every impulse. Such a mechanism has been claimed to be normal in avian muscles.[6] Some experiments were performed with this possibility in mind. The patients were given prostigmine, intramuscular in the acute and orally in other experiments. The dosage was sufficient to cause distinct signs of the action of the drug, of which especially the increase in the size of the action potentials in the acute cases should be mentioned. However no noticeable effect on the degree of facilitation was observable. It must therefore remain at this time undecided by which mechanism the facilitation is brought about.

Summary. It was found that in some patients with chronic poliomyelitis very weak muscles can be found which seem to consist of one remaining motor unit. A study of their properties has been made by recording the action potentials through skin leads.

The author is indebted to Dr. A. van Harreveld and Dr. H. E. Billig for their help and for their interest in this research.

[6] Brown, G. L., and Harvey, A. M., *J. Physiol.*, 1938, **93**, 285.

15234 P

Development of Brucella Agglutinins in Humans Following Vaccination for Cholera.*

C. WESLEY EISELE, NORMAN B. MCCULLOUGH, GRACE A. BEAL, AND WILLIAM BURROWS.

From the Department of Medicine and the Department of Bacteriology and Parasitology, The University of Chicago.

During the course of a survey for brucella agglutinins made on sera discarded from the Wassermann laboratory (C. W. E., N. B. McC. and G. A. B.), a blood bank donor was found to have a brucella agglutinin titer of 1:200. This individual was a healthy male who, 3 months previously, had been experimentally vaccinated by one of us (W. B.) with U. S. Army cholera vaccine. The possibility that this finding might be more than a coincidental occurrence led us to examine the stored sera from the cholera studies for brucella agglutinins.

Methods and Material. Thirty-four individuals were given either regular U. S. Army cholera vaccination or varying amounts of an experimental cholera vaccine consisting of extracted cell residue. Whereas the Army vaccine contained both H and O antigens, the experimental vaccine contained primarily the O antigen. The vaccines were given in 2 doses of 0.5 and 1.0 cc with an interval of one week between inoculations. Sera were collected before immunization and at 1, 2, 5 and 9 weeks after completion of the vaccination. Brucella agglutinin titers were determined by the rapid slide method of Huddleson, using the standard dilutions from 1:25 to 1:500. The subjects were divided into 4 groups:

Group I—7 subjects, routine U. S. Army cholera vaccination, 8,000 million organisms per cc.

Group II—8 subjects, experimental vaccine, 8,000 million organisms per cc.

Group III—10 subjects, experimental vaccine, 24,000 million organisms per cc.

Group IV—9 subjects, experimental vaccine, 40,000 million organisms per cc.

Five post-immunization sera from 3 individuals were further studied by reciprocal agglutinin absorption tests, using as antigens a live virulent strain of *Br. abortus* and a live 24-hour culture of *Vibrio comma*. After absorption with brucella, the sera were tested

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TABLE I.
Brucella Agglutinins in Sera of Subjects Immunized with Regular U. S. Army Cholera Vaccine.

Subject No.	Pre-immunization sera	Time after last inoculation			
		1	2	5	9 weeks
7	—	1:500P	1:200	1:200P	1:200P
10	—	1:500P	1:500	1:200	1:200
16	1:25	1:25	1:50	1:50	*
21	—	—	—	—	—
30	—	—	1:100P	—	—
32	—	1:200P	1:100P	1:50	1:25
38	—	1:100P	1:25	—	—

— Negative.
P Partial agglutination (usually complete agglutination in the next lower titer).
* Serum not available.

with cholera antigens[1] containing HO and containing only O antigen.

Results. The brucella agglutinin titers of the 7 individuals in Group I who received the U. S. Army type of cholera vaccination are presented in Table I. One of the subjects had a pre-immunization titer of 1:25, all the others being negative. Following immunization, all except one developed brucella agglutinins in titers ranging from 1:50 to 1:500. In 3 subjects, brucella agglutinins were still present at the time of the last collection of sera (9 weeks).

In Groups II, III and IV receiving varying amounts of the experimental cholera vaccine, none of the 27 individuals showed any pre-immunization brucella titers, and in only 2 subjects, low-grade post-immunization brucella agglutinin titers developed. These were both in Group IV receiving the largest amount of the vaccine, and the titers of 1:25 and 1:50 P were very transient, disappearing by the 9th week.

In the reciprocal agglutinin absorption tests, absorption with living 24-hour culture of *Vibrio comma* removed all brucella agglutinins. Sera absorbed with a live, virulent strain of *Brucella abortus* subsequently agglutinated formalinized *Vibrio comma* (HO) in markedly lowered titers.† (Serum E7, before absorption 1:1000, after absorption 1:100; Serum B32, before 1:5000, after 1:200; Serum C10, before 1:5000, after 1:200; Serum C32, before 1:5000, after 1:500; Serum D7, before 1:2000, after 1:2000.†) Agglutination tests with a boiled suspension of *Vibrio comma* (O antigen) were not significantly different from the pre-absorption tests.

