high. The "satellite phenomenon" is not observed in colonies of Ducrey bacillus. Attempts to separate Ducrey growth factors from whole blood or serum by dialysis have not been successful. The Ducrey bacillus differs significantly from *H. influenzæ* and *H. parainfluenzæ* in susceptibility to inhibition by penicillin and by tyrothricin.

Miss Elizabeth Roberts gave technical assistance in this work.

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Muscles Consisting of a Single Motor Unit After Poliomyelitis.

C. A. G. WIERSMA.

From the William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena.

During an investigation concerning the possible increase of muscle force in paretic muscles by means of increased motor nerve branching[1] the action potentials of more than 1000 different paretic muscles were obtained. Among these the records of 36 muscles in 26 different patients were remarkable for their simplicity and they resembled closely records produced by single motor units in fully[2,3,4] or partially innervated muscles,[5] though no measures were taken to insure the recording of units. The behavior of these muscles was analyzed by repeated tests.

Methods. In routine electrograms, the patient contracted the muscle as strongly as possible for 2 or 3 seconds and then released the tension quickly. Electrodes, consisting of solder pellets of 5 mm diameter were attached to the skin with tape, electrode paste being used to insure a good contact. The action potentials were amplified and recorded by a Matthews oscillograph. Nearly all of the muscles to be described were found in patients who had undergone the treatments intended to enhance the nerve branching.[1] In all cases the paresis was at least of one year standing.

Results. The short lasting, strong contractions of all muscles under consideration were accompanied by a single row of action potential spikes, (Fig. 1) which were in general, rather evenly spaced and often grew considerably in height, especially the first 10 spikes. In by far the majority of the cases the voluntary control of the muscle was good, in only a few were there signs of poor control as evidenced by an irregular and low frequency of the spikes and difficulty in maintaining the contraction even for a short time.

The maximum frequency of the spikes reached in this way has been measured for all these muscles and was found to vary between 20 and 60 per second, the mean being 40. In accordance with the data of Gibson and Mills[3] it was observed that 2 or even 3 spikes could follow each other closely, (0.01 second), after which there was usually a somewhat longer delay before the appearance of the next spike. Therefore the maximum frequency was computed by using the time interval of at least 10 consecutive spikes. Since most of the 15 different muscles used in the investigation are represented only a few times and these are only larger muscles in which the influence of the contraction of neighboring muscles on the electrogram is negligible, the material is not very suitable to show whether or not different muscles have different inherent maximum frequencies.[5] It may be significant, however, that the deltoid which is represented 5 times, showed a maximum of 60 in 3 and of 40 in 2 of these cases, and has thus a mean frequency greater than that of the mean maximum given above.

[1] Billig, H. E., van Harreveld, A., and Wiersma, C. A. G., *J. Neuropath. and Exp. Neurol.*, 1946, in press.

[2] Smith, O., *Am. J. Physiol.*, 1934, **108**, 629.

[3] Gilson, A. S., and Mills, W. B., *Am. J. Physiol.*, 1941, **133**, 658.

[4] Denslow, J. S., and Hassett, C. C., *Am. J. Physiol.*, 1943, **139**, 652.

[5] Lindsley, D. B., *Am. J. Physiol.*, 1935, **114**, 90.

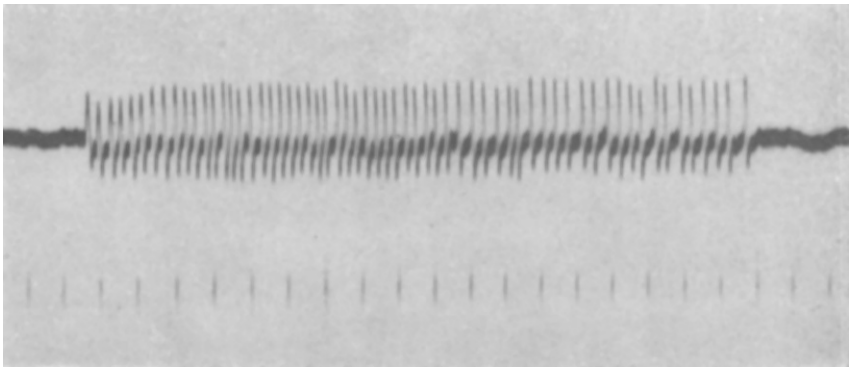

FIG. 1.
Test contraction of a rectus femoris; untreated patient. Facilitation 25%, diphasic, frequency 40 per second. As in all following pictures skin leads were used. Time 1/10 sec.

