five rats (Nos. 1 through 5 inclusive) was given one ^{3}H-antimycin food pellet (20 μg. of antimycin per pellet) each morning for 14 days. Each day after all of the treated rats had eaten essentially all of the treated pellets they were given stock, gelled diet and allowed to eat as much as desired until the evening of the same day. All food was then removed from the cages until the next morning. The remaining five control animals were fed in the same manner except they were given 5 grams of stock gel each morning instead of a treated pellet. They were not offered additional food until all of the test animals had eaten their initial food pellets. The control rats seemed to eat more readily than the test animals, so possibly the latter were able to detect the antibiotic in the food.

Each day a few crumbs from the ^{3}H-antimycin food pellet were not eaten by the treated rats. The crumbs were collected separately for each rat, and the total ^{3}H-antimycin not eaten during the 14-day period was determined from the tritium content of these crumbs. It was found that each rat had consumed over 98% of the offered antimycin.

Following the end of the 14-day treatment period, all 10 rats were allowed unrestricted intake of the gelled stock diet for 7 more days. At the end of this period all rats were killed by ether inhalation.

Feces were collected separately from each of the treated rats (Nos. 1 through 5 inclusive) and from one control rat (No. 6) on each of the 21 days of the experiment. For determination of the tritium content, the feces were dried, placed in a nichrome gauze basket (Conway *et al.* 1966) and combusted in oxygen (Kelley *et al.*, 1961; Oliverio, 1962). A fairly large amount of ash remained after the feces samples were combusted, but recombustion of part of this ash yielded no additional radioactivity.

Fecal tritium was determined separately for each animal (Nos. 1 through 5 inclusive) for each of the first 8 days of the experiment. The feces collected from these animals after the first 2 days had to be divided into two portions for combustion, since the total amount from a given rat was too great to be burned at one time. Background counts, determined from the feces of rat No. 6, did not differ significantly from normal background.

From the 10th through the 21st day, the feces from rats Nos. 1 through 5 inclusive were combined into a single lot for each day. The feces collected on the 9th day were lost. The combined feces for each day were dried, weighed, and ground with twice their weight of water in a mortar until an apparently homogeneous mixture resulted. Three weighed (0.2 to 0.4 gram each on a dry weight basis) replicate samples were removed from each mixture and the tritium in each sample determined as described above.

An attempt was made to achieve quantitative urine collection. Since the collection funnels and cages were large, the urine collected daily per rat was diluted to about 50 ml. with wash water. Consequently, the radioactivity of the diluted urine samples was so low that no accurate radioactivity determinations were obtained in this experiment. The tritium level in the carcasses of the animals was also so low that no residue measurements were made.

Second Feeding Trial. A total of 16 weanling rats was

used in this experiment. The 10 treated animals (Nos. 11 through 20 inclusive) and one control (No. 21) were kept in separate metabolism cages. The remaining five controls (Nos. 22 through 26 inclusive) were kept in separate suspended galvanized cages with wire-mesh bottoms. Each rat had a constant supply of water and was weighed each morning during the 14-day experiment. All were given stock, gelled diet and allowed to eat as much as desired during daylight hours for 3 days prior to the start of the experiment—that is, before initial dosing with the antibiotic.

Rats Nos. 11 through 20 inclusive were each given one ^{3}H-antimycin food pellet (106 μg. per pellet) each morning for 7 days. For the first 3 days of the experimental period, none of these rats was given additional food until all of them had eaten essentially all of the treated pellets. They were then given additional gelled diet *ad libitum* until the evening of the same day. All food was then removed until the next morning. From the 4th to the 7th day each individual rat was given additional food as soon as it had finished its antimycin-containing pellet. It was then allowed to eat as much as desired until the evening of the same day when all food was removed from the cages. Since some of the treated rats consistently ate the initial food pellets much more rapidly than others, their weights varied considerably, ranging from 57 to 84 grams on the 7th day of the experiment.

Rats Nos. 21 through 26 inclusive were fed exactly as above except they were initially given 5 grams of stock, gelled diet each day instead of the treated food pellet.

Five of the treated animals (Nos. 11 through 15 inclusive) and one control (No. 26) were killed with ether inhalation on the 7th day of the experiment. All remaining rats were given stock, gelled diet *ad libitum* 24 hours a day for 7 more days, then they too were killed with ether inhalation.

