Physiology. — "*On the Segmentation of Skeletal Muscles in the Frog. (Rana esculenta)*". By Dr. S. DE BOER. (Communicated by Dr. C. U. ARIËNS KAPPERS).

(Communicated at the meeting of October 31, 1925).

The question whether the fibres of skeletal muscles receive innervation from one or from different segments is a matter which has of late years engaged the attention of several investigators. On the basis of his histological researches AGDUHR [1]) came to the conclusion that in one and the same fibre of a skeletal muscle endplates of KÜHNE can be found, which are connected with nerve fibres arising from different segments. BERITOFF [2]), and also CATTELL and STILES [3]) arrived at the same conclusion after their physiological experiments, SAMOJLOFF [4]), however, basing on the results of his experiments, was induced to think that every separate muscle fibre is innervated only from one anterior root. In a previous publication I gave an extensive summary of the literature on this subject [5]), so that the above brief indications may suffice here.

The present writer studied this problem first in the M. gastrocnemius of the frog. I injected subcutaneously some drops of a veratrin solution into frogs and after the muscles had been poisoned I stimulated rhythmically one of the two innervating spinal nerves, until the veratrin curve had disappeared and twitches arose. After this an induction shock, administered to the other root, produced invariably a complete veratrin curve. If then this second root was stimulated rhythmically, until after every stimulus a twitch arose, the veratrin effect recovered itself on stimulation of the first root. That the endplates in the muscle are not the cause of the disappearance of the veratrin-effect after rhythmical stimulation of the nerve, was proved by the fact that *direct* stimulation of the muscle also produced twitches. It became evident from these experiments that the two spinal nerves innervate different parts of the M. gastrocnemious. A second series of experiments yielded the same result. I fatigued the M. gastrocnemius by applying rhythmical induction-shocks to the one root. Subsequent stimulation of the other root produced curves without signs of fatigue. (This was known before and CATTELL,

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1) AGDUHR. Anat. Anz. 49. p. 1. 1916; 52. p. 273. 1919.

2) BERITOFF. Ztschr. f. Biol. 78. p. 231. 1923; Pflüger's Arch. 205. pp. 455 and 458. 1924.

3) CATTELL and STILES. Amer. Journ. Physiol. 69. p. 645. 1924.

4) SAMOJLOFF. Pflüger's Arch. 204. p. 691. 1924.

5) S. DE BOER. The double innervation of the M. gastrocnemius. The Journ. of Physiol. Vol. 60 p. 215. 1925 and Verslag van de vergadering van de Amsterdamsche Neurologen Vereeniging op Donderdag 1 Oct. 1925.

and STILES supposed the explanation to be that on rhythmical stimulation the conductability of KÜHNE's endplates decreases. According to these investigators most muscle fibres contained KÜHNE's endplates, which were connected with the nerve fibres of the 8^{th} and the 9^{th} spinal segments). When after this the second root was again stimulated rhythmically until fatigue reappeared, the effect of the stimulation of the first root had meanwhile been considerably restored. It is seen then that during the rhythmical stimulation of the one root the fatigue recovers itself that had been previously provoked after rhythmical stimulation of the other root.

These two series of experiments have established distinctly, that in the gastrocnemius of the frog two parts of the muscle are lying side by side, each of them being innervated by one spinal nerve. However, some room was still left for the possibility that some of the muscle-fibres might be innervated from both segments. To make sure about this I registered the monophasic action-currents. To this end I first stimulated the two spinal nerves separately and after this the N. ischiadicus. It thereby appeared that after the stimulation of the N. ischiadicus the action-current curve was always equal to the sum of the two action-current curves obtained by stimulating the two spinal nerves separately [1]). This proved that none of the muscle fibres of the M. gastrocnemius receives a double innervation. Now it can be imagined that muscle fibres, innervated by the 8^{th} and the 9^{th} spinal nerves are intertwined in the muscle or that the gastrocnemius consists of two groups lying side by side, so that the muscle fibres of the one portion are not intertwined with those of the other portion.

