

# COMPARATIVE IMMUNOBIOLOGICAL CHARACTERISTICS OF NORMAL AND IMMUNE TISSUE ANTIBODIES

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There are indications in the literature that the serum of animals immunized with heterogeneous tissues acquires the ability to react positively with antigens from homologous tissues and to cause certain morphological changes in these tissues [1, 4, 5, 7, 8].

As we showed earlier [4, 5], the serum of normal animals in a majority of cases reacts immunologically with water-salt extracts of kidney tissue from the same animals.

In this connection, a comparative study of the immunobiological characteristics of the antibodies which develop in the serum of animals immunized with heterogeneous tissue and of the corresponding normal tissue antibodies is of interest.

In this paper we have attempted to compare some immunological and biological properties of immune kidney antibodies and the corresponding normal tissue antibodies.

## EXPERIMENTAL METHOD

It was found by certain authors [6, 8] that normal tissue antibodies are destroyed at 65°. Immune antibodies are resistant to this temperature. We decided to use this method to differentiate immune antibodies from normal tissue antibodies and from the antibodies which develop in the serum of the animal after immunization with heterogeneous tissue which react with extracts from homologous organs. In addition, we also wanted to study the biological activity of these antibodies in tissue culture during explanation of homologous and heterologous kidney tissue.

Adult chinchilla rabbits of both sexes weighing 2.5-3 kg and sexually mature Wistar rats were used. The rabbits were immunized with water-salt extracts of mouse and rat kidney tissue. For comparison, the serum of rabbits immunized with water-salt extracts of rat liver and lung tissues was used. The immunization of the rabbits was done according to the scheme described earlier [2]. Blood was taken from the experimental animals on the 8th to the 10th day after immunization.

The presence of antibodies in the rabbit sera was determined by the complement fixation reaction (CFR) and the gel precipitation reaction (GP) by the Ouchterlony method. In making the immunological studies, the serum was heated to 56 and 65° for 30 min. The CFR was done by the classical method at 37°. Water-salt extracts of rat kidney, liver and lung tissue prepared in a proportion of 1:8 served as antigens. For the GP in gel the antigens were prepared in a proportion of 1:4. Serum of nonimmunized rabbits having a comparatively high titer of normal tissue antibodies served as the control.

To study the biological effect of serum from normal and immunized rabbits on homologous and heterologous kidney tissue, tissue culture experiments were set up. Kidney tissue from 20-day rat and rabbit embryos and also kidneys of newborn and adult animals were taken for explanation. Culturing of the tissue was done by the method described earlier [3]. The test sera were diluted with Medium 199 in a proportion of 1:2. The results of 1-day and

TABLE 1. Complement Fixation Test for the Reaction of Normal and Immune Rabbit Sera with Antigens from Tissues of Homologous Organs

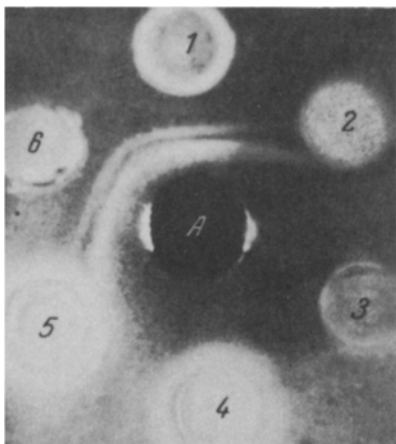
Rabbit no.	Antigens	Sera heated for 30 min											
		at 56°						at 65°					
		serum dilution											
		1:10	1:20	1:40	1:80	1:160	1:320	1:10	1:20	1:40	1:80	1:160	
1580	Kidney Liver Lungs	+++ +++ +++	+++ +++ ++(+)	+++ Γ Γ	+++ Γ Γ	± Γ Γ	Γ Γ Γ	Γ Γ Γ	Γ Γ Γ	Γ Γ Γ	Γ Γ Γ	Γ Γ Γ	
		+++ +++ +++	+++ +++ ++(+)	+++ Γ Γ	+++ Γ Γ	± Γ Γ	Γ Γ Γ	Γ Γ Γ	Γ Γ Γ	Γ Γ Γ	Γ Γ Γ	Γ Γ Γ	
288	Kidney Liver Lungs	+++ +++ +++	+++ +++ ++(+)	+++ Γ Γ	+++ Γ Γ	± Γ Γ	Γ Γ Γ	Γ Γ Γ	Γ Γ Γ	Γ Γ Γ	Γ Γ Γ	Γ Γ Γ	
		+++ +++ +++	+++ +++ ++(+)	+++ Γ Γ	+++ Γ Γ	± Γ Γ	Γ Γ Γ	Γ Γ Γ	Γ Γ Γ	Γ Γ Γ	Γ Γ Γ	Γ Γ Γ	
2436	Kidney Liver Lungs	+++ +++ +++	+++ +++ +++	+++ Γ +++	+++ Γ +++	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	
		+++ +++ +++	+++ +++ +++	+++ Γ +++	+++ Γ +++	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	
2402	Kidney Liver Lungs	+++ +++ +++	+++ +++ +++	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	
		+++ +++ +++	+++ +++ +++	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	+++ Γ Γ	

