On the Relation between the Conduction-rate, the Fibre-diameter and the Internodal Distance of the Medullated Nerve Fibre.

By

Ichiji TASAKI (田崎一二), Ken-ichi ISHII (石井謙一)
and Hidesaburo ITO. (伊藤秀三郎)

From the Physiological Institute, Keio University,
Yotuya, Tokyo.

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Since Gasser and Erlanger (3, 4) published their extensive experiments on the relationship between the conduction-rate and the diameter of the nerve fibre, there has been a large amount of work concerning this problem (Blair and Erlanger, 2; Zotterman, 15; Pumphrey and Young, 9; Hursh, 7; Gasser and Grundfest, 6 *etc.*). The method adopted by these investigators, except for Pumphrey and Young who studied on invertebrate's nerve fibres, consisted in analyzing the action potentials observed on the nerve trunk. Although these previous results seemed fully convincing, it seemed to us still worth while to re-examine this relationship on vertebrate's nerve fibres, since it is possible for us to record action currents of artificially isolated single nerve fibres and to measure the conduction-rate and the fibre-diameter directly on each fibre.

In the preliminary experiments, we have investigated the relation between the strength of induction shocks and the shock-response interval. The relation between the threshold and the fibre-size was also investigated in this connection. We have further examined the relation between the internodal distance and the fibre-diameter on the bull frog's nerve fibre.

The work described in this paper was done by Tasaki in collaboration with Ishii and Ito. But several other members of the Institute, especially Kano and Kobayashi, have aided in carrying out some part of the work. Maruhashi has also aided in preparing photographs. We express our deep gratitude for their assistance.

Method.

Isolated single nerve fibres of the Japanese Toad (*Bufo vulgaris*) or of bull frog (*Rana catesbyana*) were used. The procedures of isolation and experimentation were similar to those described in previous papers (Tasaki and others, 11, 13, 14).

Single nerve fibres were isolated from the tibial nerve at the region near the gastrocnemius or flexor digitorum muscles. The fibre-diameter was immediately measured under a high-power microscope. The preparation was then mounted on a "ridge-insulator"; the fibre was laid in two pools of Ringer's fluid as shown in Fig. 1 and the internodal stretch of the fibre was suspended in the air between two pieces of glass-capillary. Electrodes of Zn-$ZnSO_4$-Ringer gelatine-gel type (*E* in the figure) were dipped in two pools and were led to a 4-stage resistance-capacity coupled amplifier (A).

The major portion of the nerve trunk of the preparation was suspended in the air by means of silk thread attached to the proximal end of the nerve. One or two pairs of platinum electrodes (*P*) were placed on the nerve and were connected to the secondary coil (of which self inductance was 0.1 or 0.4 Henry) of an inductorium. Induction shocks were delivered to the nerve by opening the primary circuit of the inductorium with a Helmholtz pendulum. The strength of the shocks were regulated by means of the precision resistance (*R*) inserted in the primary circuit.

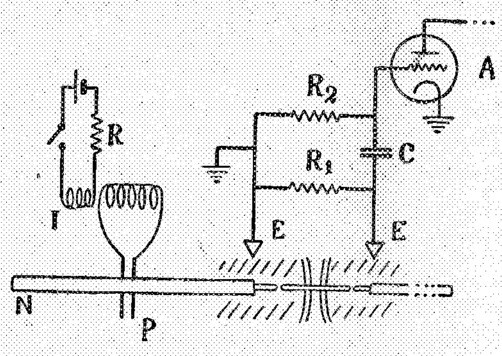

Fig. 1. Diagram showing the experimental arrangement. N, nerve; P, platinum electrodes; I, inductorium; R, resistance; R_1, 50 (or 500), R_2, 300 (or 500) kilo-ohms; C, condenser of 2 microfarads; E, non-polarizable electrode; A, amplifier.

The action currents of single fibres were amplified $1-5\times10^6$ times and were recorded with a cathode-ray oscillograph. Horizontal sweeping of the cathode-ray was executed by discharging a condensor through an appropriate resistance: the charging circuit of condenser was opened with a knock-over key of a Helmholtz pendulum and the discharge was commenced, and the current thus produced was amplified and was led to the deflection-plate of the cathode-ray tube. Figures on the screen of the tube was photographed with a "Leica".

