not appear to have been recognized. It was with some astonishment, then, that electrical stimulation of the bulbar reticular formation in the cat was found to bring completely to a halt motor activity whether induced reflexly, by brain stem mechanisms or from the motor cortex.

In the records shown in Fig. 1 A and B, the blink

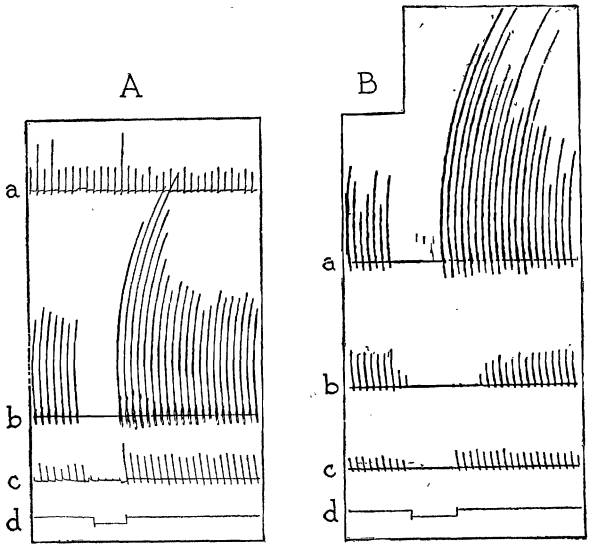

FIG. 1. A and B. Effect of bulbar stimulation (d) on flexor reflex (a), knee jerk (b) and blink reflex (c), evoked at 2 second intervals. Chlorolosane anesthesia. In all records the bulbar stimulus consisted of 60 cycle current at 3–5, R. M. S. volts.

reflex of the eyelids (c), the flexor reflex of the foreleg (a) and the knee jerk in the hindleg (b), evoked at 2-second intervals, were abolished by bulbar stimulation during the period marked by the signal (d). These reflexes, initiated respectively by tactile, nociceptive and proprioceptive stimuli, involve muscles—posturally indifferent, flexor and extensor—distributed over the length of the body. The bulbar inhibitory influence thus appears to be a general one, not limited in its action to topographically circumscribed or to functionally specific reflex acts.

The effect of bulbar stimulation upon decerebrate rigidity was observed visually with the animal supine and its legs extended in the air. Upon stimulation the limbs became flaccid and collapsed and their reflexes were lost. At the cessation of the stimulus extensor hypertonus and reflex activity promptly returned. In Fig. 2 A, flexion of the hindleg (a), induced by activating descending fibers from the motor cortex in the internal capsule (c), was abolished by stimulating the bulbar reticular formation (b).

In some of the instances illustrated (Fig. 1 A, b and c; Fig. 1 B, a; Fig. 2 A, a), the bulbar inhibitory effect was followed at the cessation of the stimulus by a subsequent augmentation of whatever motor activity was proceeding, a phenomenon attributed in other situations to the activation of intermixed inhibitory and facilitatory elements. Perhaps supporting this interpretation was the observation of purely facilitatory responses (Fig. 2 B, a) elicited from adjacent regions of the medulla.

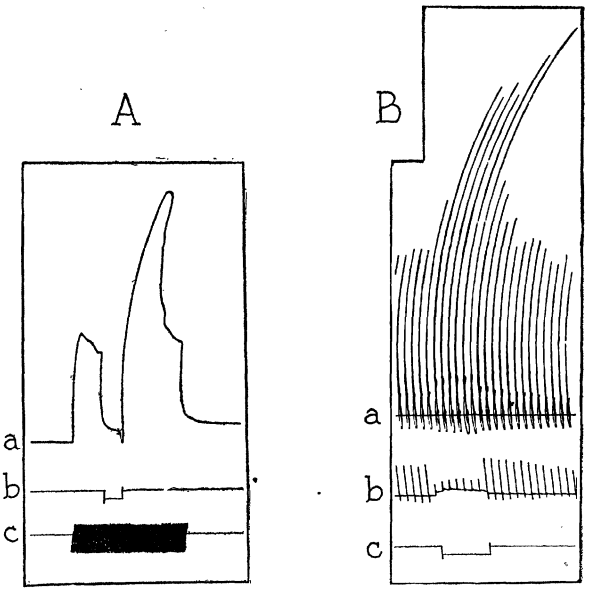

FIG. 2. A. Effect of intercurrent bulbar stimulation (b) on flexion of the hindleg (a) induced by activating the internal capsule (c) with induction shocks. Nembutal anesthesia. B. Effect of bulbar stimulation (c) on knee jerk (a) and blink reflex (b). Chlorolosane.

Retention of bulbar inhibitory and facilitatory responses after low decerebration excluded the possibility that they resulted from activating ascending pathways to higher neural levels. Similarly, they were unimpaired after decerebellation. The possibility that they were evoked by activating descending pathways simply coursing through the medulla has not been so certainly eliminated, though no comparable general effects have yet been obtained by midbrain stimulation.

