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Some Pharmacological Characteristics of Bacitracin
III. Chronic Toxicity Studies of Commercial Bacitracin in the Dog and Monkey.*

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In earlier studies of the toxicity of bacitracin,† nephrotoxic changes were oberved in the mouse, but not in the rat.[1] In further studies of the absorption, distribution, and excretion of bacitracin in the dog,[2] no apparent clinical signs of renal toxicity were observed. Because of these differences, it appeared desirable to study the toxicity of bacitracin further. The effects of prolonged daily administration of the antibiotic to dogs and monkeys were, therefore, investigated.

Comparison of a number of samples of commercial bacitracin clearly indicated that the toxicity varied independently of the activity[1] and since the toxicity 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. Sample No. B-100 was much less toxic than the others and was therefore used for the most part in the tests described below.

Experiments in Dogs. Three mongrel dogs, which had been given bacitracin previously in the course of absorption and excretion studies,[2] each weighing between 7 and 9 kg were maintained on a stock diet of Friskies‡ dog meal and water *ad libitum* for a period of 28 days. During this period, control blood counts were performed on each dog at approximately weekly intervals. The data obtained were within normal limits. During this control period, samples of urine were negative for sugar and albumin, and microscopic examination of the sediment was negative. After this period of observation, 1000 units[3] per kg body weight of bacitracin, lot No. B-100, were administered intramuscularly 3 times daily for 5 days of each week. On Saturdays and Sundays 2 doses of 1500 units per kg each were given. The antibiotic, in 2 to 3 cc of water, was injected intramuscularly using a different limb for each injection. The animals received treatment for 24 days. During this period, blood counts were performed at approximately weekly intervals. The red cell count showed no significant variations from the normal. One dog exhibited a moderate, persistent leukocytosis (15,000 per mm^3) with an increase in the polymorphonuclear leukocytes (average, 87%). The other dogs showed normal total leukocyte counts with an increase in the polymorphonuclear leukocytes (85%). Urinalyses disclosed no significant abnormalities. Blood sugar (80 to 95 mg %) and non-protein nitrogen analyses (30 to 48 mg %) after 10 and 23 days of dosing, disclosed no significant changes except in one dog in which the non-protein nitrogen ranged from 55 to 66 mg %. Without control observations, this cannot be unequivocally attributed to the influence of the antibiotic. There were no appreciable changes in body weight. On the last day of the

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† Bacitracin is the antibiotic discovered by Johnson and associates.[3] The material used in this study was kindly furnished by Dr. John T. Goorley of the Ben Venue Laboratories, Bedford, Ohio.

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‡ Carnation Company, 450 Seventh Avenue, New York, N.Y.

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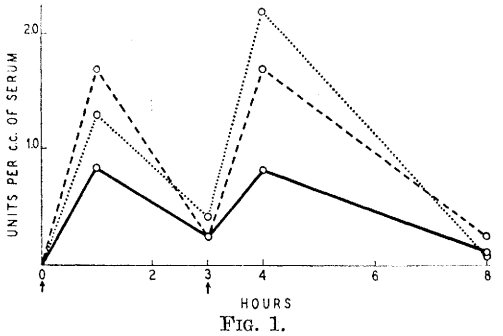

FIG. 1.

Serum concentrations following intramuscular injections of 1000 u/kg in each of 3 dogs. Bacitracin was injected at 0, 3, and 8 hours.

experiment, blood samples taken from each dog were analyzed for their bacitracin content. The data, shown in Fig. 1, indicate that measurable concentrations of the drug following the 3 intramuscular injections persisted in the circulating blood for approximately 13 hours of that day, leaving the blood free of circulating bacitracin for about 11 hours out of the 24.

At the end of the experiment all animals were sacrificed. Macroscopic examination of organs and tissues revealed no significant changes other than local induration at the sites of repeated intramuscular injection.

Microscopic examination of the tissues revealed the following: In the kidney, the cells of the proximal and distal convoluted tubules showed eosinophilia of the cytoplasm. Occasional basophilic globoid bodies were found in the convoluted tubules and a moderate number in the loops of Henle and collecting tubules. An occasional collection of round and plasma cells was found in the stroma. In one dog (No. 156) there was a coagulum in the loops of Henle and the collecting tubules.

