The phenomenon has been observed, thus far, only with active influenza virus preparations. Inactivation by ultraviolet irradiation, heat or formalin rendered the preparations innocuous. On the other hand, irradiated preparations have given evidence of interference in accord with experience gained with the influenza viruses.[5] When high concentrations of irradiated virus were injected simultaneously with active homologous or heterologous virus by the intracerebral route reduction in the incidence of convulsions was noted as compared to the controls inoculated with active virus alone.

Although only active virus has been found to elicit the phenomenon, no propagation of the influenza virus in the brain tissue could be demonstrated. When brains were harvested from mice immediately after intracerebral inoculation of PR-8 or Lee virus, or 24, 48 and 72 hours later when cerebral signs were apparent, titration of emulsions of these brains in eggs revealed that the amount of influenza virus did not increase in the brain but rather decreased to less than 0.1 per cent. of the amount of virus found in the brain immediately after inoculation. Furthermore, attempts to pass the agents from mouse brain to mouse brain at three day intervals failed in several trials. No neurological signs were noted in the second brain passage and subcultures in eggs from the third cerebral passage failed to demonstrate the presence of influenza virus.

The phenomenon described can be elicited not only by influenza viruses grown in the allantoic cavity of the developing chick, but also by strains which have been maintained continuously by mouse lung passage. Potent suspensions of infected mouse lungs and concentrates therefrom behave essentially similar to infected allantoic fluids.

Neurological signs of similar nature caused by influenza virus have been reported previously by Stuart-Harris[6] and Francis and Moore,[7] who were able to adapt certain strains to mouse brain passage. The results of these authors differ from ours in various respects: Infected chick brain tissue cultures or mouse lungs served as starting material; the virus multiplied in the brain tissue; neurological signs developed only after from 3 to 12 cerebral passages; the incubation period varied from 3 to 11 days; and only two strains (WS and Melbourne) could be established in this way, while the PR-8 strain gave negative results. In contrast to these observations we used mainly allantoic fluid preparations of influenza virus which caused the indicated signs on first passage to the mouse brain, usually within 24 to 72 hours and in exceptional cases only as late as 6 to 8 days after inoculation; the agent could not be passed from brain to brain in series, and finally, all strains of influenza virus tested gave the result, provided enough virus was present.

These apparent differences may possibly be explained by the assumption that influenza virus in sufficient concentration is toxic for the brain tissue without showing propagation in the CNS. As shown by the other workers,[6,7] only a few strains are able to multiply in the brain tissue, in which case enough virus accumulates gradually to elicit the toxic reaction. This would imply a separation of the propagating property from the toxic activity. In this regard it is of interest to note that toxic properties of another group of viruses have been observed recently by Rake and Jones.[8]

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THE INHIBITING EFFECT OF SODIUM AZIDE ON MOLD GROWTH[1]

THE growth of molds on a variety of materials is of great economic and military significance. In addition to this well-known activity, infections of man and animals, particularly of the skin, are matters of considerable importance. In view of these facts, the chance observation that high dilutions of sodium azide (NaN_3) prevented the growth of *Penicillium notatum* seemed worthy of further investigation. The inhibiting action of sodium azide on biological processes was first described by Keilin and Hartree[2] in respect to catalase. Snyder and Lichstein[3] suggested the addition of this chemical to bacteriological media employed for the isolation of gram-positive organisms from specimens of urine and feces containing a preponderance of gram-negative bacteria since its selective inhibition for the gram-negative coliform bacteria had been demonstrated by Bryan, Devereux, Hirschey and Corbett.[4]

In determining the concentration of NaN_3 which would inhibit the mold growth, modified Chapek-Dox media[5] containing varying concentrations of NaN_3

[1] From the Hygienic Laboratory, University of Michigan, Ann Arbor.

[2] D. Keilin and E. F. Hartree, *Nature*, 134–933, 1934.

[3] M. L. Snyder and H. C. Lichstein, *Jour. Inf. Dis.*, 67: 113, 1940.

[4] C. S. Bryan, E. D. Devereux, W. C. Hirschey and A. C. Corbett, *North. Am. Vet.*, 20: 41, 1939.

[5] W. Kochalaty, *Jour. Bact.*, 44: 469, 1942. Constituents: $NaNO_3$ 2.0 g, KH_2PO_4 1.0 g, KCl 1.0 g, $MgSO_4$ 0.5 g,

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.

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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.