EXPERIMENTAL BIOLOGY

A STUDY OF THE ANTIGENIC PROPERTIES OF TISSUES AND ORGANS OF ANIMALS DURING ONTOGENESIS

COMMUNICATION VI. THE STAGE-SPECIFIC ANTIGENS OF THE CHICK'S HEART

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In a previous paper [3] results were described which demonstrated that the tissues of the developing crystalline lens of the duck in early stages of embryogenesis (90-130 days of incubation) contain antigens which are characteristic of these early stages of development only, and subsequently disappear. We called these antigens "stage-specific." In the same article a number of reports were quoted from the literature which indicated the existence of certain antigenic substances only at definite stages of development.

We were able to find only one paper [5] in the literature in which the authors reported the presence of the muscle tissue of rabbit and sheep embryos of a contractile protein which was characteritic of the embryonic period of development and which was called "metamyosin" by these authors. It must be mentioned that somewhat earlier I. I. Ivanov and B. S. Kasavina [2] showed a qualitative change in the contractile proteins during ontogenesis.

In the present paper a description is given of antigens which are characteristic of definite stages of development of the chick's heart.

EXPERIMENTAL METHOD

We used the reaction of anaphylaxis with desensitization in guinea pigs [1]. In the first series of experiments we investigated the presence in the tissues of the developing heart of antigens which were characteristic of definite stages of development. The guinea pigs were sensitized subcutaneously with suspensions of heart tissue of chick embryos ("Russian White" breed) with different periods of incubation (4, 6, 8, 10, 12, 16 and 19 days) and of 3-day chicks. The heart tissue was thoroughly and repeatedly washed free from blood in physiological saline. The washing waters were examined for serum protein content by means of the ring-precipitation test. On the 21st day after the sensitizing injection all the animals were desensitized to species- and organ-specific antigens by intravenous injections of tissue extracts from the heart of an adult chicken (400 mg each).

Desensitization to organ-specific antigens of the heart arose very slowly in our experiments. For this reason, in a number of cases the animals had to be given 3-4 intravenous injections of tissue extract from the heart of an adult chicken, and only then did they become desensitized to the organ-specific antigens. Two hours after testing the completeness of desensitization to these antigens (intravenous injection of tissue extract of the heart of a chick in a dose of 500 mg) the animals were injected intravenously with the same antigens which were used to cause sensitization. The only difference was that extracts and not suspensions of the tissue were injected.

The extract was obtained in the following manner. The heart tissue was washed free from blood and then quickly ground in a homogenizer together with 4 volumes of cold physiological saline. The suspension obtained was centrifuged in the cold form for 20 minutes at a rate of 3000 rpm.

TABLE 1

The Reaction of Anaphylaxis in Guinea Pigs Sensitized by Suspensions of Heart Tissue of Chick Embryos and Desensitized to Species- and Organ-specific Antigens, in Response to Injection of a Tissue Extract of the Heart of Embryos of Various Periods of Incubation

Sensitization (subcutaneously)		Assaulting injection (intravenous) after complete desensitization to species- and organ-specific antigens		
antigen	dose in mg	antigen	dose in mg	reaction
1. Tissue suspension of the heart of chick embryos of the following periods of incubation (in days)		1. Tissue extract of the heart of chick embryos of the following periods of incubation (in days)		
4	16	4	400	+
4	16	4	400	+
4	16	4	400	+
6	16	6	400	—
6	16	6	400	—
6	16	6	400	—
8	16	8	400	—
8	16	8	400	—
8	16	8	400	—
10	16	10	400	±
10	16	10	400	+
10	16	10	400	±
12	16	12	400	+
12	16	12	400	+
12	16	12	400	+
12	16	12	400	++
12	16	12	400	+
12	16	12	400	+
16	16	16	400	++
16	16	16	400	+
16	16	16	400	++
19	16	19	400	+
19	16	19	400	+
19	16	19	400	+
2. Tissue suspension of the heart of a 3-day chick	16	2. Tissue extract of the heart of a 3-day chick	400	±
The same	16	The same	400	+
» »	16	» »	400	±
3. Technical control (sensitization not produced)	—	3. Tissue extract of the heart of chick embryos of 4-days of incubation	400	—
The same	—	The same	400	—
» »	—	4. Tissue extract of the heart of chick embryos of 12 days of incubation	400	—
		The same	400	—

