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SOME OBSERVATIONS ON THE BIOLOGICAL PROPERTIES OF BACITRACINS A, B, AND C

BY

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Newton and Abraham (1950) showed that ayfivin and bacitracin were probably identical, and that crude preparations of these antibiotics contained at least three polypeptides that were active against certain bacteria, as well as a number of other polypeptides that showed no antibacterial activity. The active peptides were called bacitracins A, B, and C. It has long been the hope that the nephrotoxicity of commercial bacitracin might be due to impurities rather than to the antibiotic itself (e.g., Meleney and Johnson, 1949). This paper records biological experiments on different constituents of crude "ayfivin."

EXPERIMENTAL

Preparation of material

A preliminary fractionation of crude ayfivin (bacitracin) was made by counter-current distribution in separating funnels, using a solvent system composed of 4 vol. amyl alcohol, 1 vol. *n*-butyl alcohol, and 5 vol. 0.05 M-potassium phosphate buffer, *p*H 7.0 (System I) (Newton and Abraham, 1950). The further purification of the individual bacitracins used for the biological studies reported in this paper was made by a series of counter-current distributions in a Craig-Post 54-chamber apparatus (Craig and Post, 1949). When bacitracins A and C were being prepared system I was used. Bacitracin B was prepared in a solvent system composed of 1 vol. amyl alcohol, 1 vol. *n*-butyl alcohol and 2 vol. 0.05 M-potassium phosphate buffer, *p*H 7.0 (System III).

The partition coefficient of A was close to unity in system I, while B had a partition coefficient of nearly one in system III.

Bacitracin A.—A concentrate containing 1 g. of material rich in A (85 per cent A) obtained from the preliminary fractionation was introduced equally into the first three tubes of the Craig-Post machine. A distribution of 150 transfers was then carried out in such a way that no bacitracin A was removed from the machine. At the end of the distribution the contents of the tubes that were shown to contain "pure A" (Newton and Abraham, 1950) were reconcentrated under conditions in which bacitracin A had been shown to be stable.

Bacitracin C.—When freshly prepared concentrates of material rich in A were redistributed in the Craig-Post apparatus about 10–15 per cent of C was obtained. However, a concentrate enriched in A which had stood in aqueous solution at *p*H 3.0 for nine months at 4° C. was found to contain little or no C. Instead, an inactive product which accounted for 10 per cent of the total material was found. The inactive product appeared in the same position on the distribution curve as bacitracin D, which is an inactive constituent of crude ayfivin having a partition coefficient of approximately 0.2 in system I.

Bacitracin B.—A concentrate containing about 30 per cent of bacitracin B was made from the appropriate fractions of the preliminary fractionation in system I. Two 130 transfer distributions in system III were required before the bacitracin B behaved like an essentially pure substance. In the first experiment 1.5 g. of the concentrate was loaded equally into the first four tubes of the Craig-Post machine. After 130 transfers had been completed, during which no B was withdrawn from the machine, the contents of the tubes that were shown to contain nearly pure B were reconcentrated. The concentrate, which contained about half of the B present in the original sample, was again subjected to 130 transfers in system III. This distribution yielded 160 mg. of " pure B " (10.5 per cent of the total material in the original concentrate).

Preparation of samples for biological studies.—It has already been shown that bacitracin is liable to lose activity when isolated in the solid state by lyophilization (Craig, Gregory, and Barry, 1949 ; Newton and Abraham, 1950).

The concentrates from the final distribution experiments were freed from potassium phosphate by extraction into *n*-butanol at *p*H 7.0, and the butanol was then removed by distillation in the presence of 0.001 N-HCl in a high vacuum. The solutions of bacitracin hydrochloride were freed from the last traces of butanol by careful extraction with *n*-hexane while still acid (*p*H 3.0) and any hexane which remained was removed by evaporation under reduced pressure. The concentration of bacitracin in the solutions was determined by the photometric ninhydrin method (Newton and Abraham, 1950). Solutions were then neutralized and adjusted to contain 0.8 per cent sodium chloride.

Antibacterial Activity

The unit of ayfivin (bacitracin) was based on the activity of a sample of crude ayfivin hydrochloride. This material was arbitrarily stated to contain 5 units per mg. (Arriagada, Savage, Abraham, Heatley, and Sharp, 1949). A solution of ayfivin containing 1 unit per ml. produced zones of inhibition 18 to 21 mm. in diameter on plates seeded with *C. xerosis*, when assayed by the cylinder-plate method (Heatley, 1944). For the purposes of assay, solutions of bacitracin were diluted so that they contained from 0.25 to 1 unit per ml. and their activity was compared with that of the standard preparations.

