were inoculated with 12 different molds. Following an incubation period of 15 days, none of the 12 strains showed indications of growth in the presence of 0.01 per cent. NaN_3, while 0.001 per cent. partially inhibited the growth of all the molds.[6] When 1½ per cent. of agar was added for the purpose of obtaining surface cultures 0.001 per cent. retarded growth, but it was necessary to increase to 0.01 per cent. in order to inhibit completely *Penicillium glaucum, Aspergillus niger, Mucor rhizopodiformis* and *Oidium albicans.*

In view of the aforementioned observations it seemed desirable to investigate the possibilities of using NaN_3 as a chemotherapeutic substance. To do this it would be most desirable to obtain infections in animals similar to the naturally occurring infections, and evaluate the changes produced following the applications of the chemical. Unfortunately, it is most difficult to produce such infections in animals, therefore attention was directed to its toxicity following intraperitoneal injections. There are references in the literature to the action of NaN_3 on animals and the consensus of opinion seems to indicate an action similar to that of the cyanides on living tissue. Physiological saline solution (PSS), in 0.3 ml quantities containing 0.30 mg NaN_3, on intraperitoneal injection into 20-gram mice was capable of producing convulsions and death in less than 20 minutes, while a single dose of 0.10 mg in 0.10 ml of PSS elicited no neurological signs. Accordingly, 0.10 ml of a solution containing 0.10 mg was administered by the same route every 2 hours for a period of 28 hours, 5 of 7 animals died during the experiment. Blood taken 10 minutes after intraperitoneal administration of 0.10 mg of NaN_3, placed in a test-tube and inoculated with *Penicillium notatum* did not contain enough of the chemical to inhibit growth. On this basis it seems that NaN_3 holds little promise for the treatment of systemic infections.

Fungicidal tests are now in progress.

J. EMERSON KEMPF
WALTER J. NUNGESTER

PENICILLIN IN OIL SUSPENSION. BACTERIOSTATIC AND SPIROCHETICIDAL AGENT[1, 2]

PENICILLIN, dissolved in water or in a glucose solution, has been used intravenously by the continuous drip method or by intramuscular injection every four hours. The purpose of the continuous administration is to maintain the necessary blood stream concentration which is constantly diminished because of the rapid elimination of penicillin. The intravenous drip method as well as frequent intramuscular injections are troublesome to the patient and necessitate the attendance of physicians or nurses and therefore hospitalization. All this implies great expense; indeed, in the case of syphilis and gonorrhea the cost of treatment becomes prohibitive. Thus the problem of reducing the speed of elimination of penicillin from the body is seen to be urgent. When back in 1938 we experimented with sulfanilamide suspensions in oil which was intramuscularly administered to mice, we were impressed with the excellent therapeutic results obtained by this method.

In experimenting with penicillin sodium, our first step was to suspend a finely pulverized sample in some dry sterile vegetable oil, such as peanut oil. It is well known that penicillin sodium dissolved in water is unstable even if kept in the refrigerator, the potency being completely lost in the course of a few days. But when we suspended penicillin in oil, we found to our great surprise that it remained stable for more than two months, as evidenced by the undiminished bacteriostatic effect. In a continuation of the experiment with this sample, there appears the possibility of its remaining intact for a very much longer period, if kept in a refrigerator. Our bacteriostatic tests, which disclosed the above-mentioned facts, were done according to the agar cup-plate method.[3] These tests indicated that 24 hours after the preparation of penicillin sodium in oil suspension, it had a bacteriostatic effect measured by clear zone around the cup of 12 mm. After 8 weeks the bacteriostatic effect was practically the same, namely, 11.5 to 12 mm.[4]

The extraordinary stability of penicillin when suspended in oil led us to the assumption that its effect in this form may be somewhat greater therapeutically than penicillin in aqueous solution, because it is less liable to destruction in the animal body and capable of more gradual absorption. All this was confirmed by our animal experiments. The blood of rabbits which

$MnSO_4$ 10 mg, glucose 40.0 g, and distilled water to 1,000 ml.

[6] *Mucor rhizopodiformis, Aspergillus niger, Aspergillus flavus, Oidium albicans, Hormodendrum compactum, Histoplasma capsulatum, Blastomyces dermatitidis, Trichophyton gypsium, Trichophyton floccosum, Penicillium notatum, P. glaucum* and *P. citrinum.*

[1] From the Dermatological Research Laboratories, Philadelphia, Division of Abbott Laboratories, North Chicago, Illinois.

[2] The author wishes to thank Drs. J. H. Stokes, H. Beerman and J. W. Lentz for their valuable advice and help in carrying out this research. The author wishes also to acknowledge with deep appreciation the assistance of Dr. M. Severac and J. C. Moetsch in the experimental work.

[3] "U. S. Food and Drug Administration Methods of Testing Antiseptics and Disinfectants," Circular No. 198, December, 1931, U. S. Department of Agriculture.

