AN ONTOGENETIC STUDY OF THE ANTIGENIC PROPERTIES OF ANIMAL TISSUES AND ORGANS

COMMUNICATION 4. AN EMBRYOGENETIC STUDY OF THE SPECIES- AND ORGANOSPECIFICITY OF A CHICKEN'S HEART

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The data from works studying the antigenic properties of the heart in embryogenesis are extremely contradictory. For example, Ebert [4], starting from results obtained from the precipitin reaction, stated that the organospecific antigens of the heart were present in chicken embryo tissues at very early stages of the embryo's development (primitive streak stage) and that they were the same as the antigens of the brain. However, the experimental results which Ebert obtained in this same work by the use of the tissue culture method contradict the above data. No heart pulsations developed in the embryos cultured on a medium to which anti-chicken heart serum had been added. Heart pulsations, however, developed in embryos cultured on a medium containing an anti-chicken brain immune serum. Based on the results which Ebert received from the precipitin reaction [4], one would expect that, due to the antigenic similarity of the heart and brain at the early stages of embryogenesis, the anti-brain immune serums would inhibit the development of the heart.

Later, Ebert [5] presented data indicating that, in his opinion, this general "cardio-cerebral" antigen complex was only found in chicken brain tissues on the 18th day of incubation.

Recently, the results of researches [6, 7] making an immunological study of the contraction proteins (myosin and actin) in a developing chicken's heart have been published. The authors confirm that the myosin and actin of a definitive chicken's heart may be found as early as the primitive streak and cephalization stages; myosin synthesis is begun long before the cellular elements which comprise the foundation of the heart begin to form (in embryos of 12-13-hour incubation). However, according to the data of Johnsen and Leone [8], definitive chicken's heart actomyosin does not appear in the embryo until after it has been incubated for 40 hours.

Therefore, the problem of the formation of the heart antigenic structure is still unsolved. This makes it difficult to understand the ontogenetic development of the antigenic properties of animal tissues.

The purpose of this work was to further investigate the formation process of the antigenic structure of the heart.

EXPERIMENTAL METHODS

We used the anaphylactic reaction with desensitization and the ring-precipitin reaction. We have already described how these reactions were conducted [1, 2]. The serums against the heart tissues of an adult chicken which were used in the ring-precipitin reaction were absorbed by the hepatic cells of the chicken

according to the method proposed by P. N. Kosyakov, V. S. Korosteleva and N. I. Kuznetsova [3].

The first series of experiments examined the antigenic species-specificity of the developing heart tissues. Guinea pigs were sensitized subcutaneously with suspensions of heart tissues from chicken embryos (Russian White species) a different stages of incubation (3, 4, 6, 8, 10, 12, 16 and 19 days), and also from an adult chicken. The heart tissues were carefully washed several times in a physiological solution to remove the blood. The washing water was examined for serous protein content by the ring-precipitin reaction. When the last specimens of washing water gave no positive reaction with the immune serum against the adult chicken serum (titer of serum according to antigen dilution, 1:50,000), the tissues were used for sensitization. On the 21st day after the sensitizing injection, all the animals were given a reacting injection of chicken serum either intravenously or intraperitoneally, depending on the dose. Large doses of the serum (500 mg) were injected intraperitoneally. The same antigen was injected intravenously into 3 nonsensitized animals (technical control).

The second series of experiments was conducted to determine the point at which the organospecific antigens of the definitive heart appeared. Animals were used which had been desensitized to species-specific antigens by an injection of serum from an adult chicken and then tested to confirm the completeness of their desensitization (intravenous injection of 300 mg of serum). Two hours after the test, all the animals were given intravenously a reacting injection of a water-salt extract from adult chicken heart tissues. The extract was also injected into 3 nonsensitized guinea pigs (technical control).

The extract was obtained in the following manner. Heart tissues, washed free of blood, were gound in a homogenizer with 4 volumes of a physiological solution. The resulting suspension was centrifuged for 20 minutes at a speed of 3,000 revolutions per minute.

EXPERIMENTAL RESULTS

All the sensitized guinea pigs responded to the chicken serum injection by a positive anaphylactic reaction (Table 1). The reaction was somewhat more pronounced in the guinea pigs sensitized by the suspensions of heart tissue from chicken embryos at the later developmental stages, in some cases, so pronounced that when the antigen was injected intravenously, the animal died. In order to preserve the animals, the injections were done intraperitoneally. There was no reaction observed in the control animals.

