The Effects of Potassium and Calcium Ions on the Transmission of Nerve Impulses through the Abdominal Ganglion of the Crayfish[1)]

By
Yoshio Watanabe

Zoological Institute, Faculty of Science, Hokkaido University
(With 10 Textfigures)

In a previous paper (Watanabe, 1958) the temporal pattern of the monosynaptic type of the ganglionic transmission was found to have the characteristic that the frequency of the efferent impulses decreases gradually. It is fundamentally most important to clarify the reason why on ionic or some other bases the efferent frequency decreases in the monosynaptic transmission.

The activity of the nervous system is greatly dependent upon the concentration of the various ions in the surrounding medium, especially of potassium and calcium ions. Changes in the spontaneous activity of the crayfish nerve cord was observed when the concentration of the external potassium and calcium ions was varied (Prosser, 1940; Roeder, 1941). The adaptation of the cutaneous tactile receptors of the frog was assumed to be produced by potassium released from the epithelial cell of the skin (Hoagland, 1936). The investigation of neuromuscular excitation revealed that removal of the calcium from the surrounding medium led to a block of the transjunctional excitation (del Castillo & Stark, 1952). Also the effects of calcium ions upon the synaptic excitation processes in the sympathetic ganglion were superficially similar to those described for the neuromuscular junctions (Bronk, 1939; Harvey & McIntosh, 1940).

In the present experiments the effects of environmental potassium and calcium on the ganglionic transmission were studied, in particular, on the temporal pattern of the impulse train transmitted through the ganglion.

Material and Methods

Preparation: The abdominal ganglion of crayfish, *Cambarus clarkii*, was used. The ventral cord dissected out from the crayfish was placed in a plastic (perspex) chamber so arranged that solutions could be exchanged easily and kept at a constant fluid surface level (Fig. 1 and Fig. 2). In intracellular recording the sheath of the ganglion was removed in order to facilitate the insertion of the microelectrode.

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Stimulation and Recording : The segmental nerve, *N. pedis sprii* or *N. dorsolaterales*, of the ganglion was lifted out of the saline bath and laid across a pair of Ag-AgCl electrodes (Fig. 1 and 2, c) for stimulation. Impulses transmitted through the ganglion were extracellularly recorded with another pair of Ag-AgCl electrodes from an isolated single efferent axon in *N. dorsolaterales*. Electric events in the synaptic region during transmission were intracellularly recorded by a glass capillary microelectrode filled with 3M-KCl solution.

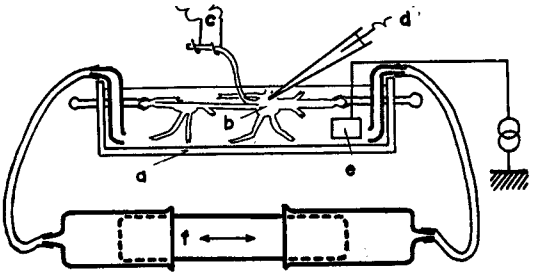

Fig. 1. Experimental chamber attached to the exchanging device of the experimental solution. a : Perspex specimen chamber. b : Ventral nerve cord of the crayfish. c : Stimulating electrodes. d : Recording microelectrode. e : Indifferent electrode. f : Syringe for exchange of solution.

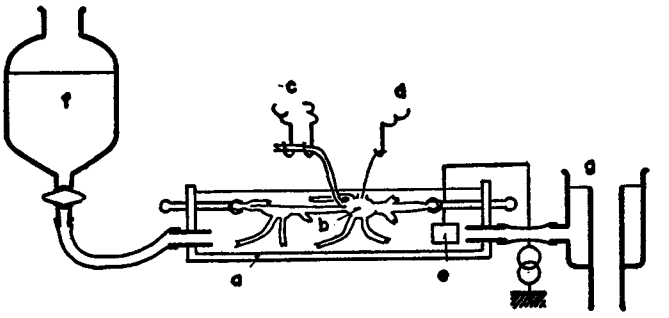

Fig. 2. Experimental chamber attached to the perfusion device of the experimental solution. a : Perspex specimen chamber. b : Ventral nerve cord of the crayfish. c : Stimulating electrodes. d : Recording electrode. e : Indifferent electrode. f : Reservoir of experimental solution, g : Device for controlling fluid level.

