

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

COMMUNICATION III. A COMPARATIVE STUDY OF THE ANTIGENIC PROPERTIES
OF THE HEART TISSUE OF THE FROG (*RANA TEMPORARIA*) AND THE TRITON
(*TRITURUS TAENIATUS*)

R. F. Averkina

From the Laboratory of the Immunology of Embryogenesis
(Head - Candidate Med. Sci. O. E. Vyazov) of the Institute of
Experimental Biology (Director - Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Received April 14, 1958. Presented by Active Member AMN SSSR
N. N. Zhukov-Verezhnikov)

The problem of the changes in the antigenic properties of tissues of the amphibia in the course of their individual development is not yet solved. The results of research in this field are very contradictory. Some authors [2, 13] consider that the antigenic properties of tissues of the amphibia do not change in the process of development. These authors' findings are not, however, in agreement with the results of other investigations [3-7, 10-12].

In order to shed light on the problem of the changes in the antigenic properties of the tissues of amphibia in the process of ontogenesis, we decided to carry out a special investigation.

We also had the aim of ascertaining the degree of interspecies similarity of the antigenic properties of the tissues of larval and adult forms of *Anura* with the analogous tissues of adult members of *Urodela*.

The immunological study of the interspecies relationships, taking into consideration the various stages of development, appeared to us to be of interest, since research in this direction has been carried out only on adult forms [2, 8, 9]. The sole exception is the work of I. L. Krichevskii [7], devoted to the comparative study of the antigenic properties of the tissues of the tadpole and the adult frog and the antigenic properties of the tissues of the triton. We have carried out a similar sort of investigation [1], differing from Krichevskii's work by the fact that we used muscle tissue and not the animals in toto.

In the present investigation a comparative study of the antigenic properties of the tissues of the heart was made.

EXPERIMENTAL METHOD

In the experiments we used the heart tissues of the tadpole, the frog (*Rana temporaria*) and the triton (*Triturus taeniatus*). As antigens we used a saline extract of the heart tissues of tadpoles taken at various stages of development (0, I-III, IV according to L. Blacher), and also saline extracts of the heart tissues of the frog and triton. Immune rabbit sera were prepared against the saline extract of the heart tissues of the frog and triton. The method of preparation of the extract and of the immune sera was described previously [1]. Complement fixation tests were carried out with the sera obtained and with all the above-mentioned antigens.

TABLE 1

Results of the Complement Fixation Test by Antisera to the Heart of the Frog and Triton with Antigens from the Heart of the Frog, Tadpole and Triton

Sera	Dilution	Antigens from the heart				The triton
		the frog	the tadpole			
			stages of development			
			0	I-III	IV	
Against the frog's heart	1 : 20	++++	++++	++++	++++	++++
	1 : 40	++++	+++	+++	+++	+++
	1 : 80	++++	++	++	++	+
	1 : 160	+++	+	+	+	h
	1 : 320	+	h	h	h	h
	1 : 640	h	h	h	h	h
Against the triton's heart	1 : 20	+++	+++	+++	+++	++++
	1 : 40	++	+++	+++	+++	++++
	1 : 80	+	++	++	++	++++
	1 : 160	h	+	+	+	++++
	1 : 320	h	h	h	h	+++
	1 : 640	h	h	h	h	+
Normal	1 : 20	h	h	h	h	h
	1 : 40	h	h	h	h	h
	1 : 80	h	h	h	h	h
	1 : 160	h	h	h	h	h
	1 : 320	h	h	h	h	h
	1 : 640	h	h	h	h	h

Note: ++++ total absence of hemolysis; +++, ++, +, ± intermediate degrees of hemolysis; h - total hemolysis; a point indicates that no test was performed.

EXPERIMENTAL RESULTS

As may be seen from Table 1,* serum against the heart tissues of the frog reacted in the complement fixation test with homologous antigen in a dilution of 1:320, and with extract from the heart tissues of the tadpoles at the above-mentioned stages of development, in a dilution of 1:160. This serum gave the weakest reaction of all with the extract from the heart tissues of the triton (1:80). The serum against the heart tissues of the triton reacted with the homologous antigen in a dilution of 1:640, with the extract from the heart tissues of tadpoles taken in stages 0, I-III and IV of development in a dilution of 1:160, and with the extract from the heart tissues of the frog in a dilution of 1:80.

