lead acetate, ammoniacal silver nitrate, antimony pentachloride in carbon tetrachloride, and boric acid-citric acid in acetone. The reaction products obtained by use of basic lead acetate, normal lead acetate, alcoholic aluminum chloride, sodium carbonate, and the boric acid-citric acid reagent give an intense fluorescence in ultraviolet light and the characteristic color test in ordinary light. This property has been used to locate and identify pigment zones on the developed chromatogram strips. Table 2 lists the visible and fluorescent colors obtained with the latter reagents.

By paper partition chromatography, coupled with the use of chromogenic sprays, one can quickly and easily obtain information as to the presence of one or more flavonoid pigments in a small quantity of plant extract and even tentatively identify the individual flavones, provided the R_f values have been previously determined for a pure sample of the pigment in question. The method makes it possible to separate mixed crystals of two or more flavonoids and to identify microquantities of an isolated pigment by running mixtures of the unknown pigment with samples of known composition on the same strip.

While the present study was in progress, E. C. Bate-Smith (*1*) reported the successful separation of anthocyanin pigments by paper partition chromatography and suggested the possibility of separating flavonoid pigments from plant extracts by similar methods. Further work with plant extracts is now in progress in this laboratory and will be the subject of a more detailed report in the near future.

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The Inhibitory Role of "Motor" Nerves

A. R. McIntyre and A. L. Bennett

Department of Physiology and Pharmacology,
University of Nebraska College of Medicine, Omaha

It is well known that immersion of a nerve-muscle preparation in Ringer's solution, deficient in calcium, results in spontaneous activity which is evident in both the nerve and the muscle. The critical concentration of calcium for frogs' sciatic-biceps femoris preparations is approximately 1 millimolar, at which concentration the nerve commences to "fire" spontaneously. When the Ca^{++} concentration is further lowered, the muscle will show spontaneous fibrillation which persists *after* failure of indirect excitation.

We have shown that the failure of indirect excitation of such preparations is caused by a partial (or possibly complete) depolarization of nerve, as evidenced by a decrease in motor-axon resting potential. Restoration of calcium promptly restores the resting potential of the axon and indirect excitability of the muscle, at the same time arresting muscle fibrillation. When a comparison is made of the effects of degeneration of motor nerve following section with those produced by gradual withdrawal of calcium ions from the intact nerve-muscle preparation, the course of events is strikingly parallel. Shortly after nerve section the response of the muscle to indirect stimulation through the distal portion of the nerve is somewhat enhanced, as is the response of the muscle to intra-arterial injections of acetylcholine.

Both of these phenomena are observed in the nerve-muscle preparation when the calcium ion concentration is slightly lowered. As the distal portion of the cut nerve degenerates, indirect excitability of the muscle is lost, and the muscle shows greatly increased sensitivity to intra-arterial injection of acetylcholine. Again, both of these phenomena can be demonstrated in the nerve-muscle preparation when the calcium is lowered to the point where loss of polarization of the nerve is reached, accompanied by failure of indirect excitability. Many of the local anesthetics have been shown to prevent depolarization of axon membranes (*1, 2*); therefore it was not surprising to find that a 1-millimolar solution of procainehydrochloride arrests fibrillation in an intact nerve-muscle preparation immersed in a calcium-free Ringer's solution. We have also shown that procaine, when added to calcium-free Ringer's solution, restores the resting potential of the immersed nerve. The cessation of muscle fibrillation accompanies this restoration and is not due to an effect of the procaine on the muscle itself because the intra-arterial injection of procaine (in concentrations sufficient to block indirect excitability in a *normal* muscle) into a *denervated* fibrillating muscle, failed to disturb its activity. It was also demonstrated that the intra-arterial injection of calcium-free solutions into denervated fibrillating muscles did not modify their activity. These findings indicate the validity of the hypothesis previously advanced by one of us concerning the inhibitory action of the normal polarized resting nerve upon muscle (*3*) and lend support to the belief that the polarized state of the terminal membrane of the normal motor axon, at rest, is inhibitory to the muscle.

Depolarization of the axon membrane or loss of the membrane following section and degeneration of the nerve results in spontaneous muscle activity. Thus passage of a "motor" impulse over a nerve does not, strictly speaking, stimulate a muscle to contract, but, as a result of the removal of the inhibiting effect exerted by the polarized end plate of the motor nerve axon, the muscle "automatically" contracts. Full details of these experiments will be published shortly.

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