Therefore, at all the developmental stages studied, the heart tissues containedspecies-specific antigens. This antigenic species-specificity evidently increased as the embryo developed.

The experimental results of the second series are also given in Table 1. No positive anaphylactic reaction was observed in the guinea pigs sensitized with suspensions of heart tissue from 3 and 4-day chicken embryos. Nor was a positive reaction observed in the control animals. In the animals sensitized with suspensions of heart tissue from embryos incubated 6, 8, 10, 12, 16 and 19 days and from an adult chicken, however, a positive anaphlyactic reaction was observed in response to the injection of a water-salt extract from the tissues of a definitive chicken heart.

Therefore, the results of this experimental series showed that the heart tissue of embryos incubated 6, 8, 10, 12, 16 and 19 days contains organospecific antigens, specific for the definitive chicken heart.

As far as the negative reactions obtained in the animals which had been sensitized with the heart tissues from 3 and 4 day embryos are concerned, we propose that they are explained not by the absence, but by the negligible amount of organospecific antigens present in the heart tissues at these stages of development. In connection with this, an experiment was conducted in which the guinea pigs were sensitized with heart tissues from embryos incubated 3 and 4 days in a dose 4 time as large as the sensitizing dose used in the preceding series of experiments (64 mg instead of 16 mg).

On the 21st day after the sensitizing injection, the animals were desensitized to species-specific antigens. All the animals reacted very strongly to the injection of serous proteins. Therefore, in order to preserved the animals, the desensitization was done in two doses (2 intraperitoneal injections of 500 mg apiece, 6 hours apart).

The results of this experiment are shown in Table 2. The guinea pigs which had been sensitized with heart tissues from embryos incubated 3 days did not respond to the injection of the water-salt extract from the definitive chicken heart tissue by a positive anaphylactic reaction. However, a positive anaphylactic reaction

TABLE 1

Anaphylactic Reaction in Guinea Pigs Sensitized With Suspensions of Heart Tissue From Chicken Embryos in Response to Injection of Serum and Extract From Adult Chicken Heart

Sensitization. Antigen—suspension of heart tissues from embryos (16 mg)	Reacting injection. Antigen—chicken serum		Test for complete desensitization. Antigen chicken serum (300 mg)	Reacting injection. Antigen—extract from adult chicken heart tissues	
	Dose in mg	Reaction	Reaction	Dose in mg	Reaction
Incubated 3 days	250	++	—	1000	—
The same	250	+++	—	1000	—
" "	250	+++	—	1000	—
Incubated 4 days	250	++	—	600	—
The same	250	++	—	600	—
" "	250	++	—	600	—
» »	250	+++	—	400	—
» »	250	++	—	400	—
» »	250	+++	—	400	—
Incubated 6 days	250	++++			
The same	500	+++	—	400	+
» »	500	++	—	400	+
» »	500	+++	—	400	+
Incubated 8 days	500	++	—	400	++
The same	500	++	—	400	+++
» »	500	++	—	400	++
» »	250	++++			
Incubated 18 days	500	++	—	400	++
The same	500	++	—	400	++
" "	500	++	—	400	++
Incubated 12 days	250	++++			
The same	500	++	—	400	++
» »	500	+++	—	400	++
	500	++	—	400	++
Incubated 16 days	500	+++	—	400	++
The same	500	++	—	400	++
» »	500	+++	—	400	++
Incubated 19 days	500	++	—	400	++
The same	500	++	—	400	++
" "	500	+++	—	400	++++
Suspension of adult chicken heart tissues	500	++	—	400	+++
The same	500	+++	—	400	+++
» »	500	+++	—	400	+++
.	300	—	.	400	—
.	300	—	.	400	—
.	300	—	.	400	—

Note: + tremor, rubbing nose and ears, dishevelled fur, panting, slight temperature decline; ++ the same symptoms, but more pronounced, the guinea pig often sneezed; +++ the same symptoms, still more sharply pronounced; convulsive kicks, cough, lying on side, but animal lived; ++++ all the symptoms very acute, animal died; - no symptoms of anaphylactic shock; • no injection.

TABLE 2

Anaphylactic Reaction in Guinea Pigs Sensitized With Suspensions of Heart Tissue From Chicken Embryos in Response to Injection of Extract From Adult Chicken Heart Tissue

Sensitization. Antigen—suspension of heart tissues from embryos (64 mg)	Desensitization. Antigen—chicken serum (1,000 mg)	Test—completed desens. Atg. 300 mg ser.	Reacting inject. Atg. 600 mg extr. adult chicken heart tissues
	Reaction	Reaction	Reaction
3 day incubation	+	—	—
The same	+	—	—
» »	+	—	—
4 day incubation	+	—	+
The same	+	—	+
» »	+	—	+

was observed in the animals which had been sensitized by heart tissue from 4 day embryos.