The room-temperature was between 20° and 25° C.

Results.

(1) *The shock-strength and the shock-response interval.* It is well-known, that the time-interval between the shock-artefact and the action current reduces as the strength of the induction shock applied to the nerve is increased. For a more exact quantitative examination of the relation between the shock-strength and the shock-response interval, toad's nerve fibres, entering into the gastrocnemius muscle, of varying sizes were used. In order to prevent undue spreading of the stimulating current, the secon-

dary coil of the inductorium, the platinum electrodes and the proximal end of the nerve were insulated as carefully as possible.

In all (seven) cases the threshold of the fibre remained constant during one series of recording action currents which lasted only about 7 minutes. Fig. 2 shows two examples of the results. In the figure the reciprocal of the shock-strength is plotted linearly in ordinates against the shock-response interval in abscissae. In barely supraliminal stimulation, the shock-response interval showed marked variation. When the shock-strength was slightly increased above the threshold, the shock-response interval was distinctly reduced and became stable (see Series 1 of the photographic records).

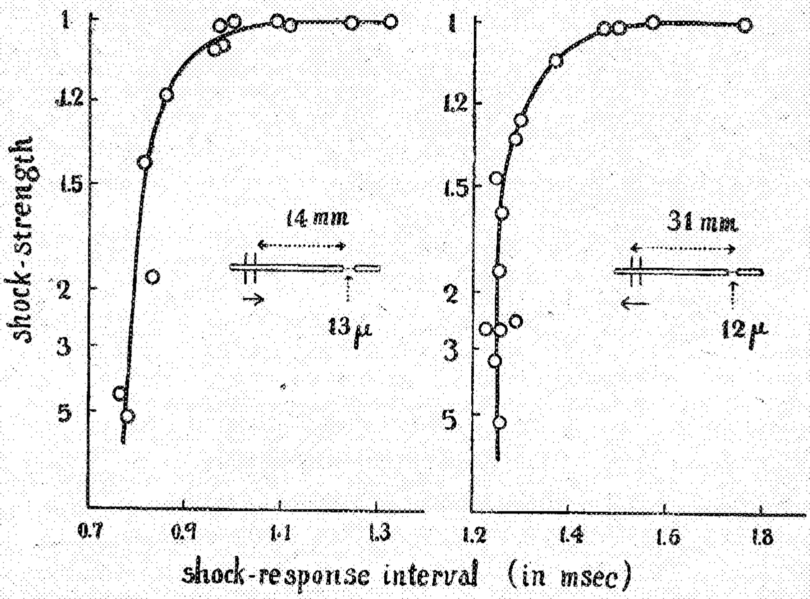

Fig. 2. Experimental results showing the relation between the shock-strength and the shock-response interval in single-fibre preparations. Conduction-distance, direction of the induction current and the fibre-diameter are shown in the diagram in each case.

Instability of the shock-response interval on threshold excitation can also be demonstrated when the action current is led directly from the site of stimulation, but in that case the effect is less marked than in the results shown in Fig. 2 (Tasaki, unpublished). The reduction of the shock-response interval by increasing shock-strength seems therefore partly referable to a greater conduction-rate in the region of nerve near the stimulating electrodes.

(2) *The shock-response interval and the conduction-distance.* The sciatic-tibial nerve of the toad has a length of about 120 mm. Induction shocks

of the strength of twice the threshold value were applied to the nerve at varying points of a single fibre preparation, the action currents of the fibre were led from the distal end of the preparation, and the shock-response intervals were measured at these points.

In Fig. 3 each circle represents the mean value of 4 to 6 measurements at each conduction-distance. It is clearly shown in this figure that the time-interval between the shock-artefact and the action current increases linearly as the distance of conduction. The conduction-rate differed from fibre to fibre, but in each fibre the impulse was conducted at a constant rate along the whole length.