This question I have set at rest. I severed the three or two spinal nerves which innervate the M. gastrocnemius, close to the spinal column; then I prepared out the N. ischiadicus together with the M. gastrocnemius and I suspended the muscle freely on the femur. If then the 8^{th} spinal nerve was faradized, the muscle bent towards the ventral side [2]); if, however, the 9^{th} spinal nerve was faradized, the muscle contracted rather in a vertical direction or it curved dorsad. Fig. 1 illustrates this result. In some cases (once in about 15 frogs) stimulation of the 7^{th} spinal nerve also produced a contraction of the M. gastrocnemius. These cases always showed after stimulation of the 7^{th} spinal nerve a curving of the gastrocnemius towards the ventral side. After stimulation of the 8^{th} spinal nerve the curvature was much less pronounced, or the direction was more vertical; after a stimulus had been applied to the 9^{th} spinal nerve the muscle pointed dorsad, or it contracted rather in a

[1]) Communicated on the 4th Tagung der Deutschen Pharmacol. Ges. Rostock von 13—15 August 1925, The Americ. Journ. of Physiol. Vol. 75. 1925 and Ned. Tijdsch. v. Geneesk. 14 Nov. 1925.

[2]) By the ventral side of the muscle is meant the upper surface of the muscle, the frog lying on its back; the under-surface is then the dorsal side.

vertical direction. Once in the course of my experiments a contraction of the M. gastrocnemius occurred after stimulation of the 10th spinal nerve. In this case the muscle pointed dorsad during the stimulation of the 10th spinal nerve; after stimulating the 9th spinal nerve it was directed vertically and after stimulation of the 8th spinal nerve it curved ventral. (See fig. 5).

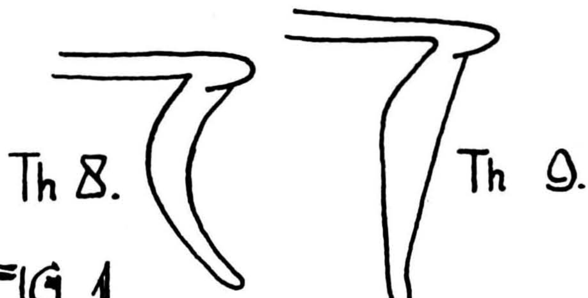

FIG. 1.

These experiments demonstrated that the fibres of the M. gastrocnemius, which are innervated by different spinal nerves, do not fall together but are arranged in groups. In order to ascertain this I slit the M. gastrocnemius lengthwise starting from the Achillestendon, and attached each part to a lever. Fig. 2 illustrates the result of this experiment. At

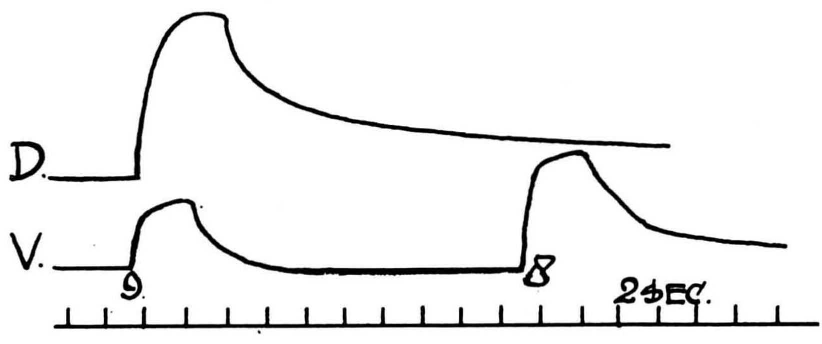

FIG 2.

8 a faradic current was applied to the 8th spinal nerve, after which the ventral part contracted, while the dorsal part did not. At 9 the 9th spinal nerve was stimulated with the consequence that the dorsal part exhibits a marked contraction and the ventral part also contracts, but less than after the stimulation administered to the 8th spinal nerve. So in this muscle the dorsal part contained fibres that were innervated only by the 9th spinal nerve. They have been isolated for the major part in the dorsal portion of the muscle, while all the muscle fibres innervated by

the 8th spinal nerve are located in the ventral portion, in which there is also a small number of the muscle fibres innervated by the 9th spinal nerve. This experiment will be all the more successful the nearer the slit is made to the dorsal side so that the ventral portion is larger than the dorsal. If, on the contrary the slit is applied nearer to the ventral side of the muscle, so that the dorsal part is larger than the ventral, the muscle fibres innervated by the 8th spinal nerve can be partially isolated. This is instanced in Fig. 3. At 9 the 9th spinal nerve received