The electronic apparatus consisted of a head amplifier with high input impedance (Watanabe, 1958), a direct coupled amplifier, a three traces cathode-ray oscilloscope attached with a recording camera, and a square-wave generator for stimulation.

Solution : The physiological solution for the crayfish, van Harreveld's solution (van Harreveld, 1936) was made up with the following ionic composition : Na^+ : 79.3 mM, K^+ : 2.9 mM, Ca^{++} : 4.7 mM, Mg^{++} : 0.6 mM, Cl^- : 131.7 mM. The experimental solutions at various potassium and calcium concentrations were made by mixing Harreveld's solution with isotonic M/4.4 KCl and isotonic M/6.6 $CaCl_2$ solution in various proportions. Even

for intracellular recording the surrounding medium was quietly exchanged while the microelectrode was inserted into a ganglionic cell (Fig. 1). In some cases of extracellular recording, the ganglion was perfused with fresh Harreveld's solution during stimulation (Fig. 2).

Results

General feature of the synaptic transmission of nerve impulses : The ganglion always sends spontaneous impulses into some efferent nerve fibres even when no stimulus is applied to it. Fig. 3 is an example of the spontaneous activity recorded intracellularly, which consists of the synaptic potential and spike in the same manner as that of the ganglionic transmission caused by stimulation of the pre-ganglionic nerve. Thus, it becomes highly probable that the ionic effect on the ganglionic transmission is similar to that on the spontaneous activity of the crayfish nerve cord (Prosser, 1940 ; Roeder, 1941).

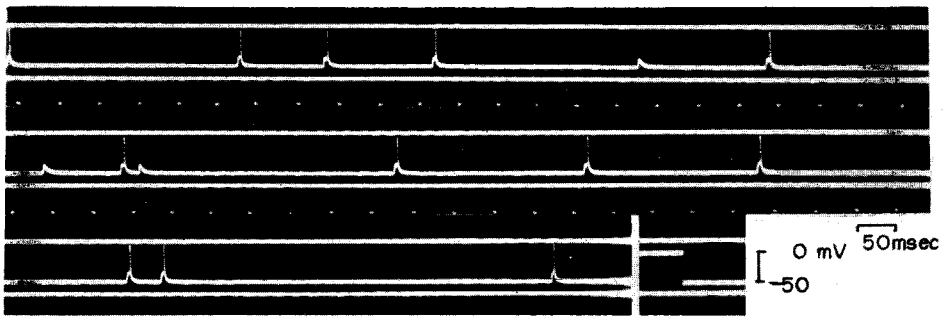

Fig. 3. Intracellular records of the spontaneous activity of a ganglionic cell.

When the synaptic response is evoked by the stimulus, the temporal pattern of the response is greatly dependent not only upon the intensity of stimulus but also upon the frequency and intervals of the repetition. The intracellular records of synaptic responses evoked by the double shocks are shown in Fig. 4. Synaptic potentials set up by two successive afferent volleys summed up temporally when the volley interval was less than 20 msec. and both the depolarization of the post-synaptic membrane and the frequency of the post-synaptic spikes increased with further reduction of the volley interval. When the successive train of the stimulus-pulses is applied to the afferent nerve, the temporal pattern of the synaptic response shows a more complex pattern than that of the response to the double shocks. Fig. 5A and B are two examples of the synaptic response transmitted through a reflex root in *N. dorsolaterales* by the repetitive stimulation at the frequency of 100 pulses/sec. In each example, I and II are the first and second response to two series of stimulations applied successively to the same root. In Fig. 6A and B, the