The uneaten crumbs from the ^{3}H-antimycin food pellets were collected separately for each rat and the antibiotic content was determined. Each test rat received a total of 739 $\pm$ 12 μg. of ^{3}H-antimycin.

Each day for the 14 days of the experiment feces were collected from each of the living, treated rats, and from one control (No. 21). The radioactivity of the control feces was determined separately each day to establish background radiation. Each day the feces collected from all the treated rats were combined into one composite sample. The 14 resultant samples were dried, weighed, homogenized, sampled, and combusted as described for the 10th through 21st day samples of the first feeding trial.

Urine was collected daily for the 14 days of the experiment from each living, treated rat and from one control rat (No. 21). The volume of the urine plus wash water varied considerably from day to day but never exceeded 50 ml. per day per rat. Each day the diluted urine from all the treated living rats was combined; then two 0.5-ml. aliquots were removed from the composite sample, placed in a scintillation vial with 18 ml. of EE-2 solution and counted. The first day composite urine sample was lost. The daily urine samples from the control rat were combined to give two samples corresponding to the 2 weeks of the experiment, and then these were used to determine background radiation.

Immediately after the rats were killed the heart, lungs, kidneys, liver, and muscle of rats Nos. 11 through 20 inclusive were examined to determine their tritium content. Since the oxygen flask combustion technique was employed, only nonvolatile tritium was measured.

Treated rats Nos. 12, 15, 19, and 20 and one control animal were used for carcass residue studies. Each carcass (minus the specific organ samples studied previously) while still frozen was passed through a meat grinder, then mixed with an approximately equal weight of water in a Waring Blendor. In each case the resultant mixture contained some small, intact pieces of skin but otherwise appeared to be homogeneous.

Three samples (0.5 to 0.8 gram fresh weight) were removed from each of the five homogenates and were weighed, dried, and combusted. This provided an estimate of the nonvolatile tritium in the entire carcass.

The volatile materials in samples of each homogenate were removed under vacuum and collected in dry ice–acetone traps. In each case, two 0.5-ml. portions of the trapped liquid were removed and placed in separate vials with 18 ml. of EE-2. Thus, a total of 10 scintillation samples, two from the volatiles of each carcass homogenate, were prepared.

Third Feeding Trial. A total of 11 rats was used in this experiment. The five treated rats (Nos. 27 through 31 inclusive) and one control (No. 32) were kept in separate metabolism cages while the remaining five animals (Nos. 33 through 37 inclusive) were kept in suspended galvanized cages. All rats (approximately 200 grams each) were given a constant supply of food and water and were weighed each morning during the 14-day experiment.

Each of the five test rats (Nos. 27 through 31 inclusive) was given 0.15 ml. of corn oil containing 1.12 mg. of ^{3}H-antimycin each morning for the first 7 days of the experiment. The remaining six animals (Nos. 32 through 37 inclusive) were given 0.15 ml. of untreated corn oil each day for the same period. All rats were then maintained for an additional 7 days during which time none of the animals received corn oil or antimycin. All rats were killed with ether inhalation at the end of this period.

Each day during the course of the 14-day experiment, one composite urine sample was collected from the five treated rats while a second sample was collected from control rat No. 32. The latter samples were later combined to give a single control urine sample. Quantitative urine collection was not attempted. The urine which was collected was diluted only slightly with water which was used to wash the urine collection jars. Each urine sample was centrifuged to remove food particles immediately after it was collected, then the supernatant was frozen for storage.

Fractionation of Urinary Radioactivity. The 24-hour composite urine sample collected from the treated rats 3 days after the initial antibiotic treatment was removed from the freezer, warmed to room temperature, and centrifuged. A white precipitate, containing essentially no tritium, had formed during the freezing and thawing procedure. Unless otherwise specified, the clear yellow urine decanted from the white precipitate was used for the following work.

Six 0.5-ml. aliquots were placed in separate scintillation vials. Two were adjusted to pH 1 with 10% HCl, two

more to pH 11 with 10% NaOH, and the remaining two were maintained untreated (pH 7). All aliquots were then lyophilized. The residue remaining in each vial was dissolved in 0.5 ml. of distilled water, then 18 ml. of EE-2 was added and the samples were counted. A fraction of each of the basic residues did not dissolve, so after the samples were counted the scintillation solution was decanted from the undissolved residues which were then dissolved in 10% HCl. The HCl solutions were counted after EE-2 was added and the observed counts included in the determination of the radioactivity of the original basic samples.