The sensitivity of a number of strains of bacteria to the bacitracins was tested by the serial dilution method. *Staphylococcus* was grown in heart extract broth, and *Streptococcus* and *Corynebacterium* in 10 per cent serum broth. In each case one drop of a 24-hour culture, diluted or undiluted, was added to each of a series of tubes containing twofold serial dilutions of the bacitracins in 2 ml. of the same medium.

Bacitracin A.—The results obtained with a specimen of " pure " bacitracin A are shown in Table I. The material used for the titration was the same as that with which the experiments on toxicity were done, the results of which are shown in Table IV.

TABLE I

ANTIBACTERIAL EFFECT OF BACITRACIN A

Organism	Dilution of culture used for inoculum	Highest dilution giving complete inhibition of growth for		Lowest dilution giving no inhibition of growth for	
		24 hr.	48 hr.	24 hr.	48 hr.
Staph. aureus (N.C.T.C. 6571)	1 : 1,000	160,000	160,000	320,000	320,000
,, ,, (C.N. 491) ..	1 : 1,000	640,000	320,000	1,280,000	640,000
Str. pyogenes (C.N. 10) ..	1 : 10	1,280,000	1,280,000	5,120,000	2,560,000
Str. haemolyticus group C ..	1 : 10	80,000	40,000	160,000	160,000
C. diphtheriae (*gravis*) ..	Neat	1,280,000	640,000	2,560,000	2,560,000

TABLE II

ANTIBACTERIAL EFFECT OF BACITRACIN B

Organism	Dilution of culture used for inoculum	Highest dilution giving complete inhibition of growth for		Lowest dilution giving no inhibition of growth for	
		24 hr.	48 hr.	24 hr.	48 hr.
Staph. aureus (N.C.T.C. 6571)	1 : 1,000	40,000	20,000	80,000	40,000
,, ,, (C.N. 491) ..	1 : 1,000	320,000	160,000	640,000	320,000
,, ,, (penicillin-resistant) ..	1 : 1,000	20,000	10,000	40,000	20,000
Str. pyogenes (C.N. 10) ..	1 : 10	1,280,000	640,000	2,560,000	1,280,000
Str. haemolyticus group C ..	1 : 10	20,000	20,000	40,000	40,000
C. diphtheriae (*gravis*) ..	Neat	1,280,000	1,280,000	2,560,000	2,560,000

Bacitracin B.—The results obtained with a specimen of " pure " bacitracin B are shown in Table II. The material used for the estimation was the same as that with which the experiments on toxicity were done, the results of which are shown in Table VI.

Conclusions.—From these results it appears that bacitracin A is about four times as potent as bacitracin B against one strain of *Staphylococcus aureus*, twice as potent against another strain, but equally powerful against a strain of *Str. pyogenes* and *C. diphtheria* (gravis).

Assayed by the cylinder-plate method against *C. xerosis* as test organism, bacitracin A had about 40 units per mg., while bacitracin B had 15 units per mg. Thus by some, but not all, criteria bacitracin A is more potent than bacitracin B.

Mode of action in vitro

Tubes containing serial dilutions of the bacitracins in heart broth were inoculated with one drop of an *undiluted* culture of *Staph. aureus* (N.C.T.C. 6571). The tubes were incubated at 37° C. and from time to time the number of viable organisms in each culture was estimated.

Bacitracin A.—Fig. 1 shows that a concentration of 1 : 40,000 or greater of bacitracin A rapidly killed all the organisms in the inoculum used, but that in a concentration of 1 : 80,000 a small proportion of the cells remained viable and eventually grew out.