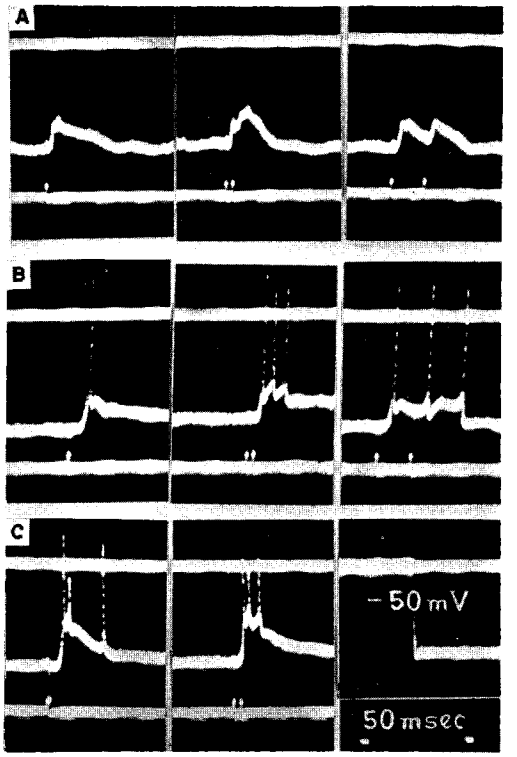

Fig 4. Intracellular records showing temporal summation of the synaptic responses to two stimuli. A shows the summation when only the synaptic potential is evoked, and B and C show the summation when the post-synaptic spikes are set up. The upper signal, the zero potential; the middle one, the ganglionic electrical responses; and the lower one, the stimuli.

graphs show the changes with time of the frequency change of the transmitted impulses shown in Fig. 5 (solid and broken lines are for the first response and second one respectively). Although these ganglionic responses belong to the simplest type (Watanabe, 1958), which have a time delay for transmission of less than 10 msec. and are unaccompanied by the discharge-bursts after the train of stimuli has ceased, the change in impulse-frequency consists of the initial steep exponential decline and the later slow decrease. Generally the responses to successive stimulations of the same reflex root are not the same in detail as is seen comparing the solid and broken lines in the frequency graph (Fig. 6).

Effects of potassium and calcium ions on the synaptic activity: In order to examine the effects of the potassium and calcium ions on the synaptic response to

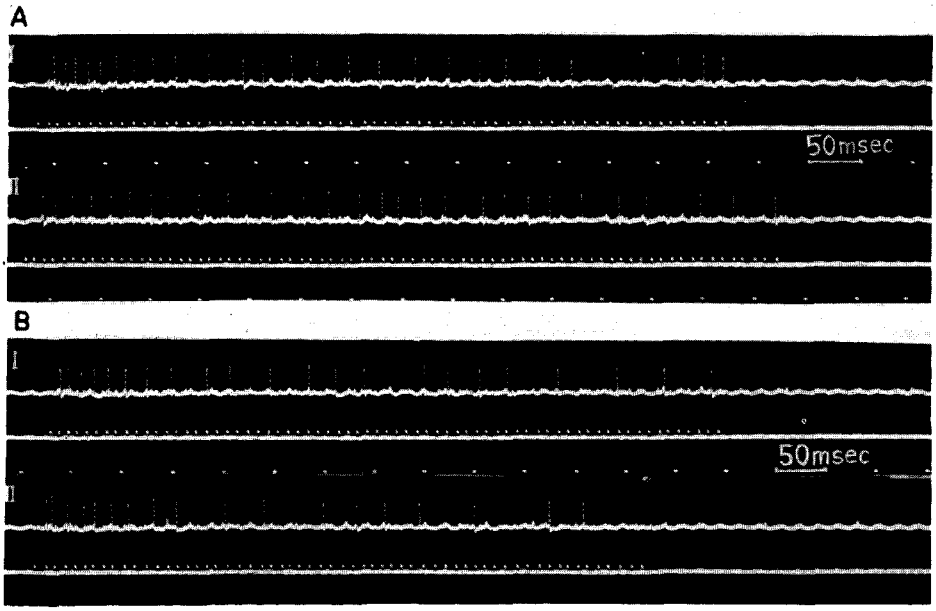

Fig. 5. A and B are two examples of the impulse-transmission through the ganglion, produced by repetitive stimuli. Each example consists of two responses to two series of stimulation, i.e. I and II show the responses to 1st and 2nd stimulation, respectively.

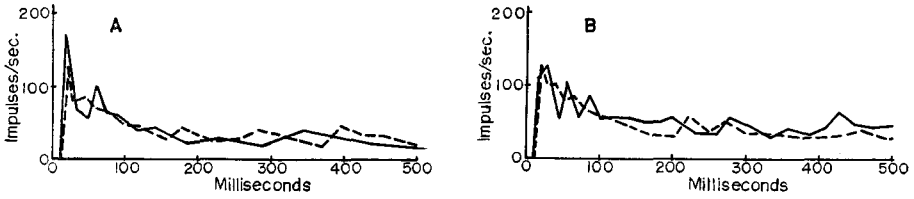

Fig. 6. The frequency change with time in the efferent impulse-trains which are shown in Fig. 5. *Abscissae* : Time from the onset of the stimulation. *Ordinates* : Frequency of the efferent impulses.

