ganic factor other than thiamine, riboflavin, pyridoxine, calcium pantothenate, nicotinic acid, inositol, *p*-aminobenzoic acid, biotin or folic acid, a substance not present in significant amounts in wheat germ, casein or yeast, but required for normal growth and ovarian development in the immature thyroid-fed rat.

Summary. The administration of liver completely counteracted the retardation of growth and inhibition of ovarian development observed in immature rats fed toxic amounts of thyroid. Wheat germ, yeast, or increased amounts of salt mixture, casein, thiamine, riboflavin, pyridoxine, calcium pantothenate, nicotinic acid, inositol, *p*-aminobenzoic acid, biotin or folic acid were ineffective in this regard. The suggestion is made that liver contains some factor other than the above required for normal growth and ovarian development in the immature thyroid-fed rat.

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Some Pharmacological Characteristics of Bacitracin.*

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Bacitracin, an antibiotic discovered by Meleney and co-workers[1] in the filtrate of surface cultures of the Tracy strain of *B. subtilis*, has become of increasing interest, particularly because of its potential value in the treatment of penicillin-resistant infections. Because of this increased interest, the following preliminary data on the toxicological and pharmacological properties of the antibiotic are presented.

Acute toxicity experiments. Fresh bacitracin† solutions used for oral and intraperitoneal administration contained the dose in 1 cc of water; solutions for intravenous and subcutaneous injections contained the dose in 0.25 to 0.50 cc per 20 g of mouse, or 50 g of rat. All samples gave clear solutions at pH 6 to 7 except No. 187, which dissolved at pH values of 5 to 6. For oral or subcutaneous administration, fine suspensions were administered because of the large doses required. Solutions of earlier samples of bacitracin were brown, but more highly purified later samples, illustrated by No. B-100, were almost colorless.

Experiments were performed with male, white, Rockland Swiss mice weighing 20 to 25 g, and male white rats of the Sherman strain maintained on a stock diet with free access to food and water at all times. Surviving animals were observed for a period of at least 7 days after acute or chronic dosing.

Results of the acute toxicity experiments are shown in Table I. Comparison of the first 4 samples, all administered intraperitoneally, indicates that the toxicity varies independently of the activity. (The fourth sample, No. B-100, is much less toxic than the other 3 and is representative of the batches now being produced). In this connection it may be noted that inactivation of the first sample under mild conditions (pH 7.0 at 37° for 8 days) did not alter the toxicity of the preparation. The LD_{50}, 200 mg per kg, did not differ significantly from the value of 240 mg per kg obtained with the active sample. Neither the scattering of the deaths, nor the toxic signs, nor the pathological lesions in mice differed appreciably

* 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 Johnson, B. A., Anker, H., and Meleney, F. L., *Science*, 1945, **102**, 376.

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

TABLE I.
Acute Toxicity of Bacitracin in Mice and Rats.

	Sample No.	Animal	Mode of administration	Activity of sample U per mg	No. of animals between LD_{16} and LD_{84}	LD_{50} mg per kg	LD_{50} units per kg	σ	Stand. dev. LD_{50}
1	187	mice*	I‡	35	170	240	8,500	3,800	410
2	188	,,	,,	13	90	650	8,500	2,000	300
3	203	,,	,,	21	113	360	7,500	2,400	320
4	B-100 new process 187	,,	,,	30	80	500	15,000	4,900	770
5	inacti-vated	,,	,,	0	40	200 $\sigma = 50$ SD.$LD_{50} = 11$			
6	203	,,	Iv§	21	75	360	7,500	1,300	210
7	203	,,	S‖	21	75	2,500	52,000	20,000	3,300
8	208WI	,,	,,	24	70	1,300	31,000	7,000	1,200
9	187	rats†	I‡	35	49	190	6,700	2,500	500

* The majority of deaths occurred during the first 24 hours, with large numbers dying during the second 24 hours, and occasional deaths thereafter.
† All deaths occurred within the first 24 hours.
‡ I = intraperitoneal.
§ Iv = intravenous.
‖ S = subcutaneous.

