Summary. Although rats grow at a rapid rate on a synthetic diet, it is possible to obtain small increases in the rate of growth by the addition of liver and liver preparations. The differences can also be obtained when the basal ration contains adequate amounts of folic acid. A larger and more consistent response to liver can be demonstrated when a corn-soybean meal ration is used. The addition of liver to the synthetic ration fed female rats increased the percentage of young surviving during the lactation period.

A factor in liver has been shown to stimulate the growth of *S. faecalis*. Assay with this organism indicates that an increased amount of the factor is excreted in the urine and stored in the liver of rats fed liver preparations. It is suggested that more rigorous methods are needed, such as prevention of coprophagy, use of natural rations or more extensive treatment of the components of the synthetic ration in order to devise suitable assay procedures.

We are indebted to Merck and Co., Inc., Rahway, N. J., for crystalline vitamins; to the Wilson Laboratories, Chicago, Ill., for liver powder 1:20 and whole liver powder; to E. R. Squibb and Sons, New York, for desiccated whole liver and lyophilized liver; to Lederle Laboratories, Inc., Pearl River, N. Y., for synthetic folic acid and several liver fractions.

15846

Some Pharmacological Characteristics of Bacitracin II. Absorption and Excretion of Bacitracin in the Dog.*

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Continuing an investigation of the pharmacological characteristics of bacitracin,[1] we have studied the absorption and excretion of the antibiotic in the dog. Because such studies are of fundamental importance in the rational use of the drug, the following data are presented.

Experimental. One female and 5 male mongrel dogs, weighing 7.5 to 11 kg, maintained on a stock diet and water *ad libitum*, were fasted over-night before use. In general, fasting dogs were given 200 cc of water by stomach tube at 9:00 a. m. and again at noon to insure an adequate diuresis. The antibiotic, a single lot designated B-100,† was administered by various routes at approximately 9:30 each morning, and sterile blood samples were taken at specified time intervals thereafter. The blood was permitted to clot and sterile samples of serum, diluted one to 3, were used directly in the assay procedure. Urine samples were collected 7 and 24 hours after administration of the drug. Occasionally 3- and 5-hour urine samples were collected in connection with distribution studies. These, and the routine 7-hour samples were taken by catheter. The 7- to 24-hour specimen was collected in a metabolism cage as a total sample. The urine samples were diluted one to 10 and passed through a Selas filter before testing. For stool analysis, a 1 g aliquot of the well mixed specimen was triturated with 50 cc of water and the clear supernatant fluid was passed through a Selas filter before testing.

The test procedure, involving the inhibition of the Chanin strain of β-hemolytic strep-

* The work described in this paper was done under a contract between the Office of the Surgeon General and Columbia University. Administration of the contract was directed by Dr. Frank L. Meleney.

1 Scudi, J. V., and Antopol, W., PROC. SOC. EXP. BIO. AND MED., 1946, **64**, 503.

† We are indebted to Dr. John T. Goorley of the Ben Venue Laboratories for the bacitracin.

tococci. Group A, was designed by Miss Balbina Johnson, and will be described by her elsewhere. In our hands, the method gave sharp end-points at .001 ± .0003 units of bacitracin per cc. In a series of 29 analyses of standard solutions containing 3.0 units of bacitracin per cc, the mean value was 2.94 ($\sigma = 0.5$). Control urine and stool filtrates gave no inhibition of the organism in the test procedure, and recovery of added bacitracin‖ was satisfactory in both cases. Recovery of bacitracin added to serum, however, was not good. In a series of 38 experiments involving bacitracin added in concentrations ranging from .03 to 1.0 units per cc of serum, the mean recovery was 42% ($\sigma = 11.3$). All serum values were, therefore, corrected by dividing by 0.42. The data submitted in the following pages are subject to these wide variations, but no other analytical method is yet available. The greatest variations occurred in attempting to duplicate analyses on different days. To keep such variations minimal and to obtain at least comparable data, all analyses in each experiment were performed in duplicate on the same day whenever possible.

