# EFFECT OF ANTICARDIAL CYTOTOXIC SERUM IN VITRO ON AURICULAR ELECTRICAL ACTIVITY IN GUINEA PIGS

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Experiments on isolated guinea pig hearts perfused with Ringer-Locke solution showed that treatment with anticardial cytotoxic serum (ACS) produced marked changes in auricular electrical activity which follow a certain pattern. The initial, stimulant effect of ACS is reflected in sinus tachycardia, an increased degree of arrhythmia, and the appearance of extrasystoles. In the second stage auricular activity is inhibited, as reflected by bradycardia, parasystole, a reduced amplitude of the P wave and, in some cases, auricular arrest. The effects of ACS were partly abolished by rinsing the preparation with Ringer–Locke solution. This was evidently because ACS acts through biologically active substances or on account of a very loose attachment of the antibody to the tissue antigen.

The investigation described below is part of a series of studies of the cytotoxic action of antibodies on excitable cell membranes conducted at the A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR [1].

This paper examines the changes in electrical activity of the auricles produced by the action of anticardial cytotoxic serum (ACS) using an extracardial recording system.

## EXPERIMENTAL METHOD

Isolated guinea pigs' hearts were perfused by Langendors's method with Ringer–Locke solution heated to 37°C. The heart was placed in a transparent plastic receiver filled with perfusion fluid escaping from the chambers of the heart. To keep the volume of perfusion fluid in the receiver constant, an outflow tube was fitted at the level of the base of the heart, through which the solution escaped at a rate of one drop per second. Stable cardiac activity was first produced by perfusing the heart for the first 15-20 min with original Ringer–Locke solution. The spontaneous electrical activity was then recorded, and 1 ml antiserum was then slowly injected into the flow of perfusion fluid.

TABLE 1. Time of Manifestation of Action of Antiserum on Auricular Electrical Activity (min)

| Initial | | | Rate changes | | Initial | | | Rate changes | |
|---|---|---|---|---|---|---|---|---|---|
| quickening of rhythm | increase in arrhythmia index | decrease in amplitude of P wave | development of bradycardia | repeated increase in arrhythmia index | quickening of rhythm | increase in arrhythmia index | decrease in amplitude of P wave | development of bradycardia | repeated increased in arrhythmia index |
| 1 | 1 | 3 | 9 | 6 | 5 | 1 | 10 | 8 | 6 |
| 2 | 2 | 5 | 5 | 9 | 1 | — | 2 | 10 | 10 |
| 1 | 1 | — | 5 | 4 | 2 | 1 | — | 10 | 5 12 |
| — | 1 | 1 | 1 | 7 | 2 | 5 | 2 | — | — |
| — | 1 | — | 3 | 4 | 5 | 2 | 1 | 9 | 6 |
| 3 | 1 | 4 | 8 | 5 | 1 | 1 | 2 | 9 | 5 8 |

Laboratory of Physiology and Laboratory of Microbiology and Immunology, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. A. Vishnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 5, pp. 21-24, May, 1973. Original article submitted on October 22, 1971.

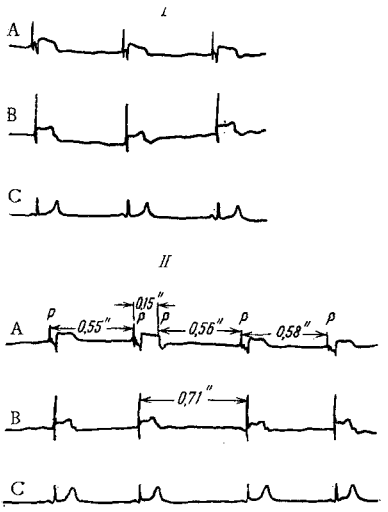


Fig. 1

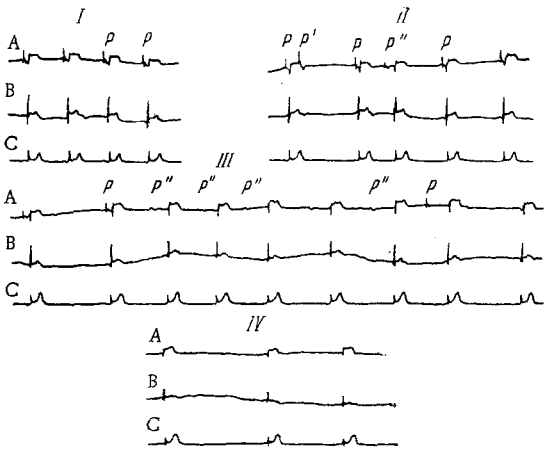


Fig. 2

Fig. 1. Initial ECG (I) and dysrhythmia of activity of main pacemaker together with auricular extrasystoles during first minutes after injection of ACS (II). A) Right-auricular unipolar ECG (RAECG) (active electrode glued to right auricle, reference electrode in Ringer–Locke solution); B) right ventricular unipolar ECG (RVECG) (active electrode glued to right ventricle, reference electrode in Ringer–Locke solution); C) ECG of isolated heart, recorded by two electrodes placed in solution bathing the heart; P) response of auricles to excitation from main pacemaker, variations in duration of P–P intervals from 0.55 to 0.58 sec; P') blocked auricular extrasystoles appearing 0.15 sec after excitation of main pacemaker and recorded only on RAECG.

