13041

Effect of CO_2 on Ameboid Changes in Motor Nerve Plates in Intercostal Muscle.*

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Protoplasmic movement has been proposed by Cajal[1] and many others as concerned in the functional activities at the synapses of the nervous system. He likewise discovered the growth cones on the terminals of embryonic and regenerating nerve fibers and attributed their extension to an ameboid movement of the growing axon. Harrison[2] demonstrated this protoplasmic movement of embryonic living nerve fibers in tissue culture which was confirmed by Lewis[3] and others.

That the variations in the size, shape, and internal structure of motor nerve plates in one structural and functional group of muscle fibers, in the same animal, during different degrees of intensity and rates of functional activity are likewise due to ameboid-like movements have not been experimentally demonstrated.

Method. The following simple experiment may be made to confirm the results reported in this paper which were repeated on 10 white rats. All of the photomicrographs (Figs. 1 to 17) were taken from the right intercostal muscles of rat No. 5. The rats were anesthetized by the intraperitoneal injection of nembutal, 25 mg per kilo. The hair was clipped with the electric clipper over the right dorsal aspect of the thorax. A one-inch vertical incision, 1 cm to the right of the mid-line of the spine, was made through the skin and muscle down to the ribs. The 6, 7, and 8 ribs and related intercostal muscles were quickly excised in one piece beginning medially 1 cm from the spine and extending 1.5 cm laterad. This excised part of the thoracic wall was run through the gold chloride technic and served as a control of the relatively normal motor end plates in the intercostal muscles of the right side. The bleeding was quickly controlled by hemostats and ligature. The skin was sutured

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1 Cajal, S. Ramon y, *Anat. Anz.*, 1890, **5**, 609.

2 Harrison, R. G., PROC. SOC. EXP. BIOL. AND MED., 1907, **3**, 179; *Anat. Rec.*, **1**, 116; *J. Exp. Zool.*, 1910, **9**, 787.