Therefore, the results of this experiment showed that the heart tissues of 4-day embryos contain a small quantity of organospecific antigens, but that there are evidently no organospecific antigens of the definitive heart contained in the tissues of the 3-day embryos. Therefore, one can assume that organospecific antigens appear in the heart tissues on or after the fourth day of incubation.

It was interesting to note that, as our histological studies showed, the morphological structure of the heart undergoes a series of essential changes in the embryos incubated 4-6 days, which confirms the observations of earlier authors (see Lillie, 1952). After the fourth day of incubation, the transformation process of the tubular heart into the four-chambered heart begins; the cardiac septa are formed (interventricular and interauricular), and the syncytial-cellular structure of the myocardium characteristic of the tubular heart changes into the syncytial-muscular structure. At this time, the laterally striated, definitive myofibrils begin to appear in the myocardium.

As Table 1 shows, the anaphylactic reaction was weakly expressed in the guinea pigs which had been sensitized by the suspension of heart tissues from embryos at the 6th day of development. A well-expressed anaphylactic reaction was observed in the guinea pigs which had been sensitized by the suspensions of heart tissues from embryos at later stages of development. The experiments showed that antigenic organospecificity increases greatly as the embryo develops.

Analogous data were obtained from the ring-precipitin reaction (Table 3). The tissues of the developing chicken heart possessed antigenic species-specificity at all the stages of embryogenesis studied. Organospecific antigens, characteristic of the definitive chicken's heart, were only found in the tissues of the developing heart after the 4th day of incubation, but then the antigenic organospecificity increased as the embryo developed.

Therefore, as a result of our studies, we have established that the heart tissues possess a well-expressed antigenic species specificity at all of the developmental stages studied. The organospecific antigens typical of the definitive chicken's heart evidently only appear after the fourth day of incubation. The appearance of the organospecific antigens precedes the fundamental morphological changes in the developing heart which occur after 4 days of incubation.

TABLE 3

Results of Ring-Precipitin Reaction Between Serums Against Chicken Blood Serum and Against Adult Chicken Heart Tissues and Antigens of Embryonic Organs at Various Stages of Development.

Serums	Antigen dilution	Antigens of embryo tissues: 4-day incubation	8-day incubation		12-day incubation			16-day incubation			Antigens of adult chicken tissues		
		Heart	Heart	Liver	Heart	Liver	Heart	Liver	Spleen	Heart	Liver	Spleen	Heart	Liver	Spleen
Against chicken blood serum (No. 725)	1 : 50	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	1 : 100	++	++	++	++	++	+++	+++	+++	++	++	++	++	++	++
	1 : 200	+	++	++	++	++	++	++	++	++	++	+	+	++	+
	1 : 300	—	+	+	+	+	++	+	+	+	+	+	+	+	+
	1 : 500	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Against chicken heart tissues (No.1059)	1 : 50	—	++	—	+++	—	+++	—	—	+++	—	—	++++	—	—
	1 : 100	—	+	—	++	—	++	—	—	+++	—	—	+++	—	—
	1 : 200	—	—	—	+	—	+	—	—	++	—	—	+++	—	—
	1 : 300	—	—	—	—	—	+	—	—	+	—	—	+++	—	—
	1 : 500	—	—		—		—	—	—	+	—	—	++	—	—
	1 : 1 000						—			—			+	—	—
	1 : 2 500												—		
Normal (rabbit No. 725 before immunization)	1 : 50	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	1 : 100	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	1 : 200	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Note: ++++ heavy ring, precipitate formation; +++ strongly expressed ring; ++ definite ring; + weakly expressed ring; - no ring.

SUMMARY

Antigenic properties of the developing chicken heart were followed up through different stages of embryogenesis, the methods of anaphylactic reaction, desensibilization and precipitation being used. At all stages of development, tissues of the developing heart manifested significant antigenic species-specificity. Organ-specific antigens peculiar to the definitive chicken's heart appear for the first time at the fourth day of incubation, and rise significantly with the development of the heart. Appearance of these antigens coincides with the beginning of morphological transformation of the tubular heart into the four-chambered one.

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