Maximum numbers occurred between 5.3 and 5.6 at 72 hr with a count of 6 million organisms per cc. Final pH was 5.2. (Fig. 3).

Strain D. This strain was received in a contaminated state with an atypical strain of *C. renalis*. It was isolated bacteria-free by a modification of the capillary migration of Stone and Reynolds.[9] The pH of strain D was essentially the same as that of Strain A. Counts were made every 24 hr. Maximum numbers occurred between pH 6.1 and 6.3 at 96 hr with a count of approximately 4 million organisms per cc. Final pH was 5.4.

[9] Stone, W., and Reynolds, F., *Science*, 1939, **90**, 91.

Strain D + Corynebacterium renalis. Maximum numbers occurred between pH 5.7 and 5.9 at 48 hr with a count of approximately 3 million organisms per cc. (Fig. 5). Two sets of controls were run, one set being uninoculated, the other with *Corynebacterium renalis*. Both sets of controls changed their pH from 7.2 to 6.8. This bacterium apparently grows in close association with *T. foetus* as the flagellates multiply more rapidly in its presence than in bacteria-free cultures. This acceleration is difficult to explain. There may be a change in the medium due to the hydrolyses of proteins and carbohydrates by the bacterium, but the nature of this is not known.

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Impedance Changes Induced in the Brain by Electric Stimulation.

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The extensive application of electric stimulation of the brain, not only experimentally but also for therapeutic purposes as in the so-called electric shock treatment of certain psychoses, makes it desirable to get a more intimate knowledge of the effects of electric stimuli and the subsequent excitation upon the state of the cells of the central nervous system. It is known that excitation of peripheral nerves is associated with decrease in D.C. resistance (Hermann,[1] Ebbecke[2]) and A.C. impedance (Lullies,[3] Cole and Curtis[4]) respectively. Since such changes may be able to throw some light upon the state of the cellular surface films (Gildemeister),[5] it seemed of interest to ascertain whether similar effects are demonstrable in the central nervous system.

For the measurement of the impedance a Wheatstone bridge arrangement energized with A.C. of from 500 to 6000 cycles was used as previously described. (Spiegel and Spiegel-Adolf).[6] Quick determinations of the balance were facilitated by a 5-stage amplifier connected not only to a telephone receiver but also to an "electric eye" so that the minimum could be detected by visual as well as by acoustic observations.

In the first series of experiments on cats under superficial ether anesthesia the electrodes were platinum wires coated with platinum black, inserted into the brain substance as in previous experiments (Spiegel and Spiegel-Adolf)[6] or platinum-Ringer electrodes placed with the smallest possible

[1] Hermann, L., *Pflueger's Arch. f. d. ges. Physiol.*, 1872, **6**, 560; 1873, **7**, 349.

[2] Ebbecke, U., *Pflueger's Arch. ges. Physiol.*, 1922, **195**, 555.

[3] Lullies, H., *Pflueger's Arch. ges. Physiol.*, 1930, **225**, 69, 87.

[4] Cole, K. S., and Curtis, H. J., *J. Gen. Physiol.*, 1939, **22**, 649.

[5] Gildemeister, M., in *Handb. d. norm. u. pathol. Physiol.*, edit, by Bethe, 1928, **8**, II, 657.

[6] Spiegel, E., and Spiegel-Adolf, M., *Am. J. Psychiat.*, 1936, **92**, 1145; *J. Nerv. and Ment. Dis.*, 1939, **90**, 188.

pressure on the surface of the brain. The stimulating electrodes were placed on the motor cortex and the testing electrodes (connected to the Wheatstone bridge) on the parieto-occipital region of the same or of the opposite hemisphere.

The convulsions following faradic stimulation of one minute duration were associated with a decrease of impedance, which was more marked if the impedance was determined with a low frequency current (547 cycles per sec.) than on measurement with a current of 5120 cycles per sec. In 10 measurements at the lower frequency the decrease of impedance varied between 0 and 16% (mean value 7.3%) while in 9 measurements at the higher frequency it varied between 0 and 7.2% (mean value 3.4%). This is in agreement with former experiments on metrazol and insulin convulsions (Spiegel and Spiegel-Adolf).[7] Since the convulsive state is associated with changes in brain circulation, which may affect these measurements, it seemed desirable to use an object in which the disturbing influence of circulatory changes could be eliminated.

A second series of experiments was, therefore, performed on surviving frog's brains. Such preparations show spontaneous discharges for as long as 3 hr after removal if kept in a Ringer's solution, as has already been demonstrated by Gerard and Libet;[8] electrical stimulation is able to change the functional state of such surviving brains as is demonstrated by the fact that their potential discharges may be altered by electrical stimulation.