Fig. 2. Changes in auricular electrical activity in late stages after injection of ACS. A, B, C) as in Fig. 1; P', P") response of auricle to excitation from various pacemakers; I) initial background (before injection of serum), sinus rhythm with distinctly functioning main pacemaker – P; II) appearance of heterotopic auricular pacemakers P' and P"; III) parasystole of auricular complexes and dissociation of contractions of auricles and ventricles; IV) disappearance of auricular activity, atrio-ventricular rhythm recorded in all leads.

The electrical activity of the heart was recorded by means of electrodes mounted in the wall of the working chamber. In some experiments surface electrodes also were glued to the various parts of the heart with MK-6 glue. In this way the ECG and the electrical activity of the atria and ventricles were recorded.

Arrhythmia of the auricular contractions was estimated quantitatively by a means of the arrhythmia index, determined as follows. The duration of ten successive P–P intervals was measured and the mean (M) found. The scatter was then determined, i.e., the difference between the maximal and minimal durations of P–P. The ratio between the scatter and the mean was taken as the arrhythmia index. Normally it varied from 0.01 to 0.09. If the duration of 10 (or more) P–P intervals remained constant, the index was zero.

The blood serum of rabbits immunized with solutions of cytoplasmic proteins from the guinea pigs' heart was used as the anticardial cytotoxic serum (ACS). The immunological parameters of ACS have been fully described previously [1]. The first portion of ACS reached the heart 40-50 sec after its injection into the perfusion fluid. In control experiments with methylene blue, to determine the dilution of antiserum with Ringer–Locke solution, it was found that after 2 min the initial ACS was diluted about six times, after 3 min by 24 times, after 4 min by 60 times, after 5 min by 128 times and after 6 min no antiserum could be detected in the working receiver.

In five preliminary experiments the ability of the heart to contract for a long time in the initial perfusion fluid without injection of ACS was tested. No significant changes in the electrical activity of the heart were observed during observations lasting 2-2.5 h.

## EXPERIMENTAL RESULTS AND DISCUSSION

At the beginning of the experiments the heart contracted in sinus rhythm. The duration of the P–P interval was 0.55 ± 0.065 sec. The arrhythmia index was 0.042 ± 0.016. After administration of the ACS marked changes occurred in the cardiac electrical activity. In the auricles the frequency of generation of the sinus beats was changed, the arrhythmia index increased, automatism of the heterotopic centers appeared, and the amplitude of the P wave was reduced. The order of appearance of these signs is important from the standpoint of early diagnosis of the development of the cytotoxic reaction. In these experiments it was as follows. To begin with the arrhythmia index usually increased. Later or (in some experiments) at the same time the frequency of generation of sinus beats increased. The amplitude of the P wave was most frequently reduced after the appearance of signs of arrhythmia. In a few cases, however, the decrease in amplitude was the first electrocardiographic sign of the action of ACS on the heart (Table 1). When identifying the beginning of development of the cytotoxic reaction, stress cannot therefore be made on any particular electrocardiographic sign, but they must all be considered.

The signs discovered also showed marked quantitative variation. The initial increase in frequency of generation of sinus beats was 13.4 ± 4.18% compared with the original frequency. The arrhythmia index increased by 11 times over the background level during this period. This was due to an increase in dysrhythmia of the main pacemaker and to the appearance of auricular extrasystoles (Fig. 1).

Immediately after this first period of changes in auricular electrical activity there was a period of relatively normal contraction when the frequency and rhythm returned almost to their initial values. The sinus rhythm then suddenly became slower and in some cases the auricles ceased to beat. The mean frequency of generation of sinus beats fell by 80.9 ± 38.2% below the initial value. The arrhythmia index in this period rose by 28 times. The second wave of arrhythmia was thus much larger, quantitatively speaking, than the first. P waves of different shapes and frequencies appeared at this time in the auricular ECG, evidence of their heterotopic origin (Fig. 1: II, III). Whereas during the first wave of arrhythmia, auricular extrasystoles usually occurred; i.e., heterotopic activity was present, in the second wave auricular parasystole was observed, with dissociation of the function of individual parts of the auricles, disappearance of impulses from the main pacemaker, and their replacement by low-amplitude P" complexes of lower frequency; i.e., active heterotopia was replaced by passive.

The amplitude of the P waves (cases with a constant pacemaker are considered) as a rule fell during the experiments.

As the heart was rinsed with Ringer–Locke solution, the auricular activity was partly restored, so that the frequency and rhythm of the auricular impulses returned partly to normal. However, the function of the main pacemaker could be taken over by another, as shown by changes in the configuration of the P wave and a decrease in amplitude and frequency of the waves. The initial cardiac activity was never completely restored.

Normal serum from unimmunized rabbits was used in the control experiments. In four of the eight experiments no changes were found in cardiac electrical activity, and in the others there was a small increase in the arrhythmia index and frequency of generation of sinus beats. However, these abnormalities were extremely slight and bore no comparison with the changes occurring after the action of ACS.

It can be concluded from these observations that the cytotoxic action of ACS led to considerable changes in the auricular ECG. These changes developed in a certain order and, as a rule, severe irreversible disturbances were present after 10 min. Partial recovery of auricular electrical activity during rinsing of the preparation with Ringer–Locke solution suggests that ACS exerts its effect through biologically active substances or through a very loose attachment of the antibody to the tissue antigen.

## LITERATURE CITED

1. B. I. Khodorov, E. G. Vornovitskii, I. I. Kolker, et al., Byull. Éksperim. Biol. i Med., No. 10, 17 (1971).