fraction of other relatives of children with this disease. That the incidence of this biochemical change diminishes in proportion to the remoteness of relationship to the affected child in approximate accordance with Mendelian ratios, suggests that this test may serve as a biological tool in identification of the carrier state. Interpretation of these findings in terms of the pathogenesis of TSD awaits clarification of the metabolic role of the various aldolases.

The authors wish to acknowledge the aid of Dr. Larry Schneck and Miss Dolores Chrapek, R.N., in procuring blood specimens. They also thank the many parents and relatives of children with Tay-Sachs' disease and other volunteers who enthusiastically donated their blood for this study, as well as Mrs. Renee Nakrinsky for typing the manuscript.

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Received July 6, 1962. P.S.E.B.M., 1962, v111.

Potentiation of Striated Muscle Contraction by Piperidylmethylandrostane.* (27886)

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Several compounds have been described which potentiate the indirectly induced twitch response of skeletal muscle. Among such compounds are some of the adrenergic amines (1), certain phenolic quarternary ammonium ions(2), physostigmine(3) and certain organic phosphorous anticholinesterase agents. Recently this laboratory reported on the potentiation and blockade of indirecly induced twitch and tetanus in the intact (rabbit) and isolated (rat) phrenic nerve diaphragm preparation as produced by the steroid, 17 (2 piperidylmethyl) 3β, 17β, androstane diol (PMA)(4). Doses of PMA which produced potentiation of the indirectly induced twitch response prolonged carbachol induced neuromuscular blockade, did not reverse curare induced neuromuscular blockade, and did not influence serum esterase activity. This report is concerned with some effects of PMA on the surface and transmembrane potentials of skeletal muscle of the rabbit.

Methods. The intact anterior tibial muscle preparation of the rabbit was used throughout the study. Mature stock rabbits were lightly anesthetized with sodium pentobarbital (30 mg/kg, IV). Bipolar, platinum, shielded electrodes were placed in the mid portion of the anterior tibial branch of the right sciatic nerve. The entire sciatic nerve was then transected proximal to the electrodes. The tendon of the right anterior tibial muscle was tied with thread, cut near its insertion, and the thread was attached to a force displacement transducer for measurement of isometric contractions. Steel drill pins were inserted into the distal ends of the

* This study was supported by ONR Contract.

femur and tibia and were externally fixed to immobilize the leg. Bipolar platinum wire electrodes fixed 3 mm apart were hooked directly into the body of the muscle for stimulating purposes. Stimuli were supplied through an isolation transformer to the nerve or muscle by a model S-4 Grass Instrument Co. stimulator. Unless otherwise indicated stimulus voltage was supramaximal and rate was 1 per second.

The fascia covering the anterior surface of the tibial muscle was removed to facilitate insertion of microelectrodes into single cells. Transmembrane potentials were obtained by the technic of Ling and Gerard(5) using glass capillary microelectrodes having tip diameters less than 0.5 μ. The microelectrodes were of the hanging type utilizing about a one cm length of tungsten wire of 1 mil diameter between the probe and the electrodes according to the method described by West(6). Surface potentials were obtained by use of microelectrodes of which the tip was broken so that it did not penetrate the cell membrane. Action potentials and tension were displayed on a dual beam Tektronix Oscilloscope or on a Sanborn Recorder.

PMA† was used as the water soluble lactate salt and all injections were made intravenously in a marginal ear vein.

Results. Fig. 1 is a recording from a single experiment showing the potentiating effect of PMA on the twitch response to indirect stimuli *via* the sciatic nerve. Onset of effect occurs within 10 seconds following injection in the marginal ear vein. Duration and intensity of effect is related to dose. Doses of 0.5 to 1.0 mg per kg produce a potentiating effect which gradually returns to normal over a period of 20 to 40 minutes. These results are similar to those obtained on the intact nerve-diaphragm preparation in the rabbit(4).

Mechanical motion of the muscle prevented the obtaining of transmembrane potentials in a single cell for any extended period. To minimize muscle movement the experiments using microelectrodes were con-

† PMA was generously supplied as Ro 2-7302/4 by Hoffmann-LaRoche, Inc., Nutley, N. J.