Results. Following oral administration in doses of 3,000 and 6,000 units per kg body weight, no detectable bacitracin (that is, less than 0.01 unit per cc of serum) was found in the blood stream, nor was any found in the urine during the 24 hours after administration of the drug. Following oral administration of 1,500 units per kg body weight to 2 dogs, less than 5% of the oral dose was recovered in the stool. Consequently, it appears that the antibiotic is largely destroyed in the gastro-intestinal tract. This finding is in agreement with the remarkable lack of oral toxicity reported in the mouse.[1] The presence of small but significant amounts of bacitracin in the stool suggests that the drug may be useful in the treatment of infections of the intestinal tract. Subcutaneous injection of the larger doses gave the blood concentrations of bacitracin shown in Fig. 1. It is of interest to note that appreciable concentrations remain in the blood as long as 7 or 8 hours after a single injection of the drug. Peak concentrations are relatively low compared to those observed following intramuscular and intravenous injection. This observation suggests, as an explanation for the low acute subcutaneous toxicity in the mouse, (1) that the balance of

‖ These were simple recovery experiments and did not include an aging study with reference to possible inactivation of the antibiotic.

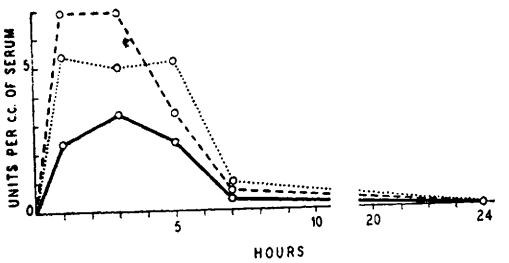

Fig. 1.
Serum concentrations following subcutaneous injection of 6000 u/kg (dot-dash lines) and 3000 u/kg (solid line) of bacitracin.

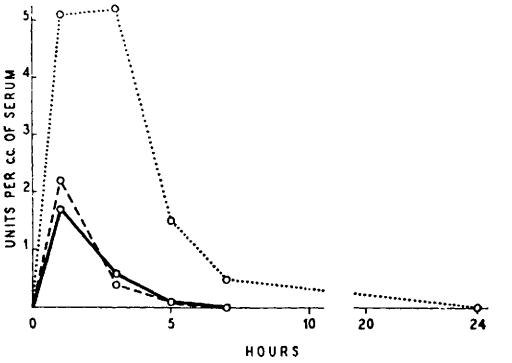

Fig. 2.
Serum concentrations following intramuscular injection of 3000 u/kg (dotted lines) and 1000 u/kg (dashed and solid lines) body weight of dog.

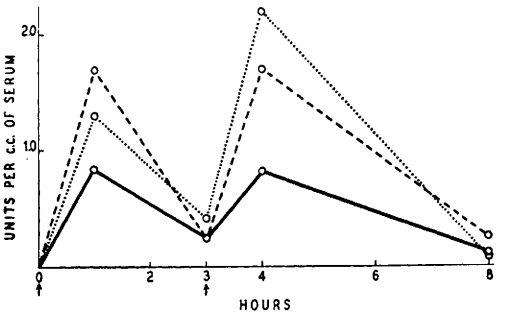

Fig. 3.
Serum concentrations following intramuscular injections of 1000 u/kg in each of three dogs. Arrows indicate times of injection.

absorption, detoxication and excretion of the drug, which keeps the blood levels low, prevents the accumulation of lethal concentrations at higher centers. Following intramuscular injections of 1,000 and 3,000 units per kg body weight, relatively higher blood concentrations were observed (Fig. 2). These were not quite so persistent as those following subcutaneous injection, but at the higher dose level an appreciable concentration remained 7 hours after dosing. Repeated intramuscular injections of 1,000 units per kg gave the data shown in Fig. 3.

None of the dogs exhibited toxic signs in the course of the preceding experiments, but following the rapid intravenous injection of concentrated solutions central effects were noted. Injection of 3,000 units per cc of solution at the rate of 3 to 4 cc per minute until a dose of 3,000 units per kg had been given, caused in order of appearance, salivation and associated signs of nausea, spastic, outstretched hind quarters, and a scoliosis owing to muscle spasm. These signs disappeared within 5 minutes after the drug was administered. With another dog given 1,000 units per kg within one minute (concentration 1,000 units per cc) evidence of nausea and muscle spasm appeared. On the other hand, when 3,000 units per kg were given over a 6-minute period in the form of a solution containing 1,000 units per cc, the only signs were those of nausea and a slight central depression, both of which disappeared rapidly. Lower doses given more slowly produced no noticeable effects. These observations suggest that at the present stage of its purification, bacitracin should be administered slowly and in dilute solution, if the drug is given intravenously. Indeed, persistence data suggest that subcutaneous or intramuscular administration may be the routes of choice. The data obtained following intravenous administration are shown in Fig. 4. Here, the blood concentration—time curves rise and fall precipitously. At a dose level of 300 units per kg body weight, a maximum of 3.6 units per cc is attained and the blood is essentially cleared of the drug in 4 hours. At higher dose levels the maxima and the persistence are increased.

