A COMPARATIVE STUDY OF THE RENAL DAMAGE PRODUCED IN MICE BY VARIOUS LOTS OF BACITRACIN¹

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In recent publications it has been suggested that bacitracin grown by the deep-tank method shows a higher degree of nephrotoxicity than the bacitracin produced by surface culture methods (1). The studies being reported were designed to compare the two types of material from a strictly objective standpoint.

EXPERIMENTAL STUDIES

Various lots of bacitracin were selected for this study on the basis of their previous clinical use and their availability. Unfortunately, many of the lots could not be employed because of confusing clinical data resulting from variable and uncertain dosage schedules, the use of several lots in the same patient and the known presence of impaired renal function in some cases prior to the institution of bacitracin therapy.

Of the surface culture type of bacitracin lots BV-112, BV-140, BV-142 and BV-144 were used. BV-112 was selected because it was one of the first production lots and the other three BV lots had all been employed clinically and were said to show little evidence of nephrotoxicity. Five lots, B-471212, B-480210, B-480512, B-480420, and B-481102, produced by deep-tank fermentation were similarly chosen for study. Lot B-471212 was one of the earliest lots produced by the deep-tank method which was employed clinically. Lots B-480210 and B-480512 were included in the study because of clinical evidence of nephrotoxicity, while B-480420 by contrast was stated to have extremely little evidence of toxicity when employed clinically. Lot B-481102 was especially prepared by the deep-tank method; a synthetic medium was used containing glutamic acid, similar to the medium described by Meleney and his associates in which the original production of bacitracin was developed by the surface culture technic.

The bacitracin was administered intravenously to mice of the same strain in accordance with the specifications set up by the FDA (October 14, 1948). These specifications require that mice weighing between 18 and 25 grams be injected intravenously with a constant volume (0.5 ml. per 20-gram mouse) within a five-second period. The following doses were injected: 50, 100, 200, 300, 400,

and 500 units per 20-gram mouse. The animals were observed for a 72-hour period. The mice receiving the three lowest doses, namely, 50, 100, and 200 units per 20-gram mouse, were under constant observation during the 72-hour period in order to obtain fresh autopsy material in the event of death. The kidneys were removed immediately after death and placed in 10% formalin. The survivors were sacrificed at the end of the experimental period and their kidneys fixed in 10% formalin. A series of 10 mice injected with saline served as the controls.

RESULTS

Evidence of gross pathology as expressed by the degree of ischemia present was noted and recorded on a four-plus graded scale at the time of autopsy. Histologic sections were made of the kidneys. These sections were stained with hematoxylin-eosin and were graded for evidence of renal changes independently (LWS) without knowledge of the gross renal classification. Very little, if any, correlation between the degree of renal ischemia grossly and the extent of renal damage microscopically was found.

In Table I the acute toxicity of the various lots of bacitracin is shown along with the biologic activity of each lot.

In Table II the incidence of microscopic renal injury in mice injected intravenously with various lots of bacitracin in dosages of 50, 100, and 200

TABLE I

The acute toxicity of various lots of bacitracin when administered intravenously to mice and the biological activity of each lot

Lot No.	LD50	Potency units/mg.
BV 112	180±24	37
CSC B481102*	205±23	35
BV 140	210±17	30
BV 144	265±34	30
CSC B480512	305±35	51
CSC B480210	307±26	37
CSC B480420	325±28	52
CSC B471212	330±25	50
BV 142	333±50	31

* Prepared on synthetic medium
BV = Ben Venue lots
CSC = Commercial Solvents lots

¹ Presented at the Second National Symposium on Recent Advances in Antibiotics Research held in Washington, D. C., April 11-12, 1949, under the auspices of the Antibiotics Study Section, National Institutes of Health, Public Health Service, Federal Security Agency.

TABLE II

The incidence of microscopic renal injury in mice treated intravenously with various lots of bacitracin

Dose units/20 gm. Mouse.....	BV112	BV140	BV142	BV144	CSC B480420	CSC B480210	CSC B480512	CSC B471212	CSC B481102*
50	2/6	3/6	3/6	2/6	4/6	4/6	2/6	4/6	5/6
100	5/6	6/6	4/6	3/6	3/6	5/6	5/6	4/6	5/6
200	6/6	6/6	4/6	5/6	5/6	5/6	6/6	5/6	6/6

* Prepared on synthetic medium
 BV = Ben Venue lots
 CSC = Commercial Solvents lots

units per 20-gram mouse is shown. In general, it will be noted there is fairly good correlation between the size of the dose and the incidence of renal damage. Analysis of these data indicates that there is no evidence of statistically significant differences between the material obtained with surface culture bacitracin and deep-tank culture. Lot B-481102 prepared on synthetic medium and grown by the deep-tank-culture method appears to be the most toxic of all the deep-culture material.

In Table III the severity of the renal injury is shown for the various lots of bacitracin and the various dosage levels employed. It is apparent that there is no statistically significant difference in the various lots with the exception of Lot B-481102 prepared on synthetic medium. This is of interest in relation to the use of these various lots clinically. Lot B-480420 has been widely employed by a number of highly competent clinical investigators who have all indicated that it has shown a very low clinical toxicity. On the other hand, B-480210 and B-480512 were reported to be

too toxic for parenteral administration. The laboratory studies in mice indicate that there is very little, if any, difference in the amount of renal damage produced in these animals by the several lots of bacitracin.

Through the cooperation of Dr. Frank L. Meleney of Columbia University, Miss Barranovsky, statistician and secretary for the bacitracin study, has supplied clinical data on the patients treated with these specific lots of bacitracin. An attempt to correlate these data with the mice data is shown in Table IV.

