contributions will be about as high as those in the reich. For those reich Germans who still draw their pay in the homeland, the contributions will be paid into the general sick fund of Katowice (Upper Silesia). Reich Germans in the Ukraine and their families in the reich will each receive a certificate enabling them to go to any doctor according to the system of free choice of doctors. By the creation of this sick fund institution a great work has been started, and the social welfare of the reich Germans in the Ukraine in the field of health insurance has been assured.

The Swedish trade union paper *Fackföreningsrörelsen* of April 24, in an article on the German labor situation, states that various regulations have been published against taking sick leave, especially in the armament factories. Press campaigns and meetings explain that to take holidays because of sickness is paramount to treason. In this connection it may be mentioned that the health condition of the German people has already been largely undermined by many years' hard work. The *Reichsgesundheitsblatt* contains sensational figures about the registered cases of illness in the reich over a period of forty-eight weeks in 1939 and in 1941:

	1939	1941
Diphtheria	128,897	154,752
Scarlet fever	119,730	226,755
Tuberculosis	69,502	88,312
Paratyphus	2,648	3,800

During the first twenty-five weeks of 1939 there were 6,135 cases of dysentery against 12,705 during the same period of 1941. Similar figures are shown for whooping cough. Compared with the difficult years preceding national socialist rule, these illnesses increased by from 500 to 800 per cent. The difficult conditions in Russia must have caused even these figures to rise greatly last year.

To increase the capacity of overworked soldiers and workers, more extensive use is being made of stimulants such as amphetamine. A luftwaffe doctor recently considered it necessary to issue a warning against such preparations in the *Deutsche medizinische Wochenschrift.*—*Journal of the American Medical Association.*

SCIENTIFIC BOOKS

POLIOMYELITIS

Neural Mechanisms in Poliomyelitis. By HOWARD A. HOWE and DAVID BODIAN. 234 pp. New York: The Commonwealth Fund. 1942. $3.50.

THE book entitled "Neural Mechanisms in Poliomyelitis" is a collection of previously published work on poliomyelitis carried out by Drs. Howe and Bodian. About 80 per cent. of the contents of the book appeared during 1941 in the *Bulletin* of the Johns Hopkins Hospital and with the exception of some cementing remarks the remainder is derived from the British publication *Brain,* from the *Journal of Experimental Medicine,* the *Proceedings* of the Society for Experimental Biology and Medicine, and the *Journal of Infectious Diseases.* While this book presents nothing new to those active in the investigation of poliomyelitis who have come to await eagerly and to digest each new publication of Drs. Howe and Bodian, it is, nevertheless, a most welcome volume because it brings together in systematic rather than chronological order the results of a series of investigations to which repeated reference will be found necessary and profitable by seasoned as well as beginning students of poliomyelitis and other neurotropic virus diseases.

A review of this book, therefore, is equivalent to an examination of the contributions which these investigators have made to our knowledge and understanding of the neural mechanisms in poliomyelitis. The work was begun about five years ago at a time when a great deal of data had already been accumulated on the behavior of many viruses (including that of poliomyelitis) with a special affinity for the nervous system. Because in addition to their obligate intracellular parasitism these viruses possess the unique property of spreading along specific nerve tracts in the peripheral and central nervous systems, it became increasingly clear that the mechanism by which they infect susceptible hosts could not be investigated properly without a thorough knowledge of the terrain over which they moved. Investigators whose primary training was in viruses had to become students of neuroanatomy before they could proceed. It was particularly appropriate and fortunate, therefore, that Drs. Howe and Bodian, whose basic training and contributions had been in the field of experimental neurology and neuroanatomy, should have entered upon the study of the neural mechanisms in poliomyelitis. In order to build upon a solid foundation much of their work consisted of a repetition and elaboration of the work of other investigators. Thus, they were able to confirm and adduce additional evidence for the following concepts: (1) that the virus of poliomyelitis moves along the axon rather than along the other structures of peripheral nerves; (2) that in the central nervous system (CNS) the virus acts only upon the neurons and not on any of the other structures, the mesodermal-glial component of the characteristic pathologic lesion occurring only in response to the neuron-virus reaction; (3) that the dissemination of the virus in the nervous