Six more replicate 0.5-ml. aliquots of the same urine sample were extracted separately with ethyl acetate after three of the aliquots had been acidified to pH 1. To each of three 0.5-ml. samples of control urine (from rat No. 32) was added 20,700 d.p.m. of ^{3}H-antimycin, and then these samples were also extracted separately with ethyl acetate. In each case, most of the solvent was removed from the ethyl acetate extract with a flow of nitrogen and the residue was dried under vacuum and counted. The aqueous phase from each extraction was lyophilized, the residue was dissolved in a mixture of 0.5 ml. of H_2O and 18 ml. of EE-2, and the resultant solution was counted.

The ethyl acetate extract from aliquots of acidified urine was examined by silica gel G thin-layer chromatography (TLC) with four different solvent systems: 4% methanol in chloroform, 6% methanol in chloroform, 20% ethanol in benzene, and butanol–acetic acid–water (3:1:1) (all percentages and proportions by volume). Samples of blastmycic acid, antimycic acid, and 3-aminosalicylic acid were chromatographed simultaneously, and the R_f's of these acids (located under long wavelength ultraviolet light) were compared with the R_f of the radioactivity on the plates (determined by scraping zones from the TLP's into counting vials, adding EE-2, then counting).

RESULTS AND DISCUSSION

The average weekly growth of the control and treated rats during the three feeding trials is shown in Table I.

The treated animals failed to gain weight normally during the second feeding trial. This fact is at least partially explained by a difference in food intake attributable to unpalatability of the test diet. A rat would frequently eat a treated pellet only slowly but rapidly consume an appreciable amount of stock diet immediately after it had finished such a pellet. During the first feeding trial, the control rats were not given additional stock diet each day until all of the treated ones had consumed the labeled antibiotic, and a difference in rate of growth was not apparent in this experiment. Treated and control animals also gained weight equally as well in the third feeding trial in which the rats were force-fed the antibiotic.

Table I. Growth of Rats Fed Antimycin

Feeding Trial[a]	Experimental Period	Average Weight of Rats, Grams/Rat	
		Control group	Treated group
1	Start of experiment	61	60
	End of first week	80	85
	End of second week	123	126
	End of third week	160	164
2	Start of experiment	55	52
	End of first week	82	69
	End of second week	130	112
3	Start of experiment	198	191
	End of first week	210	199
	End of second week	219	222

[a] Antimycin intake of test rats: trial 1, 20 μg. per day for 14 days; trial 2, 106 μg. per day for 7 days; trial 3, 1120 μg. per day for 7 days.

The feces from treated animals were softer than from the controls during the first week of both the second and third feeding trials. On the sixth day of the third trial, the antimycin-treated rats were observed to have sores around their mouths and chins. Most of the hair in the region of the sores had been lost, and inflamed lesions with scabs soon developed. The inflammations became more intense and the inflamed areas increased in size for two or three days. However, all lesions definitely appeared to be healing at the time the experiment was terminated. The sores were undoubtedly caused by the antimycin in the small fraction of the corn oil which leaked out of a rat's mouth each time it was dosed. The control animals, which received untreated corn oil, developed no sores. With the exception of the soft feces and the indicated sores, the treated animals could not visibly be distinguished from the controls.

The internal organs of both treated and control rats were examined at the end of each experiment (immediately after the animals were killed). No gross differences in the organs from the two groups were observed.

These results are in general agreement with those of Herr *et al.* (1968) who found that rats given 0.5 mg. of antimycin per kg. of body weight per day for 8 weeks and allowed to eat only antimycin-treated food consumed and gained as much as controls. Such animals appeared normal in all respects. Other rats which received 5 mg. per kg. body weight per day for 4 weeks, then 10 mg. per kg. body weight per day for an additional 4 weeks, failed to gain weight in a normal manner although food intake was normal. These rats showed soft feces within 2 weeks. However, the weights of the organs from these animals were all within the normal range and despite extensive and detailed study no histopathological changes were seen that could be attributed to the antibiotic treatment (Herr *et al.*, 1968).

