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7. We are indebted to R. Gerard and C. Fray, successively chief scientists of *Vema*, to H. C. Kohler, master of *Vema*, and to M. Tharp for their assistance. This report is Lamont Geological Observatory (Columbia University) contribution No. 415.
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23 February 1960

Succinylcholine and Muscle Excitability

Abstract. Succinylcholine lowers the resting membrane potential taken with microelectrodes similarly in nerve-scarce and innervated portions of frog sartorius muscle. Twitches to electrical excitation of the nerve-scarce pelvic end of the muscle are also rapidly reduced. The results indicate that succinylcholine probably acts generally on the muscle membrance to diminish excitability.

It is generally believed that the class of neuromuscular blocking agents known as "depolarizing blockers" act by lowering the membrane potential in the end-plate region, thereby making neuromuscular transmission ineffective (*1*). Another theory of action is that these agents decrease the sensitivity of the end-plate region to transmitter agent (*2*). Recently we found, with a microelectrode technique, that some of these agents (acetylcholine and choline) diminish the resting membrane potential, generally to the same degree, everywhere in the isolated frog sartorius muscle membrane. The potential fell to the same extent in and out of the end-plate region, and the effect was prevented by pretreating with curare (*3*). Decamethonium and other agents in this group also caused a generalized fall (*4*). Inexcitability to direct electrical and mechanical stimulation of the muscle was roughly correlated with the mean degree of membrane potential diminution and the amount of blocking agent added to the bath. The hypothesis was advanced that a generalized action of these agents and of curare occurs everywhere on the membrane of the muscle.

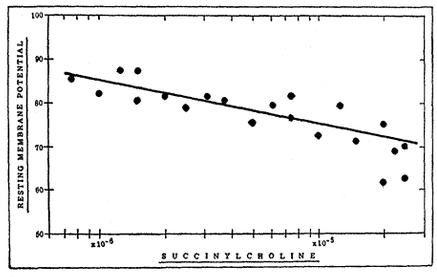

Fig. 1. Resting membrane potential and succinylcholine. Each point represents the mean resting membrane potentials of 50 to 55 fibers, taken with microelectrodes from frog sartorius muscles after adding succinylcholine in concentrations (gm/cm³) shown on the abscissa. Resting membrane potential is shown on the ordinate.

Attention was turned to other agents classified as depolarizing blocking drugs; the effect of succinylcholine is of particular interest. Single fibres were sampled at different sites along the length of frog sartorius muscles by the Ling-Gerard microelectrode technique as previously described (*3*). Each point in Fig. 1 represents the mean of approximately 50 to 55 fibers for a given muscle determined after adding different concentrations of succinylcholine. Lower mean membrane potentials were found with increased amounts of this agent. Pretreatment with curare prevented the fall in membrane potential caused by succinylcholine.

The effect of succinylcholine on the isotonic-twitch response is shown in Fig. 2. For these experiments, frog sartorius muscles were clamped and directly stimulated maximally at the pelvic nerve-scarce region with 5-msec pulses at 15 second intervals. Adequate controls showed that current did not spread to nerve endings outside the pelvic nerve-scarce region.

When succinylcholine was added to make a final concentration of 5×10^{-6} gm/cm³ (Fig. 2*A*) or 20×10^{-6} (Fig. 2*B*), the mechanical twitch response to direct electrical stimulation showed a characteristic diminution. The decrease appeared after a latency which was shorter with higher concentrations of succinylcholine (compare *A* and *B*, Fig. 2). A contracture seen as a rise in base line just after adding succinylcholine (Fig. 2*B*) was related to the amount of drug added. An occasional twitch larger than normal was also common at this time (Fig. 2*A*). The amplitude dropped, reaching a "plateau" of smaller twitch response heights which was not directly related to the concentration of the agent added. After pretreatment with curare (6×10^{-6}), succinylcholine action was blocked (Fig. 2*C*).