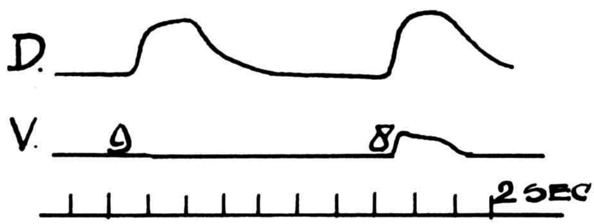

FIG 3.

a stimulation and contraction of the dorsal part ensued; at 8 the 8th spinal nerve was stimulated, which resulted in a contraction of both portions. It follows then that in this way we can isolate at will a part of the 8th or the 9th spinal muscular segment. I always found the 8th muscular segment on the ventral-, and the 9th on the dorsal side of the muscle [1]).

These experiments, then, show conclusively that the muscle fibres of the M. gastrocnemius, innervated by the two spinal nerves are not intertwined, as they revealed to us a ventral group of muscle fibres innervated by the 8th spinal nerve, and a dorsal group that receives innervation from the 9th spinal nerve.

The experiments in which also the 7th spinal nerve innervates the M. gastrocnemius, are also of interest, as is instanced in Fig. 4. The slit was applied from the Archillestendon upwards through about the middle of the muscle. At 7 the 7th spinal nerve was faradized. A contraction of the ventral part ensued, while the dorsal part remained inactive. At 8 the 8th spinal nerve was stimulated. Now both parts of the muscle contract. At 9 the 9th spinal nerve was faradized and now arises a contraction of the dorsal part, whereas the ventral portion remains inactive. It will be seen, then, that in this preparation the muscle fibres innervated by the 7th spinal nerve are all lying in the ventral part, and those that are innervated by the 9th spinal nerve in the dorsal portion.

Both parts contain muscle fibres innervated by the 8th spinal nerve.

1) When speaking of spinal segments of the muscle I mean that portion of it which is located in the M. gastrocnemius. Other portions of the same myotome are lying in other muscles.

So we see that the 7th spinal muscular segment has taken the place of the 8th. The result derived from these curves stands quite in harmony

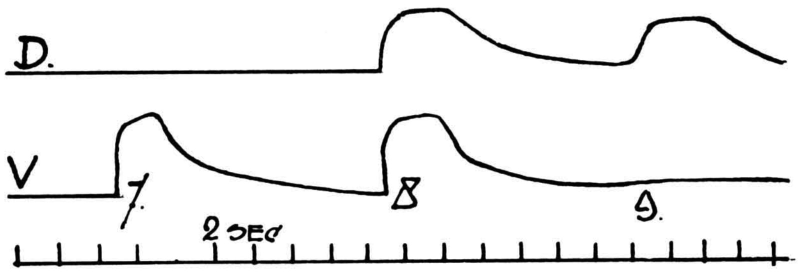

FIG 4.

with what I observed at the freely suspended muscle in those cases in which Th 7, 8 and 9 innervates part of the M. gastrocnemius. Here, indeed, we saw the same morphological changes of the M. gastrocnemius after stimulation of Th 7, viz. a curvature towards the ventral side, that I observed after stimulation of the 8th spinal nerve only in those cases, where the 7th spinal nerve does not participate in the innervation of the M. gastrocnemius. In one experiment I found that the 8th, the 9th and the 10th spinal nerves innervate the M. gastrocnemius. The curves are given in Fig. 5. They show that on stimulating the

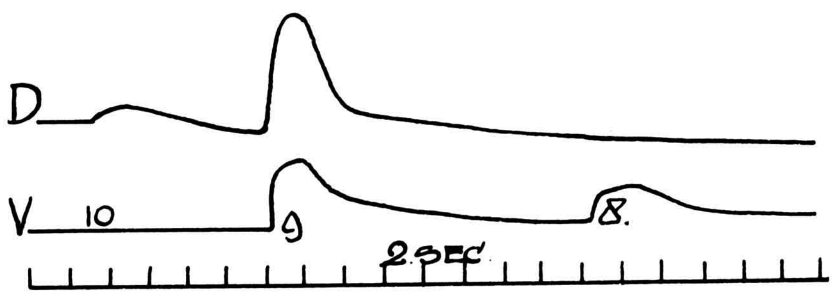

FIG. 5.