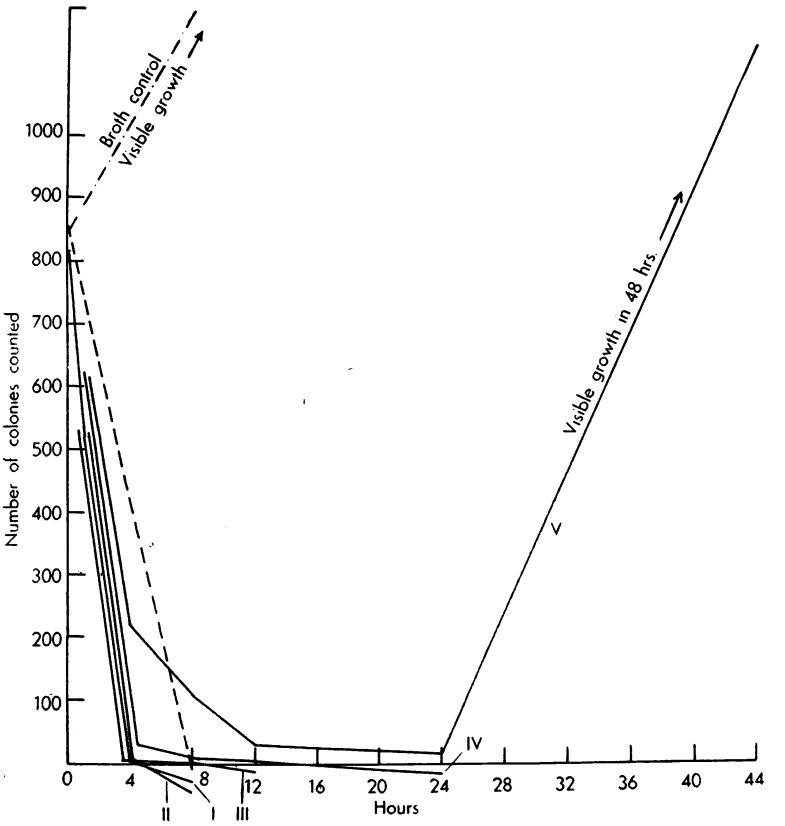

FIG. 1.—Mode of action of bacitracin A. – · – · – broth control; – – – saline control. Dilutions: I–1 : 5,000; II—1 : 10,000; III—1 : 20,000; IV—1 : 40,000; V—1 : 80,000.

Bacitracin B.—Fig. 2 shows that it was only with a concentration of 1 : 5,000 of bacitracin B that the culture was sterilized.

Conclusion.—The comparison between bacitracins A and B shows that bacitracin A is considerably more potent than bacitracin B in sterilizing cultures of *Staph. aureus.*

TOXICITY TO MICE

Only small amounts of substance have been available for testing toxicity owing to the labour involved in separating the polypeptides with the apparatus available. It has consequently not been possible to give statistically significant figures for the LD50 of the two bacitracins, but sufficient evidence has been collected to show that the toxicity of the various fractions differs.

BACITRACIN A

Acute toxicity.—All injections were given intravenously. The first experiment was done with a sample which possibly contained 15 to 20 per cent of bacitracin C

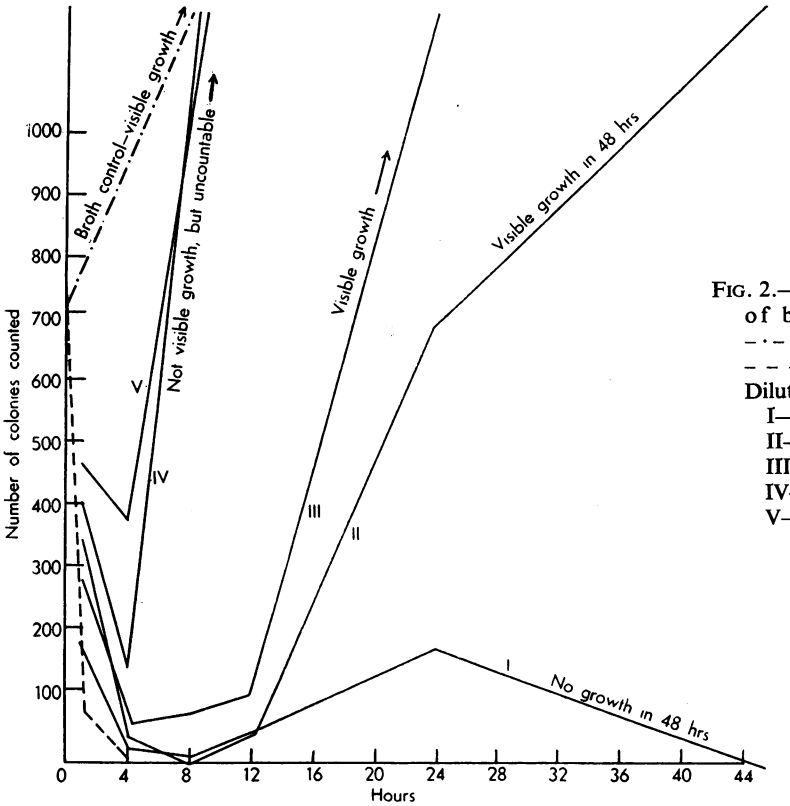

FIG. 2.—Mode of action of bacitracin B. - · - · - broth control; - - - saline control. Dilutions: I—1 : 5,000; II—1 : 10,000; III—1: 20,000; IV—1: 40,000; V—1 : 80,000.

but no bacitracin B. The results (Table III) showed that four out of the five mice used died after an interval of many hours.

