# A COMPARATIVE STUDY OF THE ANTIGENIC PROPERTIES OF TISSUES IN ANIMALS OF VARIOUS SPECIES AT DIFFERENT STAGES OF DEVELOPMENT

## ARTICLE II: A COMPARATIVE STUDY OF THE ANTIGENIC PROPERTIES OF HEART TISSUES FROM THE CHICKEN, GRASS SNAKE AND FROG

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The purpose of our research was to study how antigenic specificity forms in animal tissues and also to determine whether recurrence of the antigenic properties of tissues in less highly organized animals occurs during the process of individual development.

Earlier [1], we reported our data from a comparative study of the antigenic properties of muscle tissue from caudate and noncaudate amphibians. It was established that the muscular tissue of the tadpole has antigenic properties that differ considerably from those of the frog's muscular tissue; this difference is constituted in the fact that there are antigens present in the tadpole muscle tissue which are lacking in that of the frog.

It was also established that there is less difference between the antigenic properties of tadpole and triton muscle tissue than there is between those of frog and triton muscle tissue.

In this work, the antigenic properties of heart tissues from chicken embryos at various stages of development (5, 6, 8, 12, 14 and 18 days of incubation) and the heart of an adult chicken are compared with the antigenic properties of heart tissues from the grass snake and the frog.

The complement fixation reaction was used in the work, and set up according to the usual method.

#### EXPERIMENTAL METHODS

First, immune serum was obtained against water-salt extracts from the heart tissues of the chicken, grass snake and frog. Water-salt extracts from the heart tissues of chicken embryos incubated 5, 6, 8, 12, 14 and 18 days and from that of an adult chicken were used as antigens. The method of preparing the extract and of obtaining the immune serum was described in the first article [1]. The complement fixation reaction was set up with the serum and antigens obtained from the heart tissues of the chicken embryos and of the adult chicken. Before the reaction was set up, the amount of protein in the antigens was determined by the Kjeldahl method. After this, the amount of protein in all of the antigens was equalized by diluting the more concentrated antigens with a physiological solution.

The results of the experiments are given in Tables 1 and 2, each of which summarizes the data obtained from 60 reactions. \*

<sup>•</sup> The average amount of the experiments was computed between the three analogous immune serums and all the antigens from the chicken embryo hearts.

#### EXPERIMENTAL RESULTS

The serum against the chicken heart tissue reacted with the antigen from the heart tissues of chicken embryos incubated 4 and 6 days in a dilution of 1:80. The reaction with the antigen from the heart tissue of the chicken embryo incubated 6 days was slightly more intense. The same serum reacted with the antigen from the heart tissue of 8- and 12-day incubation chicken embryos in a dilution of 1:160. One must note that the reaction of the serum against the chicken heart tissue was slightly more marked with the antigen from the heart tissues of the chicken embryo incubated 12 days. The serum against the chicken heart tissue reacted with the antigen from the tissues of chicken embryos incubated 14 and 18 days in a dilution of 1:320, and with the antigen from the adult chicken heart tissue, in a dilution of 1:640 (Table 1).

TABLE 1

Titers and Intensity of Complement Fixation Reaction of the Serum Against the Chicken Heart Tissue with the Antigens From Hearts of Chicken Embryos at Various Stages of

Development

	Antigens						
Serum dilution	Duration of chicken embryo incubation (in days)						
	4	6	8	12	14	-18	chicken
1:20 1:40 1:80 1:160 1:320 1:640	+++	+++	-+	+++++++++++++++++++++++++++++++++++++++	+  -  - + + + + - - - - + + - - + + 	#  -+++ - -+-	+ - + - + + + + + + + + + + + + + +

Comparing the titers and reaction intensity of the serum against the chicken heart tissue with all the antigens from the chicken embryo heart tissues used in the experiment showed that the greatest difference in antigenic properties was between the heart of the adult chicken and that of the chicken embryos incubated 4 and 6 days. Then, as the chicken embryo became more developed, this difference in heart tissue antigenic properties gradually decreased. Therefore, the results of this experiment confirmed the data of other researchers [2 and others].