Comparison of the titers and the intensity of the reaction between the antiserum to the heart tissues of the frog and the extracts from the heart tissues of the frog and tadpoles showed that these heart tissues differed in their antigenic properties. The antisera to the heart tissues of the frog and triton reacted with the extract from the heart tissues of the tadpoles in various stages of development in the same dilutions and with the same intensity. Consequently the antigenic properties of the heart tissues of the tadpoles in all the three above-mentioned stages of development showed no difference in our experiments. Furthermore, comparison of the titers and intensity of the reaction of these two sera showed that the difference between the titer and intensity of their reaction with antigens of the tissues of the frog and triton was considerably greater than the difference between the titer and intensity of the reaction with antigens of the tissues of the tadpoles and triton. Thus the heart tissues of the tadpoles resemble the heart tissues of the triton more closely, by their antigenic properties, than the heart tissues of the frog.

* The Table shows the mean values of the results of 79 tests.

TABLE 2

Results of the Complement Fixation Test Using Adsorbed Antisera to Frog's Heart with Antigens from the Heart of the Frog, Tadpole and Triton

Serum No.	Dilution	Antigens from the heart of the		
		frog	tadpole	triton
3939	1 : 20	+, ++	++++	++++
	1 : 40	++++	+++	++
	1 : 80	++++	+	±
	1 : 160	++	h	h
	1 : 320	±	h	h
	1 : 640	h	h	h
1917	1 : 20	++++	++	+
	1 : 40	++++	+	±
	1 : 80	+++	h	h
	1 : 160	+	h	h
	1 : 320	h	h	h
	1 : 640	h	h	h
2119	1 : 20	++++	+	±
	1 : 40	++++	h	h
	1 : 80	++	h	h
	1 : 160	+	h	h
	1 : 320	h	h	h
	1 : 640	h	h	h

The results shown in Table 1 were obtained with unadsorbed sera. In order to exclude any possible effect of antibodies to species-specific protein in the experiments described above, we adsorbed the sera with liver tissue, washed free from formalin, from animals of the same species as those to saline extracts of whose hearts the sera were obtained. The antisera to the heart tissues of the frog failed to react after adsorption, in all the dilutions used (1:20, 1:40, 1:80, 1:160, 1:320 and 1:640), with extracts of the skeletal muscle, liver and kidney of the frog. They reacted with homologous antigen in a dilution of 1:160. We were unable to completely adsorb antiserum to the heart tissues of the triton.

We carried out tests, the results of which are shown in Table 2, with adsorbed antisera to the heart tissues of the frog and extracts of the heart tissues of the frog, tadpole and triton.

As it may be seen from Table 2, adsorbed antisera to the frog's heart (Nos. 3939, 1917 and 2119) reacted with homologous antigen in a dilution of 1:160, and with antigen from the heart tissues of tadpoles in dilutions of 1:80, 1:40 and 1:20 respectively. The same sera (Nos. 3939 and 1917) reacted with extract of the heart tissues of the triton in dilutions of 1:40 and 1:20 respectively. There was no reaction between the serum (No. 2119) and the extract of the heart tissues of the triton.

The results of the experiments with adsorbed antisera to the heart tissues of the frog and extracts of the heart tissues of the tadpole, frog and triton thus corresponded to those obtained with the analogous unadsorbed sera (see Table 1). The degree of the reaction between the sera and the extracts of heart tissues of the tadpole, frog and triton was thus determined by antigens specific for these tissues.

The results of our experiments showed that the heart tissues of tadpoles differed in their antigenic properties from the heart tissues of the frog, and more closely resembled the heart tissues of the triton than the heart tissues of the frog. It was also shown that the heart tissues of tadpoles taken in stages 0, I-III and IV were indistinguishable in their antigenic properties in our experiments.

In connection with the experimental results shown in Tables 1 and 2, the question arose whether the differences in the antigenic properties of the heart tissues of the tadpoles and frog depended on the presence in the heart tissues of the tadpoles of antigens specific for these tissues alone. It was also of interest to find out whether it was not on account of these stage-specific antigens that the resemblance between the heart tissues of the tadpoles and triton was expressed to a greater degree than that between the heart tissues of the frog and triton. For this purpose we used the anaphylaxis with desensitization test in guinea pigs. One group of guinea pigs was sensitized subcutaneously with a suspension of the heart tissues of tadpoles in a dose of 16 mg per guinea pig, and another group in a dose of 50 mg per guinea pig. On the 21st day the guinea pigs were desensitized with extract from the heart tissues of the frog and, after testing for complete desensitization, an assaulting injection of extract of the heart tissues of the triton was given. This assaulting injection served at the same time a desensitizing purpose, since after it was injected an extract of the heart tissues of tadpoles as a second assaulting injection. The results of this experiment are shown in Table 3.

As may be seen from Table 3, guinea pigs sensitized with a suspension of the heart tissues of tadpoles in a dose of 16 mg did not react in response to an assaulting injection of extract of heart tissues of the triton. In guinea pigs sensitized with a suspension of the heart tissues of tadpoles in a dose of 50 mg, severe anaphylactic shock was observed in response to the assaulting injection of extract of the heart tissues of the triton. Finally, in response to the second assaulting injection of extract of the heart tissues of tadpoles, well-marked signs of anaphylactic shock were observed once again in all the animals.

