A GIFT of $15,000 has been made by the Harshaw Chemical Co., of Cleveland, to Western Reserve University for the furtherance of research in inorganic chemistry. The fund will support two fellowships in a three-year program of research under the direction of Dr. Harold S. Booth, professor of chemistry.

SPECIAL ARTICLES

BACITRACIN: A NEW ANTIBIOTIC PRODUCED BY A MEMBER OF THE B. SUBTILIS GROUP[1,2]

IN the study of the bacterial flora of contaminated civilian wounds in the Presbyterian Hospital Unit (under the direction of the Subcommittee on Surgical Infections, National Research Council), it was found that at times organisms appeared on the blood agar plates following direct plating of the injured tissue that were not recovered from broth cultures made at the same time from the same material. This occurred most frequently when the broth cultures contained a large number of aerobic Gram-positive sporulating rods.

Many of these strains had some degree of inhibiting action on subsequent plantings of the Gram-positive cocci which appeared with them on the direct plate. One strain isolated from tissue debrided from a compound fracture of the tibia was particularly active. We named this growth-antagonistic strain for the patient, "Tracy I." When cell-free filtrates of broth cultures of this bacillus proved to possess strong antibiotic activity and to be non-toxic, further study seemed warranted. We have called this active principle "Bacitracin."

The antibiotic is formed when the strain is grown in shallow layers of tryptone broth, beef infusion broth, Savita or Amigen broth or in a synthetic medium. So far it has not been formed to any appreciable extent in submerged growth. The maximum titer is obtained if the antibiotic is harvested after three to five days incubation at 37° C. A heavy surface pellicle is formed, but the decanted medium contains the antibiotic which can be extracted with normal butanol and concentrated by steam distillation *in vacuo*. Further purification results in a grayish-white powder. It has not been obtained in the pure form to date.

"Bacitracin" is filterable through a Berkefeld or Chamberland filter. It is a neutral substance and is not precipitable from the original harvest by manipulating the pH. It differs in this respect from Gramicidin,[3] Subtilin[4] and Gramicidin S.[5] It can not be extracted by ether, chloroform, acetone or ethyl acetate. It is water soluble and withstands heating for 15 minutes at 100° C. without significant loss of titer. It does not hemolyze human or sheep's red blood cells in saline suspension. It is stable in acid solution, but unstable in alkaline solution above pH9. It resists digestion with pepsin or trypsin.

There have been no acute or delayed symptoms of toxicity when the relatively impure material, concentrated by butanol extraction to 50 to 100 times the potency of the original harvest, has been injected repeatedly by the subcutaneous or intraperitoneal routes (mice, guinea pigs) or by the intravenous route (rabbits). There has been no sign of local irritation when such preparations were injected subcutaneously into human volunteers or when applied locally to human infections or on the human conjunctiva. Blood levels have been obtained following human subcutaneous injections.

A standard "unit" for assaying potency has not yet been established. It has been convenient to designate as one "unit" the amount which when diluted 1:1024 in a series of two-fold dilutions in 2 cc of beef infusion broth, completely inhibits the growth of a stock strain of Group A hemolytic streptococcus when the inoculum used to seed the tubes is 0.1 cc of a 10^{-2} dilution of an overnight culture in blood broth. The original harvest contains as a rule two to four units per cc. Material has been obtained which assayed ten units per cc when harvested from a synthetic medium. Table 1 gives the dilutions of one unit which result in complete bacteriostasis of some of the organisms which have been tested under suitable experimental conditions.

TABLE 1

BACTERIOSTATIC ACTION OF "BACITRACIN"

Organisms	Dilutions of 1 unit giving complete bacteriostasis
B. hem. streptococcus	
Groups A, B, C, G	512–1024
Group D	16–64
Nonhemolytic streptococcus	0–64
Pneumococcus	
Types I, II and III	512–1024
Staphylococcus aureus	16–64
Other Gram-positive micrococci	16–256
C. welchii	512–768
C. septicum	512
C. sordellii	512
C. histolyticum	128
C. sporogenes	256

[1] Preliminary report.

[2] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University.

[3] R. J. DuBos, *Jour. Exp. Med.*, 70: 1–10, July, 1939.

[4] E. F. Jansen and D. J. Hirschmann, *Arch. Biochem.*, 4: 297–309, July, 1944.

[5] G. F. Ganse and M. G. Brazlini Kova, *Am. Rev. Soviet Med.*, 2: 134–148, December, 1944.