A preparation of bacitracin A that was considered to be free from bacitracins B and C was also tested. The results are set out in Table IV. From these it seems

TABLE III

ACUTE TOXICITY TO MICE OF A PREPARATION OF BACITRACIN A CONTAINING 15 TO 20 PER CENT OF BACITRACIN C

The substance was dissolved in 0.8 per cent NaCl. All mice weighed 19 g. and were injected intravenously. The units are those defined by Arriagada *et al.* (1949), not Meleney and Johnson's units

Dose			Result	Renal damage
Units	mg.	mg./kg.		
104.4	2.9	153	Died about 30 hours	Grade 2c
138.0	3.84	202	Killed 73 days in good condition	
155.5	4.32	227	Died about 48 hours	Grade 2c
172.8	4.8	253	Died between 36 and 48 hours	
172.8	4.8	253	,, ,, ,, ,, ,,	

TABLE IV

ACUTE TOXICITY TO MICE OF BACITRACIN A CONSIDERED TO BE FREE OF BACITRACINS B AND C

The material was dissolved at a concentration of 10 mg./ml. in 0.8 per cent NaCl at *p*H 7. All mice weighed 19 g. and were injected intravenously. The units are those defined by Arriagada *et al.* (1949), not Meleney and Johnson's units

Dose			Result	Post-mortem appearances	Renal damage
Units	mg.	mg./kg.			
260	6.5	342	Died at once		
260	6.5	342	Killed 9 days when sick	Clear fluid in thorax and peritoneal cavity kidneys swollen with white granular surface with cysts	Grade 2b Fig. 5 Fig. 6
260	6.5	342	Died in 22 hrs.		
260	6.5	342	Died at once		
200	5.0	263	,, ,, ,,		
200	5.0	263	,, ,, ,,		
200	5.0	263	Killed in good condition 21 days	Kidneys finely granular surface; spleen large	Grade 3
200	5.0	263	,, ,, ,,	Kidneys appeared normal; spleen large	Grade 3
200	5.0	263	,, ,, ,,	Kidneys finely granular surface; spleen large	Grade 3 Fig. 8
200	5.0	263	,, ,, ,,	,, ,, ,,	Grade 3
160	4.0	211	,, ,, ,,	Kidneys appeared normal; spleen large	Grade 3 Fig. 9
160	4.0	211	,, ,, ,,	Kidneys and spleen appeared normal	Grade 3
160	4.0	211	,, ,, ,,	,, ,, ,,	Grade 3
120	3.0	158	,, ,, ,,	,, ,, ,,	N.A.D.
120	3.0	158	Died in 34 minutes		

probable that the LD50 would lie somewhere between 5.0 and 6.5 mg. per mouse, though one mouse out of two died soon after an injection of 3 mg. As will be seen from Table V, other mice which had received a dose previously sometimes died very soon after the injection of 3.0 mg.

Chronic toxicity.—The effects of repeated intravenous injection of 3 mg. bacitracin A were noted. The drug was administered in nearly all cases at 24-hour intervals (Table V).

Bacitracin B

Acute toxicity.—All material was injected intravenously. Some material whose composition was not exactly known but which contained no bacitracin A was used for some preliminary experiments. Results:

1 mouse given 3.9 mg. intravenously survived 57 days in good condition
2 mice ,, 5.2 mg. ,, ,, ,, ,, ,, ,, ,,
2 ,, ,, 6.5 mg. ,, ,, ,, ,, ,, ,, ,,
of 2 ,, ,, 7.8 mg.

1 died at once and 1 survived 57 days in good condition.

TABLE V

CHRONIC TOXICITY OF BACITRACIN A

Toxicity to mice on repeated injection of equal amounts of bacitracin A (same preparation as used for experiment shown in Table IV). All mice weighed 18–19 g. and were injected intravenously. The units are those defined by Arriagada *et al.* (1949), not Meleney and Johnson's units

Doses				Results	Post-mortem appearances	Renal damage
No.	Total units	Total mg.	mg./kg.			
2	240	6	316	Died about ½ hr. after a 3rd dose	Kidneys pale	Grade 2c (Fig. 7)
4	480	12	631	Killed in good condition 24 hrs. after last dose	Kidneys appeared normal	N.A.D.
1	120	3	158	Died immediately after a 2nd dose	,, ,,	Grade 1
1	120	3	158	,, ,, ,,	,, ,,	Grade 2
4	480	12	631	Killed after 21 days in good order	Kidneys finely granular; spleen large	Grade 3
3	360	9	474	Killed 3 days after last dose; some air injected 1 day before death	Kidneys pale	Grade 1 (Fig. 3)
4 (+1 s.c.)	600	15	790	Killed in good order 1 day after last injection	Kidneys appeared normal	Grade 1

The acute toxicity of bacitracin B was thus apparently less than that of bacitracin A, as the LD50 would appear to be somewhere between 8 and 10 mg. per mouse.

A second experiment with a sample of bacitracin B which was homogeneous and contained neither bacitracin A nor C confirmed the view that bacitracin B was less toxic than bacitracin A. The results of these experiments are given in Table VI.

