ON THE KINETICS OF THE CHOLINESTERASE OF FROG SMOOTH MUSCLE ORGANS AFTER REMOVAL OF THE PANCREAS

L. D. Firer and Kh. S. Khamitov

From the Department of Normal Physiology, Kazan Medical Institute

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As the numerous studies of A. V. Kibyakov and his colleagues have shown [3-8, 10], the removal of the pancreas in experimental conditions disturbs the influence of the parasympathetic nerves on the organs they innervate, changes the functional condition of somatic innervation in both its central and peripheral apparatuses and causes the disappearance of the so-called "spontaneous" contractions of the smooth muscle organs. These changes are connected with disturbances in the process of acetylcholine formation, since research by S. M. Leites [9] and others has shown that the pancreas plays an important role in the regulation of fat metabolism in the body, including phospholipid metabolism. The phospholipid metabolism is also closely connected with the process of acetylcholine synthesis. However, exactly how phospholipid metabolism disturbances affect the liberation of the mediator has not yet been completely investigated.

It seemed interesting to investigate the interrelations of the mediator, or acetylcholine, with the enzymatically-active systems and also the change of these interrelations when the phospholipid metabolism is disturbed by removal of the pancreas. In this article, we present experimental data from investigation of the fluctuations of cholinesterase activity in the smooth muscles of normal and depancreatized frogs.

From the literature data, we know that there are definite correlations between acetylcholine and cholinesterase. D. E. Alpern [1] shows that intensified acetylcholine synthesis is attended by an increase in cholinesterase splitting activity. The compensation condition is characterized by such a correlation. Along with this, humoral decompensation can also occur when the activity of cholinesterase is sharply reduced under the influence of strong stimulation of the vegetative nervous system parasympathetic branch and due to the liberation of a large amount of acetylcholine. N. F. Baranova and E. N. Speranskaya [2] also observed the decrease of cholinesterase with stimulation of parasympathetic nerve fibers. B. Mendel and H. Rudney [12] show that cholinesterase is inactivated by high concentrations of acetylcholine and gives maximal activity with low concentrations of acetylcholine.

EXPERIMENTAL METHODS AND RESULTS

Cholinesterase activity was determined by the titrimetric method described by T. V. Pravdich-Neminskaya [11]. According to this method, the acetic acid which forms, under the conditions defined by the method, from the enzymatic decomposition of acetylcholine is back-titrated by a 0.01 N solution of NaOH in the presence of an indicator – a 0.02% solution of cresol red. The percentage of acetylcholine destroyed in a unit of time is calculated, which is taken as the index of enzymatic activity. Titration was done with a solution of NaOH from a microburet with a total capacity of 0.6 ml with divisions every 0.002 ml. As the experimental objects in our experiments, we used: the muscular layer of the stomach, abdominal aorta tissue, and lung and bladder parenchyma. The femoral muscle of a frog was used for purposes of comparison. The tissues were ground in a mortar with a small amount of glass sand and centrifuged in a Ringer's solution at a rotation rate of 2000 revolutions. The centrifugate was used in the experiments.

In the first series of experiments, we determined cholinesterase activity in the smooth muscle organs and in the skeletal femoral muscle of normal frogs which had not been operated upon. These studies established that, during the fall-winter period, the cholinesterase activity in the smooth muscle organs of the frog ranged between: 3.00-6.28% for the stomach, 3.19-6.37% for the abdominal aorta, 3.00-5.82% for the bladder, 3.78-7.65% for the lung parenchyma. Cholinesterase activity increased sharply in the spring period, beginning with the second half of March, returning to the normal original levels in the middle of April. The maximal cholinesterase activity for the stomach muscles was 14.56%, for the abdominal aorta, 13.65%, for the lung parenchyma, 11.83% (Fig. 1) and for the bladder, 9.46%. The activity of the skeletal femoral muscle was stable (3.27-4.10%) in comparison with the smooth muscle organs and increased slightly towards the end of March (9.37%).