The brain is held between two vertical platinum-Ringer electrodes in a glass chamber that can be filled with Ringer's solution in the intervals between the measurements (Fig. 1). In one method the stimulating electrodes are placed on the frontal ends of the hemispheres (or on the olfactory lobes). In another method the platinum-Ringer electrodes (e_1, e_2) are used for application of the stimulating current as well as for measurement of the resistance. In the latter method the brain is connected alternately to the stimulating and the bridge circuit by means of a double pole double throw switch.

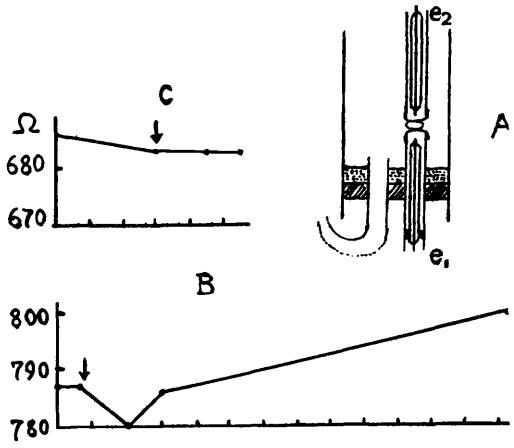

FIG. 1.

1A. Apparatus for experiments on isolated frogs' brains. e_1, e_2—Platinum-Ringer electrodes. Other details described in the text.

1B. Surviving isolated frog's brain. Changes in impedance following application of 10 make and break shocks (at ↓). Abscissa: time in minutes.

1C. Control experiment on brain killed in chloroform. Application of 10 make and break shocks at ↓.

In these experiments also stimulation of the brain was followed by a definite decrease of impedance. This was particularly evident, if there was a spontaneous tendency for the impedance of the resting brain to gradually increase so that the changes following the stimulation were in the opposite direction. In order to differentiate the physiological effect of the stimulation from purely physi-

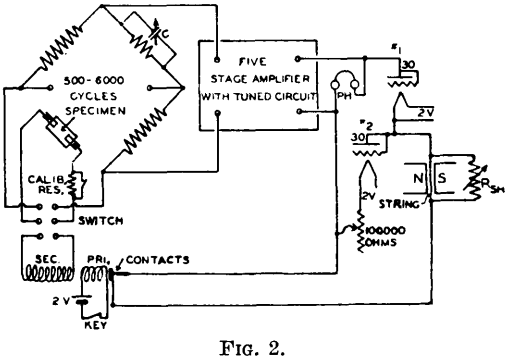

FIG. 2.

Circuit for recording of impedance changes. Details explained in text.

[7] Spiegel, E., and Spiegel-Adolf, M., *J. Nerv. and Ment. Dis.*, 1941, **93**, 750.

[8] Gerard, R. W., and Libet, B., *J. Neurophysiol.*, 1939, **2**, 153.

cal effects (such as heating, polarization), the experiments were repeated after the brain had been killed in chloroform. This was particularly important since measurements with thermocouples showed that, for instance, application of faradic (tetanizing) current through the Pt-Ringer electrodes for one minute at a coil distance of 4 cm increased the temperature on the surface of the frog's brain by from 1.0°C to 1.68°C, which change of temperature was of course by itself able to reduce the impedance of the brain. Instead of faradization we applied, therefore, single induction shocks, (10 make and break shocks), and with this type of stimulation a definite difference between the effect upon