All 34 of the vaccinated subjects developed cholera agglutinins in high titers which persisted during the 9-week post-immunization observation period. The magnitude of the cholera agglutinin response was approximately the same following the two types of vaccine. In the 3 groups receiving the varying amounts of the experimental vaccine, augmented cholera agglutinin titers did not accompany increasing amounts of vaccine. In fact, Group IV receiving the largest amount of vaccine had a somewhat poorer average cholera agglutinin response than Groups II and III receiving smaller amounts. These data will be reported in detail elsewhere.[1]

Discussion. Wong and Chow[2] reported that in 8 rabbits injected intravenously or subcutaneously with heat-killed suspensions of *Vibrio comma,* brucella agglutinins developed in unspecified titers. Of 6 humans inoculated subcutaneously or intravenously with a typhoid-cholera vaccine, 4 developed agglutinins against *Brucella abortus* in titers of 1:40 or less. Presumably, the vaccines used had lost at least some of the H antigen during heating.

The agglutination test for brucella is the most commonly used diagnostic procedure

[1] Burrows, William, unpublished data.

† In one serum, D7, the post-absorption titer was not lowered. Insufficient serum remained for further tests.

[2] Wong, D. H., and Chow, C. H., *Chinese Med. J.,* 1937, **52**, 591.

for brucellosis. Although it is well known that proven cases of the disease may at times present little or no agglutinin response, the diagnosis of brucellosis is frequently made on the basis of low-grade titers without adequate supporting data. Many clinicians consider brucella agglutinin titers in dilutions of 1:500 as almost certainly diagnostic of active infection. The development of titers as high as this following vaccination for cholera may well be expected to introduce further confusion in the clinical interpretation of this test. Cholera vaccination has been infrequently used in this country prior to the war; but large numbers of veterans are returning from the Pacific and Asiatic theaters who have been subjected to this immunization.

Studies are underway to determine the duration of the persistence of brucella antibodies following vaccination for cholera, and the possible reappearance of antibodies in the circulating blood as an anamnestic phenomenon. The possible effect of other types of immunizations on brucella antibody stimulation must also be considered.

Our preliminary data presented here indicate that there is an H antigen in *Vibrio comma* which is present also in brucella. Studies are in progress to elucidate further the antigenic interrelationships of these organisms.

15235 P

Excitability of Muscle to Direct and Indirect Stimulation During Prolonged Direct Stimulation.*

ERNST SIMONSON, AKIRA OMACHI, AND MAURICE B. VISSCHER.
From the Department of Physiology, University of Minnesota, Minneapolis.

Although electrical stimulation is used widely in diagnosis and in physical therapy and is the only practical procedure to test muscle function in animals, the effects of prolonged direct stimulation have not been studied extensively.

Alternating 60-cycle current was used in 3 independent but *in phase* circuits for stimulation through 3 pairs of electrodes, the first pair being inserted into the knee and tendon of the gastrocnemius muscle *in situ* of anesthetized rats (nembutal) and cats (dial), (D_1 electrodes); the second pair (D_2) inserted into the muscle itself at its upper and lower ends; the third pair (I) being employed for stimulation of the peripheral end of the cut sciatic nerve. The strength of the stimulus was adjusted with a rheostat and measured by a volt meter; in addition, the resistance in the muscle was measured by means of an oscilloscope in a shunt parallel to the muscle. Stimulation was continued either with D_1 or with D_2 until the muscle length reached the prestimulus resting value. During and after the continued stimulation, brief tetanic stimuli were applied at regular intervals through the 2 other pairs of electrodes. In all cases maximum stimuli were applied through a 10,000-ohm resistance placed in series with muscle and nerve. The muscle was loaded with 200 g and contraction was isotonic. Before continued stimulation the effect of summation was tested for all combinations of D_1, D_2, and I. There was no summation between D_1 and I, or D_2 and I, and a tendency to slight summation of $D_1 + D_2$.

Steel, platinum, silver, and nonpolarizable ($Cu\text{-}CuSO_{4_{1.0M}}$ - $K_2SO_{4_{sat.}}$ - $NaCl_{sat.}$ $NaCl_{0.9\%}$-Tissue) electrodes were employed in various experiments. There was no essential difference in the results to be reported. Forty-seven experiments were performed in rats and 3 experiments in cats.

With continued stimulation through D_1, there is no response to D_2 or I at the very beginning, but after a few minutes a marked

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