

ROLE OF THE ACETYLCHOLINE - CHOLINESTERASE SYSTEM
IN THE SPONTANEOUS RHYTHMICAL ACTIVITY OF THE AMNION
OF THE CHICK EMBRYO

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In the modern view the transmission of excitation from nerve to muscle is effected through the medium of the acetylcholine-cholinesterase system. Meanwhile, comparative physiological investigations have shown that acetylcholine and cholinesterase may be present in structures possessing neither nerve cells nor nerve endings. For instance, cholinesterase has been found [1, 6, 12] in the developing embryos of various animals (fishes, amphibians, birds) long before the development of their neural tube and neural plate. Acetylcholine and cholinesterase have been found [5, 7, 13] in infusorians, and it has been shown that this enzyme plays a part in the motor activity of these organisms. These findings demonstrate that the acetylcholine-cholinesterase system is characteristic not only of nervous elements, but also of other structures, and they give grounds for the suggestion that acetylcholine may also take part in the performance of spontaneous rhythmical contractions.

We were interested to study the role of the acetylcholine-cholinesterase system in the spontaneous contractile activity taking place without the participation of nervous elements. An object of this category in the vertebrates is the amniotic sac of the chick embryo. As histological investigations have shown, this contains absolutely no nervous elements. The rhythmical contractions of this structure can therefore be attributed entirely to a myogenic mechanism.

In investigations carried out in 1954 [10], cholinesterase was found in the amnion, and on this basis it was suggested that acetylcholine may also be present.

In our previous investigation we showed that the contractile activity of the amnion changes constantly and is dependent on the development of the embryo. From the 5th until the 14th day of incubation it appears, reaches a maximum, and then disappears; this process is accompanied by changes in the smooth-muscle elements of the amnion. This phenomenon may evidently explain the regular change in the frequency of the contractions during the spontaneous rhythmical activity of the amnion [2].

Kh. S. Koshtoyants put forward the suggestion that the acetylcholine-cholinesterase system plays a part in the mechanism of the spontaneous rhythmical activity of the amnion. The present research was carried out to test this hypothesis.

EXPERIMENTAL METHOD

In order to detect changes in acetylcholine synthesis during the period of development of amniotic motor activity, the acetylcholine concentration in the amnion tissue was determined by the technique of Loewi and Hellauer [11].

A weighed sample of tissue (10-50 mg) was ground with eserinized Ringer's solution and boiled, and before determination on the isolated eserinized frog's heart it was diluted $2 \cdot 10^6$ times, after which the concentration of acetylcholine was calculated in micrograms per 100 mg of tissue.

The cholinesterase activity was determined by the method of Augustinsson and Hestrin [4, 8]. For this we used a weighed sample of muscle tissue from the amnion in the same amount. This was ground on ice, 200 μ g of acetylcholine was added to it, and the mixture was incubated at 37° for 1 h. After incubation, 0.1 ml of a solution of trichloroacetic acid was added to the mixture, which was then centrifuged for 10-15 min at a speed of 4000-5000 rpm. The cholinesterase activity was expressed as the weight of acetylcholine decomposed by a 100 mg sample of the test tissue in the course of 1 h.

In view of modern ideas concerning the role of SH-groups in the synthesis of acetylcholine, we attempted to study the changes in their content in amniotic muscle tissue at the same periods of incubation. The content of SH-groups was determined by amperimetric titration, using the technique of Kolthoff, Stricks,

Changes in the Acetylcholine Concentration in the Amnion Tissue of the Chick Embryo during Development from the 5th to the 13th Day of Incubation