In Dr. Meleney's interim report (2), he stated that the dosage used with CSC deep-culture material in general was higher than that employed with the original surface culture product. In comparing the clinical toxicity of these two types of material, he states, "It is true that the dosage with the CSC lots was somewhat larger except for Lot 480420, but this cannot account for all of the difference." These animal studies indicate that the dosage may well be responsible for any differences noted in degree of clinical toxicity.

TABLE III

The severity of microscopic renal injury in mice when treated intravenously with the various lots of bacitracin

Dose units/20 gm. Mouse.....	BV† 112	BV 140	BV 142	BV 144	CSC† B480420	CSC B480210	CSC B480512	CSC B471212	CSC B481102*
50	0.50	0.50	0.50	0.50	0.75	0.50	0.50	1.38	0.70
100	0.90	0.58	0.63	0.67	0.67	0.83	1.40	0.50	1.60
200	1.25	1.92	1.50	1.30	2.00	2.40	1.33	1.40	2.50
Average of all doses	1.00	1.10	0.91	0.95	1.25	1.32	1.23	1.11	1.66

* Prepared on synthetic medium
 † See abbreviations Tables I and II
 Degrees of renal injury
 0 = Normal
 0.5 = Questionable
 1 = Slight
 2-3 = Moderate
 4 = Marked

TABLE IV

The severity of nephrotoxicity in man when treated intramuscularly with bacitracin

- 0 = No more than +1 albumin, not over 5WBC/H.P. field, no R.B.C., occ. gran. cast.
 +2 = Transient albuminuria +2 to +3, WBC = 5 to 25/H.P. field, rare R.B.C. moderate numbers of gran. casts, no prolonged rise in T.N.P.N. or BUN, no prolonged fall in P.S.P.
 +4 = Persisting nausea and vomiting, prolonged rise in T.N.P.N. and BUN, prolonged fall in P.S.P., +4 albumin, many casts, R.B.C., W.B.C., or cases in which treatment had to be stopped because of oliguria or rash.

Total dose units	BV* 140	BV 142	BV 144	CSC B480420	CSC B480210	CSC B471212
40,000-80,000	0	—	—	+2, +2	—	—
80,000-200,000	—	0,0,0, +2	—	0, +2	—	—
200,000-300,000	—	+2,0,0	—	0, +2, +2, +2	—	+2, +4
300,000-400,000	—	—	—	+2,0	+4	—
400,000-500,000	—	—	—	—	+4	0, +2
500,000-600,000	—	0	—	—	—	+2
600,000-700,000	—	+2	—	0	—	—
700,000-800,000	—	—	—	—	—	+4
800,000-900,000	—	—	—	—	—	—
900,000-1,000,000	—	—	—	—	+2	—
1,000,000-1,300,000	—	—	—	—	—	0, +2
<i>Mouse data</i>						
Nephrotoxic value	1.10	0.91	0.95	1.25	1.32	1.11
No. of mice with renal injury	15	11	10	12	14	13
No. mice treated	18	18	18	18	18	17

* See abbreviations Tables I and II

DISCUSSION

Renal pathology

The renal pathology associated with the parenteral administration of bacitracin is, of course, one of the disturbing problems associated with the widespread use of this highly effective antibiotic. It is well known that bacitracin is a complex substance, presumably polypeptide in nature, containing a large number of amino acids, several of them in significant quantities in the *d* form. Evidence to date points toward the fact that bacitracin as presently constituted is a relatively pure substance and that this nephrotoxicity may well be inherent in the molecule. There are, however, some encouraging indications that this toxicity may at least be reduced materially if not entirely eliminated.

The pathology is distinctly one of lower nephron involvement. The changes are those of degeneration of the lining tubular epithelium, its subsequent desquamation and the formation of hyaline casts. This may proceed to a point where the major portion of the tubules become blocked. In most animals surviving a 72-hour period, evidence of regeneration of the tubular epithelium can be demonstrated. In a few clinical cases of terminal

infection where bacitracin therapy was employed, similar changes were observed. Some of these cases show evidence of associated renal pathology so that it is far from certain that the bacitracin has been a factor in the death of these patients. Most of these fatal cases were suffering from subacute bacterial endocarditis caused by organisms not sensitive to either penicillin or streptomycin. Preliminary observations suggest that a synergistic relationship exists between penicillin and bacitracin in infections of this type. This may open up a field of usefulness for bacitracin in dosage levels sufficiently low to permit its use in combination with other antibiotics without fear of the development of serious renal damage.

It is apparent from these comparative studies that very little correlation exists between the gross and microscopic pathologic changes in mice. It is likewise true that no correlation exists in the degree of renal change in the several mice to whom the same dose of bacitracin was administered parenterally. Similar differences in toxicity have been shown to exist in different strains of mice. Thus it would be theoretically possible to supply data relating to the LD-50 dosage which would not only be acceptable by present FDA specifications but would also meet with the tentative requirements

suggested by the clinicians if mice from a resistant strain were employed. The obvious conclusion must be reached that the mouse is an unsatisfactory test animal for nephrotoxicity.

SUMMARY AND CONCLUSIONS

Comparative studies of the nephrotoxicity of bacitracin when grown by surface and deep-tank culture are presented. No significant statistical differences in nephrotoxicity were found with the various lots of bacitracin.

A discussion of the renal pathology reveals that the lesion is one affecting the lower nephron.

In animals surviving a 72-hour period, representing the time interval required for evidence

of acute toxicity, tubular regeneration is apparent indicating that the changes are reversible.

There is so little correlation between the gross and microscopic evidence of renal pathology that it must be concluded the mouse is an unsatisfactory test animal.

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