fection of the beans, provided their specificity is established.

Preliminary tests with beans infected with *X. phaseoli* have indicated that this method holds promise in the diagnosis of common blight as well. This procedure may be applicable to the rapid detection of both plant and animal pathogens or saprophytic organisms in various complex substrates or in simple mixtures of closely related forms.

Details of the experiments discussed above will be published elsewhere.

References

1. CRAIGIE, J. *Bact. Rev.*, **10**, 73 (1946).
2. SMITH, N. R., GORDON, R. E., and CLARK, F. E. *USDA Misc. Pub. 559*, 1946.
3. THOMAS, R. C. *Ohio Agr. Expt. Sta. Tech. Bull. 11*, 1947.
4. THORNBERRY, H. H., BRAUN, A. C., and ELROD, R. P. *Phytopathology*, **39**, 152 (1949).
5. THORNBERRY, H. H., EISENSTARK, A., and ANDERSON, H. W. *Phytopathology*, **38**, 907 (1948).
6. DELBRÜCK, M. *Adv. Enzymol.*, **2**, 1 (1942).

The Potentiation of Muscular Contraction by the Nitrate-Ion[1]

Arthur J. Kahn and Alexander Sandow

Department of Biology,
Washington Square College of Arts and Science,
New York University, New York City

It has long been known that frog skeletal muscles that have been exposed to various anions of the lyotropic series yield potentiated contractions when stimulated by such agents as cold, potassium, or electric shock (*1–5*). There is general agreement in this earlier work that the ions cause a "sensitization" of the excitatory membranes of the muscle fibers, which is then effective in increasing the mechanical response of the whole muscle. Thus, in work most directly pertinent to our own research, Chao (*4*) studied the behavior of frog sartorii exposed to a Ringer's solution normal with respect to potassium and calcium chlorides, but having all the NaCl replaced by an equivalent amount, e.g., of $NaNO_3$. Such muscles were found to quickly develop a reduction in rheobase for electric stimulation; and, furthermore, if they were stimulated with twitch shocks of a *submaximal* intensity, as determined for these muscles when previously equilibrated to ordinary Ringer's solution, their isotonic contractions became greatly augmented after only a few minutes' contact with the nitrate medium. Chao attributed the mechanical potentiation to recruitment of fibers; i.e., the lowering in threshold of the fibers of the nitrate-treated muscle permitted response in those fibers that were not excited by the submaximal shock in normal Ringer's solution, and thus, by adding their outputs to the total twitch response, caused the observed potentiated contractions. In this investigation the effects of other anions were similarly tested, and it was found that the degree of lowering of the rheobase and of the associated enhancement of shortening by submaximal stimulation was a function of the particular anion, the relative effects falling into the usual lyotropic series, $Cl < Br < NO_3 < I < CNS$.

In our research, which is reported here in preliminary form, we have used a procedure rather similar to Chao's, with the important exception that stimulation was effected by originally slightly *supermaximal* shocks. Under these conditions, irrespective of the lowered threshold of the NO_3-treated muscle, no recruitment of fibers is possible; yet such muscles produce greatly potentiated twitches approaching, or even equalling, the strength characteristic of full tetanus output. Full analysis of our results proves that the nitrate ion not only results in an increase in excitability but also causes, among other things to be discussed below, augmentation of the maximal mechanical response of which each fiber is capable.

We have studied the isometric twitch responses of excised curarized[2] frog sartorii when supermaximally shocked by the massive, transverse stimulation procedure (*6*). Records have been made by piezoelectric, cathode-ray methods (*7*) of the latent period changes and, using optical myography, of the associated developed peak tension outputs. All experiments have been done at 25°C. After 1 hr of equilibration in oxygenated phosphate buffered (pH 7.2) normal Ringer's solution, the behavior of the muscle was recorded for 2 responses separated by an interval of 1 min. This chloride-Ringer's solution was then withdrawn from the muscle chamber and replaced by a similarly buffered nitrate-Ringer's solution, through which oxygen was bubbled. In our test medium *all* the chloride was replaced by nitrate, and it is in this sense that we shall refer to our experimental medium as a nitrate-Ringer's solution. In this new environment the muscle was tested at intervals, the stimuli being again massive, transverse electric shocks of the same supermaximal strength as those applied to the muscle in normal Ringer's.