8th spinal nerve only the ventral part contracts, on stimulating the 9th spinal nerve both parts, and only the dorsal part after stimulation of the 10th spinal nerve. One question still requires further consideration. We know from segmental anatomy and above all from the work of BOLK [1]) and SHERRINGTON [1]) that the dermatomes on an extremity

[1]) See complete literature by G. VAN RIJNBERK. Versuch einer Segmentalanatomie. Ergebn. d. Physiol. Bd. 18. S. 353. 1908.

are liable to shift in a caudal or cranial direction, according as the extremity has developed itself at the cranial or the caudal side. By a new method I have determined [1]) all the dermatomes of an extremity of one and the same animal (cat), and thereby could establish the shifting of all the dermatomes. The extremity is then, as SHERRINGTON calls it, "prefixed" or "postfixed". Now the present investigation has brought to light that also the myotomes can shift in a cranial or a caudal direction. The 7th spinal segment can be substituted for the 8th and conversely the 10th can replace the 9th. This shifting may amount to half a segment, or a quarter of it, or less. In all cases, in which the 7th or the 10th spinal nerve innervates the M. gastrocnemius I found in the frog only unilateral shifting. It is clear, therefore, that shifting is not of necessity bilateral. Accordingly the position of the 8th spinal segment in the M. gastrocnemius, is not always the same and may display variations. It follows that the curving may be different after the stimulation of the 8th spinal nerve, and consequently it is clear that the myotome-parts in a muscle always present the same sequence, and can shift without disturbing this arrangement. Up to the present this had not yet been established. Once more I wish to emphasize the fact that we have to do here with fragments of myotomes. Other parts of it are lying in the other muscles.

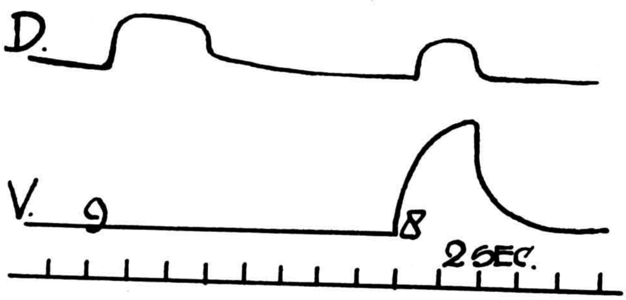

FIG. 6.

The above-mentioned experiments with longitudinal incisions in the M. gastrocnemius were also carried out with the M. Tibialis anticus longus. I cut the greater part of this muscle loose from its environment, and made a longitudinal incision down the middle between the one terminal tendon and the other. Thus the muscle was split up into a ventral-, and a dorsal part. The M. Tibialis anticus longus is innervated by the 8th and the 9th spinal nerves or by the 7th, the 8th, and the 9th. An instance of the first case is given in Fig. 6. Here we see that the stimulation of the 9th induces a contraction of the dorsal part, whereas the ventral part remains inactive. On stimulating Th 8 the dorsal part contracts

[1]) S. DE BOER. These Proceedings 18, p. 1133 and 19, p. 321.

slightly whereas the ventral part contracts considerably. Just as in the case of the M. gastrocnemius also here the ventral part of the muscle is innervated by Th 8, and the dorsal part by Th 9.

