[6] Eagle, H. and R. Fleischman: "Therapeutic activity of bacitracin in rabbit syphilis, and its synergistic action with penicillin. A preliminary report." (to be published.)
[7] Scudi, J. V., M. E. Clift and R. A. Kreuger: "Some pharmacological characteristics of bacitracin. II. Absorption and excretion of bacitracin in the dog." Proc. Soc. Exp. Biol. & Med., **65**: 9-13, 1947.
[8] Teng, P.: Work in progress.
[9] Scudi, J. V. and W. Antopol: "Some pharmacological characteristics of bacitracin." Proc. Soc. Exp. Biol. & Med., **64**: 503-506, 1947.
[10] Scudi, J. V., I. A. Coret, and W. Antopol: "Some pharmacological characteristics of bacitracin. III. Chronic toxicity studies of commercial bacitracin in the dog and monkey." Proc. Soc. Exper. Biol. & Med., **66**: 558-561, 1947.

DISCUSSION.—DR. E. P. LEHMAN, Charlottesville, Va.: I want to report briefly on some work with bacitracin that is being carried on in our laboratories by Dr. William R. Sandusky under the guidance of Dr. Meleney. This work is concerned with the use of bacitracin in experimental clostriadial infections. The experiments were performed by exposing muscle in guinea pigs, crushing it, and closing the wound. After the would was closed, varying amounts of Clostridium Welchii were injected into the area of damaged muscle and a number of the animals were treated with varying doses of bacitracin.

There were 44 control animals, of which only 17 survived. I want to point out that 26 animals died of gas gangrene with the usual picture of an enormously swollen leg and the other phenomena with which we are all familiar. One died of other causes. In the 93 animals treated with bacitracin, none died of *C. Welchii* infection. There were 25 deaths from other causes—pneumonia, persistent diarrhea, and in one or two instances intestinal obstruction as the result of intussusception. The number surviving was 68. Counting in the deaths from other causes the mortality rate is 59 per cent in the controls and 27 per cent in the bacitracin treated animals, which presents a statistically significant difference. In those animals that died of other causes there was no evidence of *C. Welchii* infection at autopsy. Leaving out the animals that died of other causes, there is no mortality in the bacitracin treated series and a mortality of 59 per cent in the animals that had no bacitracin.

At the time these experiments were done there was a good deal of disease in the guinea pig colony and it is not possible to say that this relatively large number of deaths in the experimental series had anything to do with the drug itself. The fact that they did not occur in the other series might suggest that it was a toxic factor in the drug, but I should like to hear Dr. Meleney's comment on that. The results in any event appear to suggest strongly that bacitracin has a specific effect in the prevention of clostridial infection in the guinea pig.

DR. I. S. RAVDIN, Philadelphia: I should like to review a few impressions we have obtained with the use of bacitracin in the project under Dr. Meleney that Dr. Zintel is heading in our clinic. The clinical results of systemic administration of bacitracin have been encouraging but there can be no doubt, as Dr. Meleney said, and Dr. Lockwood re-emphasized, that bacitracin is nephrotoxic for man. Dr. James Mitchie of our Section in Urology has been studying the possibilities of renal injury with this substance. Four patients have been extensively studied for evidence of renal, hepatic and blood cytology toxicity and have uniformly shown moderately severe renal tubular injury.

One patient who received 3,744,000 units of bacitracin over a period of 13 days had a 66 per cent reduction in tubular function; one month later the tubular function was still 22 per cent below the premedication value. A second patient receiving 200,000 units of bacitracin daily for 10 days had an 85 per cent reduction in tubular function:

12 days after bacitracin was discontinued the tubular function was still 66 per cent below pre-medication value. The observations of Dr. Meleney and Miss Johnson, that the nephrotoxicity of the bacitracin preparation does not parallel the antibiotic potency does suggest, as he has said, that the nephrotoxic factor is not a part of the bacitracin molecule. Until improved methods of manufacture eliminate the nephrotoxic factor, we must move very cautiously in further systemic use of this substance, for there can be no doubt that the renal injury is not a short-lived one in many of these patients, but persists over a very considerable time.