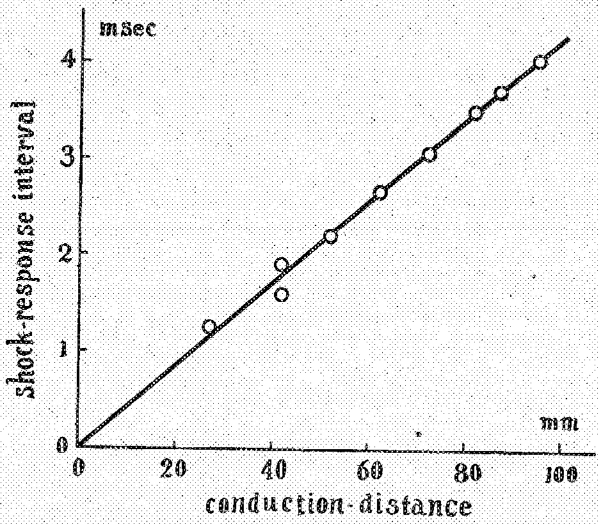

Fig. 3. Demonstration of linearity of the nervous conduction in a single nerve fibre. Diameter of the fibre, 8 μ.

(3) *The conduction-rate, the threshold and the fibre-diameter.* In this series of experiments bull frog's nerve fibres were used. A single fibre was isolated from the tibial nerve at the region just above the gastrocnemius muscle. When two nerve fibres of just the same size or of widely different sizes were accidentally isolated from a nerve trunk, the preparation was also used for the measurement, as the use of such a preparation did not seem to introduce any ambiguity into the result (see Series 5 of the photographic records). The outside diameter of the fibre was measured with a high power microscope before experimentation.

Induction shocks of the strength of twice the threshold value were applied to two points on the sciatic nerve, and the difference in the shock-response intervals was determined by using contacts of a Helmholtz pendulum. The measurement of the difference in the shock-response intervals was carried out more than twice, and the average value was taken.

The length of the nerve was measured, after observation of the action current, by hanging the nerve in the air at the end and bringing it close by a scale. The room temperature was regulated with heater to 24±1° C.

In Fig. 4, the conduction-rate is plotted in ordinates against the fibre-diameter. The sample was 49 fibres of varying size. Isolation of a single fibre of a small size (below 6 μ) was relatively difficult; but, when a fibre