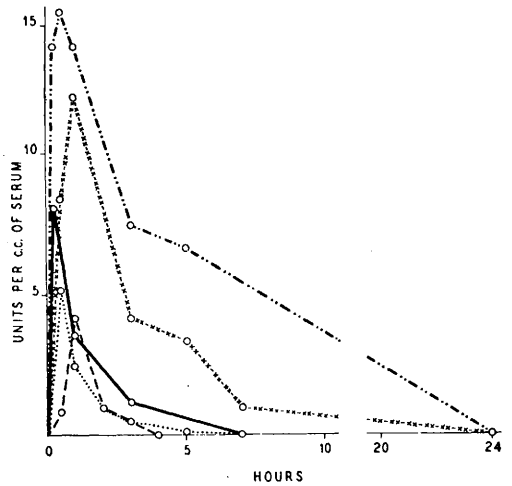

Fig. 4.
Serum concentrations following intravenous injection of 3000 u/kg (dot-dash and cross-hatched lines) 1000 u/kg (solid and dotted lines) and 300 u/kg (dashed lines),

The urinary excretion data obtained in conjunction with these experiments are presented in Table I. The lack of urinary excretion of the drug following oral administration has been considered. Following other modes of administration, the recovery of bacitracin appears, in general, to increase with the dose administered, but the recoveries are widely variable. As little as 7 or as much as 98% of the dose may be recovered in the urine. Relatively high concentrations persist in the urine for more than 7 hours after a single dose of the drug, and significant concentrations are found in the 24-hour specimen. This persistence in the urine may make the drug useful in urologic conditions.

In a study of the distribution of bacitracin between erythrocytes and plasma, 3,000 units of the antibiotic per kg body weight were injected intravenously, and after one hour, sterile blood samples were collected under oil. The samples were defibrinated and centrifuged anaerobically. Quadruplicate analyses, performed on the whole blood, serum and erythrocytes‡ gave the following average values: 8, 14 and less than 0.2 units per cc, respectively. The whole blood concentration of 8 units per cc was in good agreement with the value of 8.4 units per cc calculated from

‡ Bacitracin concentrations as high as 100 units per cc did not alter the fragility of the erythrocytes.

TABLE I.
Urinary Excretion of Bacitracin in the Dog.

Mode of administration	Dose given Units/kg	Total	7-hr urine Units/cc	Total	24-hr urine Units/cc	Total	Total	% recovery
Oral	6000	38,400	0	0	0	0	0	0
"	3000	18,900	0	0	0	0	0	0
Subcutaneous	3000	33,000	20	9,700	8	2,100	11,800	36
"	6000	63,000	35	15,500	4	1,600	17,100	27
"	6000	43,200	—	—	32	23,600	23,600	55
Intramusc.	1000	9,200	18	6,500	1	590	7,100	77
"	1000	7,400	14	1,800	2.3	400	2,200	30
"	3000	27,300	92	26,000	3	1,100	27,100	98
Intravenously	300	3,300	0.4	200	0.2	50	250	7
"	1000	7,200	—	—	3	1,300	1,300	18
"	1000	7,300	56	2,000	1.3	100	2,100	29
"	3000	21,000	0.6	300	2.3	8,900	9,200	44
"	3000	18,600	72.0	4,300	9.0	3,600	7,900	42

the serum concentration. These findings are in agreement with results obtained by adding bacitracin to whole blood, cells, and serum *in vitro*. Two types of experiments were performed. In the first, 6.66 units of bacitracin were added per cc of serum.§ The average recovery in triplicate experiments was 5.5 units per cc. The original red blood cells were then added to the serum and the reconstituted blood was incubated for 10, 30 and 60 minutes, and the serum was separated. Triplicate analyses of the serum gave an average value of 5.2 units per cc. Thus, none of the bacitracin was taken up by the cells. As an additional control, 5 units of bacitracin was added per cc of red cells. Following hemolysis triplicate analyses gave an average recovery of 5.1 units of bacitracin per cc of cells. In the second type of experiment, bacitracin was added to whole blood and the system was incubated for 30, 60 and 120 minutes. In one series of experiments, 2 units of bacitracin were added per cc of whole blood. The average recovery from serum in 6 analyses was 2.2 units per cc, and the cells contained less than 0.1 unit per cc. In another series of experiments, 6 units of bacitracin were added per cc of whole blood, and again the system was incubated for the same periods of time. The average recovery from serum in 3 determinations was 5.2 units per cc, and the red blood cells contained less than 0.2 unit per cc. Thus, it may be concluded that bacitracin does not penetrate the red blood cell. In connection with these findings, it was of interest to calculate the *approximate* volume of distribution of the drug in the dog. In 4 analyses, the average volume of distribution after intravenous or intramuscular injection was 40% of the body weight as shown in Table II.