Table 2 shows that the serum against the heart tissue of the grass snake reacted with the antigen from the heart tissues of the chicken embryos incubated 5 and 6 days in a dilution of 1:320, with the antigen from the heart tissue of the chicken embryo incubated 8 days, in a dilution of 1:160, with that from the heart tissue of the embryos incubated 12 and 14 days, in a dilution of 1:80, and with the antigens from the heart tissue of an 18-day incubated embryo and from the adult chicken heart, in a dilution of 1:40. Comparing the titers and reaction intensity of the serum against the grass snake heart tissue with the antigens from the chicken embryo heart tissue and from the adult chicken heart showed that the antigenic properties of the hearts from the chicken embryos incubated 5 and 6 days were more similar to those of the grass snake heart. Then, in the same way, as the chicken embryo developed, this similarity decreased.

In the next series of experiments, results were obtained from complement fixation reactions between the serum against the frog heart and the antigens from the heart tissues of chicken embryos at the same stages of incubation as were used in the reaction with the serum against the grass snake heart. The serum against the frog heart tissue reacted with the antigen from the heart tissues of chicken embryos incubated 5, 6 and 8 days in a dilution of 1:160, with the antigen from embryos incubated 12 and 14 days, in a dilution of 1:80 and with the antigen from the adult chicken heart tissue, in a dilution of 1:40 (Table 3).

Comparing the titers of the reaction of the serum against the frog heart tissue with the antigens from the chicken embryo heart tissues and with the antigen from the adult chicken heart showed that the antigenic properties of the heart tissue from chicken embryos at the earlier stages of incubation were more similar to those of the frog heart tissue than were those of the heart tissue from embryos at later stages of development.

TABLE 2

Titers and Intensity of Complement Fixation Reaction Between Serum Against Heart of Grass Snake and Antigens From Hearts of Chicken Embryos at Various Stages of Development

Serum dilution	Antigens							
	Duration of chicken embryo incubation (in days)							
	5	6	8	12	14	. 18	Adult chicken	
1:20	- -+++	- - + - -		-   -	-11-		-  -	
1:40	-+-+-+-	+++	-   -	++	-  -		-1	
1:80		-+ -+-		-				
1:160	. +	-11-	- -				**	
1:320			-		-			
1:640	-			_		-	-	

TABLE 3

Titers and Intensity of Complement Fixation Reaction Between Serum Against Frog Heart and Antigens From Hearts of Chicken Embryos at Various Stages of Development

Serum dilution	Antigens							
	Duration of chickenembryo incubation (in days)							
	4	6	8	12	14	- Adult chicken		
1:20	-[			-1	-[]-	-}		
1:40	-  -		-  -	+		-1-		
1:80	-  -	-1-		,				
1:160	-  -	-	<del>-</del> :		**			
1:320	<u>-</u> :			<del>-</del>		-		
1:640	-	4	**	-	÷1 1			

Therefore, the results of our experiments showed that, during the development process of the chicken embryo, the antigenic properties of its heart tissue become increasingly more similar to those of an adult chicken's heart. The antigenic properties of heart tissue from chicken embryos incubated 5 and 6 days are more similar to those of the grass snake and frog hearts. As the chicken embryo develops, this similarity decreases.

Based on the marked antigenic similarity of the heart tissues from chicken embryos at the earlier stages of development with the heart tissues of the grass snake and frog, one can propose that, at these stages, the antigenic properties of the bird's ancestors, which were somewhat similar to contemporary reptiles and amphibians, are reflected.

### SUMMARY

Antigenic properties of the heart tissues of chicken embryo of 5, 6, 12, 14, 18 days, of mature chickens, reptiles (Naotrix natrix) and amphibia (Rana ridibunda) were comparatively studied by the complement fixation test. With the development of the chicken embryo antigenic properties of the heart tissues approach more and more those of the mature chicken. In the more early embryonic periods they resemble those of the grass snake and frog. With the development of the chicken embryo this similarity becomes less and less pronounced.

## LITERATURE CITED

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- [4] Konyukhov B. V., Byull. Eksptl. Biol. i Med., 1956, Vol. 42, No. 9, pp. 55-60.\*

<sup>•</sup> Original Russian pagination. See C. B. translation.