TABLE VI

ACUTE TOXICITY TO MICE OF BACITRACIN B CONSIDERED TO BE FREE OF BACITRACINS A AND C

All mice weighed 20 g. and were injected intravenously. The units are those defined by Arriagada *et al.* (1949), not Meleney and Johnson's units

Dose			Result	Post-mortem appearances	Renal damage
Units	mg.	mg./kg.			
88.5	5.9	295	Killed in good condition after 21 days	Kidney appeared normal; spleen large	Grade 3 (Figs. 10 and 11)
88.5	5.9	295	,, ,, ,,	Kidney pale; spleen large	Grade 3
115.5	7.7	385	,, ,, ,,	,, ,, ,,	,,
115.5	7.7	385	,, ,, ,,	Kidney finely granular; spleen large	,,
115.5	7.7	385	,, ,, ,,	Kidney normal; spleen large	,,
150.0	10.0	500	,, ,, ,,	,, ,, ,,	,,
150.0	10.0	500	Died in 4 minutes		
150.0	10.0	500	Died in 1 minute		

TABLE VII

CHRONIC TOXICITY OF BACITRACIN B

Toxicity in mice on repeated injection of equal amounts of bacitracin B (same preparation as used for experiment shown in Table VI). All mice weighed 18–20 g. and were injected intravenously. The units are those defined by Arriagada *et al.* (1949), not Meleney and Johnson's units

Dose				Result	Post-mortem appearances	Renal damage
No.	Total units	Total mg.	mg./kg.			
4	300	20	1,100	Killed in good condition 24 hrs. after last injection	Kidneys large and mottled	Grade 2a (Fig. 4)
6	267	18	890	,, ,, ,,	Kidneys normal	Grade 1
6	267	18	890	,, ,, ,,	Kidneys finely granular; spleen large	Early Grade 3

Chronic toxicity.—The results are shown in Table VII of giving three mice repeated daily injections of bacitracin B.

Symptoms following injection of both bacitracins A and B

The animals that received the larger doses from which they recovered became disoriented immediately after the injections and sometimes jumped about. A stage of excitement lasting a few seconds passed into one in which the animals were completely quiescent as though at least partially paralysed. In the course of an hour or so they gradually recovered and were able to move around freely. An interesting feature was the rapid development of great engorgement of the blood vessels of the ears, and presumably also of other parts of the body that could not be so easily seen.

There was no apparent difference between the behaviour of mice made sick by bacitracin A and of those made sick by bacitracin B.

Bacitracin C

Only a few observations were made on a specimen rich in bacitracin C. The results shown in Table VIII suggest that bacitracin C is more toxic than the other two bacitracins, but more observations are required.

Histological Observations on Lesions in the Kidney

The kidneys of 27 mice were examined and renal damage of varying degree was found in all but two animals.

Group A.—This group consisted of 12 mice that were killed or died before the tenth day after the beginning of administration. The kidneys from one mouse were normal.

Grade 1.—The earliest change from the normal is dilatation and epithelial damage in the ascending loop of Henle and the second convoluted tubule. Casts may or may not be present. Epithelial changes may consist of mere flattening of cells, but

TABLE VIII

ACUTE TOXICITY TO MICE OF A PREPARATION OF BACITRACIN C

All mice weighed 20 g. and were injected intravenously. The units are those defined by Arriagada *et al.* (1949), not Meleney and Johnson's units

Dose			Result
Units	mg.	mg./kg.	
18.75	0.75	37.5	Killed after 57 days in good order
27.5	1.1	55	,, ,, ,,
27.5	1.1	55	,, ,, ,,
27.5	1.1	55	,, ,, ,,
27.5	1.1	55	,, ,, ,,
37.5	1.5	75	Died in 3 minutes
37.5	1.5	75	,, 2 ,,
37.5	1.5	75	Killed after 57 days in good order
37.5	1.5	75	Found dead after 7 days
75.0	3.0	150	Died in 2 minutes

where damage is more severe and the dilatation of the second convoluted tubule greater the epithelium may be deficient in some places. Casts with cellular debris and nuclear fragments may be present in the lumen (Fig. 3). There is little change in the first convoluted tubule or in the glomerulus.

Grade 2.—The next grade of damage shows alteration in the first convoluted tubule as well as more severe lesions in the second convoluted tubule (Figs. 4, 5, 6, and 7).

In the second convoluted tubules there is considerable dilatation almost amounting to cyst formation in some cases (Figs. 5). The epithelial cells are flattened, may be deficient or may show necrosis, the lumen is filled with hyaline casts and some nuclear fragments are present in the casts. Desquamated cells or nuclear fragments may form conspicuous basophil casts (Figs. 5 and 6).