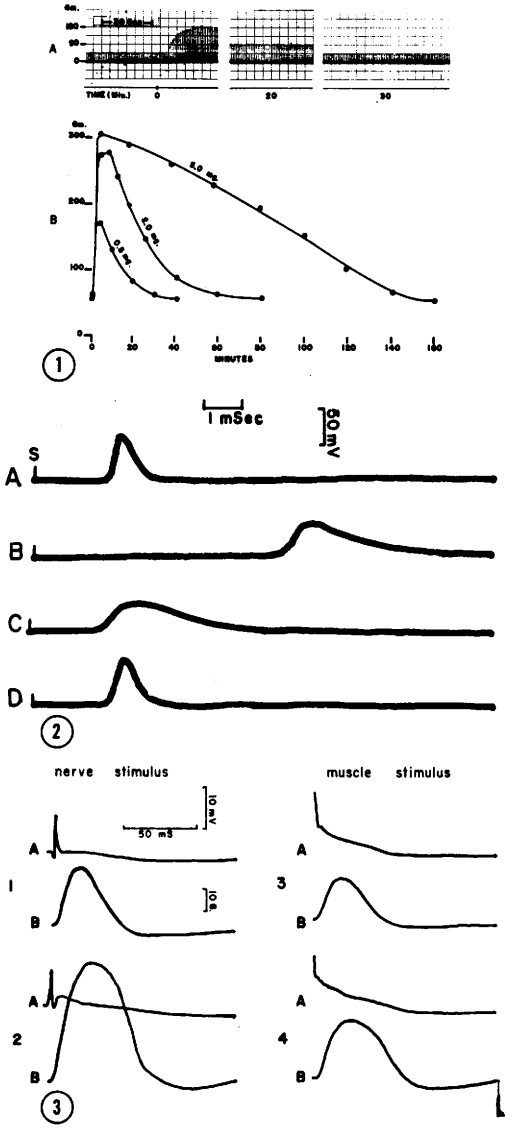

FIG. 1. A. Effect of PMA (0.5 mg/kg) on indirectly induced isometric twitch response of intact anterior tibial muscle of rabbit. Stimuli continuous, rate 1/sec, 0.4 V. PMA injected intravenously at 0 time. B. Graphs of response of above preparation to 0.5, 2.0 and 5.0 mg/kg doses of PMA.

FIG. 2. Copies of a sequence of transmembrane potentials. S indicates stimulus artifact as obtained on 2nd channel of dual beam scope. A is control, B, C, and D obtained respectively at 3, 8 and 30 min after PMA 0.5 mg/kg. B reconstructed from photo taken at one-half (2 millisec/cm) sweep speed used in A, C and D to enable visualization on scope. Indirect stimulus, rate 2/sec, 0.4 V.

FIG. 3. Surface action potentials (A) and tension (B) following indirect stimulation (1 and 2) and direct stimulation (3 and 4) of anterior tibialis muscle of rabbit. Scope sweep triggered by stimulus. Control (1 and 3), 5 to 8 min after PMA (2 and 4).

ducted with submaximal stimuli. Occasionally the microelectrode would impale and remain in a cell for about a half minute. Impalement of a cell was not uncommonly accompanied by a transient 2 to 3 second period in which trains of transmembrane potentials could be observed. Occasionally this was followed by electrical quiescence in the presence of continued nerve stimuli although the tip of the electrode seemed to remain in the cell as indicated by the magnitude of the resting potential. The magnitude of the action potential was of the order of –70 to –90 millivolts and its duration was of the order of 1 to 1½ milliseconds. Surface potentials were 20 to 30 millivolts and recordings from single cells could be readily obtained for periods as long as 30 to 60 minutes during repeated twitch responses at a rate of 1 to 3 per second.

Fig. 2 is a copy of a sequence of transmembrane potentials obtained before, and at intervals of 3, 8 and 30 minutes after administration of 0.5 mg per kg of PMA. All tracings are from cells within an area of about 2 mm on the surface of the anterior tibial muscle. The stimulus artifact was recorded on the second beam of the oscilloscope and was added to the tracings of the action potential to indicate the time relationship between stimulus and cell action potential. The figure shows that PMA resulted in an increased interval of time between the stimulus and the action potential and that the duration of the action potential is prolonged. The lengthened conduction time returns to nearly normal in the 8 minute recording but the prolonged action potential continues to be present and only gradually returns to normal over the following 10 to 20 minutes. Similar results were obtained on 6 rabbits in which successful recordings were obtained following 8 injections of PMA.