The liver appeared to contain considerable glycogen. There was no evidence of cellular damage. The bone marrow was hyperplastic. The spleen showed large follicles and the pulp contained numerous macrophages with brown pigment and megakaryocytes. No significant changes were found. The thyroid appeared hyperplastic. The colloid was vacuolated and in places basophilic. Small nests of epithelial cells were found in dog No. 156. The muscle at the site of injection showed edema and numerous polymorphonuclear leukocytes which extended between the muscle fibres.

Experiments in Monkeys. After a control period of one to 3 weeks, during which time routine blood counts and urinalyses were performed, 5 monkeys were given bacitracin. Monkeys No. 1 and No. 44, each weighing 3 kg, were given 1500 units of bacitracin, lot No. B-100, per kg of body weight morning and evening for 5 days of each week. On weekends the total dose of 3000 units per kg was given at one time. All injections were given into the posterior muscle group of the right thigh, thus concentrating any local effect there might be. In order to compare the effect of 2 different lots of bacitracin, monkeys No. 2, No. 30, and No. 46 were similarly dosed with bacitracin lot No. B-102. The animals in both groups were dosed for 37 and 39 consecutive days, respectively.

During the period of treatment, blood counts were performed at approximately weekly intervals. The red cell counts showed no significant variation from those of the control period. One monkey, No. 1, showed a definite leukopenia. This animal, at autopsy, exhibited widespread nodular lesions, even though all animals were tuberculin negative at the outset of the experiment. The white counts in the other 4 animals remained in the normal range. During the period of treatment 3 of the 5 monkeys showed a fluctuating, but definite eosinophilia. In contrast with the normal data reported by Downey[4] all animals during both the control and treatment periods showed a lymphocytosis.

Urine samples were collected in metabolism cages and were, therefore, subject to errors of contamination. Nevertheless, it is of interest to note that the urine samples were negative for albumin and sugar during the control period, but after the animals had been on test for 3 weeks, albumin appeared in the urine of 4 of the 5 monkeys. Sugar appeared in the urine of all 5 animals in amounts graded qualitatively from 1 to 3 plus.

Blood sugar (71 and 62 mg %) and non-

[4] Downey, H., *Handbook of Hematology*, P. B. Hoeber, New York, 1938.

protein nitrogen (31 and 32 mg %) analyses in 2 monkeys just prior to the termination of the experiment were within the normal range, (sugar 60-80 mg % and non-protein nitrogen 30-40 mg %). Approximately 2 to 5 hours after the last dose of the drug just before the animals were sacrificed, samples of blood and spinal fluid were withdrawn and analyzed for their bacitracin content. The blood concentrations were 6.6, 0.75, 0.18, 8.1, and 6.6 units per cc and the spinal fluid concentrations were 0.34, .009, .08, and .34, and .34 units per cc, respectively, indicating that the antibiotic does not readily pass the blood brain barrier in the normal monkey.

Gross examination of the organs revealed in monkey No. 1, firm grey nodules 1 to 5 mm in diameter in the lungs, liver, spleen, kidney, and heart. The kidneys of the other monkeys were moderately enlarged and edematous. The cut surface lipped over the capsular edge. The capsule stripped with ease, revealing a smooth surface. The kidneys exhibited a diffuse deep yellow color with an orange tint. The muscle at the site of injection was edematous and, in places, there were small areas of necrosis. No other gross abnormalities were noted.

Microscopic examination of the kidney in the monkeys disclosed prominent epithelial elements in the glomeruli; and, in some cases, these contained an abundant clear cytoplasm. The cytoplasm of the convoluted tubules was eosinophilic (hematoxylin-eosin stain). There was coagulum in the loops of Henle and the collecting tubules; at times, globoid bodies were present. In the loops of Henle some of the cells were vacuolated. In the tubules of monkeys No. 1 and No. 2 necrotic cells could be found only very infrequently. No necrotic cells were found in the kidneys of any of the other monkeys. There was an occasional collection of round cells in the medulla. No excess fat could be demonstrated by Sudan stains in frozen section. Best's carmine stain revealed no glycogen.