Relatively small drops in membrane resting potentials were found with amounts of succinylcholine which were

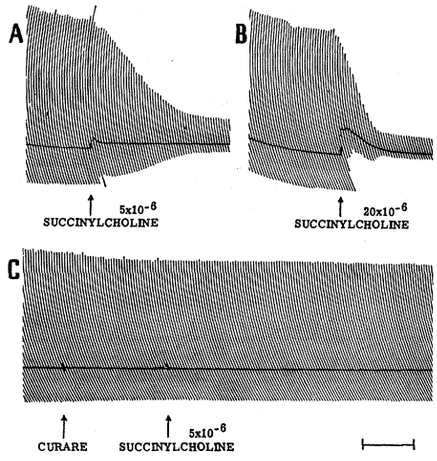

Fig. 2. Twitch responses and succinylcholine. Twitch heights to maximal direct stimulation at the relatively nerve-scarce pelvic end of isolated frog sartorius muscle are shown. Stimulation every 15 sec with 5-msec pulses. *A*, after 5×10^{-6} (gm/cm³) succinylcholine; *B*, 20×10^{-6}; *C*, curare 6×10^{-6} added and then 5×10^{-6} succinylcholine ineffective. Bar represents 5 min.

effective in diminishing direct excitability. This suggested that succinylcholine does not produce its block of excitability by simply lowering resting membrane potential. Jenerick and Gerard had shown that the sartorius muscle membrane could support an action potential until the resting membrane potential was lowered by KCl to a critical level of 52 to 57 mv (*5*). The inference drawn of an excitation block applies as well to similar data obtained with acetylcholine, choline, and decamethonium, where membrane potential falls were reported (*3*), but usually above the critical level of 52 to 57 mv.

A much greater degree of depolarization in the end-plate region, which, from Burns and Paton's work with external electrodes (*1*), was to be expected with microelectrode recordings, was not found for succinylcholine, acetylcholine, choline, or decamethonium. Nor does the theory of Thesleff that these depolarizing blocking agents decrease the sensitivity of the end-plate regions (*2*) indicate that a conduction block to direct muscular excitation would be expected.

Our findings suggest that the excitation mechanism of the membrane is interfered with or the membrane-contractile link (*6*) is blocked. The lowering of membrane potential is probably a coincident phenomenon with a generalized membrane action of succinylcholine on the muscle fiber (*7*).

S. Ochs
B. Annis*
A. K. Mukherjee†
Department of Physiology, Indiana University School of Medicine, Indianapolis

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* Fellow of the Indiana Neuromuscular Research Laboratory.

† Associated in an earlier portion of this work done at University of Texas, Medical Branch, Galveston. Present address: Department of Physiology, Presidency College, Calcutta, India.

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Deuterium Analysis—a Simple and Precise Method

Abstract. By means of reaction with calcium hydride in a generator of simple design, the water samples are converted into H_2 and HD. With hydrogen as carrier gas, the greater thermal conductivity of HD produces a peak whose size is linearly related to the deuterium content of the original water.

Described below and depicted in Fig. 1 is a simple, inexpensive apparatus which analyzes water samples (without special purification) for deuterium content with a precision that is generally better than the mass-spectrometer and falling-drop procedures currently in use (*1*).

The mixture of HOH, DOD, and HOD (about 0.1 ml) contained in reservoir bulb *B* (Fig. 1) is allowed to drop slowly onto granules of calcium hydride contained in cartridge *C* suspended in evacuated tube *A*. This generates a mixture of only H_2 and HD and since the separation factor (*2*, *3*) for the reaction of mixed waters with calcium hydride is nearly 1.00, the HD content of the gas corresponds almost exactly to the deuterium content of the water sample. Three-way stopcock *D* connects the gas generator to double-arm manometer *E* and the gas sampler of the gas chromatography equipment *F*. After evacuation of the manometer-sampler system, the system is sealed off with stopcock *G*, through which the system is connected to a vacuum pump. The gas mixture from *A* is now introduced through *D* into the evacuated sampler and manometer and the pressure throughout is adjusted to atmospheric pressure by manipulation of the leveling bulbs. The mixed gases are now released into the carrier stream of the chromatograph.