That bacitracin is not freely diffusible in the animal organism was further demonstrated by comparison of the concentrations in the serum and spinal fluid of the monkey. In a series of 5 monkeys, used in chronic toxicity studies which will be reported later, serum concentrations of 6.6, 0.75, 0.18, 8.1 and 6.6 units per cc were found. Analysis of spinal fluid taken at the same time gave concentrations of 0.34, .009, .08, .34 and .34 unit per cc of spinal fluid, respectively.

Summary. The absorption and excretion of bacitracin have been studied in the dog. Only small fractions of the oral dose were recovered in the stool. Since none was found in the blood or urine after oral administration and little was found in the feces, it appears that the antibiotic was largely destroyed in the gastro-intestinal tract. Following parenteral administration of large single doses, significant concentrations of the drug persisted in the blood stream for as long as 7 or 8 hours. Other than slight and transient central effects produced by excessively rapid intravenous injection, the animals showed no apparent signs of toxicity in spite of the fact that doses as high as 6,000 units per kg body

§ Recoveries of added bacitracin at these higher concentrations was uniformly better than 42%. The data in this section therefore are given directly without any correction factor.

TABLE II.
Apparent Volume of Distribution* of Bacitracin in Male Mongrel Dogs.

Dog No.	Wt kg	Total dose in units	No. hr after inj.	Plasma conc. units/cc	Total urinary output in units	Apparent volume of distribution in liters	% body wt
156	6.4	19,200†	3	7.5	2,200	2.3	36
156	6.4	19,200†	5	6.7	4,300	2.2	35
151	7.3	7,300†	3	1.3	2,000	4.1	56
229	9.2	27,600‡	7	0.44	26,200	3.2	34

* The total dose, minus the fraction excreted, divided by the plasma concentration gives the number of liters of body water in which the bacitracin appears to be distributed. The apparent volume of distribution is expressed in terms of body weight.
† Dose administered intravenously.
‡ " " intramuscularly.

weight were administered. The recovery of the drug in the urine appeared, in general, to increase in direct ratio to the dose administered, but the recoveries were widely variable. Significant concentrations of bacitracin persisted in the urine for more than 7 hours after administration of a single dose of the antibiotic. Bacitracin is not freely diffusible. It did not penetrate the red blood cell, nor did it enter the spinal fluid freely.

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Effect of Muscular Activity on Curarization in Rabbits.

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In studying the curariform action of a group of quaternary quinine derivatives by the rabbit head-drop method, one of us[1] observed that if rabbits struggled following a single intravenous injection of these paralyzing substances, head-drop seemed to occur more rapidly than usual. More recently, in the course of studies with *d*-tubocurarine, it was observed that shortly after recovery from, or even during, convulsions induced in rabbits by the injection of cocaine or veratrine, the amount of *d*-tubocurarine required to produce "head-drop" was markedly reduced. Torda and Wolff[2] have demonstrated the presence of a curare-like agent in the serum of blood obtained from the hind limbs of anesthetized cats following tetanic stimulation of the sciatic nerve or following passive exercise of the extremities. In view of these findings, quantitative studies have been made of the synergistic effect of exercise on curarization, using the rabbit "head-drop" assay method.

Method. Several types of muscular exertion were investigated.

(1) *Running.* Rabbits were exercised in a long corridor for 5 minutes, in response to gentle stimuli from the experimenter, who followed the animal at a rate no faster than a normal walk. Animals, thus exercised, showed signs of exertion such as increased respiration, but, of course, were by no means exhausted. This method proved more satisfactory than were attempts to exercise rabbits in a treadmill.

(2) *Violent exertion.* When the hind legs of rabbits were grasped and held off the floor, the animals made violently active attempts to escape; these repetitive efforts were punctuated by brief intervals of rest. One-half-

1 Chase, H. F., Lehman, A. J., and Rickards, E. E., *J. Pharm. and Exp. Therap.*, 1944, **82**, 266.

2 Torda, C., and Wolff, H., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 242.