[4] C. Nielsen, of the Abbott Laboratories, North Chicago, Illinois, in a personal communication reported that suspensions of penicillin in peanut oil were found to be stable even at incubator temperature, that is, at 37° C. In his experiments the original bacteriostatic values remained the same after incubation for 30 days.

were given intramuscular injections of penicillin oil suspensions remained bacteriostatic for a longer period of time than when penicillin was administered in aqueous solution. In these experiments all the rabbits received intramuscularly 4,000 Oxford units of penicillin per kilo. When the penicillin was administered in aqueous solution, the blood became bacteriostatic for only one hour.[5] This was evidenced by the absence of growth of *Staphylococcus aureus* in broth culture mixed with rabbit blood. But bloods obtained one and one half, two, three, four and five hours later did show growth. On the other hand, when the blood of other rabbits which received penicillin in oil suspension was tested, the results proved that it was bacteriostatic one, one and a half and two hours after intramuscular injection, as determined by the absence of growth. Three, four, five and six hours afterwards, growth was observed—thus indicating the cessation of bacteriostatic effect.

The next experiment involved the treatment of rabbit syphilis by intramuscular injections of penicillin sodium suspended in oil. Rabbits were infected intratesticularly with a suspension of *Spirocheta pallida,* according to the method previously described by us.[6] One syphilitic rabbit was given intramuscularly 2,500 Oxford units per kilo body weight of penicillin sodium in aqueous solution twice a day consecutively for 8 days, totaling 40,000 units. The testicles were not free of spirochetes until the 14th day, and the complete healing of the lesions occurred at the end of 42 days. Two other rabbits were each administered intramuscularly 5,000 Oxford units per kilo of penicillin in oil. In this case, however, each animal was given penicillin only once a day for a period of 8 days, comprising again a total of 40,000 units. In both rabbits, the testicular lesions became negative for spirochetes in less than 10 days and the testicles appeared normal at the end of 35 days.

From the above it may be concluded in a preliminary way that penicillin in oil suspension is therapeutically somewhat more active in experimental rabbit syphilis than penicillin in aqueous solution. The superiority of the oil suspension, however, lies chiefly in the fact that the administration of the drug can be reduced to only one treatment a day. This experimental study opens the way to a method by which syphilis patients can be treated with penicillin in oil by a greatly simplified procedure. One daily intramuscular injection may thus suffice. This study further suggests a possibility of replacing the intravenous drip method and similar procedures by a few intramuscular injections of penicillin in oil to be used in the treatment also of various bacterial infections.

GEORGE W. RAIZISS

[5] Rabbit's blood was diluted with broth culture in the proportion of 1 to 20. This culture was seeded with a resistant strain of *Staphylococcus aureus* using one 4 mm loop of a 1–1,000 dilution.

[6] George W. Raiziss, *et al., Am. Jour. Syph. and Neurol.,* 19: 473, October, 1935.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD FOR THE COLORIMETRIC DETERMINATION OF PHOSPHORUS[1]

IN 1920 Bell and Doisy[2] devised a method for the colorimetric determination of phosphorus. This consisted in adding sulfuric and molybdic acids to the unknown ortho phosphate solution and reducing the phosphomolybdic acid thus formed by means of hydroquinone in a carbonate-sulfite medium. The resulting blue solution was read in a colorimeter against a standard. Briggs[3] improved the method by changing the order of adding the reagents. Fiske and Subbarow[4] considerably improved the method by substituting aminonapthol sulfonic acid for hydroquinone. Since the reduction of phosphomolybdic acid by aminonapthol sulfonic acid proceeds relatively slowly at room temperature, Lohmann and Jendrassick[5] have advocated heating the reactants to 37° C. for from 5 to 7 minutes. Allen[6] has employed amidol (2,4 diaminophenol dihydrochloride) in place of aminonapthol sulfonic acid, since this is a very powerful reducing agent. Kuttner *et al.*[7, 8] have used stannous chloride as the reducing agent, and this compound has been employed successfully by Youngburg and Youngburg.[9] Bodansky[10] found that trichloracetic acid caused deviations from Beer's law when the stannous chloride procedure was employed. He published a table of corrections.

While the Allen method has a number of minor dis-

[1] From the Biochemistry Laboratory, Cornell University, Ithaca, N. Y.

[2] R. D. Bell and E. A. Doisy, *Jour. Biol. Chem.,* 44: 55, 1920.

[3] A. P. Briggs, *Jour. Biol. Chem.,* 53: 13, 1922; 59: 255, 1924.

[4] C. H. Fiske and Y. Subbarow, *Jour. Biol. Chem.,* 66: 375, 1925.

[5] K. Lohmann and L. Jendrassick, *Biochem. Zeits.,* 178: 419, 1926.

[6] R. J. L. Allen, *Biochem. Jour.,* 34: 858, 1940.

[7] T. Kuttner and H. R. Cohen, *Jour. Biol. Chem.,* 75: 517, 1927.

[8] T. Kuttner and L. Lichtenstein, *Jour. Biol. Chem.,* 86: 671, 1930.

[9] G. E. Youngburg and M. V. Youngburg, *Jour. Lab. Clin. Med.,* 16: 158, 1930.

[10] A. Bodansky, *Jour. Biol. Chem.,* 99: 197, 1932.