3 Lewis, W. H., *Am. J. Anat.*, 1907, **6**, 461.

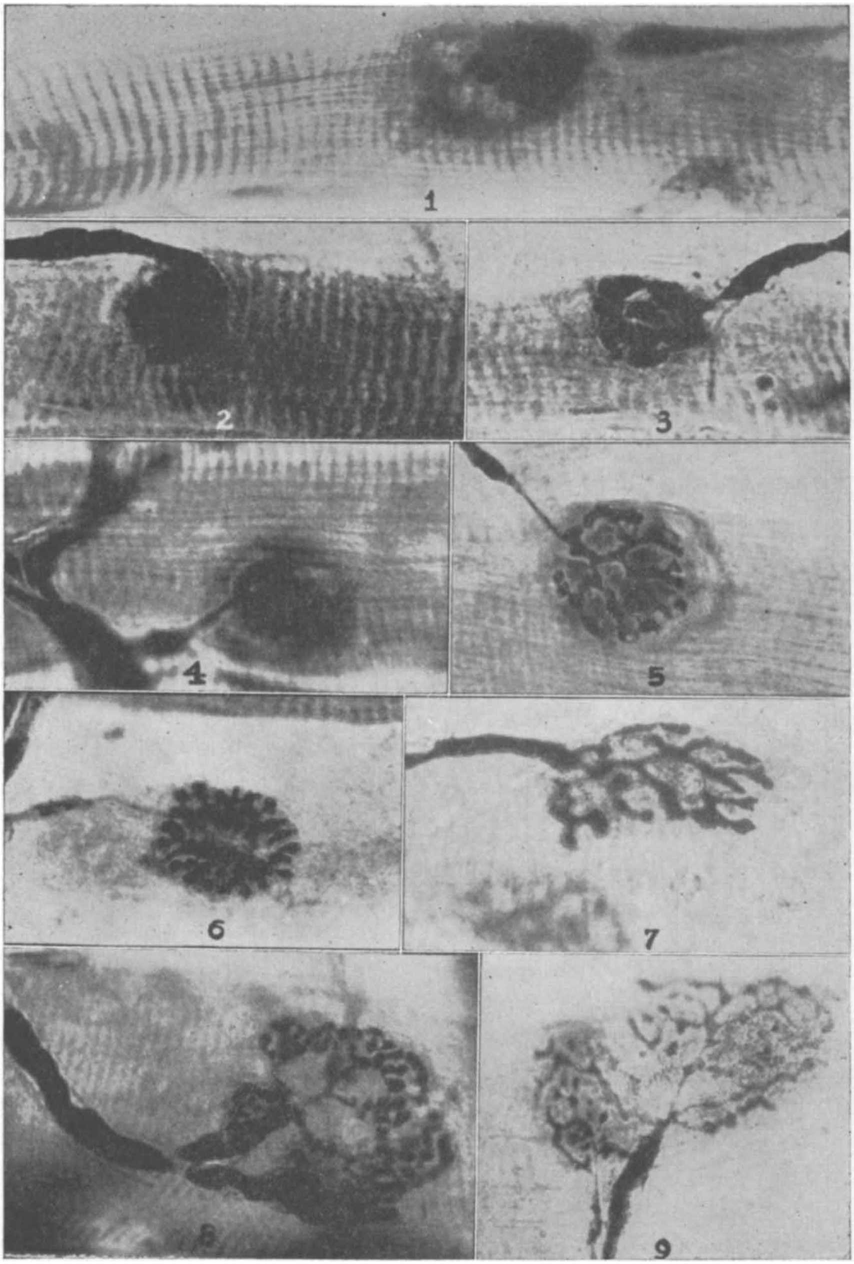

FIG. 1 TO 9.

Photomicrographs, teased normal motor nerve plates in intercostal muscle fibers, white rat No. 5, Fig. 1 to 7; carbon dioxide stimulation, Fig. 8 and 9, rat No. 5; gold chloride; $\times$ 700.

over the thoracic gap and the animal immediately placed for 2 to 10 minutes in a jar, the air of which contained roughly 15% of carbon dioxide. The rat was removed from the jar and the third and fourth intercostal muscles of the right thoracic wall were then quickly excised and run through the gold chloride technic for microscopic study of the teased muscle fibers and motor end plates. After again suturing the skin the animal was allowed to breathe normal air for a period of four hours and then decapitated. The ninth, tenth, and eleventh right intercostal muscles were then excised, subjected to the gold chloride technic, and teased.

Results. The respiratory rate after nembutal anesthesia in the white rat varied from 30 to 50 per second. During excision of the piece from the right thoracic wall the rate increased to that of 60 to 80 per minute. This was the reason for making the first incision through the ribs along the dorsal aspect, thereby quickly severing the nerve supply to the excised muscles before the respiratory rate increased. These muscles were used as relatively normal controls. The rat was then placed in carbon dioxide. The respiratory rate varied from 130 to 150 per minute. When the rate of labored breathing fell below 100 per minute the rat was taken out of the jar. The second excision of the right third, fourth, and fifth ribs was quickly made. The respiratory rate fell to 60 per minute within 30 minutes while the rat breathed normal air and remained at this rate until the animal was decapitated 3½ hours later. By this simple means the histologic changes in the same group of muscle motor nerve plates in the same animal were observed under different degrees of intensity of functional activity stimulated by the chemical method of carbon dioxide.

The motor nerve plates from the right intercostal muscles in the relatively normal controls excised prior to carbon dioxide stimulation varied from the retracted state with wide fronds (Fig. 1), 25 microns in diameter to the expanded state (Fig. 7), 52 microns in diameter. These were the extremes in the dimensions of 1000 normal nerve plates. The retracted motor end plates with wide coarse fronds are related to coarse widely spaced cross striations (Figs. 1 to 3). The expanded plates with narrow elongated processes are related to fine, closely-spaced cross striations (Figs. 5 to 7). This finding confirms the classification of muscle fibers by Hines[4] into coarsely and finely striated. There was a closely graded series of transitional stages in the structure of the muscle fibers between these two extremes with variations in the number of

[4] Hines, M., *Am. J. Anat.*, 1931, **47**, 1.

