logous N-guanylarsanilic acid (II) was obtained in the form of its monohydrate, as lustrous transparent colorless prismatic crystals, $C_7H_{10}AsN_3O_3 \cdot H_2O$, which lost weight and became white and opaque when heated *in vacuo*. This opaque product, which gave analytical figures agreeing with those calculated for $C_7H_{10}AsN_3O_3$, when heated darkened around 250–260°, gradually turning black but was still unmelted at 350°.

The same guanylarsanilic acid (II) was obtained by the interaction of arsanilic acid, the methyl sulfate addition product of thiourea and alkali, according to the Rathke procedure, as utilized by Wheeler and Merriam[4] for the synthesis of *o*-guanidinobenzoic acid from anthranilic acid. The hydrolysis of this guanyl arsanilic acid to carbarsone (III) is now being investigated.

Preliminary experiments have also been carried out in the reduction of the guanylarsanilic acid to the corresponding 4,4′-diguanidinoarsenobenzene (IV), but the product awaits final purification and identification. The synthesis of other guanidino arsenicals, including derivatives of both tri- and pentavalent arsenic, is also under way and will be reported later.

Full experimental details and analytical results will be published elsewhere.

MARSTON T. BOGERT
WILLIAM C. STICKLER
ORGANIC CHEMICAL LABORATORIES,
COLUMBIA UNIVERSITY

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A STANDARD PENICILLINASE PREPARATION

IN 1943, Lawrence[1] reported that Clarase was capable of inactivating penicillin, and suggested this enzyme preparation as an agent in the sterility test for penicillin. Later, both Stanley[2] and Lawrence[3] showed that the penicillin destroying power of Clarase was due to a bacterial air-borne contamination in the preparation of a specific lot (1351) of the enzyme. Clarase, Lot 1351 of the Takamine Laboratories, Clifton, N. J., is now used in the Official Food and Drug Administration sterility test for penicillin. In a great number of determinations in our laboratories, the use of Clarase has been found to present a number of difficulties. The preparation of the Clarase solution is lengthy and the solution is difficult to sterilize by filtration. From 24 to 36 hours are required to inactivate the penicillin which is to be tested for sterility. A more powerful penicillin inactivating agent which could easily be sterilized and standardized appears therefore very desirable.

Harper[4] prepared penicillinase preparations from paracolon bacilli for use in the sterility test and for inactivating penicillin in blood samples. Ungar[5] described a penicillinase preparation produced from the culture medium of a strain of *B. subtilis*. The preparation was used successfully for both the penicillin sterility test and exudates of body fluids from patients under penicillin treatment. However, neither of the investigators gave quantitative methods of standardizing their preparations. If a material is to be used routinely as a penicillin inactivating agent, it should be of known activity.

Using the bacterium NRRL-B569[6] and a modified procedure of Benedict and Schmidt,[7] we have prepared a very active penicillinase preparation which was sterilized by filtration and standardized according to the method of McQuarrie and Liebmann.[8] Aliquots containing 2,000 penicillinase units were lyophilized and sealed in sterile bottles. Bottles were stored until needed, then diluted with sterile water. The resulting solution was aseptically added to the penicillin sample to be tested for sterility.

The penicillinase was tested for its effect on bacteria likely to be present in unsterile penicillin preparations or in blood samples. In all cases the enzyme preparation had no effect on the growth of these organisms. In fact the growth in those tubes to which penicillinase had been added was more luxuriant than in those to which Clarase had been added in parallel tests. This would indicate the fact that either the nature of the enzyme itself or its associated impurities serve as growth-promoting substances. This may be of distinct advantage in the test.

Samples of penicillin were tested for sterility by both the official FDA method and by FDA method with 100 units of our penicillinase substituted for the Clarase. When Clarase was used a minimum of 36 hours was required for complete destruction of the penicillin. With our penicillinase preparation, less than one hour was required. By adding higher concentrations of the enzyme, an almost immediate destruction can be effected.

Preliminary studies with blood samples from animals infected with *Staph. aureus* indicate that the

[4] Wheeler and Merriam, *Am. Chem. Jour.*, 29: 491, 1903.
[1] C. A. Lawrence, SCIENCE, 98: 413, 1943.
[2] A. R. Stanley, *ibid.*, 99: 59, 1944.
[3] C. A. Lawrence, *ibid.*, 99: 15, 1944.
[4] G. J. Harper, *Lancet*, ii: 569, 1943.
[5] J. Ungar, *Nature*, 154: 236, 1944.
[6] Kindly supplied by Dr. R. D. Coghill, of the U. S. Northern Regional Research Laboratories.
[7] Private communication from Dr. R. G. Benedict, of the U. S. Northern Regional Research Laboratories.
[8] E. B. McQuarrie and A. J. Liebmann, *Arch. Biochem.*, in press.

penicillinase preparation may successfully be used for inactivating penicillin in such materials, thereby allowing the penicillin sensitive organisms to grow on the culture medium. The procedure is similar to the one using para-amino benzoic acid for the testing of sulfonamides in body fluids.