The average daily excretion of fecal radioactivity (expressed as μg. ^{3}H-antimycin) by individual rats from the second through the eighth day of the first feeding trial ranged from 2.63 ± 0.36 for rat No. 1 to 4.25 ± 0.67 for rat No. 2. The daily excretion of fecal radioactivity by the entire test group, expressed as per cent of daily dose, for both the first and second feeding trials is presented in Figure 1. The excretion level rose rapidly at the beginning of both experiments but declined equally rapidly when administration of the antibiotic was discontinued. The maximum daily excretion during both experiments corresponded to roughly 20% of the daily dose. It is inferred, therefore, that at least 80% of the ingested anti-

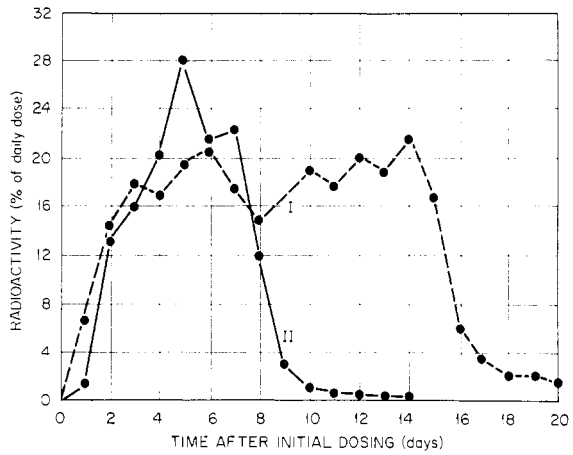

Figure 1. Radioactivity in the feces of rats fed ^{3}H-antimycin admixed with the diet

Curve I—first feeding trial: 20 μg. per rat per day, days 1 through 14; none, days 15 through 20. Curve II—second feeding trial: 106 μg. per rat per day, days 1 through 7; none, days 8 through 14

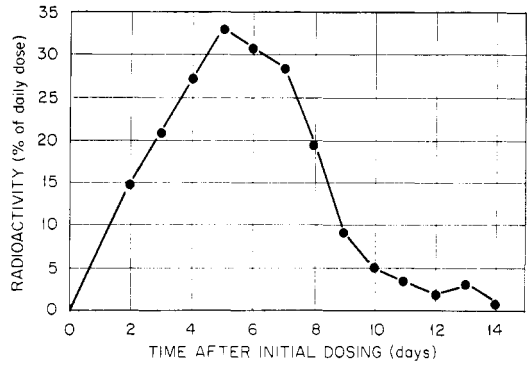

Figure 2. Radioactivity in the urine of rats fed ^{3}H-antimycin admixed with the diet

106 μg. per rat per day, days 1 through 7; none, days 8 through 14

mycin was absorbed by the animals in each feeding trial even though the dosage levels were widely different.

This observation was somewhat unexpected in view of the relatively low oral toxicity of antimycin. The oral LD_{50} value for rats has been reported to be 28 ± 6 mg. per kg. body weight (Herr *et al.*, 1968) as compared to values of 1.6 ± 0.28 (Herr *et al.*, 1968) and 0.81 (Reif and Potter, 1953) by I.P. injection. Possibly ingested antimycin is extensively decomposed prior to absorption.

The daily excretion data for urinary radioactivity (determined for the second feeding trial only) are presented in Figure 2. The values presented for the last 4 days are based on results with greater than a 10% standard deviation arising from statistical counting errors. Excretion in the urine followed the same general pattern as in the feces but corresponded to a peak of about 30% of the daily dose.

Solvent fractionation studies on urine obtained during the third feeding trial are summarized in Table II. The values listed were obtained on lyophilized samples and therefore refer to nonvolatile radioactivity only. This amounted to about 80% (9,058 ± 138 d.p.m. per 0.5 ml. of urine) of the total (11,366 d.p.m. per 0.5 ml. of urine) regardless of the pH at which the volatiles were removed. Consequently, the volatile radioactive material was neither acidic nor basic and most probably was ^{3}H-water.

Since over 85% of ^{3}H-antimycin added to control urine (pH 7) could be extracted with ethyl acetate while only 15% of the nonvolatile radioactivity was extracted from test urine under similar conditions (Table II), it was concluded that less than 15% of the total radioactivity in the urine of the experimental animals could be due to intact antimycin.