from those observed with the active material. Since the toxicity of the sample was not altered by destruction of the active principle, it seems reasonable to assume that further purification of bacitracin will lead to a lowered toxicity. The third sample (203) gave identical LD_{50}'s after intravenous and intraperitoneal injections. The acute subcutaneous and oral toxicities of bacitracin were much lower than the intraperitoneal toxicity: for example, sample No. 203 was about 7 times as toxic when given intraperitoneally as when given subcutaneously. There was no direct comparison for the eighth sample (208 WI) but here again the subcutaneous toxicity represented by the LD_{50} was notably low. Given orally in amounts increasing up to 3.75 g per kg or 131,000 units per kg, sample No. 187 failed to produce death. At 7.5 g per kg 2 mice out of 5 died. These deaths may have been influenced by the high osmotic concentration of the solutions administered. The oral and subcutaneous findings are similar to those observed by Molitor *et al.*[2] in a study of streptomycin.

The acute intraperitoneal toxicity of sample 187 was significantly greater in rats than in mice. There were no deaths subsequent to the first 24 hours after the administration of the drug to rats. Further, renal lesions, described below, were striking in mice but were minimal in rats. The intravenous toxicity of bacitracin appeared to be greater in rats than in mice. Sudden, convulsive deaths could be produced regularly at doses as low as 4000 units per kg of sample No. 187 intravenously.

Signs of acute toxicity were similar regardless of the sample of bacitracin used. Following intraperitoneal injection of lethal doses, mice exhibited hyperirritability and clonic convulsions followed by an increasingly severe depression. Central respiratory paralysis appeared to be the cause of death in both mice and rats. Similar effects were produced more rapidly by intravenous injections. No acute toxic effect other than

[2] Molitor, H., Graessle, O. E., Kuna, S., Mushett, C. W., and Silber, R. H., *J. Pharm. Exp. Ther.*, 1946, **86**, 151.

apathy was noted after oral or subcutaneous administration of the impure antibiotic. Complete blood counts were made in all mice prior to, and serially in surviving mice for 8 days after the intraperitoneal injection of B-100. Although large doses, grouped around the LD_{50}, were given, no abnormalities in blood morphology were observed.

Chronic toxicity experiments. Groups of 25 mice were given bacitracin (No. 187) subcutaneously at doses of 2, 20, and 50 units per 20 g mouse‡ every 4 hours for 5 days. The total dose administered during the 5-day period was 3,000, 30,000 and 75,000 units per kg of mouse. None of the animals died during the period of treatment nor in the following 7 days. The animals appeared normal and made average weight gains of 5% of body weight during the 5 days of treatment and 10% during the ensuing 7 days, regardless of the dose given.

Rats weighing 80 to 90 g were given 6,000 units per kg of bacitracin intraperitoneally. One week later surviving animals were divided into 2 groups of 10 each. Animals in the first group were given, subcutaneously, 650 units per kg per day, 5 days of each week for 13 doses followed by 12 additional doses intraperitoneally. Animals in the second group were maintained on the same schedule but the chronic doses were doubled. Rats in the first and second groups received a total of 22,250 and 38,500 units of bacitracin per kg, respectively. With the exception of local areas of alopecia at the site of injection all animals appeared grossly normal. During the 5 weeks of treatment, animals of the first group grew from an average of 88 to 177 g. Those in the second group grew from 84 to 150 g, suggesting that the larger doses impeded growth to a slight extent.

Pathological changes in mice and rats following single or repeated doses. Gross examination of the organs following acute deaths showed congested splanchnic vessels and emphysematous lungs. Mice which survived large doses for more than 24 hours lost weight and exhibited enlarged, edematous, ischemic kidneys. Kidney involvement appeared maximal 3 to 5 days after intraperitoneal injection of bacitracin, and then appeared to regress. With the exception of adrenal enlargement, remaining organs were normal grossly. At the site of subcutaneous injection, induration, congestion and hemorrhage were often found with the early preparations; product B-100, manufactured by a new process, caused considerably less irritation.