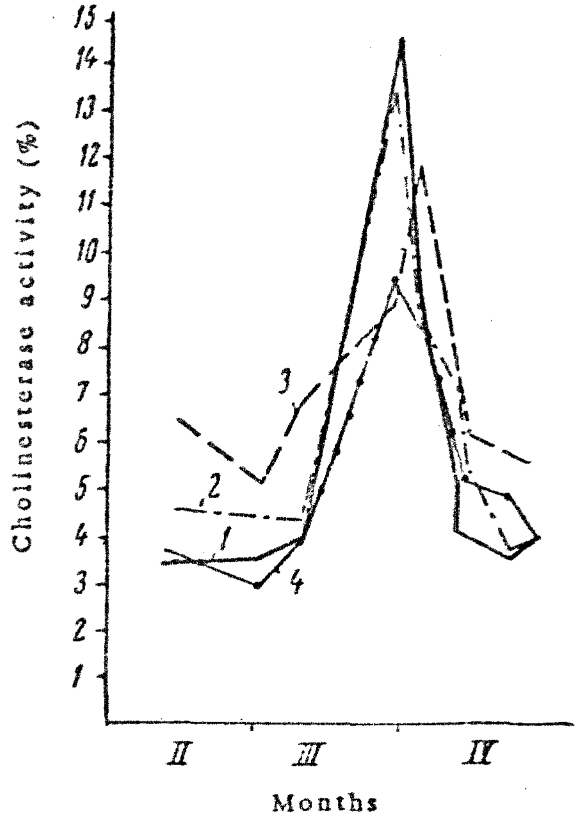

Fig. 1. Dynamics of changes in the cholinesterase activity of the smooth muscle organs of a frog at different months of the year.
1) muscular layer of the stomach; 2) abdominal aorta; 3) lung parenchyma; 4) bladder.

In a series of experiments, we injected 3-4 ml of eserine in a concentration of 1 : 10,000 into the subcutaneous posterior lymph sac of spring frogs (60 minutes before the experiment). The eserine injection caused a sharp decrease in cholinesterase activity, which ranged between the following limits: 2.21-5.73% for the stomach muscles, 2.28-6.37% for the abdominal aorta, 2.91-7.01% for the bladder, 3.00-7.19% for the lung parenchyma, and 3.55-6.55% for the skeletal muscle. Consequently, eserine preliminarily injected into the body of a spring frog lowered cholinesterase activity considerably; its magnitude under these conditions approximated the original, normal level, or was even somewhat less than the cholinesterase activity in the frogs of the fall-winter period.

The experiments described above make it possible to suggest the presence of seasonal fluctuations in the cholinesterase activity, which places it in direct dependence on the physiological phenomena which occur in the frog's body during the spring period after the winter hibernation.

In a second series of experiments, the pancreas was first removed from the frogs by operation. The experiments were done on frogs of the fall-winter period on the 2nd-14th day after the operation. It was established that cholinesterase activity in the depancreatized frogs gradually increased, beginning the second day after the operation. The cholinesterase activity of the stomach muscles and lung parenchyma was observed to increase until the 9th day after the operation, achieving a magnitude of 9.01-9.10%. Then the activity decreased and, after the 13th post-operative day, was reduced to the original normal range (Fig. 2, a, c). The change in the cholinesterase activity of the abdominal aorta was characterized by two sharp increases on the 3rd and 10th days after the operation (7.87% and 14.92%). After this, the activity decreased and only began to be reestablished after the 13th post-operative day (Fig. 2, b). A slower increase in cholinesterase activity was observed in the bladder tissues; by the 8th day after the operation, its magnitude equaled 6%, then the activity decreased and subsequently returned to the normal level (Fig. 2, d). A slight increase over normal cholinesterase activity (3.27-4.10%) was observed in the skeletal musculature (4.76%).

In order to prove that the changed cholinesterase activity in the organs of the depancreatized frogs was actually connected with the reduced amount of acetylcholine forming in the body, we conducted a third series of experiments in which depancreatized frogs were given a compensatory injection of pharmacological acetylcholine. The experiments, which were conducted on the days in which the greatest changes in cholinesterase