PMA induced both prolongation of the conduction interval and delayed repolarization as observed in surface action potential recordings. Fig. 3 shows copies from films of a sequence of recordings in which the surface action potential and tension were recorded simultaneously on the dual beam oscilloscope during control conditions and 5 to 8 minutes after injection of 0.5 mg per kg of PMA. The sweep was triggered by the stimulus potential, and the muscle was stimulated alternately, either indirectly through the nerve or directly to the muscle. The figure indicates that the twitch response to both direct and indirect stimuli is potentiated by PMA and that the cell depolarized only once in response to each stimulus, that is, trains of action potentials following a single stimulus were not observed. Fig. 3 also indicates that both contractile tension and duration of the contraction are increased by PMA.

Discussion. PMA differs from the cardiac glycosides mainly by the absence of an unsaturated lactone ring in the C-17 position. Levy(7) found that PMA exerts effects similar to quinidine but has no activity characteristic of the strophanthus glycosides in studies on isolated rabbit atria. Lendle and Merker (8) in a review of the literature on the extra cardiac actions of digitalis cite evidence that in the isolated rat nerve-diaphragm preparation low concentrations of the cardiac glycosides produce an increase in indirectly induced twitch tension. Guttman and Cattell (9) have shown that frog muscles maintained in air or oxygen after treatment with ouabain show a short period of increased twitch tension but this is followed by loss of excitability. Del Pozo and Pardo(10) showed in cats that K-strophanthoside increased amplitude of contractions of ischemic gastrocnemius-soleus-plantarus muscles to direct and indirect stimuli.

The methodology used in the current experiments involves measurement of single cell action potentials whereas the isometric contractions represent the entire muscle bundle response. If one can assume that the cell under observation is representative of the response of the muscle bundle, then prolongation of the repolarization phase of the action potential as produced by PMA is associated with potentiation of the twitch tension. Currently no information is available on the influence of drug-induced prolonged repolarization on the mechanical tension of the intact, striated, single muscle cell. Evidence is available which suggests that physostigmine and the phenolic quaternary ammonium ions pro-

duce potentiation of indirectly induced twitch tension by an effect on the motor nerve terminal resulting in repetitive discharge following a single stimulus(2,3). In the current experiments indirect stimulation of the muscle occasionally resulted in trains of transmembrane potentials but these were transient and were observed immediately following impalement of the cell. Although some irregularities occurred in the after potential, such trains of action potentials were not observed as an effect of PMA at a time when potentiation of muscle twitch was evident.

Summary. The potentiating effect of piperidylmethylandrostane on twitch response of intact, striated, anterior tibial muscle was shown to occur following both direct stimulation of the muscle and indirect stimulation *via* its motor nerve. By use of the microelectrode technic evidence was obtained which indicates that the potentiating effect is accompanied by an increase in conduction time of indirectly induced cell depolarization, and an increased duration of transmembrane and surface potentials of single muscle cells.

The advice and assistance of Dr. T. C. West and the technical assistance of Miss Jeanne Nelson is gratefully appreciated.

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Received July 11, 1962. P.S.E.B.M., 1962, v111.

Biosynthesis of Seleno-Compounds from Inorganic Selenium by Sheep.*† (27887)

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Substitution of selenium for sulfur in biosynthetic processes has been postulated for a considerable period. The effects of these seleno-substituted compounds on the biological system and their function in the metabolic pathways are obscure. It is well known that small amounts of selenium may produce acute or chronic selenosis and death by its toxic effects(1), and that trace amounts of selenium prevent various disease syndromes in different species of animals(2).

Monogastric animals do not utilize inorganic sulfur compounds to any extent for synthesis of the essential sulfur amino acids and sulfur amino acids must be supplied by the diet. Evidence of microbial biosynthesis of cystine from S^{35} (elemental and sulfate sulfur) in the rumen of sheep and the presence of radioactivity in wool proteins has been reported(3). Highly significant correlations were found between the number of distorted fibers and selenium content of the wool(4).

Ruminants could supply the evidence whether selenium can substitute for sulfur in the biosynthesis of amino acids and to what extent this substitution can take place. The protein selected for investigation was wool fiber. Wool is particularly well suited for the study of protein synthesis because the catabolic breakdown encountered in other tissues is negligible in this protein.

* This work was supported in part by a research grant from AEC.

† Approved for publication by the Director of Experiment Station Journal No. 193.