

well apply to the seasonal sexual development of other vertebrates as well. The results of the above experiment point to an increase of Vitamin D or some kindred substance as the specific dietary factor. Other experiments, planned to determine the exact mechanism, are in progress.

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Anomalous Behavior of Isolated Muscle Fibers Toward Certain Chemical Stimuli.

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The rhythmic twitching induced in skeletal muscle by sodium chloride solutions is well known. It has also been known for a long time that the sodium salts of the calcium-precipitating anions induce even stronger twitching and that potassium chloride, citrate and oxalate are also effective, although potassium salts in addition cause a characteristic contracture. It was rather surprising, therefore, to find that the single isolated muscle fiber, when immersed in any of these solutions, did not twitch at all nor did it undergo contracture. The behavior of the isolated fiber was so different from that of the whole muscle that an attempt was made to discover the cause of this paradox.

Single muscle fibers were isolated from the adductor magnus muscle of the frog (*Rana pipiens*) in the usual frog Ringer's solution, buffered with di-sodium phosphate. Ordinarily, no attempt was made to preserve the nerve ending and the fiber was cut at both ends in the process of dissection. The isolated fiber then differs from the fiber in the intact muscle in that its nerve ending may be lacking, that is, it may have been located in a part of the fiber which was discarded; and also in that its ends are cut. This fiber responds quite normally to electrical and ultraviolet stimulation. It twitches in response to a condenser discharge or an induction shock and shortens on faradic stimulation or ultraviolet irradiation. However, its reaction to chemical stimulation is lacking in so far as the chloride, citrate and oxalate of sodium and potassium are concerned. Moreover, it does not respond to acetylcholine in Ringer's solution. In this respect my results corroborate those of Keil and Sichel.¹

¹ Keil, E. M., and Sichel, F. J. M., *Anat. Rec.*, 1937, **70** (Supplement No. 1), 112.

By more careful dissection, it was possible to isolate muscle fibers with the nerve ending intact. In the frog, the motor nerve ending is rather diffuse, so that it was not assumed to be present unless a portion of the myelinated fiber was identifiable also. Such preparations were treated with isosmotic sodium citrate or with acetylcholine in Ringer's solution (1:10,000). It was found that the presence of the nerve ending made no difference, and in no case was there any response to immersion in either of these solutions.

Fibers were also isolated intact in the semitendinosus muscle, in the manner described by Asmussen.² These were also immersed in isosmotic sodium citrate or acetylcholine-Ringer's solution. Again there was no reaction, although in one case, in which the fiber was immersed in acetylcholine-Ringer's solution, the nerve ending was also intact so that the fiber was exactly as in the undissected muscle. Keil and Sichel¹ concluded that the failure of the isolated fiber or small bundle of fibers to react to acetylcholine-Ringer's solution was due to the lack of the nerve ending. The results reported here do not support this conclusion because the isolated fiber does not react to this solution even when the nerve ending is seen to be present. Moreover, it is generally conceded that sodium citrate, which causes twitching in curarized muscles, acts on the muscle substance itself. Therefore, this salt should cause twitching in the isolated fiber whether the nerve ending is present or not. That it does not do so suggests that the same factor may be in operation in the failure of the isolated muscle fiber to react either to sodium citrate or to acetylcholine solutions.

Work is now in progress on the effects of various concentrations of acetylcholine and the salt solutions as well as on the effect of localized application of these solutions. The recent results of Buchthal and Lindhard³ indicate that such localized applications of acetylcholine to the region of the motor end plate are effective in producing a twitch. However, they worked on small bundles of fibers, in which the single fibers were not completely isolated. Experiments on the size of the bundle of fibers which will react to these chemical stimulants of muscle, which will be reported in detail later, indicate that under certain conditions as few as 4 fibers, in a bundle, will react to acetylcholine or sodium citrate solutions. On the other hand, single isolated fibers have never been observed to twitch in any of the solutions used, although altogether several hundred individual fibers have been observed.

² Asmussen, E., *Arch. f. d. ges. Physiol.*, 1932, **230**, 263.

³ Buchthal, F., and Lindhard, J., *J. Physiol.*, 1937, **90**, 82P.