a single afferent volley, the surrounding medium of the ganglion was exchanged with the experimental solution by a device described above during intracellular recording. Height of the synaptic potential changed when the ionic concentration in the surrounding medium was varied as is shown in Fig. 7, where A and B are the potassium and calcium effect respectively ; the synaptic potential falls with increase in K-concentration and rises with increase in Ca-concentration. Since it is supposed that such changes in ionic concentration have influence not only upon the junctional region but also upon the pre- and post-synaptic nerve fibre, the

changes in the resting and action potentials of the pre-synaptic fibre and that in the height of synaptic potential were measured one after the other and the results are illustrated in the same figure. It is concluded that the height of the synaptic potential had a close parallel relation to the activity of the pre-synaptic nerve fibre at various K-concentration; on the other hand, the synaptic potential becomes higher with the increase in Ca-concentration in the surrounding medium, although there is no change in the pre-synaptic nerve activity.

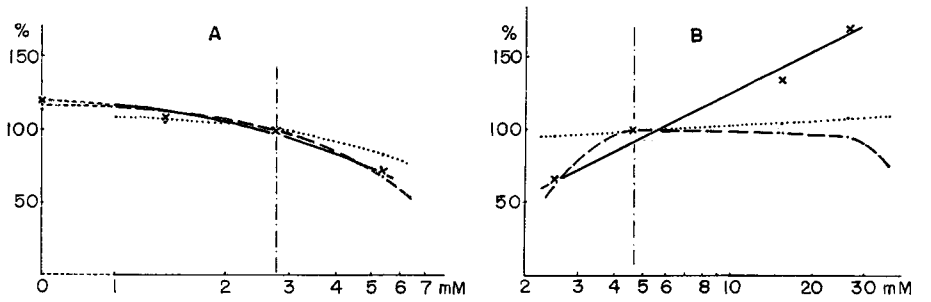

Fig. 7. Changes in the height of the post-synaptic potential (the solid line) when potassium (A) and calcium (B) concentrations in the surrounding medium were varied, and changes in the amplitude of the action potential (the broken line) and the resting potential (the dotted line) of the pre-synaptic nerve fibre. In each graph, the vertical broken line indicates the normal concentration of potassium (or calcium) ions in Harreveld's solution. *Abscisae*: Ionic concentration (mM) in the experimental solution. *Ordinates*: Ratio of the experimental value to the normal one (in percent).

Since the frequency of the post-synaptic spikes is varied with the height of the synaptic potential as is illustrated in Fig. 4, ionic effects on the ganglionic transmission of the nerve impulse train were studied in detail. In Fig. 8 and Fig. 9 the manner of change in the frequency of the impulses transmitted through the

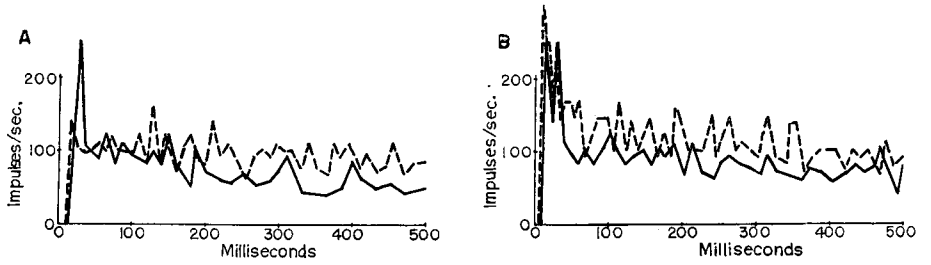

Fig. 8. Graphs show changes in frequency of the ganglionic responses when potassium concentration in Harreveld's solution is varied from 2.9 mM, i.e. normal concentration, to 5.4 mM (A) and 10.9 mM (B). In each graph, the responses in Harreveld's solution (broken line) and the experimental solution (solid line) are shown together. *Abscissae*: Time from the onset of the stimulation. *Ordinates*: Frequency of the efferent impulses.

ganglion are compared graphically between K- and Ca-excess solutions (solid line) and the normal Harreveld's solution (broken line); the change in the frequency of the post-synaptic impulses is in the same direction as that of the synaptic potential, both decreased with the increase in K-concentration but increased with the increase in Ca-concentration. However the relation between the ionic concentration and the impulse frequency transmitted was not so simple as the relation between ionic concentration and the synaptic potential. It may be partly owing to the complexity of the temporal pattern of the ganglionic response to the repetitive afferent stimulation.