On the other hand, a much larger portion, about 90%, of the nonvolatile radioactivity was extracted at pH 1 (Table II). This fraction was examined by thin layer chromatography (TLC) in four different solvent systems. A typical TLC plate, developed with 20% ethanol in benzene, is shown in Figure 3. In this system blastmycic, antimycic, and 3-aminosalicyclic acids, known hydrolytic products of antimycin (Liu *et al.*, 1960), have R_f values below 0.20. The distribution of radioactivity on the plate was 66% in zone 1, 19% in zone 2, and 15% in zone 3. Zone 4 was used to determine background radiation. By comparing the radioactivity in zone 3 with the total radioactivity on the plate it appears that a maximum of 15% of the total radioactivity was present in the hydrolytic products examined.

Table II. Fractionation of Urinary Radioactivity[a]

Urine Treatment	Nonvolatile D.P.M. Extracted	Nonvolatile D.P.M. Unextracted
pH 7 from treated rats; extracted four times with ethyl acetate	1,278 1,236 1,276	6,789 7,014 7,295
pH 1 from treated rats; extracted four times with ethyl acetate	7,040 7,127 7,170	886 800 969
pH 7 from treated rats; lyophilized; residue extracted three times with 100% ethanol	7,012 6,940	1,416 1,279
pH 7 from control rat; add 20,700 d.p.m. ^{3}H-antimycin; extract four times with ethyl acetate	17,306 17,718 17,742	1,882 1,849 1,613

[a] Urine from five rats each (except the control) given 1.12 mg. ^{3}H-antimycin per day for 3 days prior to urine collection. Small (0.5 ml.) aliquots of a 24-hr. composite sample were used. The individual values were obtained in replicate trials.

The results of these fractionation studies indicate that the antimycin metabolites excreted in the urine can be subdivided approximately as follows: (a) nonvolatile 80%, volatile 20%—probably ^{3}H-water; (b) neutral (extractable at pH 7), 15% of nonvolatile—may or may not include intact antimycin; (c) acidic plus neutral (extractable at pH 1), 89% of nonvolatile—products derived primarily from the aromatic portion of the molecule but not containing basic nitrogen; up to 15% of this fraction possibly consisting of blastmycic acid, the only one of the three known hydrolysis products examined which is

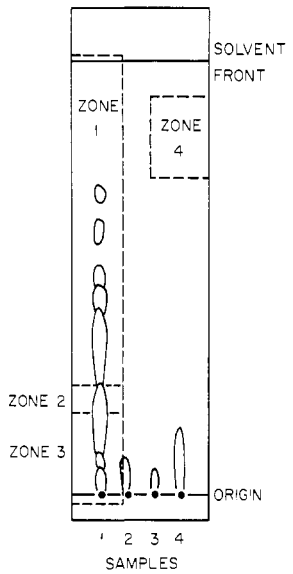

Figure 3. Thin-layer chromatogram developed with 20% ethanol in benzene

Samples: 1, an ethyl acetate extract of acidified urine from rats each given 1.12 mg. of ^{3}H-antimycin per day for 3 days prior to urine collection; 2, blastmycic acid; 3, antimycic acid; 4, 3-aminosalicylic acid. The spots were located by viewing under ultraviolet light

readily extractable from acidic solution by fat solvents (Liu *et al.*, 1960); and (d) highly water-soluble material not readily extractable with ethyl acetate, 11% of nonvolatile.

Table III summarizes the nonvolatile tritium levels in specific tissues of rats killed during the second feeding trial, while Table IV shows the volatile and nonvolatile tritium levels in homogenates prepared from the carcasses of some of the same animals. The volatile tritium levels in the carcasses of the animals killed on the 14th day of the experiment are based on results with greater than 10% but not over 15% standard deviation arising from statistical counting errors.

The radioactivity was distributed throughout the test animals but was higher in heart, kidney, and liver than in muscle. A similar pattern of distribution was observed in fish (Ritter and Strong, 1966). The data in Tables III and IV also indicate that the radioactivity decreased very markedly and rapidly as soon as the dosing with antimycin was discontinued. The average values for rats 16 through 20 (killed after 7 days without dosing) ranged from 20.5% (heart) to 38.7% (muscle) of the corresponding values for rats 11 through 15 (Table III). Similar reductions also occurred in the entire carcass.