The first convoluted tubules may be affected in various ways. This may take the form of an increased eosinophilia of the cytoplasm, with granularity (Fig. 4). Globules of eosinophil material may appear in the cytoplasm in the form known as hyaline droplet degeneration classed as grade 2a. In other cases (grade 2b) interstitial cells have proliferated in some areas and young connective tissue cells with young fibroblasts surround some of the first convoluted tubules and glomeruli (Fig. 5) most frequently in the superficial layers of the cortex. The tubules show evidence of collapse and the lumen is almost closed. Some glomeruli show thickening of Bowman's capsule. In kidneys with this degree of damage (grade 2a and b) mitotic figures are usually demonstrable in the epithelium.

The next group (grade 2c) represents the most severe renal damage (Fig. 7). The first convoluted tubule shows epithelial degeneration and contains granular casts while frank necrosis of the epithelium of the second convoluted tubule is present. In this group there is no evidence of epithelial regeneration, no mitotic figures being found, nor is there evidence of proliferation of connective tissue.

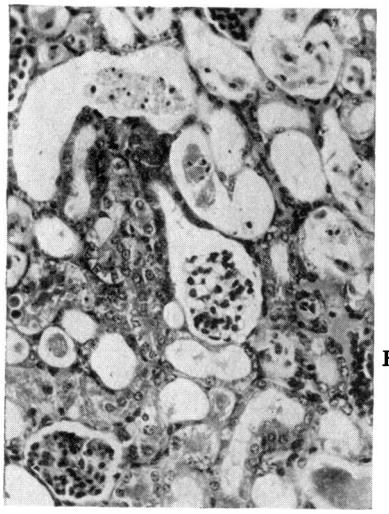

FIG. 3

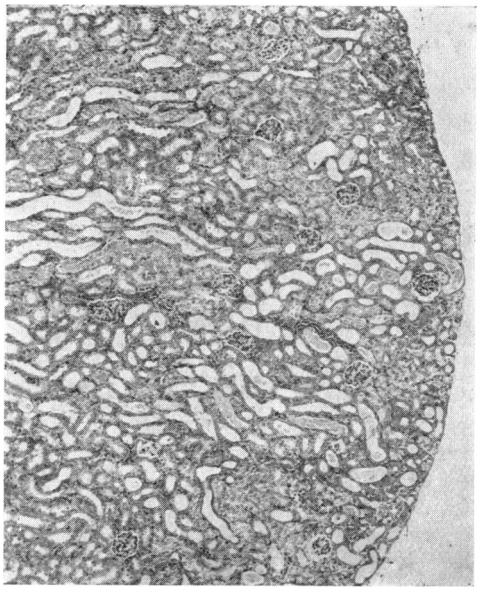

FIG. 4

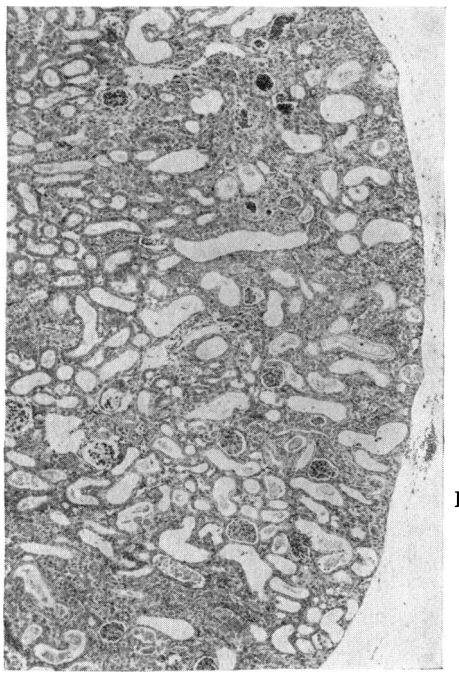

FIG. 5

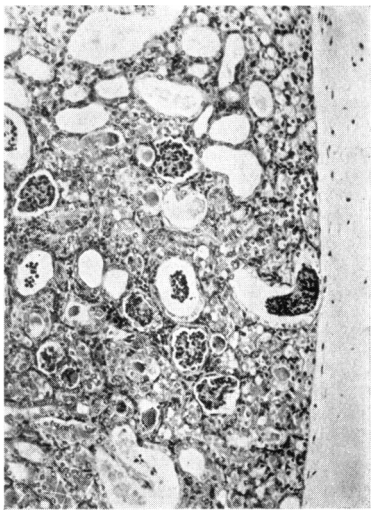

FIG. 6

FIG. 3.—See Table V. Three intravenous injections of bacitracin A at 24-hour intervals. Second convoluted tubules are widely dilated, epithelium is flattened, degenerated or absent. Casts with nuclear fragments are present. Little change is noted in the first convoluted tubules or glomeruli. Grade 1. Haematoxylin and eosin. × 305.