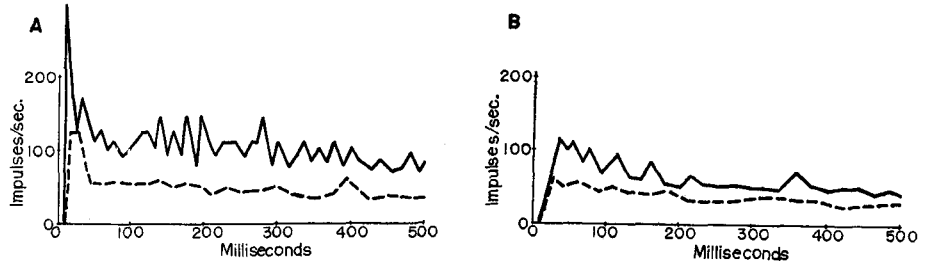

Fig. 9. Graphs show changes in the frequency of the ganglionic responses when calcium concentration in Harreveld's solution is varied from 4.8 mM, i.e. normal concentration, to 8.7 mM (A) and 15.9 mM (B). The other lines mean the same as those shown in Fig. 7.

Perfusion effect of the external medium: After long continuous stimulation, the ionic composition of the medium closely surrounding the preparation or of the fluid in the ganglionic tissue space may be changed as a result of excitation of the nervous tissue accompanied by the leakage of K-ions from nerve cells (Cowan, 1934; Hodgkin & Huxley, 1947; Keynes, 1951). Then the ganglionic transmission is observed during perfusion of the preparation with fresh Harreveld's solution in order to keep a constant ionic composition in the surrounding medium or in the fluid of the tissue space as is shown in Fig. 2. The typical responses of the perfused ganglion are illustrated in Fig. 10 together with the responses recorded while the ganglion was not perfused. The average curves for the decrease in frequency of the transmitted impulses were obtained by calculation from the observed values of the frequency using the least squares method. The frequency of the responses of the ganglion which was not perfused decreased more rapidly than that of the perfused ganglion. Consequently it may be considered that some substance which obstructs the ganglionic transmission accumulates in the surrounding medium or in the tissue space during the excitation of the nervous tissue and that it can be removed partially by the perfusion.

When Harreveld's solution surrounding the ganglion was replaced with raw egg white and the transmission of the nerve impulses through the ganglion was

observed, a complete conduction block occurred through the ganglion as the result. The immersion of the nerve fibre in egg white did not induce any appreciable change in volume as it did in the muscle fibre (Tamasige, 1951), but the ganglion never recovered from the conduction block after the immersion in egg white for over a few ten seconds.

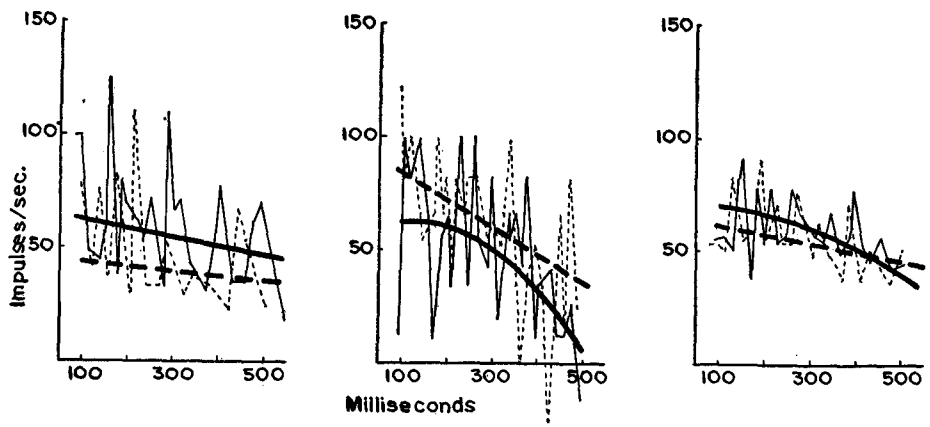

Fig. 10. Changes in rate of fatigue in the ganglionic responses when the preparation was perfused (broken line) and not perfused (solid line) with Harreveld's solution. The thin and thick lines indicate respectively the observed values of the frequency of the efferent impulses and the mean values calculated by the least squares method from them.