From the data in Table IV it may be calculated that rats No. 12 and 15 contained in the entire carcass radioactivity equivalent to a total of 154 and 193 μg. of the original ^{3}H-antimycin, respectively (ignoring samples removed for Table III analyses). On the same basis the bodies of rats No. 19 and 20 contained the equivalent of only 48 and 50 μg., respectively. Each of these animals had consumed a total of 739 ± 12 μg. of antimycin. Thus over 90% of the tritium, whether present in intact antimycin or—more probably—in various metabolic degradation products, was eliminated from the body within a period of 1 week after administration of the labeled antibiotic was discontinued. These results demonstrate quite conclusively that antimycin is not a cumulative poison, but that it is rapidly degraded by and eliminated from the animal body.

Table III. Nonvolatile Radioactivity in Specific Tissues of Rats Administered ^{3}H-Antimycin in the Diet[a]

Rat No.[c]	Nonvolatile Radioactivity Equivalent to μg. of Antimycin per Gram Fresh Weight[b]				
	Kidney	Heart	Muscle	Lung	Liver
11	1.15	1.32	1.26	0.58	1.42
	1.19				1.34
					0.95
12	1.33	1.52	0.68	0.56	1.19
	1.35				1.21
					1.16
13	1.29	1.67	0.83	0.52	1.43
	1.24				1.48
					1.45
14	1.32	1.49	0.47	0.59	1.12
	1.34				1.20
					1.18
15	1.26	1.56	0.52	0.49	1.21
	1.08				1.20
					1.16
Av. of rats 11 through 15	1.26	1.51	0.75	0.55	1.25
16	0.29	0.27	0.22	0.15	0.38
	0.27				...
					...
17	0.32	0.33	0.30	0.14	0.32
	0.31				0.32
					...
18	0.28	0.30	...	0.15	0.32
	0.26				...
					...
19	0.34	0.31	0.34	0.14	0.36
	0.32				0.36
20	0.34	0.33	0.31	0.17	0.34
	0.32				0.34
					0.32
Av. of rats Nos. 16 through 20	0.31	0.31	0.29	0.15	0.34

[a] Second feeding experiment.
[b] The left kidney, right kidney, lungs, and heart of each rat were each used for single activity determinations. A muscle sample was taken from the right hind leg of each rat. The liver from each rat was homogenized with water and then three replicate samples were examined.
[c] Rats numbered 11 through 15 and 16 through 20 inclusive were killed and the tissues analyzed at the end of the 7th and 14th days of the experiment, respectively.

Table IV. Volatile and Nonvolatile Radioactivity in Carcasses of Rats Administered ^{3}H-Antimycin in the Diet[a]

Replicate Samples	Radioactivity Equivalent to μg. Antimycin per Gram Fresh Weight[b] Rat No.			
	12	15	19	20
	VOLATILE			
1	1.78	1.49	0.13	0.20
2	1.67	1.54	0.14	0.25
	NONVOLATILE			
1	0.72	0.94	...	0.24
2	0.71	0.90	0.26	0.26
3	0.73	1.03	0.24	0.26

[a] Second feeding trial.
[b] Based on radioactivity of whole body homogenates (minus specific tissue samples—Table III). Rats 12 (63 grams) and 15 (78 grams) were killed after 7 days and 19 (123 grams) and 20 (122 grams) after 14 days.

Table V. Recovery of Radioactivity from Rats Administered ^{3}H-Antimycin in the Diet[a]

Fraction	Rats Killed on 7th Day	Rats Killed on 14th Day
	——Per Cent of Dose——	
Feces	17.6	20.3
Urine	22.2	27.0[b]
Tissues, volatile ^{3}H	15.4	3.0
Tissues, nonvolatile ^{3}H	8.1	3.8
Not recovered	36.7	45.9

[a] 739 μg. antimycin consumed per rat.
[b] Urine from days 11 through 14 not included.

A tritium balance for the second feeding trial is presented in Table V. About 20% of the ingested radioactivity appeared in the feces during the 14-day experiment while another 27% appeared in the urine. The volatile and nonvolatile tissue levels are based on the carcass homogenate studies. One third to one half of the radioactivity could not be accounted for and was presumably lost from the animals as water. Part of the missing tritium could also have been lost from the urine by evaporation before the urine samples were collected each day.

A quantitative tritium balance was not possible for the first feeding trial since activity levels were often too low to be determined accurately with the techniques employed. However, the fact that the tissue tritium levels were low indicated that there was no accumulation of antimycin and that its elimination must have been rapid.

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