The results of a representative experiment are presented in Fig. 1. The inset at the top of this figure is a diagrammatic sketch of a typical photographic record obtained with our apparatus. The curved line, corresponding to the deflection of the cathode-ray beam in a single sweep, gives the mechanical changes that appear during the twitch latent period. The arrow at the left indicates the instant of stimulation. Following this moment 2 time intervals are presented: L_R, which measures the duration of a mechanically quiescent period —i.e., the latency of the immediately following latency relaxation (LR); and L, which measures the time interval of the mechanical latent period for positive tension development. After the termination of L, the record shows in the upward deflection the very beginning of the contraction period. Also on this sketch are indicated 2 tension variables: R, measuring the depth of the LR;

[1] Aided by a contract between the Office of Naval Research, Department of the Navy, and the New York University (NR113-300).

[2] We are very grateful to E. R. Squibb & Sons for generously supplying us with the *d*-tubocurarine chloride used in these experiments.

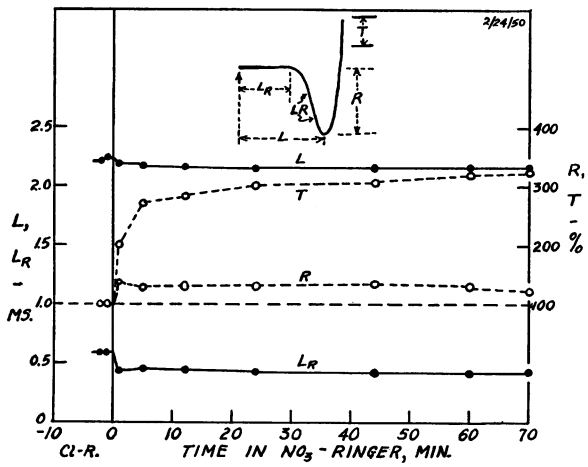

FIG. 1. Effect of nitrate-Ringer's solution on the latent period and peak-developed tension responses of the isometric twitch of the frog sartorius muscle.

and T (upper right corner), which measures the peak tension developed by the contraction. (The upper horizontal line represents the light beam of the optical myograph at a level representing the muscle's resting tension, and the lower line corresponds to the displaced position of the beam at peak of contraction.)

The solid graphed lines give the changes in L_R and L, with the time scaling indicated in ms on the left, vertical axis. Both these time intervals decrease by 0.1–0.2 ms immediately upon immersion of the muscle in the NO_3-Ringer's, and these decreases are maintained throughout the remainder of the 70-min exposure to the experimental medium. These are very small diminutions, but they are definite and have been repeatedly obtained in every experiment performed. The variations in R and T (dashed lines), with their scaling (right vertical axis) in per cent of the chloride-Ringer's outputs, also occur very soon after the muscle is put into the nitrate-Ringer's; the increase in R is about 50%, whereas T is maximally potentiated to about 325%—i.e., to a value about as large as the maximum tetanus tension. Thus, as compared to the chloride-Ringer's behavior, the muscle, when placed in the modified salt solution, quickly develops a small but certain speeding up of the latent period events, a considerable deepening of the latency relaxation, and a quite enormous increase in developed tension. Furthermore, although the data are not shown in the figure, the nitrate-induced changes are all rapidly reversed when the muscle is restored to a normal Ringer's bath.

The highly potentiated peak tensions of the nitrate-Ringer's muscles cannot be accounted for by recruitment of fibers, since the supermaximal shocks we used throughout ensured that even in the chloride-Ringer's all the available fibers of the muscle were stimulated. Nor can these potentiations be a consequence of a sort of tetanization that would occur if the single shock we used evoked a series of action potentials, as occurs, for example, in a veratrinized muscle. For a study of the electrical response of the muscle after exposure to the nitrate-Ringer's (using wire-electrode stimulation and conventional recording technique, with the muscle in a moist chamber) proves that only a single, evidently normal, action potential occurs. This result is shown in Fig. 2, which presents the action potential response of the chloride-Ringer's muscle in the upper record, and of the nitrate-Ringer's muscle in the lower. The much greater tension output of the experimental muscle is incidentally demonstrated also by comparing the tension beam deflections of the 2 muscles in the upper right portion of each record. Our results thus prove that the responses in the nitrate-Ringer's solution are potentiated maximal twitches in which each fiber produces a peak tension of the order of its maximal tetanus strength.