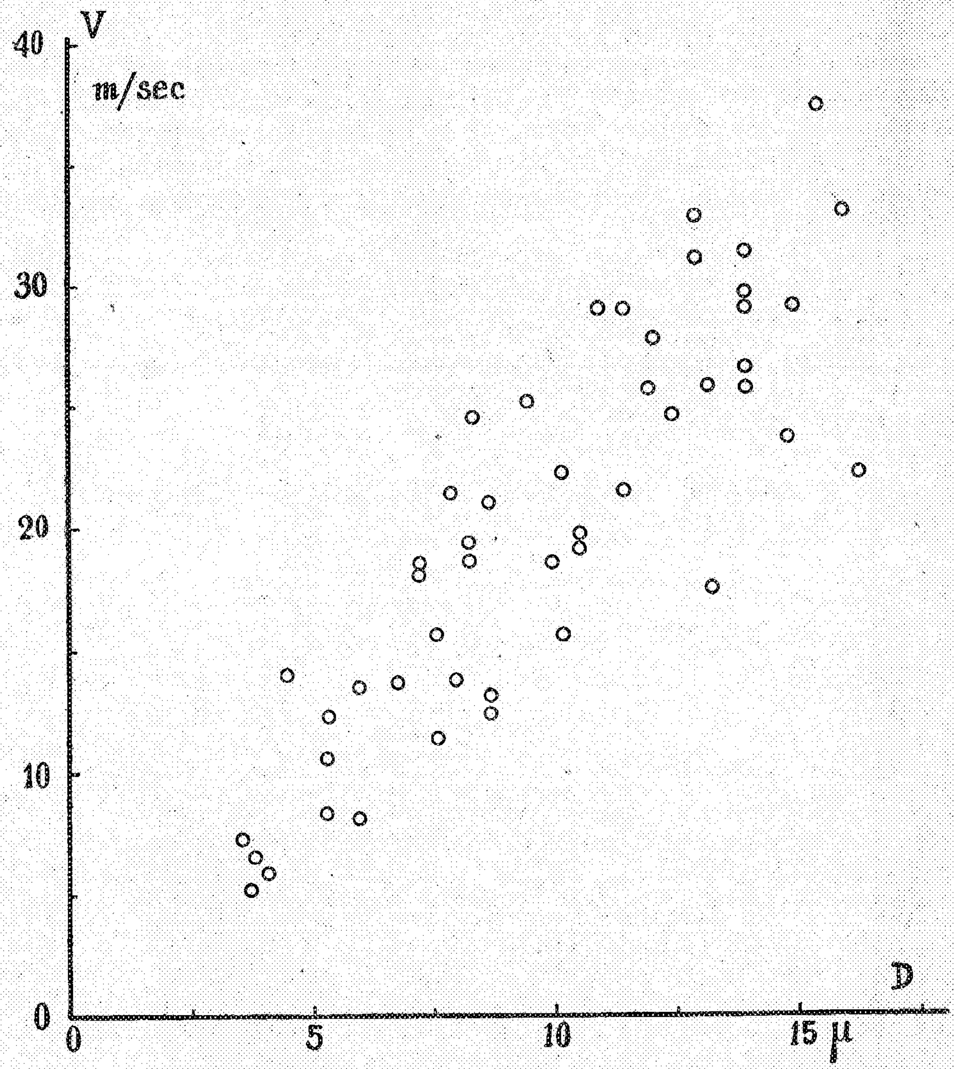

Fig. 4. Conduction-rate V (ordinates) plotted against fibre-diameter D. Data from 49 single fibres of the bull frog. 24° C.

of this size was successfully isolated, we never failed to observe the action current of the fibre.

It is clearly seen in this figure that the conduction-rate increases, in a statistical sense, proportionally as the fibre-diameter. The coefficient of correlation between these two values was 0.92. The method of average equation showed that the slope of the straight line connecting these points was 0.146. In other words, the relation between the conduction-rate V and the fibre-diameter D could be expressed by the formula:

$$V = 0.146\,D.$$

Fig. 5 shows the relation between the threshold strength and the fibre-diameter. In the ordinates the reciprocal of the threshold strength (the resistance in the primary circuit of an inductorium) was plotted. They were of those fibres used for the measurement of the conduction-rate, but several cases (in which an inductorium of a different type was used)

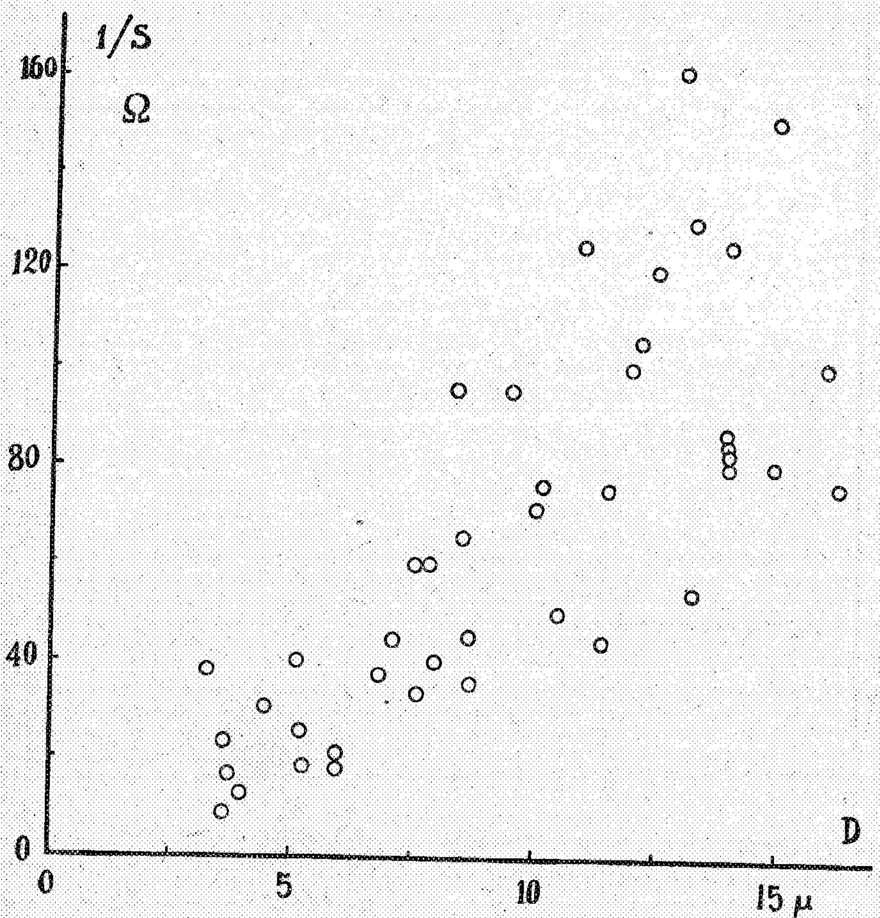

Fig. 5. Reciprocal of the threshold strength 1/S (ordinates) plotted against fibre-diameter D. Bull frog's single fibres.

were excluded. The interpolar distance of the platinum electrodes was 2.5 mm.