Analysis of the results obtained showed that in the guinea pigs sensitized with a suspension of the heart tissues of tadpoles and desensitized to antigen of the heart tissues of the frog, anaphylactic shock took place in response to the injection of extract of the heart tissues of tadpoles. The heart tissues of tadpoles therefore contain antigens which are absent from the heart tissues of the adult frog (stage-specific antigens). It could also be concluded from the results of this experiment that in animals desensitized to antigen of the frog's heart, an anaphylactic reaction was also observed in response to the injection of extract of the heart tissues of the triton. The stage-specific antigens of the heart tissues of tadpoles thus possessed a resemblance to the antigens of the heart tissues of the triton. These antigens were presumably present in the heart tissues of tadpoles in small amounts, for we found them only in those cases in which a comparatively large dose of tissue (50 mg per guinea pig) was used for sensitization. Finally, in accordance with the results obtained, signs of anaphylactic shock were again observed in the guinea pigs after desensitization to antigen from the heart tissues of the frog and triton, in response to the injection of extract from the heart tissues of tadpoles. The heart tissues of tadpoles thus contained stage-specific antigens also, which did not resemble the antigens of the heart tissues of the triton. These antigens were present in the heart tissues of tadpoles evidently in larger amounts, for it was sufficient to sensitize guinea pigs with a dose of 16 mg in order to detect them.

It therefore followed, in the first place, from the results of our experiments that the heart tissues of tadpoles differed considerably in their antigenic properties from the heart tissues of the frog; in the second place that this difference depended on the presence in the heart tissues of tadpoles of stage-specific antigens, which were absent from the heart tissues of the frog; in the third place that the heart tissues of tadpoles resembled more closely in their antigenic properties the heart tissues of the triton than the heart tissues of the frog; in the fourth place that the closer resemblance between the antigenic properties of the heart tissues of the tadpole and the triton than that between the heart tissues of the frog and the triton was due to the presence of stage-specific antigens in the tadpoles. The fact of discovery in tadpoles of antigens absent from the adult frog and similar to the antigens of another species of animal, lower in its organization, may be explained, we are inclined to believe, as a manifestation of antigenic recapitulation. Finally, it followed from our experiments that the heart tissues of tadpoles also contained stage-specific antigens which had no resemblance to the heart tissue antigens of the triton.

The experiments thus showed that the interspecies relationships of the antigenic properties of larval tissues are quite different from those of the analogous tissues of the adult animal of the same species.

SUMMARY

The complement fixation test and the anaphylaxis reaction with desensitization were employed in guinea pigs. It was shown that the cardiac tissues of tadpoles differ from those of frogs by their antigenic properties (particularly as a result of the presence of the antigens in tadpoles, which are absent in the adult frogs, i.e.

of the stage-specific antigens). Cardiac tissues of tadpoles are more similar by their antigenic properties to the cardiac tissues of Triturus taeniatus than the cardiac tissues of frogs at the expense of the stage-specific antigens, which are similar to the cardiac antigens of Triturus taeniatus. Stage-specific antigens with no similarity to the antigens of cardiac tissues of Triturus taeniatus were revealed in the cardiac tissues of tadpoles.

LITERATURE CITED

- [1] R. F. Averkina, Byull. Éksptl. Biol. i Med. 41, 2, 70-73 (1956).*
- [2] A. Boyden and G. K. Noble, Am. Museum Novitates. 606, 1 (1933).
- [3] H. Braus, Arch. Entwickl. Mech. Org. 22, 564 (1906).
- [4] R. M. Clayton, Nature, 168, 120 (1951).
- [5] R. M. Clayton, Embryol. a. Exper. Morph. 1, 25 (1953).
- [6] R. A. Flickenger and G. W. Nace, Exper. Cell Res. 3, 393 (1952).
- [7] I. L. Krichevskii, Zbl. Bakt. 72, 81 (1914).
- [8] G. H. Nuttall, Blood Immunity and Blood Relationship, New York Press, 1904.
- [9] Cited by Philippson and G. H. Nuttall, 1904.
- [10] J. L. Spar, J. Exper. Zool. 123, 467 (1953).
- [11] P. Uhlenhuth, Haendel. Ergebn. wissenschaft. Med. 2, 1-4 (1910).
- [12] P. Uhlenhuth and K. Wurm, Ztschr. Immunitätsforsch. 96, 183 (1939).
- [13] K. Wilkoewitz and H. Ziegenspeck, Botanisch. Arch. 22, 227 (1928).

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