human disease and on the behavior of strains of virus of human or recent human origin in cynomolgus monkeys and chimpanzees have revealed rather different patterns of virus behavior, which appear to depend as much (or more) on certain qualitative differences between viruses derived from human beings and those thoroughly adapted to rhesus monkeys, as on the varying characteristics of the terrain in the different hosts. One may wonder, therefore, to what extent the neural mechanisms worked out with M.V. virus in rhesus monkeys may apply to the disease in human beings or experimental infection with other strains of virus. From what has been observed already, however, it would appear that regardless of what these other strains of virus may do in other tissues before invasion of the nervous system, the available evidence is still in favor of the view that that invasion occurs along specific peripheral nervous pathways and that the subsequent dissemination within the CNS is governed by the neuronotropism of the virus of poliomyelitis. The investigations of Drs. Howe and Bodian supply an excellent foundation for the future attack on the many unsolved and intriguing problems presented by the neural mechanisms in poliomyelitis and other neurotropic virus diseases, and as Dr. Thomas M. Rivers has indicated in a foreword to this book, it is highly desirable that virus workers in this field train themselves in "neurobiology" and that "neurobiologists" come to regard neurotropic viruses as a suitable *terra incognita* to invade.

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SPECIAL ARTICLES

ON PENICILLIN[1]

PENICILLIN, an antibacterial agent produced by the mold *Penicillium notatum,* was discovered by Fleming[2] in 1929. A chemical study of penicillin and the pigments produced by this mold was undertaken by Clutterbuck *et al.*[3] Last year the remarkable chemotherapeutic effect of purified penicillin, coupled with a low toxicity was reported by Chain *et al.*[4] A few months ago a more complete report by Florey and his collaborators[5] was published, including a method of purification.

Work on penicillin by our group was started over a year ago. In view of the practical importance of the subject it was thought advisable to publish some of our data at this incomplete stage.

For the routine test in this work a serial dilution method was used, employing 15- to 18-hour cultures of strain C203Mv, a group A hemolytic streptococcus. In some experiments this test was supplemented by the Oxford method,[5] in which the width of a zone of growth inhibition formed by the action of the agent on a heavily seeded agar plate culture is measured.

The culture fluid for the mold was a modified Czapek-Dox medium. In part of this work, the penicillin containing medium was supplied by the Charles Pfizer Company.[6]

Compared to other naturally occurring bactericidal substances like pyocyanine, gliotoxin, gramicidin or actinomycin, the isolation of penicillin proved rather difficult. This is due to the great instability of the agent and to the simultaneous production by the mold of many yellow pigments of similar chemical properties, which however are practically inactive as bactericidal agents.

In our procedure the culture medium is adjusted to pH 3–4, saturated with ammonium sulfate and extracted with chloroform. The active agent is removed from the concentrated chloroform extract by phosphate buffer at pH 7.2. Extraction with chloroform and buffer is repeated and the less acidic pigments separated from the most active fraction by chloroform extraction at different acidities. Penicillin is obtained from the concentrated extracts either as the free acid by precipitation from petroleum ether, or as the ammonium salt by saturation of a chloroform-benzol solution with dry ammonia gas. If the precipitation of the free acid is slow, it separates in yellow thick whetstone shaped crystals. The ammonium salt forms a dark yellow microcrystalline powder. In solution, penicillin, especially the free acid, is rather rapidly inactivated. The ammonium salt is more stable. In

[1] From the Departments of Ophthalmology and Medicine, College of Physicians and Surgeons, Columbia University, the Institute of Ophthalmology, the Edward Daniels Faulkner Arthritis Clinic, Presbyterian Hospital, New York, and the Research Division, Schering Corporation, Bloomfield, N. J. The authors submitted this paper on January 30, 1942, to the Committee on Medical Research of the Office of Scientific Research and Development who requested that publication be deferred pending official decisions on policy.

[2] A. Fleming, *Brit. Jour. Exp. Path.,* 10: 226, 1929.

[3] P. W. Clutterbuck, R. Lovell and H. Raistrick, *Biochem. Jour.,* 26: 1907, 1932.

[4] E. Chain, H. W. Florey, A. D. Gardner, N. G. Heatley, M. A. Jennings, J. Orr-Ewing and A. G. Sanders, *Lancet,* 2: 226, 1940.

[5] H. W. Florey, E. P. Abraham, E. Chain, C. M. Fletcher, A. D. Gardner, N. G. Heatley and M. A. Jennings, *Lancet,* 2: 177, 1941.

[6] We thank the Charles Pfizer Company for this material.

dry form both acid and salt keep only *in vacuo*. This procedure has given uniform results and a yield of over 50 per cent. of the original potency.