The liver contained considerable glycogen. No cellular damage was seen. The spleen showed no significant changes. The bone marrow was hyperplastic. In the lung of monkey No. 44 there were foci of a sub-acute pneumonia. The testes showed no spermatogenesis. Since the age of the animals is unknown, this finding cannot be evaluated. The thyroid in monkey No. 1 showed slight glandular hyperplasia. The intestine in monkey No. 30 was ulcerated and showed a granulomatous reaction in the wall, and parasites resembling oxyuris were found in the lumen. In the adrenals, the reticularis was dense. The muscle at the site of injection contained collections of round cells and polymorphonuclear leukocytes which extended for a considerable distance between the muscle fibres. Small areas of necrosis were present. In monkeys No. 1 and No. 2, sarcosporidia were found.

In monkey No. 1 the nodules in the lung, liver, spleen, kidney, and heart were granulomatous and contained numerous epithelioid cells. No acid-fast bacilli or other organisms could be demonstrated in the tissues with Ziehl-Neelsen stain. The etiological factor for these lesions was not ascertained.

In contrast with the striking kidney lesions in the mouse,[1] the kidney in the rat[1] and the dog did not vary appreciably from the normal. The renal lesions in the monkey were insignificant in comparison with those of the mouse.

Additional Experiments. Since bacitracin is harvested from bacterial cultures, it was of interest to determine whether or not the crude materials at hand were anaphylactogenic. Accordingly, attempts were made to sensitize each of 6 guinea pigs with 5 mg (150 units) of bacitracin administered subcutaneously as a single dose. One month later a shocking dose of 10 mg (300 units) was administered intracardially with no evidences of anaphylaxis, but additional experiments would be required to demonstrate that none of the phenomena of anaphylaxis can be caused by bacitracin.

Bacitracin solution (lot B-100 at a concentration of 6000 units per cc of distilled water) was injected intradermally in doses of .05 and .10 cc into different areas of the

shaved abdominal skin of each of 5 rabbits with no visible reaction within the ensuing 4 days.

A solution of 1200 units of bacitracin per cc of normal saline at pH 7.0 was instilled into the conjunctival sac of rabbits and the lids were held closed for 2 to 5 minutes. Even though the residual solution was not washed out, only faint evidences of irritation were noted in each of the 5 rabbits used. The faint reddening of the conjunctiva disappeared within 4 hours in all animals.

It is to be noted that the foregoing results were obtained with relatively impure commercial bacitracin concentrates as currently produced. Somewhat different results may be obtained with pure materials.

Summary. 1. Following prolonged daily administration of crude bacitracin concentrates in dogs and monkeys, no significant changes in blood morphology were observed. 2. Injection of bacitracin solution (6000 units per cc) into the shaved abdominal skin, and instillation of bacitracin (1200 units per cc) into the conjunctival sac of the rabbit caused little irritation. 3. Repeated intramuscular injection of 1000 units of bacitracin per kg 3 times each day for 23 days into the dog produced local induration. 1500 units per kg twice a day for 45 days in the same area in monkeys produced both induration and small areas of necrosis. 4. In the dog, urine samples remained negative for sugar and albumin while in the monkey, sugar and albumin appeared in the urine as the animals continued on test. 5. Large doses of bacitracin, approximating the LD_{50}, produced damage to the renal tubules, with tubular necrosis in the mouse. In the rat and the dog, the lesions were, insignificant, while in the monkey, necrotic cells were found in two instances only, and then in comparatively insignificant numbers as contrasted with the mouse.

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Comparison of Intestinal Lengths and Peyer's Patches in Wild and Domestic Norway and Wild Alexandrine Rats.*

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The two most common species of rats in the world, the Norways and the Alexandrines or roof rats, have very different resistance to poisoning with alpha naphthyl thiourea (ANTU) and show very different symptoms. Alexandrines have an LD_{50} of 250 mg/kg which is more than 30 times as high as the LD_{50} of the Norways, 6.9 mg/kg.[1] Furthermore, in the latter species ANTU produces a marked pulmonary edema and pleural effusion, while in the former species it causes no detectable change in the lungs or in any other organs.[2] At present these species differences in toxicity and physiological effects remain unexplained. The observation[2] that herbivorous animals such as rabbits, guinea pigs, meadow mice, prairie dogs, ground squirrels, and monkeys have a high resistance to ANTU poisoning and show no lung effects, while carnivorous or omnivorous animals such as dogs and pigs do show a marked pulmonary edema and pleural effusion and a relatively low resistance, may throw some light on this problem,† The Alexandrine rat superficially resembles the Norway rat; often inhabits the same houses and buildings; and in general

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