# THE EFFECT OF REMOVAL OF THE PANCREAS AND ADMINISTRATION OF ACETYLCHOLINE ON THE PERIPHERAL DIVISIONS OF THE REFLEX ARC IN COLD-BLOODED ANIMALS

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The pancreas plays an important role in the activity of the body. The evidence which we have goes to show that, besides its well-known importance in carbohydrate metabolism, it takes part in phospholipid metabolism [7, 11, 12] and in the associated acetylcholine metabolism [5].

It was shown in previous research [1, 2, 3] that interference with acetylcholine metabolism by means of removal of the pancreas causes a diminution of some of the functional properties of the neuromuscular apparatus. Administration of pharmacological acetylcholine prevented the development of these changes. These experiments were performed exclusively with the use of myographic recording.

In the present communication we give the results of electrophysiological investigations of the common nerve trunk, the myoneural junction and the proprioceptors of the skeletal muscles of the frog after removal of the pancreas and administration of small doses of acetylcholine to the animal after operation.

## EXPERIMENTAL METHOD

The research was carried out on a nerve-muscle preparation of the gastrocnemius muscle and the sciatic nerve, and on the sciatic-tibial nerve trunk of the frog (Rana ridibunda) on the 5th-9th day after pancreatectomy. Acetylcholine was injected daily, starting on the 3rd day after operation, into the posterior lymph sac (0.5 ml of a 1:10,000 solution). Stimulation of the motor nerve was effected by means of an electronic device. The action currents of the nerve and muscle were recorded on a two-channel cathode oscillograph. Altogether 185 experiments were performed, including 110 to investigate the nerve trunk, 25 the myoneural junction and 50 the proprioceptors. In a series of experiments all the investigations were carried out on a single nerve-muscle preparation. In these cases the proprioceptors were studied first, followed by the myoneural junction and lastly, by the nerve trunk.

## EXPERIMENTAL RESULTS

After removal of the pancreas the following changes were observed in the activity of the above-mentioned elements of the somatic nervous system.

a) Investigations on the nerve trunk. The development and spread of a wave of excitation in the nerve were preserved for different lengths of time after the operation. The degree of excitation was altered insignificantly and irregularly. In some cases an increase in the excitation of the nerve trunk was also observed. The velocity of spread of the waves of excitation in the most rapidly conducting fibers (A,$\alpha$) fell slightly, and on the 6th-9th day after operation its average value was 28.5 m/sec instead of 32 m/sec in the animals not subjected to operation (Fig. 1 a, b). The velocity of conduction of excitation in the other groups of nerve fibers

was not determined, but drawing out and splitting up of the action currents were systematically observed (Fig. 1, b), demonstrating that the velocity of spread of excitation in the more slowly conducting fibers ($\beta$) had decreased to an even greater extent.

The optimal rate of excitation, determined by the lowest frequency causing a reduction in the action currents of the nerve [6], fell to 50-70 per second (normal 80-150 per second). The limits of transformation of the action currents (maximum rhythm) were also displaced toward lower frequencies and had values of 210-270 per second (normal 400-450 per second) (Fig. 1 c, d).

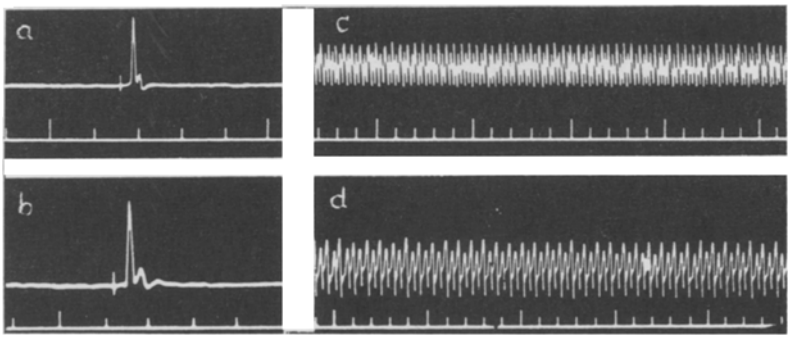


Fig. 1. Action currents in the sciatic nerve of the frog. Velocity of spread of the impulse in a normal frog (a) and in a frog on which the operation was performed 7 days before the experiment (b). Limits of transformation (alternating rhythm) in a normal frog (c) and in a frog undergoing operation 7 days before the experiment (d). Frequency of stimulation 550 and 270 per second respectively. Time marker 0.01 second.