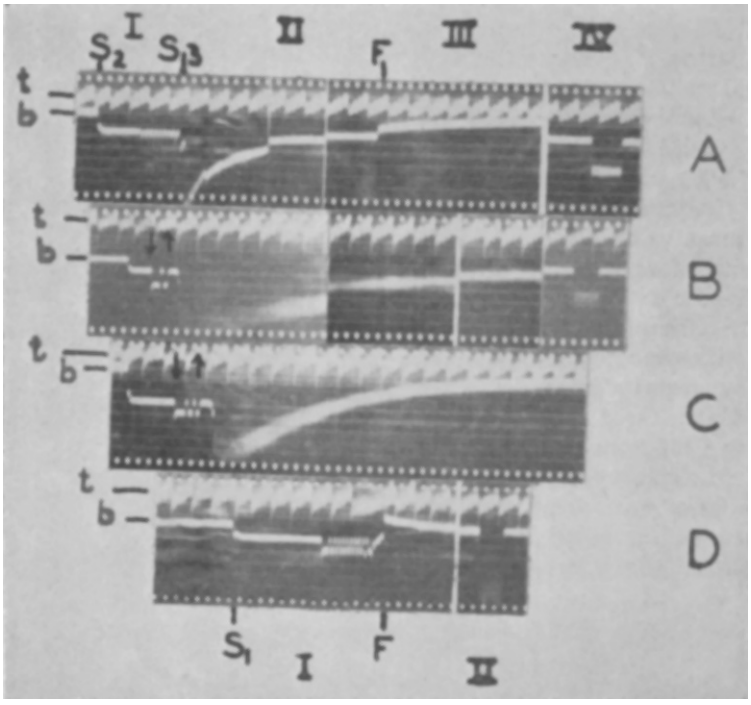

FIG. 3.
Stimulation of Isolated Surviving Frogs' Brains.

A. I. Application of 10 make and break shocks with shunt at 10 Ω (S_2); immediately after stimulation, shunt 1000 Ω (S_3).
II. 2 seconds later (shunt 1000 Ω).
III. 4 seconds later (shunt 1000 Ω), then full sensitivity of string (from F).
IV. Standardization with 20 Ω (shunt 1000 Ω).
B. I. Application of one make and break shock (shunt 1 Ω), then full sensitivity.
At ↓ brain disconnected from bridge and connected to stimulating coil, at ↑ brain reconnected to bridge. Application of stimulus between ↓ and ↑.
II. 6 seconds later.
III. 7 seconds later, full sensitivity.
IV. Standardization with 15 Ω.
C. Application of 2 make and break shocks (shunt 1 Ω), then full sensitivity. ↓ and ↑ as in B.
D. Control experiment on brain killed in chloroform.
I. Application of 10 make and break shocks, shunt 1 Ω (S_1), then full sensitivity of the string (from F).
II. Standardization with 15 Ω (full sensitivity).
t = time in seconds; b = balance position.

the surviving and that upon the dead brain could be demonstrated. While on the latter no or only minimal changes of impedance in either direction could be detected, there was a definite decrease on the surviving brain. (Fig. 1).

It soon became apparent that the changes of conductance took place rather quickly, and that the beginning of this change escaped the measurement, even if special measures were taken to shorten the balancing of the bridge (*e.g.*, setting, during the stimulation, the variable resistance and capacitance at the approximate expected values). The changes of impedance were, therefore, recorded by a string galvanometer in the following way. (Fig. 2).

The zero points of the bridge were connected to a 5-stage amplifier (with a filter circuit tuned to the frequency of the bridge current). The output of the amplifier was connected to head phones to facilitate preliminary bridge balancing and also through a vacuum tube rectifier, with zero balancing circuit, to a string galvanometer. First the balance position of the bridge and its eventual shift were recorded. Then a one or 10 ohm shunt was applied to the string galvanometer to greatly reduce its sensitivity. (Fig. 3). The brain was disconnected from the bridge and connected to the stimulating coil by means of a double pole double throw switch, and make and break shocks were applied. After the stimulation was completed, the specimen was reconnected to the bridge as quickly as possible, and the sensitivity of the string was restored. Thus changes in the impedance of the brain were recorded 1-2 sec following the stimulation, and the gradual return to the previous value could be followed. After each stimulation experiment the circuit was standardized; the bridge was first balanced while a certain additional resistance (*e.g.*, 15Ω) was in series with the brain. One first recorded this balance position of the string and then its deflection after the additional resistance had been removed.

After a single make and break shock the deviation was 1.2-2.5% of the initial value (*e.g.*, deviation 18-37Ω, initial value 1460-1480Ω), and the string returned to the original position after 14-27 sec. (Fig. 3B). After 10 make and break shocks in various preparations changes from 3-10% and a return to the initial value in 9-38 sec were observed. (Fig. 3A). Repetition of the same procedure in control experiments on brains killed in chloroform gave practically no changes in the balance position of the string. (Fig. 3C). Since the string was deflected in the same direction whether the impedance of the specimen increased or decreased from the balance position, it was necessary to establish in which direction the impedance changes actually occurred. This was repeatedly done with the bridge and phones as detector, and it was found that we dealt with a decrease of impedance after stimulation. Studies of the spinal fluid are in progress (with Drs. M. Spiegel-Adolf and E. W. Ashkenaz) which seem to indicate that these changes of impedance are at least partly due to impairment of the brain cells.

Summary. The convulsions following faradic stimulation of the brain are associated with decrease of its impedance (experiments on cats under superficial ether anesthesia). A similar change could also be demonstrated following single induction shocks applied to isolated, surviving frog's brains, where the disturbing influence of the circulation was eliminated, while such a change failed to appear in control experiments on dead brains.