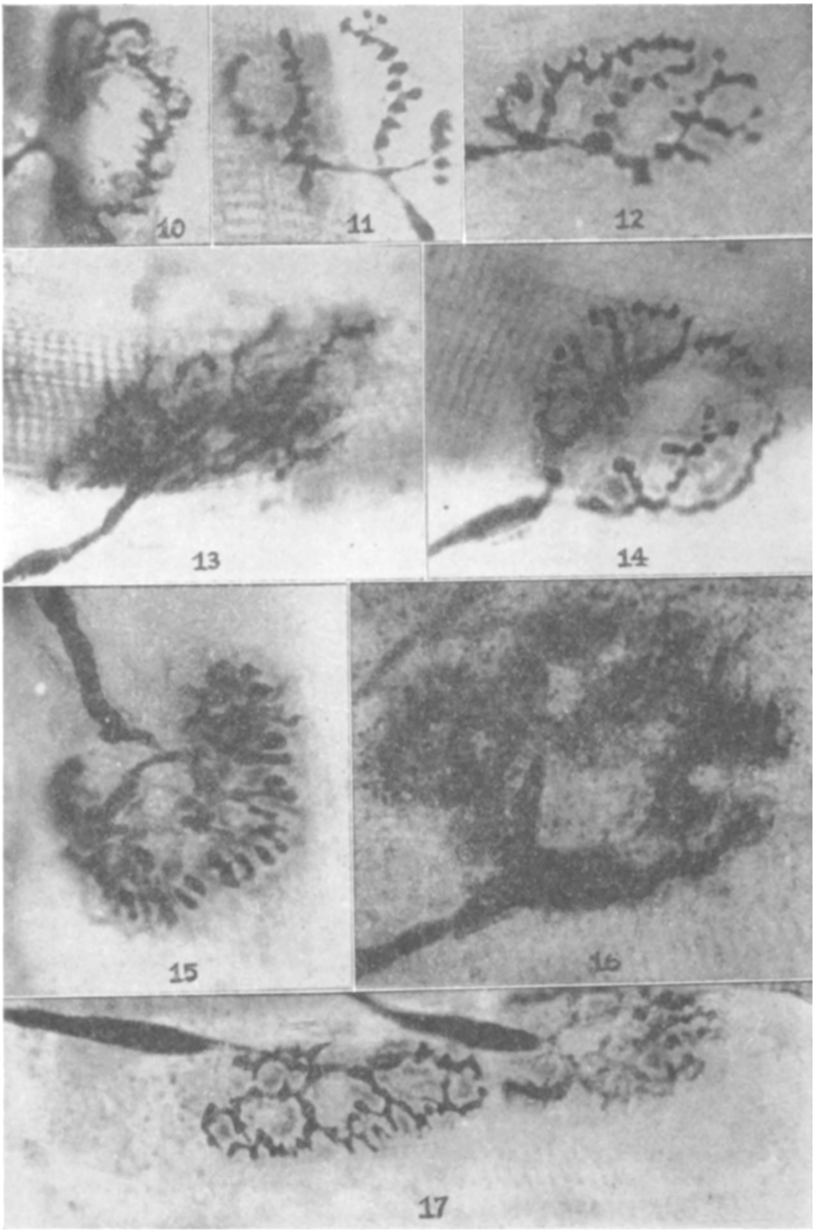

FIG. 10 TO 17.