Signs: + tremor, rubbing of the nose and ears, dishevelling of the fur, shortness of breath, slight fall of temperature; ++ the same signs, more severely shown, the guinea pig often sneezes; ± signs of anaphylactic shock are shown indistinctly; — no signs of shock.

EXPERIMENTAL RESULTS

A positive anaphylaxis reaction was observed in guinea pigs sensitized with suspensions of heart tissue of embryos of 4, 12, 16, and 19 days incubation after the injection of extracts from heart tissue of embryos (Table 1). Meanwhile, in guinea pigs sensitized with suspensions of heart tissue of embryos of 6 and 8 days incubation no signs of anaphylactic shock were observed. After injection of the assaulting dose of antigen (tissue extracts from the hearts of 4- and 12-day embryos) into nonsensitized animals (technical control) no positive anaphylactic reactions were obtained. In guinea pigs sensitized with suspensions of heart tissue of embryos of 10 days incubation and of 4-day chicks the results were mainly indefinite (±). The guinea pigs sensitized with tissue suspensions of the heart of embryos of 4, 12, 16 and 19 days of incubation gave a marked anaphylactic reaction which was assessed as + and ++.

The results obtained demonstrate that the tissues of the developing heart at a definite period of embryogenesis contain antigens which are specific for that stage of development alone – stage-specific antigens. These antigens are present in the heart tissue of embryos of 4 days of incubation, disappear in embryos of 6 and 8 days of incubation, reappear in embryos after 10 days of incubation and disappear once more after hatching.

During embryogenesis, therefore, 2 periods are observed in the course of which it is possible to find stage--specific antigens. The question naturally arises at once, are these the same stage-specific antigens or are the disappearing stage-specific antigens replaced in the course of development by new antigens. In order to answer this question we carried out a second series of experiments.

Four guinea pigs were sensitized by tissue suspensions of the heart of embryos of 4 days of incubation. On the 21st day after the sensitizing injection all the animals were desensitized to species-specific (by intraperitoneal injection of a dose of 500 mg of chick serum) and organ-specific antigens (by intravenous injection of tissue extract of chick's heart in a dose of 500 mg). Two hours after testing for complete desensitization to organ-specific antigens all the animals were injected intravenously with 500 mg each of tissue extract of the heart of embryos of 14 days of incubation. In no animal was a positive anaphylactic reaction observed (Table 2). Thus, the results of this series of experiments demonstrate that the stage-specific antigens present in the heart tissues of 4-day chick embryos are different from the stage-specific antigens found in the heart tissues of embryos of 12-19 days of incubation.

TABLE 2

The Reaction of Anaphylaxis in Guinea Pigs Sensitized by Suspensions of Heart Tissue of Chick Embryos of 4 Days of Incubation and Desensitized to Species- and Organ-specific Antigens, in Response to the Injection of a Tissue Extract of the Heart of Embryos of 14 Days of Incubation

Sensitization (subcutaneously)		Assaulting injection (intravenous) after complete desensitization to species- and organ-specific antigens		
antigen	dose in mg	antigen	dose in mg	reaction
Tissue suspension of the heart of embryos of 4 days of incubation	16	Tissue extract of the heart of embryos of 14 days of incubation	500	–
The same	16	The same	500	–
" "	16	" "	500	–
" "	16	" "	500	–

The same signs as in Table 1.