The growth of the spikes of the action potentials during the contraction was present in all cases and often very pronounced. In records in which the action potentials were practically monophasic this increase was usually larger than with di- or polyphasic spikes. In the latter there was an increase of more than 50% in 14 of the 27 records, and in only 1 record was the increase as small as 20%. In the monophasic group 13 out of 26 records showed an increase of more than 100% and all but one of more than 50%. One muscle was outstanding in the amount of facilitation obtained. The first top was always very small and the later ones were at least 10 times as large. (Fig. 2).

The amount of facilitation was found to be dependent on several factors, in any one muscle. Relatively little difference was encountered when on different days similar contractions were performed with similar electrode placements, unless the state of fatigue of the muscle was different. At any given time with the electrodes fixed, the most important factor was the frequency of the impulses. Up to a certain limit the growth is faster and larger the more rapidly the spikes follow each other. However, above a certain frequency, which seems to differ for different muscles, the potentials become smaller. This does not interfere with the facilitation process, any spike which happens to be separated by a sufficient time interval from a foregoing one will be large. In a fatigued muscle the growth is much smaller and may, soon after the start of the contraction, stop and be replaced by a decrease in height, even when the frequency is low. In a few instances the muscle was in tone both before and after the test contraction. In these cases little or no facilitation took place, which is explained by the supposition that during the tonic period facilitation had developed to such an extent, that the increase in frequency has no longer a pronounced effect.

In a limited number of experiments the contractions were maintained for long times. It was found that in most cases the spikes after their initial facilitation start to decline confirming Smith[2] and may become very small indeed. (Fig. 2). In others the frequency becomes very irregular which in turn gives irregular action potential heights. In one case 5 minutes contraction did not result in any noticeable decrease. Though the rate of the spikes was rather low, ($\pm$ 25), other muscles with even lower rates showed profound fatigue after much shorter times.

Once a muscle has been fatigued, the result remains noticeable for a long time. During the first minutes after relaxation the action potentials remain small, after longer rest periods the growth is at first normal but diminishes soon and the maximum height is never reached. This may well play a part in the difference of facilitation obtained in different muscles. In one instance a muscle

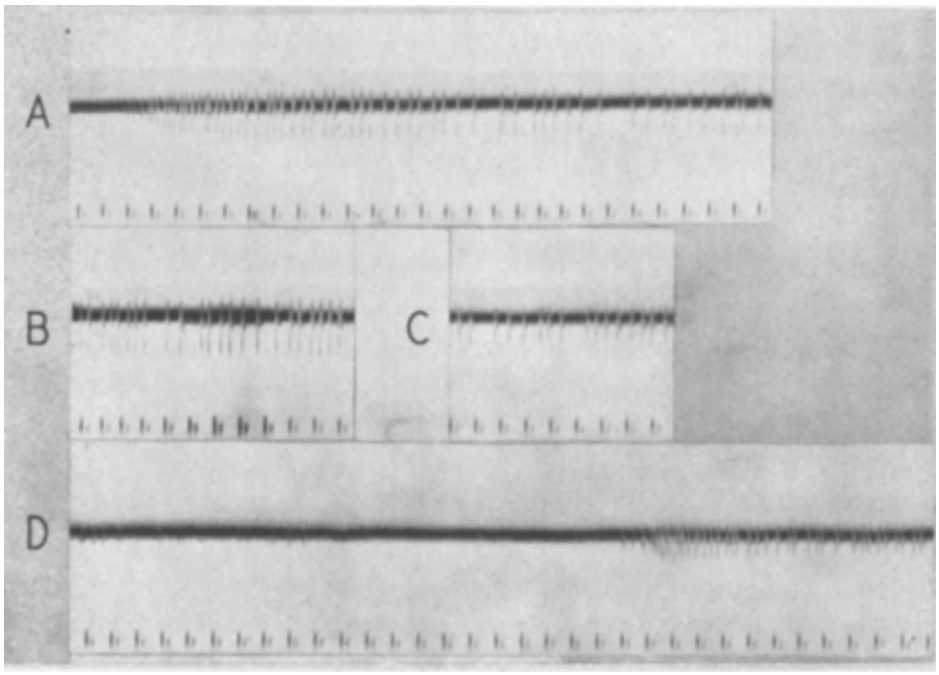

FIG. 2.
Deltoid, treated. Long lasting contraction. A. Start. Notice pronounced facilitation and triphasic spikes. B. After 15 seconds, maximum facilitation is reached. C. After 30 seconds, the height of the spikes is diminished. D. After 90 seconds. The contraction ends with muscle action potentials just visible. After 1 second rest they reappear and facilitate, but diminish quickly. Time 1/10 second.

which had been rested for a long time reached maximum peak height after one minute, whereas the same muscle normally reached its maximum after 30 seconds and already began to diminish in height at one minute. If, therefore a patient has used the muscle to a great extent, *e.g.* in walking, the rest provided during the investigation time may not be long enough to overcome the after-effects of this use.