Photomicrographs, teased motor nerve plates in intercostal muscle fibers, white rat No. 5, stimulated with carbon dioxide; gold chloride; $\times$ 700. Ameboid expansions of processes of end plate in respiratory superactivity.

muscle cross striations which agreed with the observations reviewed by Cobb,[5] Needham,[6] Hines,[7] and Hinsey.[8]

The motor nerve plates from the right intercostal muscle stimulated by carbon dioxide to superactivity had in 1000 measurements 341 plates that varied from 30 to 58 microns in diameter. The largest motor nerve plates expanded by carbon dioxide stimulation of the respiratory center reached a diameter of 105 microns (Fig. 16). In 1000 measurements of the diameters of stimulated motor nerve plates, 659 varied from 58 to 87.5 microns and had 2 to 6 elongated, discrete processes with periodic enlargements. These long, attenuated processes were separated by wide meshes (Figs. 8 to 17) and related to fine, closely spaced cross striations. In some places the protoplasmic projections of the end plates formed anastomoses (Figs. 12 and 17). There were 2 motor nerve plates on one muscle fiber; these were the 2 terminals of the 2 medullated branches of a single axon (Fig. 17). The sole plate of Kühne formed a dark granular rim around the retracted and slightly expanded normal motor plates (Figs. 1, 4, and 5). The granular sole plate disappears in the greatly expanded end plates related to fine, closely-spaced striae (Figs. 9 and 17). There was a reduction by 80% in the number of motor nerve plates expanded beyond 55 microns in diameter in the right 9, 10, 11 intercostal muscles after rat No. 5 was again breathing normal air for 3½ hours. During this time the respiratory rate fell from 150 to 60 per minute.

Summary. The retracted motor nerve plates with wide, short projections were related to coarse, widely spaced cross striations, whereas expanded nerve plates with thin, elongated and moniliform processes were longer and separated by wider meshes than the retracted ones. 60% of the expanded motor nerve plates in the intercostal muscle accelerated in the transmission rate of the nervous impulses by carbon dioxide became twice the size of the relatively normal plates. Their processes were longer and separated by wider meshes than those of the normal controls. This morphologic expansion and extension of the processes of the plate by the proposed ameboid motion under the stimulus of the demand of increased functional activity evidently favors the transmission of the nerve impulse to the receptive muscle substance by increase of the surface area of the motor nerve plate. The differences in the histologic structure of

5 Cobb, S., *Physiol. Rev.*, 1925, **5**, 518.

6 Needham, D. M., *Physiol. Rev.*, 1926, **6**, 1.

7 Hines, M., *Quart. Rev. Biol.*, 1927, **2**, 149.

8 Hinsey, J. C., *Physiol. Rev.*, 1934, **14**, 514.

motor nerve plates and muscle cross striations may be arranged in a graded series and are evidently determined by the active and inactive fibers in the fractional contraction of the same muscle. These structural changes favor the chemical theory of impulse transmission.

13042

Effect of Angiotonin and Renin on Glomerular Circulation in Frog Kidney.

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Angiotonin is the substance produced by the interaction of renin and renin activator in the plasma.[1, 2, 3] Corcoran, Kohlstaedt and Page expressed the opinion that angiotonin is a highly active vasoconstrictor which raises the blood pressure without simultaneously depressing the cutaneous temperature. They reported the effect of angiotonin on the kidney to be predominantly a vasoconstriction of the efferent arterioles of the glomeruli.[4, 5, 6] Hill and Andrus[7] noted an increase in the amplitude of the heart beat, a decrease in coronary flow, but no consistent effect on the rate of the perfused heart of the cat when angiotonin was added to the perfusate. However, renin had no significant effect. Page and Helmer[8] observed tolerance to successive doses of angiotonin in normal dogs, but if the kidneys are removed, the degree of tolerance becomes slight.

The purpose of this investigation was to study by direct microscopic observation the effect of angiotonin* and of renin* on the

1 Kohlstaedt, K. G., Page, I. H., and Helmer, O. M., *Am. Heart J.*, 1940, **19**, 92.

2 Kohlstaedt, K. G., Helmer, O. M., and Page, I. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 214.

3 Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 29.

4 Corcoran, A. C., Kohlstaedt, K. G., and Page, I. H., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 244.

5 Corcoran, A. C., and Page, I. H., *J. A. M. A.*, 1941, **116**, 690.

6 Corcoran, A. C., and Page, I. H., *Am. J. Physiol.*, 1940, **130**, 335.

7 Hill, W. H. P., and Andrus, E. C., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 213.

8 Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 495.

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