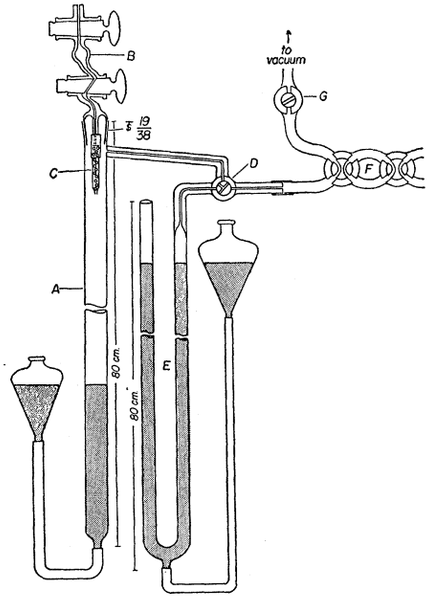

Fig. 1. Apparatus used to convert deuterium into H_2 and HD.

No attempt is made to separate hydrogen from HD (*4*). Instead, the recording katharometer of the chromatograph is used to measure the well-known (*1*) difference between the thermal conductivity of HD and that of hydrogen, the function of the column (Burrell High Activity Charcoal) being to separate volatile impurities from the hydrogen isotopes. Hydrogen is used as the carrier gas so that the size of the peak traced by the recorder depends only on the quantity of HD in the gas sample. This gives the method great sensitivity and we have detected D_2O at twice the background level (0.017 mole percent).

From 0 to 10 mole percent deuterium—the region of greatest interest for tracer studies—the relation of peak height to mole percent deuterium in the original water is strictly linear (*3*) (Fig. 2). Series of replica samples analyzed during the same day often agree within an estimated standard deviation of 0.3 (relative) percent while replicas analyzed on different days usually have a standard deviation of 0.5 to 1.0 percent, depending on deuterium content. Thus our results compare favorably with conventional methods which have a "precision" of 0.5 to 3 percent (*1*). We find that the calibration line holds within the above error over the life of a tank of hydrogen (about a week), if the hydrogen flow is left undisturbed night and day and correction is made for change in atmospheric pressure. Since the calibration curve is a straight line passing through the origin (by least squares), a single run with a standard solution serves for recalibration. Analytical precision may be improved by recalibrating immediately after running an unknown using a standard solution of nearly the same deuterium content. We are currently developing the sensitivity and precision of our method and expect that both may be improved considerably.

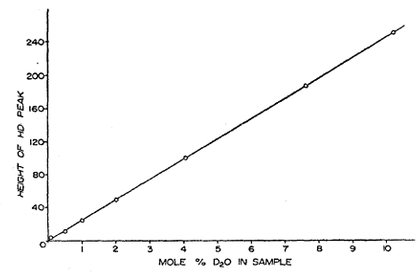

Fig. 2. Each peak height shown on this curve is an average taken from the heights of 6 to 12 curves obtained from 2 to 4 samples run on 2 to 4 different days. They have been corrected for changes in barometric pressure. Peak heights for this system are more reproducible than peak areas measured with a planimeter.

A standard Burrell Kromotog K-2 was used with a hydrogen flow of 40 ml/min, a cell current of 310 ma and a 2½-m column, at room temperature. A 20-ml gas sampler thermostatted at 50°C was employed in order to get large peaks for the less concentrated heavy water solutions. Better results for solutions with more than 2 mole percent of heavy water can be obtained by using a smaller sampler. Indeed, a great advantage of the method is the flexibility made possible by the use of different-sized gas sampler chambers for different HD concentrations, which permits the use of high recorder sensitivity and high precision over the whole H_2–HD range from 0 to 100 percent except at the extreme ends. The standard solutions used for the calibration line were prepared from deaerated, distilled water and deuterium oxide (General Dynamics, 99.9 percent). The exact deuterium content of every sample was established with a 25-ml pyknometer (*1*) at 25°C.

Reproducibility depends mainly on the freshness of the calcium hydride surface (Metal Hydrides, Inc.), the constancy of carrier gas flow, and the scrupulous avoidance of leaks during evacuation. The latter are easily detected both with the manometer and by the appearance of air peaks on the recording (*5*).

EDWARD M. ARNETT, MICHAEL STREM, NORBURT HEPFINGER, JONATHAN LIPOWITZ, DAVID MCGUIRE
Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania

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