In another series of experiments repeated contractions of very short duration were studied. In such contractions each ensuing contraction starts with a higher action potential than the previous one (Fig. 3) which shows that purely mechanical factors are not responsible for the increase in height. In this type of contraction the frequencies reached tend to be higher than during the "standard" contractions and may become high enough to seriously interfere with the gradual growth of the action potentials. (Fig. 3). At least in these muscles it seems possible to obtain frequencies in the nerve fibers which are too high for the muscle fibers to follow normally.

In a number of selected cases mechanograms were obtained from these muscles by the application of a heart apex beat recorder over the contracting belly. In some cases but not in all, single twitches caused by a single impulse were obtainable,[3] in these it was found that higher contractions and action potentials occurred after previous facilitation by a tetanic contraction. Summations of 2 and more impulses could be nicely demonstrated with this method. (Fig. 4).

In some of the cases needle electrodes were put into the substance of the muscle. The resulting action potentials differed only in voltage from those obtained from the intact skin.

Discussion. As in all cases of human muscles it is not certain that the data presented are caused by the action of single motor units.[3] Especially the growth of the

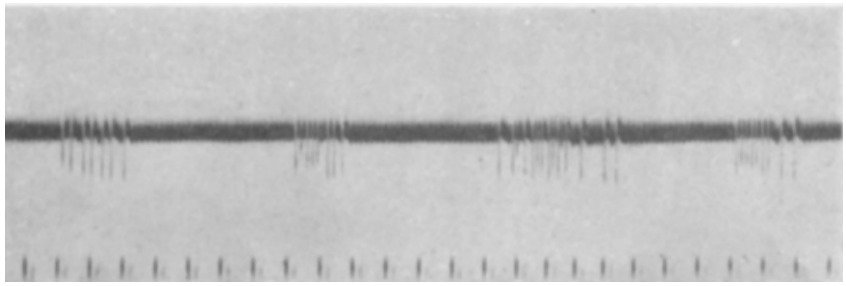

FIG. 3.
Lower peroneal, treated. Series of short lasting contractions. In the first of the series the facilitation is regular; in the following three the frequency is higher and the spikes are irregular. Each of the later contractions starts with a higher spike than the previous one. Time 1/10 sec.

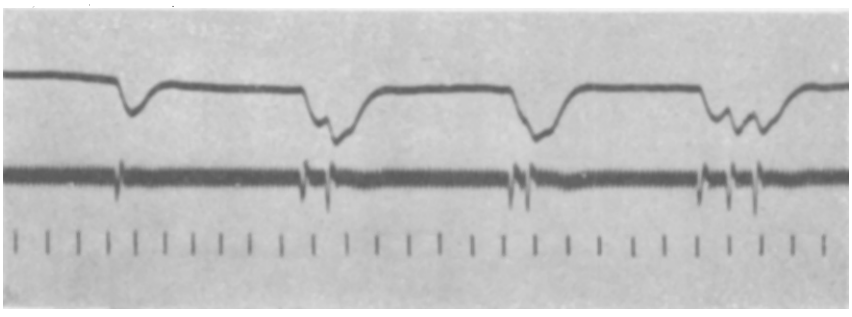

FIG. 4.
Gluteus maximus, treated. A series of voluntary twitches, the first of which is single. Upper line, mechanogram, middle electrogram, lower time 1/10 sec.

action potentials which from the evidence presented cannot be ascribed to artifacts, might well be considered as against the presence of single motor units. The alternative would be that a number of nerve fibers would always fire at the same time, but would have different thresholds. Such a mechanism might possibly be brought about by the connection of a number of motorneurons with a single pyramidal fiber. If this were the case the number of motorneurons so connected should be large in order to explain the smooth increase of the growth of the potentials. No evidence was ever obtained which showed that these hypothetical central units could split themselves into component parts, and they would therefore form a serious handicap to the amount of central control of the contractions. Furthermore in all cases the contracting part of the muscle was a single band, whereas if different axons were involved one would expect that several contractile bands would occur in at least some of them.

If then these bands are considered as single units it remains to be discussed what the growth in the action potentials represents. There are two likely explanations. The first is that facilitation represents the increased spreading of the action potential over the muscle fibers. In freshly reinnervated muscles such a process might well take place, the first impulse of a series would be limited to the neighborhood of the end plate and successive impulses would spread more and more to the ends of the muscle fibers. However, as recovery went on, it would be expected that facilitation become less and less pronounced. This was not observed; in patients which have been studied for one year or more no difference in the amount of facilitation was found between the first and the last test. The other, and perhaps more likely possibility is that only part of the muscle fibers respond on a single nerve impulse and that considerable facilitation is necessary before all fibers contract with

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.

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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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