Discussion

In the neuromuscular junction the amount of transmitter substance liberated from the nerve ending depends upon Ca-concentration in the surrounding medium (del Castillo & Stark, 1952) and also it has been considered that the neuronal junction is in a similar situation (Brink, 1954). In the present experiment, dependance of the height of the synaptic potential upon the Ca-concentration may be attributable to the same phenomena. On the other hand in a medium of altered K-concentration, the nerve impulse is modified in amplitude before its arrival at the nerve ending, and consequently the height of the synaptic potential varies, because it is in close parallelism with the presynaptic activity in response to the afferent volley; thus excess potassium in the external medium depresses the height of the synaptic potential although the mechanism differes from that of excess calcium.

In order to analyse the temporal pattern of the efferent impulses, it is necessary to know ionic effects upon the post-synaptic region where the propagating action potentials are set up, because the change in the firing level of the post-

synaptic spike has equal significance to the change in height of the synaptic potential. Therefore the firing levels in several experimental solutions were accurately measured, but the membrane potential of the post-synaptic region could not be accurately determined because of irregular fluctuation of the potential level caused by the unstable condition of the microelectrode insertion. But the resting potential of the post-synaptic membrane could be estimated as about 50 mV, which is less than that of the nerve fibre; this may indicate that the synaptic region is under specific conditions as described by Bullock (1957). No appreciable change could be observed in the resting potential of post-synaptic membrane by alteration of K-concentration in the external medium.

The lower resting potential of the post-synaptic membrane than that of the post-synaptic axon membrane may be attributed to the difference in permeability between the membranes and to the accumulation of the potassium ions which leaked out of the activated synaptic region. It is probable under such conditions that excess potassium added to the surrounding medium does not decrease further the resting potential of the synaptic membrane, while excess calcium in the external medium does not produce a marked change in the resting membrane potential for it has the antagonistic effect against excess potassium ions as the permeability increaser. And also there was no detectable change in the firing level of the post-synaptic spike. In other words the threshold of excitation of the post-synaptic axon membrane did not change with alternation of potassium and calcium concentration in the surrounding medium, unlike that of the presynaptic axon membrane or of the muscle fibre membrane as described by Tamasige (1951) and Jenerick & Gerard (1953). At the neuronal junction, the fall of the synaptic potential induced by excess K or Ca deficiency resulted thus in the decrease in frequency of the post-synaptic spikes.

When the frequency of the stimulation is relatively higher (at 100/sec.), the synaptic potentials may gradually decline as the result of the accumulated potassium ions leaking out of the excited nervous tissue, because the amount of potassium ions leaking from the junctional region during evokation of the synaptic potential is larger than that of the axon during activity of the nerve fibre (Eccles, 1957). Consequently it is considered that, if the excess potassium ions are removed from the external medium by perfusion, the rate of fatigue of the ganglionic response becomes smaller; while in the egg white the fatigue is very rapid because the potassium ions leaking out of the excited ganglion can not diffuse into the surrounding protein of high molecules structure, and the potassium content in the egg white is more than that of Harreveld's solution.

Summary

The effects of potassium and calcium concentration in the surrounding medium upon the ganglionic transmission of the nerve impulses were studied.

1. The temporal summation of the synaptic responses set up by two afferent volleys was observed. When the volley interval was less than about 20 msec., the height of the synaptic potential was increased, or the frequency of the post-synaptic spikes increased if the synaptic potential had risen above the firing level.

2. Change in potassium concentration effected the amplitude of the presynaptic volley and the height of the synaptic potential; these as well as the frequency of the post-synaptic spikes decreased with increase in K-concentration.

3. Change in calcium concentration had an effect upon the height of the synaptic potential even though it had no effect upon the post-synaptic nerve fibre. The height of the synaptic potential, and the frequency of the post-synaptic spikes, increased with increase in the Ca-concentration.

4. Fatigue of the ganglionic response was prevented by perfusion of the preparation with Harreveld's solution but it became very rapid after replacement of Harreveld's solution with the egg white. Probably the fatigue was a result of the excess potassium leaking out of the excited nervous tissue.

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