Day of incubation	Wt. of tissue (mg)	Concentration of acetylcholine obtained in experiment	Amt. of acetylcholine in tissue sample	Wt. of acetylcholine in 100 mg tissue (μg)
5th	8,5	$1 \cdot 10^{-13}$	0,0000001	2
	12	$1 \cdot 10^{-13}$	0,0000001	2
	11	$1 \cdot 10^{-13}$	0,0000001	2
	11	$1 \cdot 10^{-13}$	0,0000001	2
6th	8	$1 \cdot 10^{-13}$	0,0000001	2
	9	$1 \cdot 10^{-13}$	0,0000001	2
	8	$1 \cdot 10^{-13}$	0,0000001	2
	11	$1 \cdot 10^{-13}$	0,0000001	1
7th	16	$1 \cdot 10^{-12}$	0,000001	10
	16	$1 \cdot 10^{-12}$	0,000001	10
	20	$1 \cdot 10^{-11}$	0,00001	100
	15	$1 \cdot 10^{-11}$	0,00001	100
8th	20	$1 \cdot 10^{-11}$	0,000001	100
	15	$1 \cdot 10^{-13}$	0,0000001	1
	20	$1 \cdot 10^{-11}$	0,00001	100
	18	$1 \cdot 10^{-11}$	0,00001	100
	18	$1 \cdot 10^{-11}$	0,00001	100
	20	$1 \cdot 10^{-11}$	0,00001	100
	20	$1 \cdot 10^{-11}$	0,00001	100
	20	$1 \cdot 10^{-11}$	0,00001	100
10th	30	$1 \cdot 10^{-13}$	0,0000001	1
	25	$1 \cdot 10^{-13}$	0,0000001	1
	40	$1 \cdot 10^{-13}$	0,0000001	0,4
	25	$1 \cdot 10^{-13}$	0,0000001	1
	29	$1 \cdot 10^{-13}$	0,0000001	1
13th	50	$1 \cdot 10^{-13}$	0,0000001	0,4
	50	$1 \cdot 10^{-13}$	0,0000001	0,4
	45	$1 \cdot 10^{-13}$	0,0000001	0,4
	49	$1 \cdot 10^{-13}$	0,0000001	0,4

and Morren, as improved by Turpaev and Nistratova [3]. The result was calculated per 100 mg of tissue. The points on the curve (as in the determination of the cholinesterase activity) are mean values of all the experiments carried out during a 24 h incubation period.

EXPERIMENTAL RESULTS

The results of the first series of experiments to determine the acetylcholine in the amnion using the isolated frog's heart are given in the table. This shows that the acetylcholine level in the amnion on the 5th-6th day of incubation was very low, it began to rise on the 7th day, reached its maximum on the 8th day, and then began to fall again. On the 13th day of incubation the acetylcholine content of the amnion reached its lowest level.

In the second series of experiments we investigated the cholinesterase activity (Fig. 1). On the 5th day of incubation it was maximal, and during the subsequent development of the embryo it fell sharply, reaching 25% of its initial value on the 8th day. No further decrease in cholinesterase activity took place after the 8th day of incubation; it remained at the same level (8th-10th day) or actually rose slightly (11th-12th day).

The results of the experiments to determine the content of sulfhydryl (SH-) groups are given in Fig. 2. The curve shows the changes in the content of SH-groups in 100 mg of tissue in the course of a given period of development (from the 5th until the 13th day). The highest content of SH-groups in the tissue was observed on the 5th day of incubation, after which it fell gradually until the 8th day, when it fell particularly sharply (by 50% of its initial value). During the subsequent period of development the content of SH-groups fell more gently: from the 8th to the 13th day it fell by approximately a further 25%.

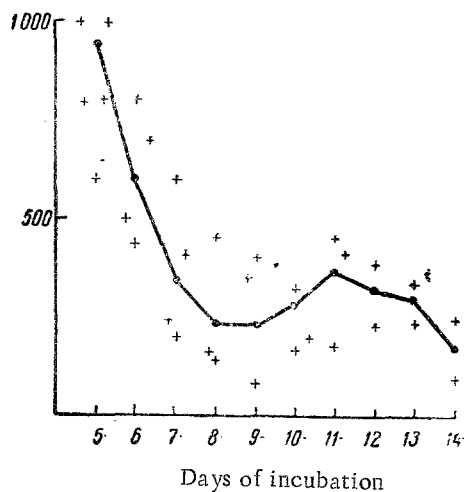


Fig. 1. Changes in the cholinesterase activity in the amnion of the chick embryo (in micrograms per 100 mg tissue per hour).