Histologically, renal damage in the mouse was striking. With moderate involvement, the cytoplasm of cells of the convoluted tubules was filled with fine eosinophilic granules, particularly in the free portion of the cell; in places it appeared as though this portion of the cell was being cast into the lumen. The loops of Henle, particularly the descending limbs, were filled with deeply staining eosinophilic material usually in the form of casts or globules. Similar material was also present in the collecting tubules. The loops of Henle and collecting tubules were markedly dilated. With severe involvement there was tubular necrosis so that identification of the individual tubule was difficult. There was necrosis of the convoluted tubules which appeared to involve the distal convoluted tubules and, occasionally, the proximal tubules. The lumina were filled with necrotic cells, nuclear debris and calcium particles. Similar material was found in the loops of Henle and collecting tubules. Rarely a tubule was calcified. Bowman's space was distended with coagulum but the glomerular tuft proper appeared uninvolved. Mice in which appreciable tubular necrosis was found were either dying or were sacrificed when moribund.

Mice which survived 6 days after administration of the drug showed marked dilation of the collecting tubules and, to a less extent, the loops of Henle. The collecting tubules contained eosinophilic globoid bodies or casts which completely filled the lumen and had the appearance of coagulated serum. At times basophilic bodies were found. Tubular calcification was not frequent. After 10 to

‡ This may be assumed from the work of Meleney *et al.* to represent 1, 10, and 25 times the therapeutic dose.

12 days a less pronounced picture was observed. There were small depressed subcapsular areas composed of collapsed tubules, and a rare atrophic glomerulus. Cystically dilated tubules and collections of monocytic cells about vessels were seen. Some of the tubules were lined with basophilic cells and mitosis was occasionally observed. The nuclei of the tubular cells varied considerably in size and staining quality. With repeated subcutaneous doses varying from 2-50 units per 20 g mouse, renal lesions observed one week after the final injection were inconspicuous, consisting for the most part of occasional tubular casts. Necrotic tubules were infrequent.

The thyroids appeared moderately hyperplastic.

In the rat renal lesions were minimal and necrosis of cells rare.

Other pharmacological data. The best available preparation of bacitracin (No. B-100) was used in gathering the following data. A 5% solution containing 1500 units per cc was found, by means of the freezing point depression, to be approximately isotonic with frog-Ringer solution. The contractility and rhythmicity of the frog's heart perfused by the Straub technic were not altered by the addition of 15 units of bacitracin per cc of frog-Ringer solution. Higher concentrations often caused diminished contractility and progressive slowing.

Tests with the isolated guinea pig ileum indicated that bacitracin contains a minute amount of a histamine-like substance; for example, 100 mg or 3,000 units of preparation B-100 contained the equivalent of approximately 3 μg of histamine diphosphate. Bacitracin-induced contractions were essentially abolished by first adding 1 cc of 1-100,000 pyribenzamine hydrochloride to the 50 cc bath used in the experiments.

Intravenous injection of bacitracin solution into the dog, anesthetized by intravenous pentobarbital, caused an acute transient fall of blood pressure immediately followed by an equally transient rise; for example, 10 mg or 300 units of lot B-100 per kg body weight caused a fall of 30-40 mm of Hg followed by a rise of 10-20 mm of Hg above the preinjection level of the blood pressure. Each effect lasted only a few seconds. Either benadryl or pyribenzamine essentially abolished the action of equidepressor doses of histamine diphosphate, but neither altered the depressor effect of bacitracin. Hence, histamine is not the cause of the depressor effect. This depressor effect, unlike that of acetylcholine, was not prevented by atropine sulfate. The pressor phase of the diphasic blood pressure response and the vagal heart beats associated with it disappeared after the injection of pyribenzamine.

Summary. The acute, intraperitoneal toxicity of bacitracin concentrates was greater in rats than in mice. The subcutaneous LD_{50} in mice was 4-7 times the intraperitoneal LD_{50}. Lethal results were produced only with immense oral doses. Damage to the renal tubules, with tubular necrosis after very large doses, was observed in mice whereas in rats there was little kidney involvement. The toxicity of bacitracin, as measured by the LD_{50}, appeared to be independent of the antibiotic activity. Concentrates of the antibiotic contained a minute amount of an histamine-like substance which was not responsible for the definite depressor effect caused by the intravenous injection of 10 mg (300 units) per kg into the dog.

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