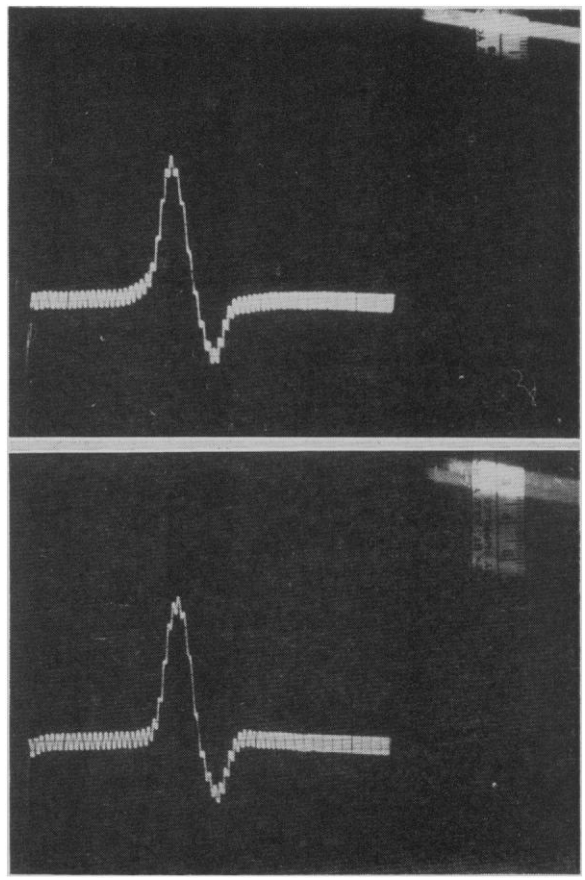

FIG. 2. Records of action-potential and peak-developed tension responses in isometric twitch of frog sartorius muscle: in chloride-Ringer's (upper photograph), and in nitrate-Ringer's (lower photograph). A 5,000 c/sec timing wave is superimposed on each action potential record.

Following Chao's procedure, we have also studied the effects of submaximal shocks (50% of maximal), as determined in chloride-Ringer's, on muscular responses in the nitrate-Ringer's. Here, again, all electrical responses consist of a single action potential, which, however, is much larger in the nitrate medium, thus indicating recruitment of fibers made possible by the nitrate-induced increase in excitability. But the mechanical response of these muscles is very much larger than would be expected if only recruitment of mechanically normal fibers were

involved. Hence, our results in general prove that the nitrate ion does not merely reduce the threshold, but—and this is the essential new finding of our research—it also can somehow modify the events of the latent period and increase the contractile strength of each excited fiber.

It is significant that the effect of nitrate on the mechanical behavior, both of the latent period and of the peak tension development of the contraction period, occurs so rapidly after immersion of the muscle in the nitrate-Ringer's solution. This indicates that the nitrate ion could not have had sufficient time to penetrate into the muscle fibers and thus modify the mechanical response by a direct action on the contractile system. We therefore infer that this ion directly affects only the surface—i.e., the excitatory membrane of the fibers—and that this alteration leads, in turn, to the observed changes in the mechanical response by a modification of the mechanism by which excitation of the membrane by the electric shock is coupled to the inner contractile system.

That the membrane is altered is clear. This is proved by the increase in excitability. But it is also demonstrated by the fact that the nitrate ion slightly increases the resting potential (*5*) and considerably enhances the permeability (*8*) of muscle cells. Probably, as Chao suggests (*2, 3*), the increased excitability is a consequence of the greater dispersibility of the membrane under the influence of nitrate, and the possibility that this structural change may underlie the other differences in membrane properties we have mentioned is not excluded.