Arising from these findings it appears that the tissues of the developing chick's heart contain 2 different groups of stage-specific antigens which are present at different stages of development.*

In 1952, Schjeide [6] reported that in the serum of chick embryos of the 10th day of incubation antigens

* It is possible that in this particular case the tissues contain only one stage-specific antigen. However, lacking appropriate accurate data, we shall speak of groups, without begging the question of the number of component antigens.

were present which were not to be found in the serum of the adult bird. In view of the extremely high sensitivity of the anaphylactic reaction, we began to wonder whether the stage-specific antigens present in the heart tissues of 12-19-day embryos were not the antigens of the blood serum of embryos of these same periods of incubation. We thought that despite the thorough washing of the pieces of heart tissue to free them from blood serum, some small trace of serum proteins may nevertheless remain and thereby bring about sensitization of the animals to these antigens. In order to answer this question we made a comparative study of the antigenic properties of the heart tissue and the serum of embryos of 14 days of incubation. In the heart tissue of embryos of this period of development there is evidently the greatest number of Group II stage-specific antigens. In the first place it was necessary to find out whether stage-specific antigens are present in the serum of embryos of 14 days of incubation, and if so, in what amount.

With this object we carried out a third series of experiments. Four guinea pigs were sensitized subcutaneously with serum from embryos of 14 days of incubation. On the 21st day after the sensitizing injection all the guinea pigs were desensitized to the antigens of the serum of the adult chicken, after which they were injected intravenously with the serum of 14-day embryos. In addition the same antigen was injected into 3 nonsensitized guinea pigs (technical control).

The results of the experiments showed (Table 3) that 3 guinea pigs gave a positive anaphylactic reaction in response to the injection of the serum of 14-day chick embryos and in one guinea pig signs of anaphylactic shock were indistinct (±). Meanwhile no positive reaction was observed in the control animals. Thus, the results of the experiments showed that in the blood serum of embryos of 14 days of incubation stage-specific antigens are present in small quantity.*

In order to solve the problem whether Group II stage-specific antigens are present in the heart tissues or whether this group is a mixture of serum antigens, we carried out a fourth series of experiments. Four guinea pigs were sensitized with suspensions of thoroughly washed heart tissue from embryos of 14 days of incubation, free from blood serum. On the 21st day after the sensitizing injection the animals were desensitized to species and organ--specific antigens by the intraperitoneal injection into each guinea pig of 500 mg of serum and 400 mg of extract of heart tissue of an adult chicken. Two hours after testing the completeness of desensitization to organ-specific angigens (by intravenous injection of an extract of the heart tissue of the chicken in a dose of 500 mg) all the guinea pigs were injected intravenously with an extract of the heart tissue of 14-day embryos. In addition,this same antigen was injected into 3 nonsensitized guinea pigs (technical control).

The results of the experiment showed (Table 3) that in all the experimental guinea pigs a well-marked positive anaphylactic reaction was observed in response to the injection of an extract of heart tissue of 14-day embryos. In the control guinea pigs no signs of anaphylactic shock were observed. Thus, the results of the fourth series of experiments showed that the heart tissues of embryos of 14 days of incubation contain a considerable quantity of the stage-specific antigens.

As has already been pointed out above, in the third series of experiments as sensitizing and assaulting injections we used the serum of embryos of 14 days of incubation,but in the fourth series of experiments, suspensions and extracts of the heart tissues of embryos of the same period of development. Contamination by serum antigens of the suspensions and extracts used in the fourth series of experiments was evidently very slight. However, the degree of the anaphylactic reaction to stage-specific antigens in the guinea pigs of the fourth series of experiments was higher than in those of the third series. It therefore may be thought that the positive anaphylactic reaction in the animals of the fourth series of experiments was caused by the stage-specific antigens present in the heart tissues of 14-day embryos and not by mechanical contamination by the antigens of the serum of the same embryos.