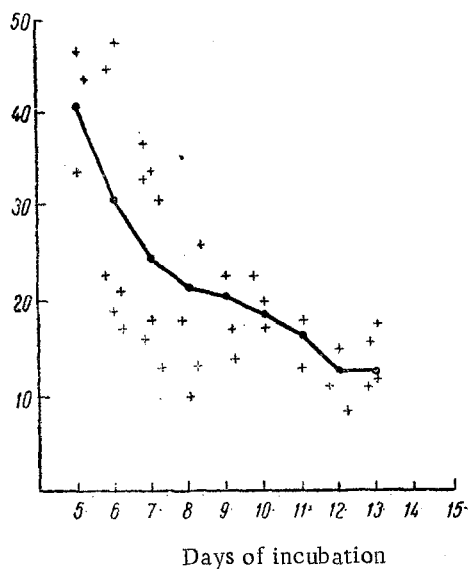


Fig. 2. Changes in the content of SH-groups in the amnion of the chick embryo (in micrograms per 100 mg of tissue).

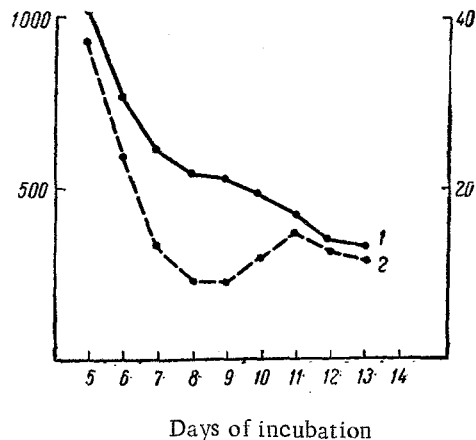


Fig. 3. Parallel changes in the content of SH-groups (1) and the cholinesterase activity (2) in the amnion of the chick embryo.

The results of these experiments revealed characteristic changes in the acetylcholine-cholinesterase system taking place in the amnion at different stages of its development. For instance, the amount of acetylcholine found in the amnion on the 5th day of incubation was small, corresponding to its high content of cholinesterase. In the process of development of the amnion, the content of acetylcholine rose, and this was accompanied by a decrease in the cholinesterase activity. On the 8th day of incubation the highest acetylcholine content and the lowest cholinesterase activity in the tissue were found. In the course of subsequent development there was a decrease in the acetylcholine content and a slight increase in the cholinesterase activity. On the 13th day of incubation a very low cholinesterase activity and a very small acetylcholine content were found in the amnion tissue.

Analysis of the curve of the changes in the SH-groups from the 5th until the 13th day of incubation shows that their total content fell continuously. During the first three days of development this decrease in SH-groups, like the fall in cholinesterase activity, was steepest, and thereafter it was more gradual, whereas the cholinesterase activity rose slightly. From the 11th to the 13th day of incubation the changes in these indices again followed a parallel course. Comparison of the curves (Fig. 3) suggests the existence of correlative relationships between the changes in cholinesterase activity and the content of SH-groups at corresponding periods of development.

We have previously shown [2] that during the period of development of the embryo from the 6th until the 14th day the rhythmical activity of the amnion undergoes regular changes in the frequency and strength of its contractions. Hence it was natural to suggest that these changes were based on biochemical processes closely associated with excitation and, consequently, with changes in the acetylcholine content of the tissue. Comparison of the results confirms that the frequency of the amniotic contractions and the acetylcholine content of the tissue changed in a parallel manner during the period from the 5th to the 13th day of incubation. This demonstrates that the acetylcholine-cholinesterase system plays a part in the production of the spontaneous rhythmical contractions of the amnion.

SUMMARY

A biphasic change was seen to occur in the acetylcholine content of the amnion muscular tissue during the development of the chick embryo (5th - 13th incubation days). From the 5th to the 8th day the amount of acetylcholine exhibited a considerable rise; from the 8th to 13th day a marked reduction of the acetylcholine content was revealed in the tissue. Within the same period the cholinesterase activity is also seen to change, showing a marked reduction from the 5th to the 8th day, a slight rise on the 11th day, this being followed again by a drop.

The change of the acetylcholine content and of the cholinesterase activity is accompanied with reduction of the SH-group concentration in the tissue; a sharp one from the 5th to the 8th day, then becoming smoother from

the 8th to 13th day. It is pointed out that the changes in the acetylcholine—cholinesterase system are linked with those in the frequency of spontaneous rhythmical contractions of the amnion.

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