A decrease thus took place in the lability of the nerve trunk when determined both at the optimum and the maximum frequency of stimulation. As has been shown by myographic and electrophysiological investigations of single tetanic contractions [4, 9] carried out under similar conditions, this decrease in lability was associated with a strengthening and drawing out of the subsequent processes.

b) Investigations on the motor nerve endings. Direct determination of the lability of the myoneural junction by an electrophysiological method showed that transformation and a lowering of the amplitude of the action currents of the muscle were observed even at a frequency of 35-50 stimuli per second. Complete disappearance of the electrical activity (pessimum) took place at frequencies of stimulation of 60-80 instead of 100-110 per second in the unoperated animals. The stability of the myoneural junction was sharply reduced. In response to tetanic stimulation of the motor nerve at a frequency of 35 per second, the action currents of the muscle completely disappeared after only 5-15 seconds of stimulation (in the unoperated animals after 40 seconds and over). These findings of a decrease in the lability of the myoneural junction after removal of the pancreas confirmed the results of experiments on warm-blooded animals previously described by us [3].

c) Investigations on the proprioceptors. The degree of excitation of the proprioceptors, determined by the magnitude of the stretching of the muscle to cause minimum electrical activity of the nerve connected with the muscle, fell slightly (average value in normal conditions 3.3 g, after removal of the pancreas 4.2 g). The frequency of impulsation depended on the load, and when this was twice the threshold weight the average frequency was 108 impulses per second (120 in the unoperated frogs), and in response to a standard 100 gram stretching, on the average 191 impulses per second (232 in the unoperated frogs). A fall in the adaptation of the receptors was also observed. The frequency of impulsation at the end of the first minute of stretching of the muscle in response to twice the threshold load was 31.8% of the initial value, and in response to a standard 100 gram load, 84.6% (in the unoperated animals 27.7 and 75% respectively). The course of adaptation was altered: in the unoperated frogs the greatest fall in the frequency of impulsation was observed in the first 10 seconds of stretching, but after removal of the pancreas, at 30 seconds (Fig. 2 a, b).

d) The effect of acetylcholine. The systematic injection of small doses of acetylcholine to the animals after operation led to normalization of the functional state of the neuromuscular apparatus. Under these circumstances an obvious fall in the excitation of the nerve trunk was observed. Experiments carried out at the same

time (in February) showed that after removal of the pancreas the degree of excitation corresponded, on the average, to 0.5 of a scale division of the stimulator, and after the animal had received an injection of acetylcholine, to 1.4 divisions. An increase also took place in the resistance of the nerve to alteration and in the optimum and maximum frequencies to 100-150 and 300-400 stimuli per second respectively. At the same time a diminution of the aftereffects was observed, as shown by disappearance of the ability to develop single tetanic responses.

The resistance of the myoneural junction to tetanic stimulation was increased. The limits of the pessimum and of the disappearance of the action currents of the muscle were displaced toward higher frequencies. The adaptation of the proprioceptors was accelerated, its value at the end of the first minute of stretching by twice the threshold load being 23%, and by a 100-gram load 72% of the original frequency. The adaptation curve acquired the typical character of the unoperated animals (Fig. 2, c). Thus after removal of the pancreas, the character of the proprioceptive impulsation became close to the impulsation of muscles in tetany, and after injection of acetylcholine to the impulsation of the proprioceptors of tonic muscles [8].

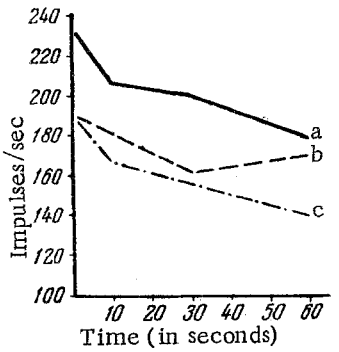


Fig. 2. Curves of adaptation of the proprioceptors of the gastrocnemius muscle of the frog. a) Unoperated; b) after removal of the pancreas; c) in response to the compensatory injection of acetylcholine to the operated animal. Along the abscissa — time of stretching, in seconds, along the ordinate axis — frequency of impulsation. Load 100 g.

It must be pointed out, however, that clear differences were observed in the reaction of the elements of the reflex arc to administration of acetylcholine. Whereas in the nerve trunk, the injected acetylcholine behaved almost exclusively as a factor enhancing its functional state, overdosage of the drug had a pessimal effect on the nerve endings.

The results obtained showed that removal of the pancreas affected the functions of the various elements of the somatic nervous system to a different degree. It caused more substantial changes in the nerve and myoneural junction, and less so in the receptor apparatus of the skeletal muscles.

The electrophysiological investigations carried out provided direct proof of the fact that after removal of the pancreas the waves of excitation in the elements of the somatic nervous system became drawn out, and a stagnating parabiotic process developed in them, characterized by diminished lability, unchanged or even increased excitation, decreased velocity of conduction of the nerve impulse and lowered adaptation of the receptors. The systematic administration of acetylcholine to animals after operation produced an increase in the functional properties and normalization of the functional state of the nerve tissue.

The results obtained confirmed A. A. Ukhtomskii's hypothesis [10] that in the somatic nervous system acetylcholine is a regulator of the functional state of the nerve tissue, and can thus be regarded as a trophic factor of the somatic nervous system [1].

## SUMMARY

Preliminary (several days before the experiments) removal of the pancreas in frogs caused a certain decrease in the rate of the excitation spread and reduction of the nerve stem lability. Both the lability and the stability of the myoneural connection are decreased as well as the excitability, the frequency of impulsation and adaptation of the proprioceptors of the neuromuscular appratus. Systematic administration of acetylcholine to the operated animals prevents the development of the mentioned changes in the preparation.

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