has been shown that during this period thymus hormones can no longer be detected in the circulation [4]. Rats were thymectomized at the age of 3 months. Two months later the animals were given an intraperitoneal injection of 0.5 ml NDV, and their serum interferon titer was determined after 24 h. Rats undergoing mock operations were used in the control.

The interferon titer in the animals of both groups was 1:256.

The results suggest that the constant presence of thymus hormones is not essential for the interferon-forming activity of the lymphocytes in the adult organism. Stimulation of interferon production by ASF in the period of early postnatal development is linked in all probability with the action of humoral factors of the thymus contained in it, which are responsible for the formation of the immunocompetent system and for immunological maturation. Thymus hormones are evidently differential factors, the quantity of which in the early stage of postnatal development, i.e., during the period of formation of the immunocompetent system, does not correspond to the number of cells requiring these substances for their further differentiation toward immunocompetence.

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ALLERGIC REACTIONS OF PULSATING HEART CELLS IN CULTURE

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The method of culturing and the characteristics of growth in culture of pulsating human embryonic heart cells are described. The effect of homologous antiheart antibodies and of a complex of ragweed allergen and antiragweed antibody on the contracting heart cells of chick and duck embryos in culture was investigated. Under the influence of these factors pulsation of the heart cells slowed and weakened and they developed vacuoles.

KEY WORDS: *culture of heart cells*; *pulsation*; *antiheart antibodies*; *antiragweed antibodies*.

The study of allergic reaction of the heart in the intact organism is difficult because of complex neurohumoral influences for which it is sometimes difficult to make allowance. The use of cultures of heart cells enables these side effects to be eliminated and the direct action of the immunological factor on heart cells to be studied.

A characteristic feature of heart cells in culture is that they preserve their contractile power. Because of this feature, not only the morphological changes, but also functional changes in these cells in response to stimulation can be investigated. Cultures of heart cells are thus an interesting model for the study of allergic reactions.

The effect of antibodies and, in particular, of antiheart antibodies on cells in culture has been studied in only a few investigations [4, 9, 11, 12].

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Chick [1, 3, 5], duck [4], and rabbit [10] embryonic hearts, and the hearts of newborn rats [6-10, 13] and guinea pigs [11] have been used for the culture of pulsating heart cells. Cultures of human heart cells are particularly interesting. In the accessible literature only one paper could be found in which the study of an organ culture of human embryonic hearts was described [2]. The authors cited did not study its histological characteristics.

The object of this investigation was to obtain a culture of pulsating human heart cells and to study the effect of antiheart and antiragweed antibodies (along and together with ragweed pollen allergen) on pulsating heart cells of chick and duck embryos.

EXPERIMENTAL METHOD

Culture of Human Embryonic Heart Cells. The hearts of 6- to 10-week human embryos taken during surgical termination of pregnancy were placed in sterile flasks containing isotonic sodium chloride solution. The subsequent manipulations were carried out in a special bench-top box with careful observance of the rules of asepsis. The hearts were freed from the vascular bundle and pericardium, rinsed with medium 199, and cut into pieces not more than 0.5 mm in diameter. The attempt was made to use mostly areas containing the conducting system with a high degree of automatic activity (the region of the sinus nodosus, the atrioventricular septum). The minced heart tissue was covered with 0.15% trypsin (Difco) solution and placed for 10-15 min on a magnetic mixer. The top layer of fluid was decanted, for according to observations by other workers [10] and our own observations, it contains endo- and epicardial cells and also cells injured during mincing of the myocardium. The remaining pieces of heart tissue were covered with a fresh portion of trypsin and again placed on the magnetic mixer for 20 min. The resulting suspension of cells and of very small, but not completely trypsinized fragments of heart tissue was washed three times with medium 199 during centrifugation (1500 rpm for 5 min). The resulting residue was diluted with nutrient medium made up in Gey's solution with the addition of amino acids, vitamins, calf fetal serum, and antibiotics (the formula for the medium was taken from Mark and Stresser [10]). The cells were seeded into Rose's Chambers, the bottom wall of which consisted of a coverslip and the top wall of polystyrene 150-200 μ thick, permitting diffusion of oxygen and carbon dioxide. The cultures were grown in an incubator at 37°C.

Culture of Chick and Duck Embryonic Heart Cells. The hearts of 6- to 10-day chick and 10- to 12-day duck embryos were minced, washed in Hanks' solutions to remove blood, and incubated in 0.3% trypsin (Difco) solution at 37°C for 30 min. The trypsinized tissue was additionally pipeted and thrice washed in Hanks' solution by centrifugation at 2000 rpm for 5 min. The resulting suspension of heart cells was resuspended in medium consisting of equal volumes of Hanks' solution, homologous plasma, and homologous embryonic extract and seeded in Rose's chambers. The chambers were incubated at 37°C.

Growth of the heart cells (human and avian) in culture was observed under the phase-contrast microscope and by microfilming; pulsation of the cells was recorded by photoelectrometry.