Our principal interest, however, lies in the influence of nitrate on the mechanism of excitation-contraction (*E-C*) coupling. Our results indicate that this ion has its initial effect on the excitation part of this process. This influence, however, is not on the reactions responsible for the action potential, since the electrical response of the excitatory membrane of each fiber is unaffected. It would therefore seem that some other feature of the excited membrane is altered by the nitrate ion, and that this occurs in such a fashion as to change the subsequent course of the coupling mechanism. The time course of *E-C* coupling extends from the instant of stimulation to the onset of tension change—i.e., it is coincident with the events of the latent period, or at least with the earlier phases of this interval. Hence, it is not surprising that under the influence of nitrate we record latency alterations—the shortening of L_R and L, and the deepening of the latency relaxation. Previous work (*7*) has shown that the *LR* is an expression of the contractile material undergoing activation for its positive tension development. It is therefore evident that these latency mechanical changes may reflect the modified course of the latter portion of *E-C* coupling, during which the chain of events initiated by the action of the stimulus on the membrane becomes linked to the contractile material, and in such manner, enables the fiber-tension output to be so greatly potentiated.

Needless to say, the full elucidation of the mechanisms discussed above requires the performance of much more research. We plan to study the effects of other anions and of a variety of other agents (e.g., of the cholinesterase system) that are believed to play a role in, or to affect, excitability mechanisms. Some insight into the complexity of the processes is indicated in already reported results (*9, 10*) concerned with the effects of the potassium ion. This agent in relatively small concentrations acting for prolonged periods, or in larger concentrations acting for short durations, increases R up to 300% or more, potentiates T about 25%—effects that are irreversible—and reversibly depresses the action potential. Here, as in the NO_3 effects, the various changes are due to the direct action of K on the excitatory membrane. Yet, as can be seen by comparing the noted actions of these two ions, considerable differences exist regarding the quantitative alterations in R and T, the action potential behavior, and the possibility of reversal of the induced changes. It is felt, however, that a systematic study of these phenomena will shed some light on the fundamental mechanisms of excitation-contraction coupling, and, since this process activates contraction, it is hoped that any knowledge gained concerning it may also help in furthering comprehension of the contraction process itself.

References

1. LILLIE, R. S. *Protoplasmic Action and Nervous Action.* Chicago: Univ. of Chicago Press, 1932.
2. CHAO, I. *Am. J. Physiol.*, **109**, 550 (1934).
3. *Ibid.*, 561.
4. ———. *J. Cell. Comp. Physiol.*, **6**, 1(1935).
5. HOBER, R. *Physical Chemistry of Cells and Tissues.* Philadelphia: Blakiston, 1945.
6. SANDOW, A. *J. Cell. Comp. Physiol.*, **24**, 221 (1944).
7. ———. *Ann. N. Y. Acad. Sci.*, **47**, 895 (1947).
8. CHAO, I. *Chinese J. Physiol.*, **11**, 253 (1937).
9. SANDOW, A., and KAHN, A. J. *Fed. Proc.*, **8**, (1), 107 (1949).
10. KAHN, A. J., and SANDOW, A. *Ibid.*

The Mechanism of Action of Organic Mercury Compounds on Cytochrome Oxidase

Sister M. Angelice Seibert, Cornelius W. Kreke, and Elton S. Cook

Division of Chemistry and Biochemistry, Institutum Divi Thomae, Cincinnati, Ohio

Several organic mercury compounds (phenylmercuric nitrate [PMN], phenylmercuric hydroxide [PMOH], and *p*-chloromercuribenzoic acid [benzoate]), known to react with the –SH group, have been shown to inhibit the cytochrome c–cytochrome oxidase system at the oxidase portion of the chain (*1*). Since Barron (*2*) and others have considered this oxidase not to be sulfhydryl-dependent, it was suggested that the inhibition of the oxidase (a crude rat heart preparation) was nonspecific and might be due to the particulate nature of the preparation.

Investigations presented in this note, in which a solubilized sodium desoxycholate preparation of cytochrome oxidase (*3*)[1] was used, show that this partially purified enzyme is also inhibited by the mercurials. The in-

[1] The cytochrome oxidase was generously supplied by S. J. Cooperstein, of Western Reserve University.