The threshold value seemed to be influenced by the amount of con-

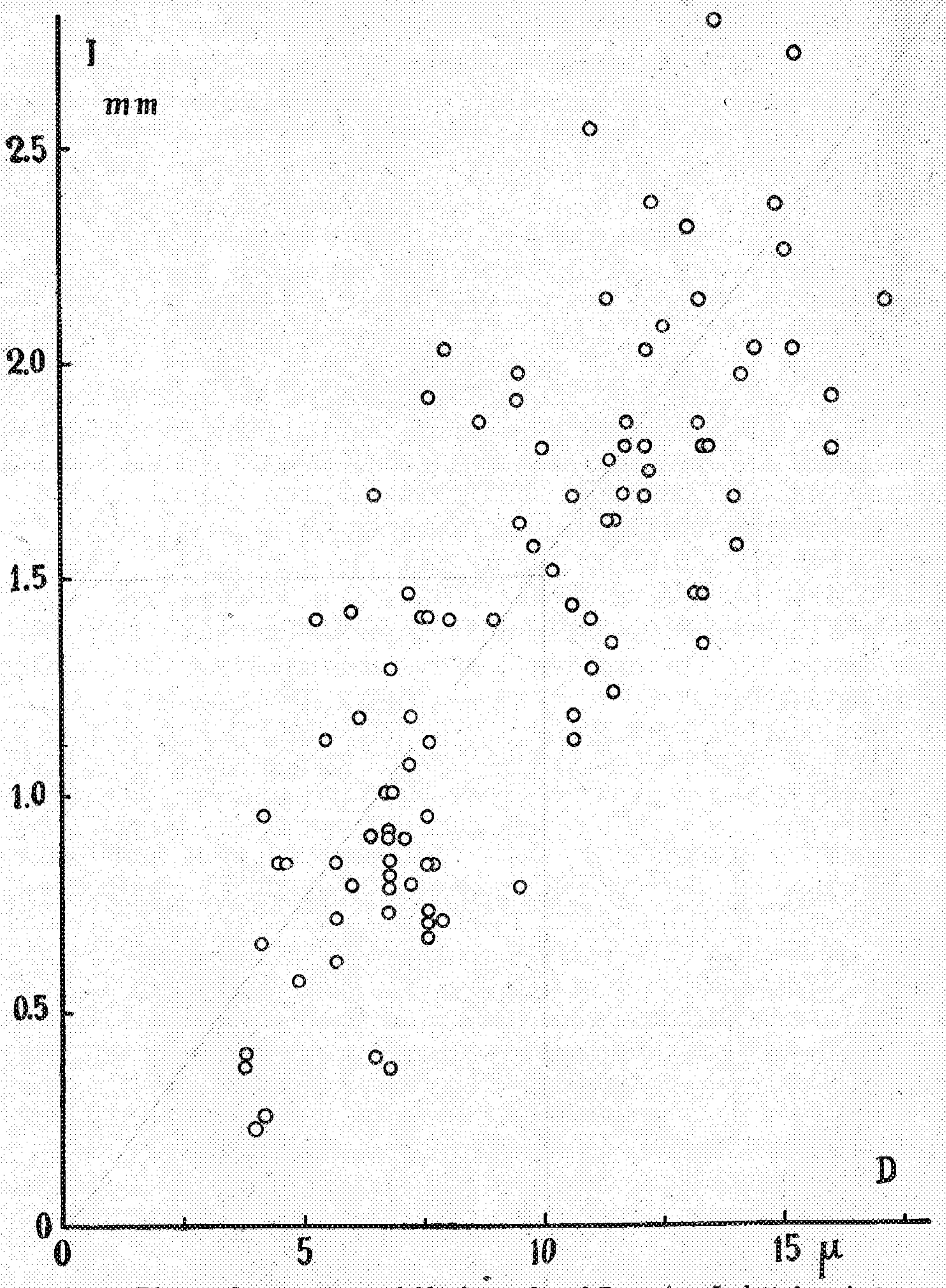

Fig. 6. Distance between two neighboring nodes of Ranvier l plotted against fibre-diameter D. Data from 100 fibres of the bull frog.