SUMMARY

(1) Standardization of penicillinase has been made possible by the method for its assay.

(2) A purified, dried and sterile penicillinase has been found to be a penicillin-inactivator superior to Clarase for the penicillin sterility test.

(3) Preliminary studies show this penicillinase preparation may be used for inactivating penicillin in exudates of body fluids.

A. J. LIEBMANN
E. B. MCQUARRIE
D. PERLSTEIN

PENICILLIN TREATMENT OF CROWN GALL

CRUDE penicillin, produced in this laboratory,[1] has cured crown gall on Bryophyllum. The penicillin assayed 2 to 6 Oxford units per cc and was obtained from an improved strain of *Penicillium notatum* contributed by the Northern Regional Research Laboratory, Peoria, Ill. It was made almost automatically and cheaply through the use of a modification of the apparatus described by Clifton.[2] The galls for study resulted from hypodermic inoculations of Bryophyllum with a pure culture of *Agrobacterium* (*Phytomonas*) *tumefaciens*. They were of the "soft gall" type.

From the first hypodermic injections of crude penicillin, just below the galls, the only effect observed was a checking of growth of the gall above the needle punctures, which resulted in an accentuation of the irregularity of the surface of the gall. That effect was interpreted to mean incomplete lateral diffusion of the penicillin in the gall and to indicate as necessary a different method of application.

Penicillin-soaked antiseptic cotton was wrapped around galls and thereafter frequently wetted with crude penicillin. The result of that procedure was the retarded growth and browning of the minute elevations or "pimples" on the surface of the gall. The protective layers of the surface of the elevations appeared to be sufficiently thin for the inward penetration of the penicillin, but elsewhere the drug obviously was kept out of the internal tissues of the gall.

Next, the gall under the cotton wrapping was punctured in numerous places with a sterile needle and soon the tissues began to die and turn brown. Apparent complete destruction of the gall followed. Normal tissues of the stem were somewhat injured where the penicillin-containing cotton wool remained in contact with the surface of the stem, but internally only the gall tissues were affected.

Crown gall is particularly destructive in the Southwest, where the alkaline reaction of the soil, long-growing season, irrigation and heavy transpiration in an arid atmosphere favor the disease.

Penicillin should prove valuable in treating galls on nursery stock and also on set trees and other plants in which the galls are limited to the crown and aerial parts. Cure of the first infected tree in an irrigated orchard frequently would save the entire planting where irrigation would otherwise carry the bacterium and spread the disease. Galls are often seen first at the crown where they may be treated; later they appear on the roots as a result of the downward spread of the gall bacterium. Cure of the crown gall within reach would save not only further spread of infection to the roots of the same tree but, more important, spread of the germ over the field by irrigation water.

The cost of the crude penicillin used in our experiments has been slight. The medium fed to the fungus costs approximately 2 cents per quart and the galls that were cured required a tablespoonful or two of crude penicillin.

Noteworthy is the fact that penicillin apparently destroys, in the case of the crown-gall bacterium, a gram-negative organism. Gram-negative bacteria, in general, have been reported[3] as relatively resistant to penicillin. Interesting, too, is a comparison of the action of crude penicillin on crown gall (often likened to cancer of animals and man) with the reported[4] ineffectual penicillin injection of mice with sarcoma.

J. G. BROWN
ALICE M. BOYLE

PLANT PATHOLOGICAL LABORATORY,
ARIZONA AGRICULTURAL EXPERIMENT STATION, TUCSON

[1] J. G. Brown and Alice M. Boyle, *Phytopathology*, 34: 760–761, August, 1944.

[2] C. E. Clifton, SCIENCE, 98: 67–70, 1943.

[3] For example, see A. D. Gardner, *Nature*, 146: 837–838, December 28, 1940.

[4] Margaret Reed Lewis, SCIENCE, 100: 314–315, October 6, 1944.

BOOKS RECEIVED

KIRSCHENBAUER, H. G. *Fats and Oils. An Outline of Their Chemistry and Technology.* Illustrated. Pp. 154. Reinhold Publishing Corporation. $2.75. 1944.

SAHYUN, MELVILLE and OTHERS. *Outline of the Amino Acids and Proteins.* Illustrated. Pp. 251. Reinhold Publishing Corporation. $4.00. 1944.

WALLING, S. A., J. C. HILL and C. J. REES. *Nautical Mathematics and Marine Navigation.* Illustrated. Pp. ix + 221. Macmillan Company. $2.00. 1944.

The price quoted for the abridged edition of Organic Chemistry by Louis F. Fieser and Mary Fieser is $4.00 not $6.00, as incorrectly stated in the issue of SCIENCE for November 10.