A morphological study which we carried out showed that in the period when the Group I stage-specific antigens are found,the process of transformation of the tubular heart into a four-chambered organ is beginning. At this period of development a high tempo of growth is observed in the heart tissues, and in its histological structure the myocardium resembles in its main features the myocardium of embryos of earlier periods of incubation (there

* As a result of cross anaphylactic reactions with antigens of serum and heart tissue of 14-day embryos it was shown that the stage-specific antigens of the serum and also of the heart tissue of the embryos are evidently qualitatively the same. However, the qualitative similarity of the antigens could not serve as a convincing argument in support of any theory of their localization.

are many mitoses in the myocardium and it has a syncytial cell structure with a small number of transversely striated myofibrils). It may be thought that the presence of stage-specific antigens in the heart tissues of embryos of 4 days of incubation is connected with the particular morphological and physiological features of development of the heart in early stages of embryogenesis, when it is still tubular in structure. We may point out that the susceptibility of the developing heart of the chick embryo to external agents changes considerable after 4 days of incubation [8].

TABLE 3

The Reaction of Anaphylaxis in Guinea Pigs Sensitized with Serum and Suspensions of Heart Tissue of Chick Embryos of 14 Days of Incubation and Desensitized to Species- and Organ--specific Antigens, in Response to the Injection of Serum and of Extract of Heart Tissue of Embryos of the Same Period of Incubation

Sensitization (subcutaneously)		Assaulting injection (intravenous) after complete desensitization to species- and organ-specific antigens		
antigen	dose in mg	antigen	dose in mg	reaction
1. Serum of chick embryos of 14 days of incubation. . .	100	1. Serum of chick embryos of 14 days of incubation. . . .		
The same	100	The same	400	+
" "	100	" "	400	±
" "	100	" "	400	+
2. Technical control (sensitization not produced).		" "	400	+
		" "	400	−
3. Suspension of heart tissue of 14-day chick embryos. . .	16	" "	400	−
		" "	400	−
The same	16	2. Extract of heart tissue of chick embryos of 14 days of incubation.	500	+
" "	16	The same	500	++
" "	16	" "	500	++
" "	16	" "	500	+
4. Technical control (sensitization not produced).	−	" "	500	−
The Same	−	" "	500	−
		" "	500	−

The same signs as in Table 1.

It has not yet been possible to observe what these definite morphological peculiarities in the development of the heart may be, in the period when the Group II stage-specific antigens are found (12-19 days of incubation). However there are reports in the literature which demonstrate that at this period of development the heart tissue is undergoing essential biochemical changes. Thus, for instance, Moog [4], when studying the activity of apyrase (adenylpyrophosphatase) in the developing cardiac muscle of chick embryos beginning with 12 days of incubation, established that the apyrase activity rises sharply on the 16th day of incubation and then falls again at the moment of hatching. The apyrase activity in embryos of 16 days of incubation is 400% higher than that of 12-day embryos. These findings show that the intensity of metabolism in the heart muscle of embryos of 16 days of incubation is increased. Similar results showing increased enzyme activity in cardiac muscle during the second period of embryonic development were obtained by Sippel [7] with another enzyme – succino-dehydrogenase. It is possible that the presence of Group II stage-specific antigens in the developing heart is connected with the particular biochemical changes in the tissues at this period of development.

Thus, as a result of a study of the antigenic properties of the developing heart, 2 groups of stage-specific antigens have been demonstrated. The presence of stage-specific antigens in the heart tissues is connected with the particular morphological and physiological features of development of this organ at the various stages of ontogenesis.

SUMMARY

Anaphylactic reaction with densensitization was employed in guinea pigs to show that there are 2 different groups of stage-specific antigens in the chick's heart. The first group of antigens is present in the embryonic cardiac tissues during the first 4 days of incubation, while the second appears in the developing heart 10 days after incubation and disappears after hatching. Existence of stage specific antigens in the cardiac tissues is connected with definite morphologico-physiological peculiarities of development of this organ in different stages of ontogenesis.

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* Original Russian pagination. See C. B. translation.