FIG. 4.—See Table VII. Four intravenous doses of bacitracin B at 24-hour intervals. Second convoluted and collecting tubules are dilated. Eosinophilic cytoplasm of first convoluted tubules appears dark in the photograph. Grade 2a. Haematoxylin and eosin. × 90.

FIG. 5.—See Table IV. Single intravenous dose of bacitracin A. Second convoluted and collecting tubules are widely dilated. Some casts with nuclear débris in quantity are present on the left. First convoluted tubules are compressed and there is some increase in connective tissue. Grade 2b. Haematoxylin and eosin. × 90.

FIG. 6.—Higher magnification of same kidney as in Fig. 5. Note casts in dilated second convoluted tubules and increase in connective tissue with thickening of Bowman's capsule of glomerulus on the right. Grade 2b. Haematoxylin and eosin. × 145.

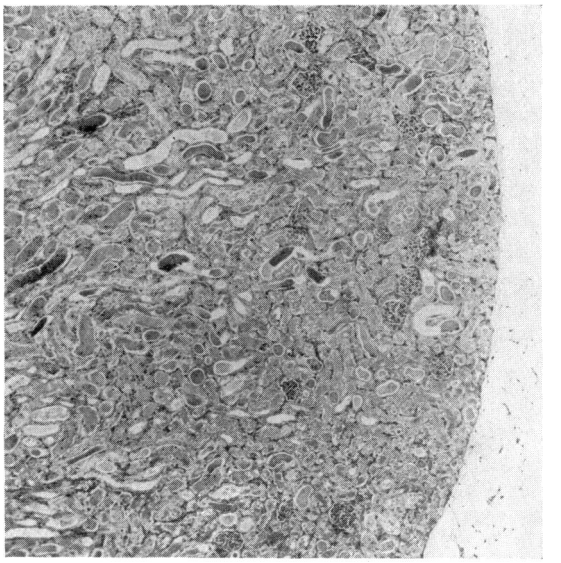

FIG. 7.—See Table V. Two intravenous doses of bacitracin A at 24-hour intervals. Widespread retention of casts in dilated second convoluted tubules. The casts are mostly hyaline but nuclear fragments are present in some. The epithelium of the second convoluted tubules is flattened or deficient. The ascending loop of Henle and collecting tubules are also affected. First convoluted tubules show epithelial degeneration. There is no proliferation of tubular epithelium or of interstitial connective tissue. Grade 2c. Haematoxylin and eosin. × 90.

The kidneys of all except one of the mice in group A can be assigned to one of these grades of damage. The exceptional animal received five doses of bacitracin B at 24-hour intervals and was killed 24 hours after the last dose. Changes in the second convoluted tubule are minimal, the cells of the first convoluted tubule show eosinophilia. In some parts of the superficial layers of the cortex there is diminution in size of the first convoluted tubules with relative increase of interstitial tissue, leading to a definite depression in the cortical surface. These patches show condensation of connective tissue round the capsule of the involved glomeruli and between the adjacent tubules, and are separated from the next patch by normal cortical tissue. The similarity of distribution and the scar-like character suggests that this animal should be classed with some of the animals killed at 21 days.

Group B

Fifteen mice were killed at 21 days. Nine had received bacitracin A in single doses from 3 mg. to 6.5 mg., and one mouse receiving 3 mg., showed no evidence of renal damage. The six mice that had had bacitracin B all showed renal lesions.

The lesions in the whole group follow the same pattern (grade 3). Scarred areas at present in the superficial cortex causing depression on the surface. The cortex between these areas is normal. The scarring, which involves one, two, or more nephrons, runs radially towards the medulla (Fig. 8). In some sections the damaged area includes the outer zone of the medulla. The interstitial cells in the scarred area proliferate (Figs. 9, 10, and 11). The accumulations of cells are greatest round the glomeruli. Collections of similar cells can be found in relation to the larger blood vessels in the cortico-medullary region or even more remote from the scarred area in the cortex. There is a suggestion that after bacitracin A more adult fibroblasts are present than after bacitracin B.

No exact parallel has been shown to exist between the severity of the renal damage and the amount of the antibiotic given, nor is there any constant difference