EXPERIMENTAL RESULTS

Characteristics of Cultures of Pulsating Human Heart Cells. Immediately after filling of the chambers the cells and very small pieces of incompletely detrypsinized heart tissue were in a suspended state and did not pulsate. After 24 h many of these structures were adherent to the wall of the chamber. The fragments of heart tissue appeared "rounded" and were beginning to be overgrown with cells. Cells adherent to the glass were proliferating and forming structures resembling fibers. Individual heart cells were connected with one another by processes and they formed sheets of cells.

The structures listed above, forming the culture of human embryonic heart cells, were able to perform their contractile function, but the number of pulsating cells in such a culture was relatively small compared with cultures of heart cells from other species of animals. The frequency of the pulsations of the various structures of the human heart cell culture varied mainly from 12 to 25 per minute. This frequency is much slower than that for cultures of pulsating heart cells from other animals. The frequency and character of the contraction of the cells and of the sheets of cells and fiber-like structures were stable; the frequency of pulsation of individual fragments of heart tissue varied during the course of the 24-h period by only 3-5/min.

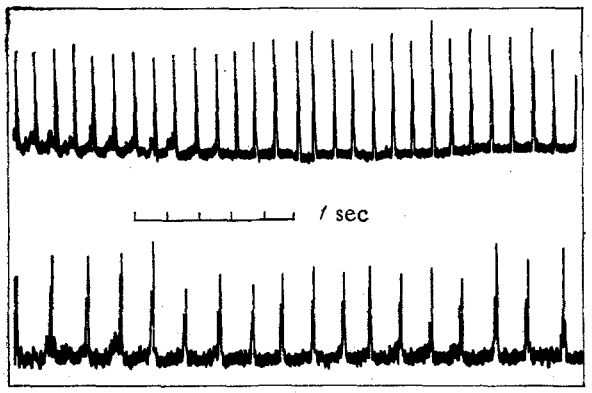

Fig. 1. Changes in pulsation of heart cell under the influence of combination of ragweed allergen and antiragweed antibody. Top curve) original contractions; bottom curve) contractions after treatment with combination of ragweed allergen and antiragweed antibody.

After 5-7 days the contractions of all the structures began to weaken and to decrease in number to a few per minute. However, the number increased once again after a change of nutrient medium and often returned to the original level.

Study of Some Allergic Reactions of Heart Cells in Culture. About 1000 experiments were carried out on 3- to 5-day cultures of pulsating heart cells from mainly chick and some duck embryos. A detailed description of the growth of these cells in culture was given previously [3, 4]. The effect of antiheart and antiragweed antibodies on the heart cells in culture was investigated. In the initial state the number of contractions of individual cells varied mainly from 30 to 80 (mean 54 ± 2.9).

The effect of antiheart antibodies on pulsating heart cells was studied in experiments in which plasma containing homologous complement-fixing antiheart antibodies was added to the culture medium; the method of their preparation was described previously [4]. The number of contractions of the heart cells was reduced 10-20 min after addition of the antiheart antibodies and the decrease continued during the first hour of the experiments. The number of contractions per minute fell on average by 16 ± 1.2 ($P < 0.001$). The pulsations became superficial and often arrhythmic. The changes observed persisted in most cases during 6-8 h of the experiments. The number of vacuoles in the cells increased sharply.

In the control investigations in which plasma of intact birds or of birds sensitized with homologous liver was added to the cultures, a small but not significant increase in the frequency of pulsation of the cells was found.

Homologous complement-fixing antiheart antibodies, by their direct action on pulsating heart cells, thus had a marked injurious effect.

The effect of antiragweed antibodies on heart cells in culture was studied in two series of experiments. In series I the effect of antiragweed antibodies obtained by immunization of hens with ragweed pollen [3], was studied. The culture fluid was replaced by medium containing antiragweed antibodies. Under these circumstances a small but not statistically significant increase was observed in the number of contractions, similar to that after an ordinary change of nutrient medium. The character and frequency of the contractions remained unchanged during the next 6-8 h of observation.

Consequently, antiragweed antibodies themselves has no significant effect on pulsation of the heart cells in culture.

In the experiments of series II the effect of a combination of ragweed allergen and antiragweed antibody was studied. One or two drops of extract of ragweed pollen (antigen) was added to a culture grown in medium with antiragweed plasma. In the control experiments the same antigen was added to heart cells grown in ordinary medium. The number and depth of the contractions of the cells in the control were unchanged. After addition of ragweed pollen extract to the experimental cultures the frequency of contractions of the structures observed showed a marked decrease within 20 min (Fig. 1). The mean number of contractions fell

by 19 ± 0.7 ($P < 0.001$). The contractions of the heart cells became more superficial and arrhythmic. These changes as a rule continued throughout the period of observation (6-8 h). By the end of the first day the number of vacuoles in the cytoplasm of the cells was considerably increased.

The results of the experiments of series II show that a combination of ragweed allergen and antiragweed antibody has a marked injurious action on contracting cells.

It can be concluded from an analysis of these results that heart cells participate directly in the allergic reactions studied and that, therefore, they may play the role of one of the target cells in the sensitized organism.

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