nective tissue arround the nerve and showed greater variation than the conduction-rate. It is clear that the threshold strength, measured with electrodes placed on the nerve trunk, increases as the diameter of the fibre decreases; but the relation between the reciprocal of the threshold strength and the fibre-diameter did not seem to be linear.

(4) *The internodal distance and the fibre-diameter.* It has been shown by several previous investigators that the distance between two neighboring nodes of Ranvier increases proportionally as the fibre-diameter (8, 10). We have attempted to examine this relationship on bull frog's nerve fibres.

The connective tissue sheath of the tibial or sciatic nerve was removed and the nerve fibres were separated from one another with fine needles. The internodal distance was measured with a low power microscope and the fibre-diameter with a high power microscope on fresh materials. It was possible to carry out measurement on 3 to 6 fibres in a nerve trunk.

Fig. 6 shows the result; the sample was 100 fibres of varying size. The coefficient of correlation between the fibre-diameter and the internodal distance was 0.62. The linear relation between these two values could be expressed by the formula:

$$L = 2.05\,D,$$

where L represents the internodal distance (in mm) and D the fibre-diameter (in μ). The coefficient 2.05 was determined by the method of average equation.

(5) *Observation on multi-fibre preparations.* In a preparation in which several nerve fibres were left uncut in the operated region of the nerve,

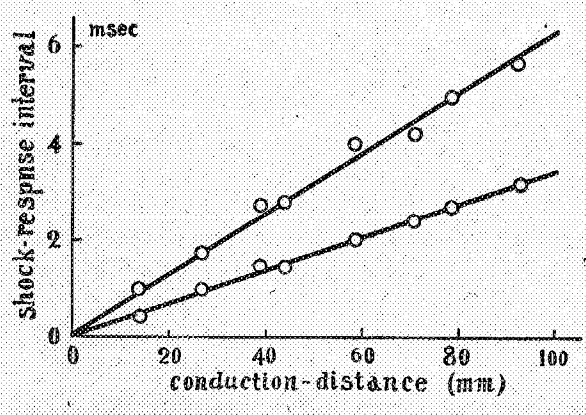

Fig. 7. Shock-response interval as a function of conduction-distance in a 2-fibre preparation. Diameter of the fibres, 12 and 6 μ (See Series 5 of the photographic records).

a strong induction shock applied to the nerve evokes multiple responses corresponding to the number of the living nerve fibres. When the strength of the shock is reduced step by step, the responses of the nerve fibres drop out one after another. The smaller fibres, which we know develop weaker action currents at longer shock-response intervals, have higher thresholds, and their responses drop out when the shocks are made weaker. As there was practically no exception to this order in dropping out of the responses, it was concluded that the wide variation of the thresholds in the results shown in Fig. 5 was due mainly to the variation in the amount of connective tissue which surrounds the nerve (see Series 2–4 of the photographic records).

Fig. 7 and Series 5 of the photographic records demonstrate the difference in the conduction-rates of two nerve fibres in a preparation. The strengths of the induction shocks were slightly greater than the threshold values for the second (*i. e.* the smaller) action current. In each fibre the shock-response interval increased with increasing conduction-distance.

Discussion.