A considerable increase in stability of the solutions was obtained by acetylation or benzoylation of the ammonium salt. The free acids of the acyl derivatives form fine needles which have about the same *in vitro* activity as the mother substances.

The analysis of penicillin best fits the formula $C_{14}H_{19}NO_6$ or $C_{14}H_{17}NO_5+H_2O$. The Oxford authors have stated that their preparations are nitrogen free. All our highly active and pure preparations, including the acyl derivatives, analyze for one N atom. Chromatographic adsorption or treatment with charcoal did not lower the N content. Penicillin is strongly dextrorotatory and has an absorption maximum at 2750 A°.[7]

Biologically our preparations are inactive against *E. coli*. The minimal concentration showing activity against 2 to 3 million hemolytic streptococci per cc is at a dilution of 1:32 million. This corresponds to about 240 Oxford units per mg. The Oxford standard has an activity of 42 units per mg.

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THE REVERSAL OF PNEUMOCOCCUS QUELLUNG BY DIGESTION OF THE ANTIBODY WITH PAPAIN

IN a recent study[1] we have been able to remove the antibody by means of digestion with the proteolytic enzyme papain and recover several neutralized biologic agents in the active state. Among the agents studied was Pneumococcus, Type I, and it was found that it regained its virulence for the mouse (even after gross overneutralization) when the suspension of neutralized pneumococci was treated with papain.

Since there is no clear evidence as to the nature of the Quellung phenomenon, we thought it worth while to investigate whether the reactivation of the pneumococci was accompanied by reversal of the Quellung as a result of the action of papain, since such a result would indicate that the Quellung merely represents the addition of antibody with no further mechanism needing to be postulated. In fact, Etinger-Tulzynska,[2] on the basis of experiments wherein the Quellung reaction was reversed by means of heat treatment, postulated that the Quellung phenomenon was merely a visualization of the capsule that was already there. Nungester and Kempf[3] have likewise claimed that there is a reversal of the Quellung upon the addition of specific soluble substance, which in fact may represent the removal of antibody, although this involves assuming that the antibody was "pulled" off the pneumococcus by the soluble polysaccharide.

Our investigations were carried out in the following manner: Equal volumes of twenty-four hour tryptose broth culture of Pneumococcus Type I and undiluted antibody (rabbit serum) were mixed and a small portion was immediately removed and examined under the microscope for Quellung, which was plainly evident in a few minutes. The remainder of the mixture was incubated at 37° C. for thirty minutes, after which time a small portion of activated papain was added to half of the quelled pneumococcus suspension, and as a control papain, which had not been activated, was added to the other half. It was noted that, in the presence of the active papain, in a few minutes the clumps of agglutinated pneumococci were dispersed and went into a homogeneous suspension. Examination of the digested mixtures after about thirty minutes' incubation indicated a complete loss of the Quellung, while the control mixture with inactive papain remained agglutinated and swollen.

In order to ascertain whether the de-quelled pneumococci were capable of being re-quelled, after the digestion had taken place, the bacteria were spun down in the angle centrifuge and washed five times with saline in order to remove the enzyme. More serum was then added and Quellung occurred almost immediately. Thus, the cycle has been completed; it has been possible to reverse the Quellung by means of the removal of antibody and to re-quell the same pneumococci by the addition of more antiserum.

This experiment indicates that the Quellung merely represents the addition of antibody, an explanation which has been suggested.[4] We can bolster this theory by pointing out that from the quantitative point of view it is possible for the antibody to account for the increase in size, simply by its own volume. This is possible since we now have knowledge of the amount of antibody which the very same culture pneumococcus takes up.[5]

White[6] gives the size of the pneumococcus as from 0.5–1.2 μ. Taking as an average a diameter of 0.8 μ, or a radius of 0.4×10^{-4} cm, the volume of a sphere

[7] We thank Dr. H. Darby for the spectrographic analysis.

[1] G. Kalmanson and J. Bronfenbrenner, *Federation Proceedings*, 1: 2, 179, 1942.

[2] R. Etinger-Tulzynska, *Ztschr. f. Hyg. u. Infectsionkr.*, 114: 769–789, 1933.

[3] W. J. Nungester and A. H. Kempf, *Proc. Soc. Exp. Biol. and Med.*, 43: 705–706, 1940.

[4] A. D. Hershey, *Jour. Immunol.*, 42: 485–513, 1941.

[5] A. D. Hershey, *Jour. Immunol.*, 39: 383–396, 1940.

[6] B. White, "The Biology of the Pneumococcus," p. 31. Oxford University Press. 1938.