The action of "bacitracin" on additional species has been ascertained through use of the blood agar plate-penicylinder method. When *C. novyi* was the test organism, the zone of inhibition obtained was comparable to that with *C. histolyticum*. Certain strains of anaerobic nonhemolytic streptococcus were inhibited to the same extent as beta hemolytic streptococcus. Other strains were more resistant. The anaerobic staphylococci were very susceptible to its inhibiting action. Gonococci and meningococci also showed a zone of inhibition of growth in chocolate agar-penicylinder plate tests under increased CO_2 tension. *E. coli, Ps. aeruginosa* and *B. proteus* were not inhibited in agar plate-penicylinder tests or in serial dilution in broth tests.

The *in vivo* protective action of "bacitracin" has been tested against hemolytic streptococcus C203 M. V. in mice. When mice were injected intraperitoneally with 10,000 M. L. D. of an overnight culture of this streptococcus in blood broth, followed immediately by an intraperitoneal injection of one to two units of "bacitracin" in a cell-free filtrate of the antibiotic as harvested, approximately 80 per cent. of the mice survived. When the mice were injected with 10,000 M. L. D. intraperitoneally, followed immediately by a subcutaneous injection of one to two units of "bacitracin," 30 to 40 per cent. of the mice survived. If mice so injected received additional doses of one to two units subcutaneously every three hours for 36 hours, 80 to 90 per cent. survived.

Guinea pigs have been protected against the development of gas gangrene when 1 cc and 0.5 cc of an overnight culture of *C. welchii* or *C. septicum* combined immediately before injection with 50 to 100 units of "bacitracin" in 1 cc of distilled water, was injected into the thigh muscle. These pigs received additional doses of 50 to 100 units of "bacitracin" subcutaneously every three hours for 36 hours. Some swelling and oedema developed at the site of injection of the gas gangrene organisms, but in 80 per cent. of the pigs, this gradually receded and the pigs were alive and well two weeks after the injection. The control pigs injected with a like inoculum of organisms all died within 12 hours. There was no sign of local toxicity from the subcutaneous injection of the "bacitracin," nor was there any toxic general reaction in the experimental pigs or the antibiotic controls.

"Bacitracin" has been used locally to treat a relatively small number of human infections due to hemolytic streptococcus and staphylococcus. The results have been comparable to the response of similar cases to local penicillin. Not enough material has been available as yet for systemic treatment. Attempts are now being made to produce this antibiotic by large-scale commercial methods.

SUMMARY

A new antibiotic "bacitracin" has been recovered from a strain of the *B. subtilis* group of organisms. It is neutral, water-soluble, non-toxic and relatively heat stable. *In vitro* it is active chiefly against Gram-positive organisms, but the gonococcus and meningococcus are susceptible to its action. It is also active *in vivo* against experimentally produced hemolytic streptococcal infections in mice and gas gangrene infections in guinea pigs. Clinical use in hemolytic streptococcal and staphylococcal infections in man have given encouraging results.

BALBINA A. JOHNSON
HERBERT ANKER
FRANK L. MELENEY

DEPARTMENTS OF SURGERY AND BIOCHEMISTRY,
COLLEGE OF PHYSICIANS AND SURGEONS,
COLUMBIA UNIVERSITY

SEROLOGIC EVIDENCE OF CYTOPLASMIC INTERCHANGE DURING CONJUGATION IN PARAMECIUM BURSARIA[1]

THE preliminary experiments reported by the authors[2] on antigenic changes in cultures of *Paramecium bursaria* have been expanded to include serologic examinations of conjugants at various intervals after the onset of the mating phenomenon and at various times following the conclusion of the event. The new work has been done very largely with two strains of the ciliate: one of these is colored brilliantly green by zoochorellae inclusions; the other is colorless. (The color difference is so striking that no difficulty whatever was experienced at any time in determining to which strain either representative belonged.) These two strains are antigenically distinct: each reacts quickly and extensively in homologous antiserum; neither reacts at all in antiserum for the other unless it has undergone conjugation with the other.

The development of a gelatinous precipitate in and about the ciliary zone and the occurrence of ciliary tangling (with eventual loss of action) was taken as positive evidence of antigen-antibody reaction when it appeared within two hours after the paramecia were placed in antiserum. The examinations were made in hanging drop preparations with the "high dry" (4 mm, 44X) objective and 10X ocular of the microscope.

The examination of a large number of paramecia has revealed that about 95 per cent. of all recent (within one hour) ex-conjugants contain some antigenic substance common to their mates which was not previously present within themselves. Moreover, a study of other ex-conjugants has revealed that the

[1] This work was aided by a grant from The Rockefeller Foundation.

[2] J. A. Harrison and E. H. Fowler, *Jour. Immunol.*, 50: 115, 1945.