The experimental results above described showed that (a) the conduction-rate, (b) the internodal distance and (c) the threshold for induction shock vary regularly as the fibre-diameter. In the next place, we will discuss the relationship between the fibre-diameter and other characters of the nerve fibre.

d) *The internodal conduction-time.* The above stated experimental results (Figg. 4 and 6), showing that both the conduction-rate V and the internodal distance L vary proportionally as the fibre-diameter D, indicates immediately that the internodal conduction-time is independent of the fibre-diameter. From the two equations relating V, L and D for bull frog's nerve fibres, we obtain

$$T = L/V = 0.071,$$

where T represents the internodal conduction-time in milliseconds. As the nervous transmission from a node of Ranvier to the neighboring one is effected through re-stimulation by the action currents developed at the node, the internodal conduction-time represents the latent period in excitation of a node by action current of the neighboring. It is now concluded that this latent period is independent of the fibre-size.

e) *The threshold of the node.* Though the threshold strength measured with electrodes placed on the nerve trunk varied as the fibre-diameter (Fig. 5), the threshold value measured with electrodes dipped in the pools of Ringer on both sides of the ridge-insulator (*i. e.* measured with voltage applied between two neighboring nodes) was found to vary only slightly as the fibre-size. The change in the threshold in the experimental result of Fig. 5, obtained with electrodes on the nerve trunk, could therefore be referred to the change in the internodal distance as the fibre-size. The interpolar distance of the platinum electrodes placed on the nerve trunk was 2.5 mm.; this corresponds to the internodal distance of large fibres. In the smaller fibres two or three internodal segments are involved between these two electrodes. It is clear that, when the potential difference is applied across several internodal segments, stronger shocks are needed to excite the fibre (*cf.* Tasaki, 11).

f) *The absolute strength of the action current.* The fact that in small fibres the internodal distance is short, makes it difficult to measure accurately the maximum strength of the action current in those fibres. The width of the "ridge-insulator" can not be made much less than 0.5 mm. It is however evident that the action current is weaker in smaller fibres than in larger fibres (see Series 2–5 of the records). An impression is received that the action current varies linearly as the fibre-diameter. In the "rapid motor" fibre of the toad (about 11 μ in diametar), the peak value of the action current was about 2×10^{-9} amp., and in the toad's "slow motor" fibre (about 6 μ) it was about 1×10^{-9} amp. (Tasaki, 12).

As the internodal distance decreases linearly as the fibre-diameter, the resistance of the axis-cylinder between two neighboring nodes of Ranvier should increase directly as the diameter. From this it follows that, if the action-electromotive force devoloped at the plasmic membrane of the node has a maximum value of 100 millivolts regardless of the fibre-size, the action current must decrease linearly as the fibre-diameter.

g) *The duration of the action current and the absolutely refractory period.* The action current of a nerve fibre, observed after application of a concentrated narcotizing solution to the portion of the nerve fibre in the distal (right) pool of Ringer in the experimental arrangement of Fig. 1, has a temporal configuration reproducing the course of the electromotive force developed at the plasmic membrane of the node. The duration of such "mononodal" action currents varies inversely as the fibre-diameter. In the toad's rapid motor fibre (11 μ) it lasted about 1.5 msec. and in the slow moter fiber (6 μ) it was about 5 msec. long (Tasaki, 12).

The absolutely refractory period seems to have, as Adrian (1) has already pointed out, a duration appoximately the same as the action current. The refractory period of the rapid moter fibre of the toad was about 2 msec. and that of the slow motor fibre was 5 to 9 msec (Tasaki, unpublished).

h) *Susceptibility to narcosis.* We have re-examined the old experiment of Gasser and Erlanger (5) showing that the smaller fibres were more susceptible to narcotics than the larger. A dilute narcotizing solution (3 to 5% urethane-Ringer or 0.1% cocaine-Ringer) was applied to the nerve trunk of a multi-fibre preparation. The length of the narcotized region was 5 to 15 mm. The experimental result we obtained showed that the time to failure of conduction in each fibre was practically independent of the fibre-diameter.

In one occasion we have examined the effect of compression block. We could not find any order in the susceptibility to compression block according to the fibre-size.

Summary.

1) The relation between the shock-response interval and the strength of the induction shock was investigated on toad's single nerve fibres (Fig. 2).

2) The relation between the conduction-rate V (in m/sec) and the fibre-diameter D (in μ) was found to be expressed by the formula $V=0.146D$ (bull frog's nerve fibre at 24° C).

3) The internodal distance L (in mm) varied linearly as the fibre diameter D (in μ): in bull frog's nerve fibre $L=2.05D$.

5) The internodal conduction-time was in bull frog's nerve fibres 0.07 msec., regardless of the fibre-diameter (24° C).

5) The relation between the threshold strength and the fibre-size was investigated.

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