

## LETTERS TO THE EDITORS

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## Function of Krause's Membrane

A. V. HILL<sup>1</sup> has shown that ordinary diffusion of a chemical substance is too slow to explain the rapidity with which the contraction in the interior of a striated muscle fibre is set off by the action potential, which is a change in the potential difference across the surface membrane; some more rapid link must therefore exist. Krause's membrane (*Z* line, telophragma) is a possible anatomical basis for such a link<sup>2</sup>, since it extends continuously across the fibre and is attached to the interior of the sarcolemma<sup>3,4</sup>.

We have tested this possibility by depolarizing very small areas of the surface of fresh fibres isolated from frog muscles. A micropipette with a squared-off tip, diameter about 2 microns, filled with isotonic sodium chloride, was firmly applied to the fibre surface and fed with pulses of current of  $\frac{1}{2}$ -sec. duration, making the interior of the tip about 20–40 mV. negative with respect to the Ringer bath. Fig. 1 shows that, when this was done opposite an *A* band, there was no contraction, while if the pipette was opposite an *I* band (with *Z* at its centre), that *I* band alone shortened, the contraction spreading only a short way in. In neither case was it possible to initiate shortening with pulses which hyperpolarized the membrane. Greater inward spread was obtained with a 4-micron pipette (Fig. 2), which is the largest that could be used without overlapping more than one *Z* line. The *I* band to which this pipette was applied now shortened for a distance of about 10 microns inward from the surface, while neighbouring *I* bands (1.5–3 microns from the central *Z* line) did not shorten and were, in fact, passively stretched.

The fibres had ceased to give propagated electrical activity by the time we made these observations, but were still capable of powerful local twitches in

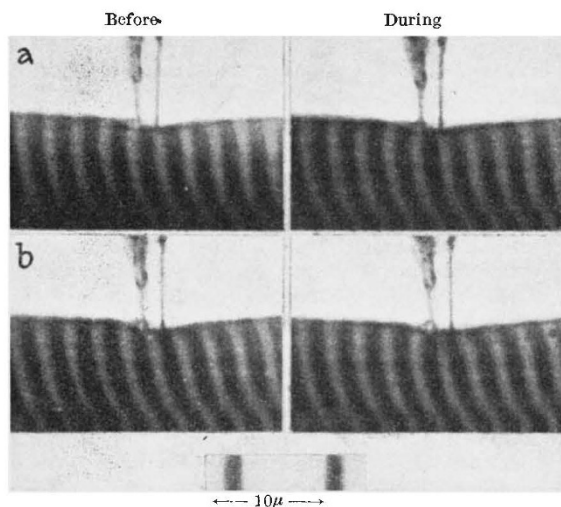


Fig. 1. Micropipette applied to edge of an isolated fibre from the semitendinosus muscle of the frog. Polarized light, compensated so that *A* bands appear dark. In (a), pipette is applied opposite an *A* band, in (b) opposite an *I* band. Left, before, and right, during, a pulse of current depolarizing the membrane opposite the tip. Contraction in (b) only, and then affecting only one sarcomere. In other experiments which were not recorded photographically, the contraction spread about 4 microns inwards.

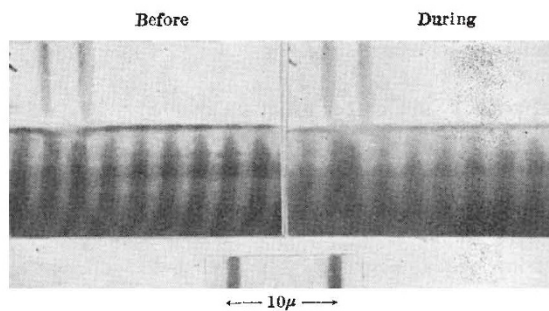


Fig. 2. As in Fig. 1, b, but with larger pipette. Contraction spreads about 10 microns inwards, but there is no spread to the adjacent sarcomeres, where the *I* bands are, in fact, stretched.

response to depolarization of a larger area of membrane. The current applied with the micropipette would not be expected to set up a propagated action potential even in an excitable fibre, because of the smallness of the area depolarized.

We consider that these results are strong evidence that the influence of membrane depolarization is conveyed to the interior of the fibre by spread along some structure in the *I* band; from the anatomical situation, this must almost certainly be Krause's membrane.

This work was done during the tenure of a fellowship by one of us (R. E. T.) from the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service.

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<sup>1</sup> Hill, A. V., *Proc. Roy. Soc., B*, **136**, 399 (1949).

<sup>2</sup> Tiegs, O. W., *Aust. J. Exp. Biol. Med. Sci.*, **1**, 11 (1924).

<sup>3</sup> Heidenhain, M., "Plasma und Zelle," **2**, 613 (Fischer, Jena, 1911).

<sup>4</sup> Draper, M. H., and Hodge, A. J., *Aust. J. Exp. Biol. Med. Sci.*, **27**, 465 (1949).

### Reaction of Starfish Spermatozoa to Histidine and Certain Other Substances considered in Relation to Zinc

STARFISH spermatozoa are generally immobile in sea-water suspension. However, Metz<sup>1</sup> has reported that they become intensely active when they are suspended in egg albumin-sea water. His experiments were chiefly concerned with the agglutination reaction, and further it was shown (Metz and Donovan<sup>2</sup>) that most of the amino-acids and peptides were also capable of agglutinating the sperm, when fertilizin was added. Apart from the problem of agglutination, we were interested in the induced motility of these spermatozoa in albumin-sea water, and have examined the effects of various amino-acids on their motility and metabolism.

It was found that L-histidine exerts a marked effect on the motility of these spermatozoa. It is very striking to take a small amount (0.5 ml.) of the diluted sperm (1:20) of the starfish, which are almost motionless, and observe instantly active movements on addition of 0.5 ml. of histidine-sea water ( $2 \times 10^{-3} M$ , pH 8.0). At the same time the effect of histidine on the initial oxygen uptake of these spermatozoa was examined by the usual Warburg technique, at 20° C. and with air in the gas phase. As shown in Fig. 1, histidine sharply increases the oxygen uptake, the effect being progressively increased when sea water containing  $10^{-4}$ ,  $10^{-3}$  or