At this preliminary stage, the relation of the bulbar mechanism described to other motor components of the nervous system can only be conjectural, but the duality and widespread distribution of the effects of its activation certainly suggest its potential importance in the regulation of motor activity.

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RELATIONSHIP OF PENICILLIN THERAPY TO BRAIN INVOLVEMENT IN EXPERIMENTAL RELAPSING FEVER[1]

PREVIOUS papers[2, 3, 4] on the therapeutic efficacy of

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penicillin in experimental relapsing fever have reported the use of the old rat passage strain of *Borrelia recurrentis* (*novyi*). The heavy inocula used have resulted in massive infections in time intervals which are never experienced in natural infections. Reported "cures" have not taken into consideration the well-known fact of brain involvement in experimental relapsing fever.[5]

The 45 rats employed in the treatment phase of our experiment were infected by intraperitoneal injections of 0.01 cc of onset blood (1 to 5 spirochetes per 100 dark fields) from tick (*O. turicata*) infected rats or from rats not more than six onset blood passages away from tick infection. Incubation periods ranged from 2 to 5 days and maximum infections were attained in 4 to 7 days. The infection status was established by the daily examination of uniform dark-field preparations of tail blood (0.01 cc of a 1:20 dilution).

The infection in the late treatment group of 25 rats was allowed to progress to late first attack or to early or late relapse stages (2 to 18 days after dark-field onset) before treatment was begun. Treatment was begun in the early treatment group of 20 rats on the day that spirochetes were first observed in dark-field preparations (1 spirochete per 176 to 6 spirochetes per 100 dark-fields). The 45 animals were subdivided into the following 9 groups of 5 each on the basis of infection status and treatment dosage. Rats of approximately equal weights were distributed among the various groups:

Late Treatment Group:

Group I—Treated with 7,600 Oxford units of penicillin beginning 2 to 6 days after dark-field onset.

Group II—Treated with 7,600 Oxford units of penicillin beginning 14 to 15 days after dark-field onset.

Group III—Treated with 3,800 Oxford units of penicillin beginning 9 to 18 days after dark-field onset.

Group IV—Treated with 1,900 Oxford units of penicillin beginning 12 to 15 days after dark-field onset.

Group V—Controls not treated. Dark-field onset 14 to 15 days before treatment of test animals was begun.

Early Treatment Group (Treatment was begun on day of dark-field onset):

Group VI—Treated with 7,600 Oxford units of penicillin.

Group VII—Treated with 3,800 Oxford units of penicillin.

Group VIII—Treated with 1,900 Oxford units of penicillin.

Group IX—Controls not treated. Dark-field onset on day treatment was begun in test animals.

All animals were treated concurrently by the intraperitoneal route, at intervals of 4 hours for 72 hours. Groups I, II and VI received an initial dose of 800 units followed by 17 doses of 400 units each. Groups III and VII received an initial dose of 400 units followed by 17 doses of 200 units each. Groups IV and VIII received an initial dose of 200 units followed by 17 doses of 100 units each.[6]

The 20 animals in Groups VI through IX were examined microscopically at intervals of 1, 5 and 25 hours after the initial dose of penicillin and daily thereafter for 10 days from the date of infection. The results of these examinations tend to confirm the previously reported ability of adequate doses of penicillin (in our case between 6,000 and 12,900 Oxford units per kg of rat) to clear the blood stream of experimentally infected animals of microscopically demonstrable spirochetes within 5 to 6 hours. Whether or not this 5-hour blood clearing dose constituted a curative dose in these animals remains to be established. These rats continued to receive penicillin, and 7 of the 8 later proved to be cured. The 8 rats which showed blood clearing (5 of Group VI and 3 of Group VII) received total penicillin dosages equivalent to between 38,400 and 81,700 units per kg body weight. The 7 rats (2 of Group VII and 5 of Group VIII) which either failed to clear or relapsed received total penicillin dosages equivalent to between 9,400 and 26,700 units per kg body weight. The five control animals (Group IX) showed 2 to 28 spirochetes per 100 dark-fields at the 5-hour examination. These infections progressed to maxima of 3 to 40 spirochetes per field.

Seven days after the termination of treatment the 44 surviving rats were sacrificed and the brains from these animals were inoculated intraperitoneally to fresh rats. Uniform dark-field preparations from these brain passage rats were examined daily for 10 days.

The 25 brain passage rats from Groups I through V (late treatment rats) all became positive, indicating that either the brain or blood or both still harbored spirochetes. The total penicillin dosage received by the test animals in this group ranged between 5,100 and 48,100 Oxford units per kg. Seven of the 10 animals in Groups I and II received more than 40,000 units of penicillin per kg, but were not rendered negative to brain passage.

In the early treatment group, the brains from 7 of the 8 rats which showed microscopic blood clearing failed to infect the rats to which the brains were passed. Thus it would appear that adequate penicillin

[2] F. R. Heilman and W. E. Herrell, *Proc. Staff Meetings Mayo Clinic*, 18: 457–467, 1943.

[3] Donald L. Augustine, David Weinman and Joan McAllister, SCIENCE, 99: 19–20, 1944.