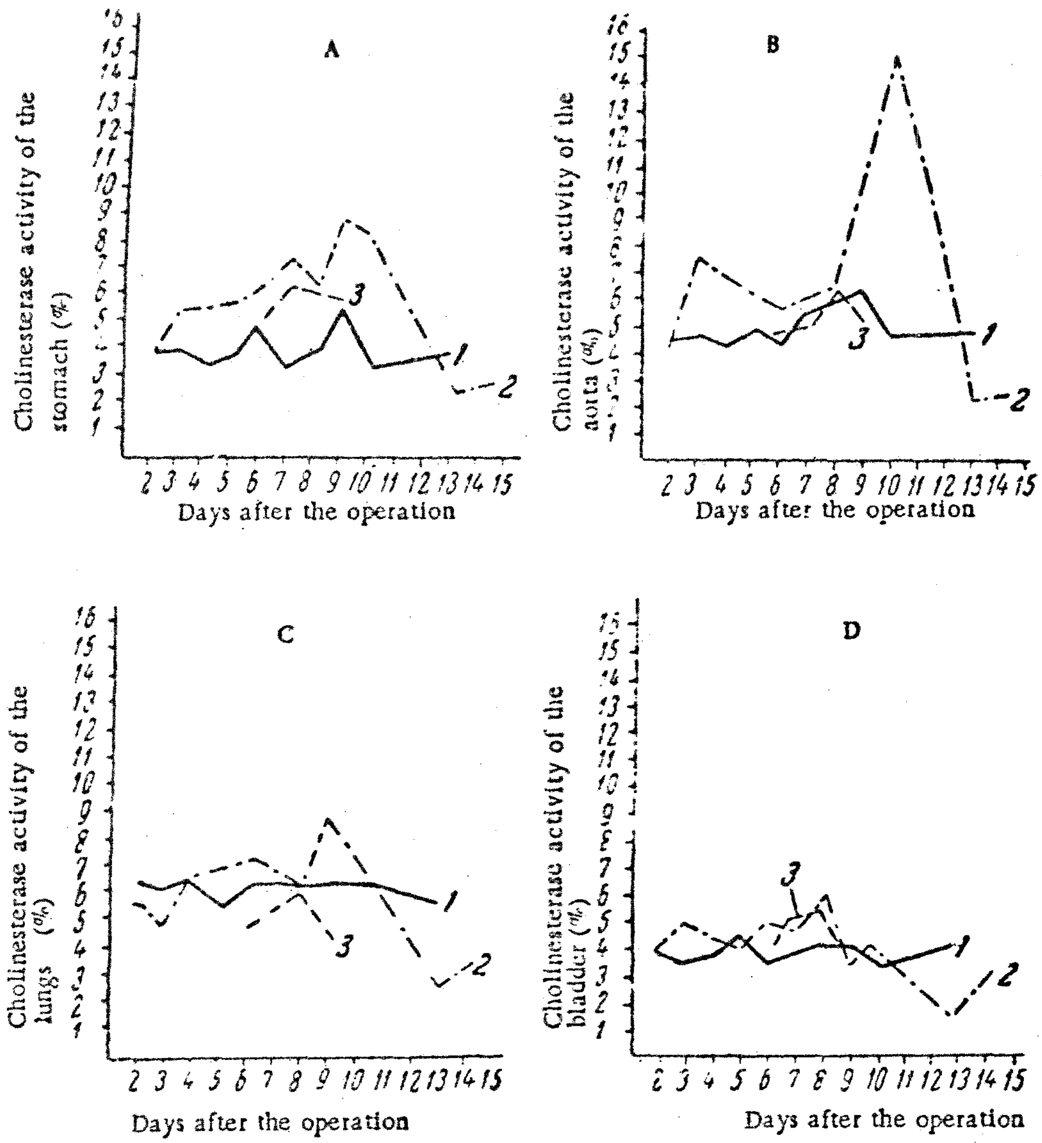

Fig. 2. Dynamics of changes in cholinesterase activity of smooth muscled organs of control frogs which had not been operated upon (1), of depancreatized frogs (2) and of depancreatized frogs which had been given a compensatory injection of acetylcholine (3).

content occurred (6th-9th days), showed that cholinesterase activity was almost normal or even slightly less than normal (see Fig. 2, a, b, c, d).

Our observations indicate that the removal of the pancreas in frogs causes considerable changes involving the increase of cholinesterase activity in the tissues. These changes in cholinesterase activity coincide in time with the disturbances of parasympathetic and somatic innervation which have been observed in many of our laboratory's works. From the experimental data obtained, we can conclude that, with the inadequate hormonal functioning of the pancreas, an increase is observed in cholinesterase activity which is probably the essential feature in the disturbed activity of the parasympathetic and somatic nervous systems which occurs in the body of depancreatized animals.

SUMMARY

Experimental data of the study of cholinesterase activity in the smooth muscles of normal and depancreatized frogs are presented. Variations in the activity of cholinesterase of smooth muscle organs of frogs are insignificant during the autumn-winter season. However, the activity of cholinesterase considerably increases during the spring season (March-April).

Increased activity of cholinesterase is, likewise, observed in depancreatized frogs. It coincides with the time of derangement of the parasympathetic influence on the organs and tissues during the postoperative period. Compensatory introduction of acetylcholine to operated frogs prevents the increase of cholinesterase activity.

It is probable that increased cholinesterase activity in tissues and organs of frogs is one of the factors of parasymphathetic disturbance in depancreatized frogs.

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