DR. CHAMP LYONS, New Orleans: Beginning rather cautiously a year ago when this problem was first presented to us, namely, that of deciding between bacitracin therapy for penicillin-resistant infections and the extension of other methods, we proposed to try combined therapy. We had already begun to vary the interval of dosage, and for the past eight months we have routinely used a dosage of penicillin every eight hours, as prescribed by Dr. Altemeier, with complete satisfaction to ourselves.

Instead of treating penicillin-resistant infections with bacitracin, we elected to use mixed thrapy, because the evidence in the literature suggests very strongly that any antibiotic of bacillary origin will have inseparably produced in that mixture some nephrotoxic constituents. Until that problem is solved we have decided not to use any antibiotics of bacillary origin. We have used mixtures of penicillin, streptomycin and sulfadiazine, with an eight hour interval for doses of penicillin and streptomycin for penicillin-resistant infections with, I believe, quite satisfactory results.

DR. FRANK L. MELENEY, New York (closing): I appreciate very much the interest shown in the presentation, and thank the discussors for bringing up these points. Certainly the toxicity of bacitracin is of great importance and concern. I want to say a few words to indicate why I think this is not a factor of the bacitracin itself, but one of the byproducts of manufacture. In the first place, the material we made in our laboratotry with a synthetic medium showed no evidence of toxicity whatsoever.

The manufacturers began the preparation of bacitracin with the same method which we had used in the laboratory and one of them sent us a small quantity of material which likewise showed essentially no toxicity. However, the yield per cc. in this medium was not satisfactory from a commercial standpoint and the manufacturers began using a soy-bean medium. In this medium bacitracin was produced in five to ten times as high a titre per cc. All commercial lots so far have been made in this medium but, along with the bacitracin, toxic factors came through in the final product. However, the material made by the Ben Venue Laboratories by means of surface culture in bottles produced only transient albuminuria and temporary rises in retained nitrogen. We were becoming quite complacent about the importance of toxicity inasmuch as we were able to obtain a clinical response without appreciable evidence of kidney damage. Most of the cases reported in this paper were treated with bacitracin from this source. However, recently we have been using bacitracin made by the Commercial Solvents Corporation by the deep-tank method and, while we have been told that in all other respects the manner of manufacturing is essentially the same, this material is certainly giving more frequent and more prolonged toxic symptoms. Furthermore, different lots vary considerably in their toxicity. This is true of lots having the same potency titre. We know that the toxicity is greatly modified by the salt content. In our animal tests bacitracin dissolved in physiologic saline is very much less toxic than bacitracin dissolved in distilled water.

Bacitracin has not yet been obtained in a pure state but chemical analyses indicate that it is made up of a number of amino acids, some of which are natural while others are unnatural. The toxicity may likely be in the unnatural amino acids which

may be either removable or neutralizable. When bacitracin is put in the ultracentrifuge, certain heavy products are carried down which are toxic, while the bacitracin remains evenly distributed. Furthermore, a dializing membrane will hold back certain toxic products which are obviously not a part of the antibiotic itself. The toxicity problem will be very intensively studied in the course of the next two or three months.

It is surprising to me to hear Champ Lyons say that almost all of the infection problems can be met by combined therapy of the sulfonamides with either penicillin or streptomycin. Certainly many infection problems come to me which have failed to respond to all of these agents. In fact, many of the cases reported in this paper are in this category. Dr. Lehman's report on the work of Dr. Sandusky with gas gangrene is of particular interest and clearly indicates that bacitracin is able to prevent the development of gas gangrene in experimental animals. The late deaths of some of the survivors may be indicative of toxicity of this particular lot of bacitracin, or it may be due to some factor associated with the culture material used in the injection. The control animals did not live long enough to show these late effects. Until this toxic problem is solved it is essential that the systemic treatment of bacitracin should be administered only in those units which are properly set up for its appraisal and in those cases which have not responded to the other available methods for the treatment of infections.