[4] Harry Eagle and Harold J. Magnuson, *Pub. Health Rep.*, 59: 583–588, 1944.

[5] A. Buschke and H. Kroo, *Deutsch. Med. Wschr.*, 49: 1435–1436, 1923.

[6] We wish to thank Dr. J. W. Foster, of Merck and Company, for sending a vial of standard penicillin and Miss Grace Beal, of the Brucellosis Research Project of the Clayton Foundation, the University of Texas, for checking our penicillin dosages.

dosage given early in experimental relapsing fever (*B. recurrentis* var. *turicatae*) infection will not only cure the blood stream involvement but will prevent brain involvement in a great majority of the cases. The fact that the 1 positive brain passage in this group of 8 animals occurred in a rat which received 45,000 units of penicillin per kg, whereas in two other instances 38,400 and 41,300 units per kg prevented brain involvement, indicates that even early treatment with apparently adequate dosage will not prevent brain involvement in all cases. Also the fact that brain passage was positive in the 7 late treatment rats of Groups I and II which received more than 40,000 units per kg is strong presumptive evidence that adequate dosage for early treatment is not adequate dosage for late treatment. The question of whether or not brain involvement can be cured with any dosage of penicillin remains to be solved.

This is a preliminary report, and complete details and additions will be published elsewhere. We believe that the question of brain cure or prevention of brain involvement in spirochetoses is a most important consideration and that the results of our experiments reemphasize the need for early treatment and adequate dosage in relapsing fever therapy.

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PRECIPITATION AND AGGLUTINATION TESTS WITH THE HEMOLYTIC STREPTOCOCCUS. TITRATION OF "M" AND "T" ANTI-BODIES IN HUMAN SERA

THE determination of the predominating "M" and "T" antigens by means of precipitation or agglutination tests is the accepted means of identifying hemolytic streptococci as to type in epidemiological studies of human infection. Since such investigations are frequently subject to error due to a multiplicity of antigens in the strains isolated or to the presence of cross infections, it seemed possible that the type implicated in any given infection might be more readily ascertained if it could be shown that type-specific antibodies are produced in the patient's serum in sufficient strength for identification by means of either precipitation or agglutination tests using antigens of known specificity.

In the course of studies at the U. S. Naval Training Station at Newport, R. I.[1] in 1941, blood sera from naval recruits were made available for the determination of antibody content. Forty samples of serum were tested for specific agglutinins by slide agglutination. Nine were from cases of upper respiratory infection—either sore throat or scarlet fever, and eleven were from cases of rheumatic fever. In each instance, the hemolytic streptococcus isolated from the throat was typed by agglutination at the time of hospitalization. Twenty sera from well recruits were included as controls. In a few cases hemolytic streptococci were isolated from the throat cultures in this group also, and the type established.

The sera were tested in dilutions ranging from 1–1 to 1–2,000, from six to eighteen dilutions being set up in each case as needed. All cultures for the agglutination tests were from the type collection of the Department of Preventive Medicine of the Harvard University Medical School. Since "T" antigen[2] is apparently constant and unrelated to colony morphology, it seemed justifiable to use these cultures without continuous mouse passage. Suspensions were prepared from 20- to 22-hour cultures grown at 37° C. in 5 cc broth prepared according to the formula of Swift and Hodge.[3] Before use, the supernatant fluid was pipetted off and the sedimented cells resuspended to a uniform density in a small volume of broth. The slide agglutination technique of Griffith[4] was followed, readings being made after a brief agitation of the serum dilution-suspension mixtures. Control suspensions were included on each slide to eliminate any possibility of error due to spontaneous agglutination. The type cultures used formed smooth suspensions with the exception of types 6 and 13, which frequently had to be prepared from cultures grown at room temperature to ensure stable suspensions.

Cross reactions were common in all the sera and the more sensitive suspensions showed agglutination in relatively high dilutions. Agglutination in dilutions 1–5, 1–10 and 1–20 were frequent. However, agglutinins of sufficient strength or specificity to indicate an antibody response related to either present or past infection could not be demonstrated. In no instance could correlation be shown between serum agglutinins and the streptococcus type isolated from the throat cultures. Heterologous agglutinins were present to the same or even higher titre than those of homologous type. Moreover, the control sera showed cross reactions of the same magnitude and complexity.

Although these experiments seemed to indicate that the presence or absence of "T" antibodies in human sera have little significance, it was hoped that comparable tests for "M" antibodies would show more conclusive evidence of correlation between antibody content

[1] Captain R. M. Lhamon (M.C.), U.S.N.; Commander R. W. Huntington (M.C.), U.S.N.R.; Lieutenant S. M. Wheeler (M.C.), U.S.N.R., and T. Duckett Jones, M.D. To be published.

[2] Rebecca C. Lancefield, *Jour. Exp. Med.*, 71: 521–550, 1940.

[3] Homer F. Swift and B. E. Hodge, *Proc. Soc. Exp. Biol. and Med.*, 30: 1022–1023, 1933.

[4] F. Griffith, *Jour. Hyg.*, 34: 542–583, 1935.