system is influenced by the specific tract connections of the affected neurons rather than upon the mere contamination of neighboring cells with virus; (4) that certain regions of the CNS are more resistant than others to the virus of poliomyelitis; (5) that in animals sacrificed in the preparalytic stage the regions affected vary with the central connections of the primary neurons involved in different portals of entry; (6) that even late in the paralytic stage of the disease produced by the M.V. virus in rhesus monkeys the olfactory bulbs are not affected except when invasion of the CNS has occurred along the olfactory pathway; (7) that when a thoroughly adapted monkey strain of poliomyelitis virus is instilled into the nose (thus reaching also the alimentary tract) of rhesus monkeys, paralytic poliomyelitis results only when the olfactory connections are intact, and (8) that the distribution of lesions in the central nervous system of human beings is essentially the same as that found in experimental animals infected by other than olfactory pathways.

Among the many new observations made by Drs. Howe and Bodian, the following are important as much for what they have revealed as for the new leads to future investigations that they have created. Their data suggest that, as in the case of some other neurotropic viruses, there is a latent period between the time axons are exposed and the time poliomyelitis virus begins to move in them towards the cell body, and that the original amount of virus entering the axon seems to reach the cell body without multiplication in the axon. Taking into account the probable latent period they estimated the rate of progression of one strain of poliomyelitis virus in the sciatic nerve of the rhesus monkey as 2.4 mm per hour on the assumption that the rate of progression is uniform. While one can not accept this as an established fact because the endpoint depends upon the result in a single monkey and the assumption of a uniform rate of progression has not yet been checked by experiment, there is, nevertheless, presented a method by which the rate of progression of various strains of poliomyelitis virus can be determined. They demonstrated the limited protective barrier of the pia by showing that while virus suspended in physiological salt solution and dropped on the uninjured pia failed to produce the disease, virus suspended in distilled water and administered in the same manner resulted in paralytic poliomyelitis. They presented the first really conclusive evidence that the alimentary tract can be a portal of entry for poliomyelitis virus by showing that it is possible to produce the paralytic disease in chimpanzees with surgically severed olfactory tracts when *virus of human origin* is given by mouth or by stomach tube.

Perhaps the most significant observation that has emerged from the labors of these investigators concerns the change in the behavior of certain susceptible neurons when their condition or metabolism is altered by severing their axons, particularly when this is done close to the cell body. The fate of a mitral cell in the olfactory bulbs, or an anterior horn cell in the lumbar cord, invaded by the M.V. strain of virus, is, under ordinary circumstances, complete necrosis. However, although no change in their reaction to this virus seems to occur for about a week after the axons of these cells are interrupted, they appear to become increasingly refractory thereafter, the maximum refractory state being reached at about three weeks and persisting apparently until effective regeneration has occurred. The effects observed were somewhat as follows. When the axons of certain groups of anterior horn cells were interrupted by cutting or freezing one sciatic nerve or by root section, and the monkeys, after a suitable interval, were given poliomyelitis virus by the intracerebral or intranasal routes, it was found that the nerve cells with the interrupted axons were spared, while extensive destruction of neighboring nerve cells had occurred. Similarly, when the olfactory tracts were cut, especially when this was done close to the bulbs, and virus was given intranasally after a suitable interval, the typical destruction of the mitral cells and the other associated lesions failed to take place. Drs. Howe and Bodian are inclined to interpret these data as indicating that the cells have become refractory to the effects of the virus, *i.e.*, that the virus reached the cells but was unable to destroy them. It would seem, however, that the existing evidence could also be interpreted to mean that the virus fails to invade the nerve cell because of its altered condition. I am more favorably inclined to the opinion expressed in the following sentence which appeared in their original communication but not in the book: "At the present time it is unknown whether the nerve cells are invaded by virus but not destroyed, or whether they no longer offer a foot-hold for it." In some experiments with another neurotropic virus I found that the centripetal movement of virus in the axon is dependent upon certain influences from the cell body. One would be justified, therefore, in considering the possibility that the spread of virus towards a nerve cell across a synapse may similarly be under the influence of the cell body toward which the virus is moving.

Drs. Howe and Bodian began their work at a time when critical investigators, guided by the available data, believed that the course of events in the human disease was essentially similar to that occurring in rhesus monkeys infected with the M.V. strain of virus. Recent studies, however, on the natural history of the

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.