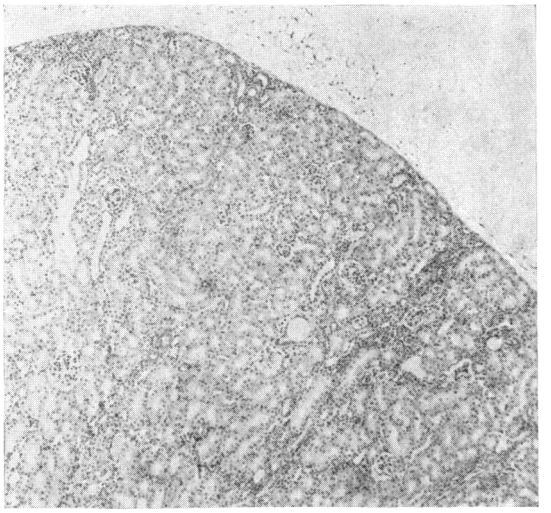

FIG 8

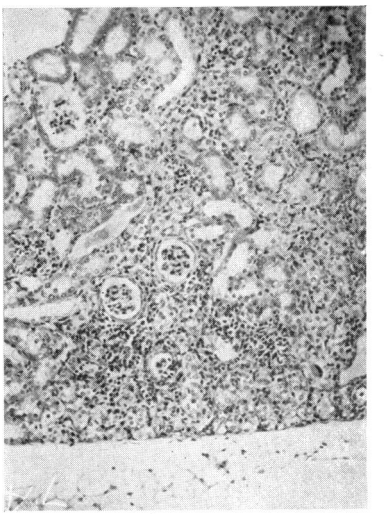

FIG. 9

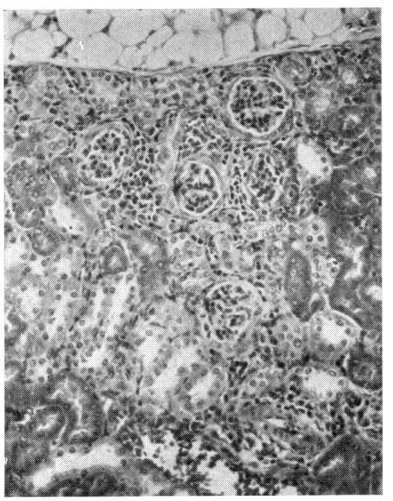

FIG. 10

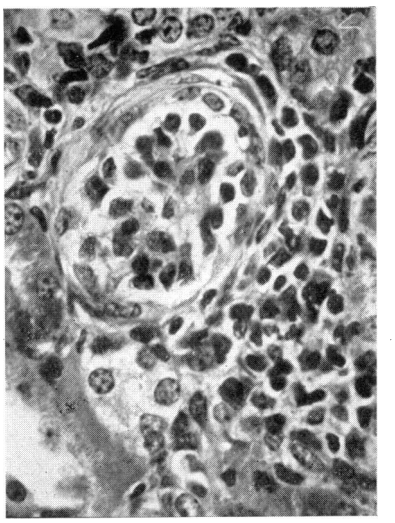

FIG. 11

FIG. 8.—See Table IV. Single intravenous dose of bacitracin A. Scarring is maximal in the outer zone of the cortex, but in this preparation the alteration can be seen to extend inwards through the cortex. (Cf. Fig. 9.) Grade 3. Haematoxylin and eosin. × 90.

FIG. 9.—See Table IV. Single intravenous dose of bacitracin A. Loss of renal tissue in the superficial layer of the cortex. Replacement by connective tissue cells with fibril formation round the glomeruli. (Cf. Fig. 8.) Grade 3. Haematoxylin and eosin. × 145.

FIG. 10.—See Table VI. Single intravenous dose of bacitracin B. There are glomerular adhesions in the subcapsular zone and an increase of connective tissue cells and fibrils. In the superficial layers of the cortex the tubules have disappeared. Grade 3. Haematoxylin and eosin. × 225.

FIG. 11.—See Table VI. Higher magnification of same kidney as in Fig. 10. A glomerulus showing an increase in the connective tissue fibrils round the capsule with adhesion of the tuft. Grade 3. Haematoxylin and eosin. × 860.

between the renal damage produced by bacitracin A and B. The initial lesion is in the second convoluted tubule, that is in the distal part of the nephron. Similar results are obtained with crude ayfivin produced by the A5 strain of *B. licheniformis* grown on the glucose ammonium-lactate medium of Hills, Belton, and Blatchley (1949). A different strain of *B. licheniformis* grown on an ammonium-lactate medium (Callow, Glover, Hart, and Hills, 1947) gives rise to an antibiotic material licheniformin II. Ayfivin and licheniformin II have been shown to be indistinguishable by counter-current distribution between solvents (Newton and Abraham, unpublished) and they produce identical lesions (Ross, unpublished).

Discussion and Summary

The observations recorded in this paper make it reasonably certain that the nephrotoxicity of commercial preparations of bacitracin is due in part at least to the antibacterially active constituents. There is thus little hope of obtaining a preparation of bacitracin which does not have the capacity to damage the kidneys.

For therapeutic purposes it does not appear to be worth trying to separate bacitracin A from bacitracin B, for although the latter is somewhat less toxic than the former, it is less potent against certain bacterial species. The little data so far available suggest that bacitracin C has a greater acute toxicity than the other two bacitracins.

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