However, here also Th 7 can partake of the innervation, as appears from Fig. 7. On stimulating Th 7 there arose a contraction of the

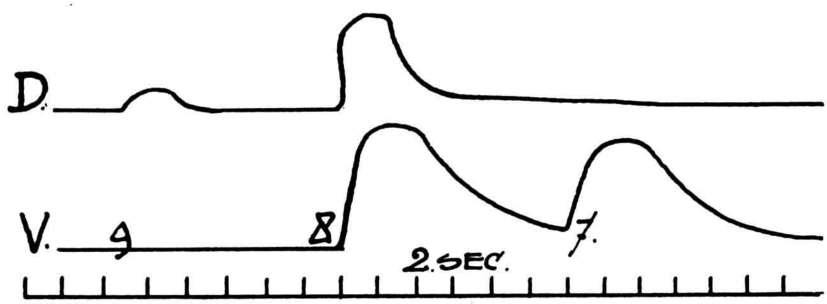

FIG. 7.

ventral part and on stimulating Th 8 a marked contraction of the ventral and the dorsal part ensued, whereas stimulation of Th 9 yielded a small contraction of the dorsal part. It will be seen, then, that also in the M. tibialis anticus longus the myotome fragments can shift without disturbing the serial arrangement, so that Th 7 takes the place of Th 8. Hence these relations appear to be similar to those established by us for the M. gastrocnemius.

Also in this muscle there are no muscle fibres which receive a double innervation. I demonstrated this by means of the string galvanometer and found that the monophasic action-current curve, obtained after stimulation of Th 8 added to the one produced by stimulating Th 9, was equal to the action-current curve obtained by stimulating the N. ischiadicus. Just as in the present experiments with the gastrocnemius, I used also here only muscle preparations that were innervated exclusively by Th 8 and Th 9.

Next I continued my experiments with the M. rectus femoris and the M. gracilis major. In the muscle I made longitudinal incisions after I had cut loose the lower portion from its environment, and thus I split up the muscles into a medial and a lateral part. Next I stimulated the 8th and the 9th spinal nerves and registered the contractions of the medial and the lateral part on a smoked drum. It hereby appeared that the medial part of these muscles is innervated by the 8th spinal nerve and the lateral part by the 9th. In both muscles it will be necessary to ascertain by means of the string galvanometer whether in these muscles part of the muscle receives innervation from both spinal nerves.

By the methods worked out by me heretofore I purpose to prosecute my experiments also with other muscles, while at the same time I shall endeavour to collect data also from other animals.

The above results do not entitle me to draw conclusions for other muscles, as it may very well be possible that fibres of muscles with an intenser function possess a plurisegmental innervation. In a previous investigation I could establish, that the skin areas with a more intensive function are innervated from more segments than cutaneous areas with a less intense function [1]). Thus I could establish that the "overlap" of the dermatomes is more marked on the ventral side than on the dorsal side of the body. This tallies with the stronger function of the skin on the ventral side and with the smaller sensory circles of WEBER in situ on the ventral than on the dorsal side. In previous publications [1]) these relations were dwelt upon at length. Therefore we have also to consider the possibility that muscles with a more intensive function may possess fibres with a plurisegmental innervation. In this direction I shall proceed with my investigations.

When we stimulate one of the two innervating anterior roots of the freely suspended gastrocnemius, it will depend on the position of the segmental parts of the muscle concerned how much the muscle inclines towards the ventral side or whether it will point in a dorsal or in a vertical direction. If the 8th muscular segment is located along the ventral border, then a typical and pronounced curving will appear ventrad after stimulation of the 8th spinal nerve. If, on the contrary, this muscular segment extends dorsad, the curving will be less pronounced after stimulation of the 8th spinal nerve. The frequent morphological change in the various gastrocnemii implies that the position of the muscular segment is not stationary but vacillating.

The fact that the ventral or medial side of the muscles (gastrocnemius, tibialis anticus longus, rectus femoris, M. gracilis maior) receive innervation from more cranial parts of the central nervous system than the dorsal side, is perfectly in keeping with the experience of Prof. BOLK [2]). This investigator found for the upper leg and the lower leg that "the medial muscles in these groups originate from the more proximal (higher) segments, and the lateral muscles from the more dorsal (lower) ones".

My own investigation showed that this pronouncement applies not only to the entire muscle but also to parts of it.

1) See S. DE BOER, These Proceedings **18**, 1915, p. 1133 and **19**, 1916, p. 321. Ned. Tijdschr. v. Geneesk. Jaargang 1916, II and Psychiatrische en Neurol. Bladen 1918. (Feestbundel WINKLER).

2) L. BOLK, De Segmentale Innervatie van romp en ledematen bij den mensch, Haarlem, F. Bohn 1910.