Normal rabbit sera

Sera of rabbits immunized with kidney tissue antigen

TABLE 1 (cont.)

Sera heated for 30 min												
		at 56°					at 65°					
Rabbit no.	Antigens	serum dilution										
		1:10	1:20	1:40	1:80	1:160	1:320	1:19	1:20	1:40	1:80	1:160
Sera of rabbits immunized with liver tissue antigen												
2446	Kidney Liver Lungs	++++ ++++ ++++	++++ ++++ ++	++++ ++++ +	++ ++ +	+	+	++++ ++++ +	++++ ++++ +	++ ++ +	+	+
50	Kidney Liver Lungs	++++ ++++ ++++	++++ ++++ ++++	++++ ++++ ++++	++++ ++++ ++++	++ ++ ++	++ +	++++ ++++ +	++++ ++++ +	++++ ++++ +	++ ++ +	+
Sera of rabbits immunized with lung tissue antigen												
2459	Kidney Liver Lungs	++++ ++++ ++++	++++ ++++ ++	++++ ++++ ++	++ ++ +	++ +	+	++++ ++++ +	++++ ++++ +	++ ++ +	+	+
47	Kidney Liver Lungs	++++ ++++ ++	++++ ++++ ++	++++ ++++ ++	++++ ++++ ++	+	+	++++ ++++ ++	++++ ++++ ++	++++ ++++ ++	++ ++ +	+



Gel precipitation reaction of serum of rabbit, immunized with rat kidney, with antigens from tissues of its own organs. In central hole (A) — rabbit serum, in holes 1 and 6 — kidney tissue antigen, in holes 2 and 3 — liver tissue antigen, in holes 4 and 5 — lung tissue antigen.

3-day cultivation were determined with respect to the nature of the growth of the explantates, its intensity, and the morphological structure of the growth zone.

## RESULTS

Thirty four rabbits were used in the experiment, 11 of them were immunized with antigens from kidney tissue, 9 with antigens from liver tissue, and 8 with antigens from lung tissue. One of the typical protocols of the experiment is presented in Table 1. From this table it is seen that sera of rabbits immunized with antigens from heterogeneous kidney react +++ with antigens from homologous kidneys in a dilution of 1:320, with antigens from tissue of homologous livers ++ and ++++ in a dilution of 1:20 and with antigens from homologous lung tissue +++ in a dilution of 1:40.

Antiliver serum reacted in comparatively high titers with antigens from the homologous animal tissue. The maximum titer of the antisera was 1:160 (++ reaction). Antilung serum also reacted positively with antigens from the homologous animal tissue (1:80 and 1:160, +++ and ++++). The antisera reacted in high titers with the antigens from heterogeneous tissues to which the rabbits were immunized (++++1:640 and ++ and +++ 1:1280). However, sometimes the antiserum reacted ++ and +++ with heterogeneous antigens in a dilution of 1:160. Here, it was noted that such serum also reacted with antigens from the homologous animal tissues at a low dilution.

As we showed in a previous paper [5], the serum of normal rabbits also can sometimes react in a comparatively high titer with antigens from tissues of homologous organs. In the present work sera from 6 normal rabbits were studied in the presence of normal tissue antibodies. Sometimes the sera reacted in the complement fixation reaction with antigens from tissues of homologous kidneys +++ in a dilution of 1:80. Heating these sera for 30 min at 65° caused them to stop reacting with antigens from their own tissues even at a dilution of 1:10.

Immune rabbit sera after heating at the same temperature continued to react with antigens from heterogeneous tissues at approximately the same titer as before heating. These sera, heated at the stated temperature, also reacted with antigens from their own organs. However, it should be noted that antikidney, antiliver and antilung sera reacted in all cases with antigens from kidney tissue. Antiliver serum reacted with antigens from homologous liver tissue, but did not always react with antigens from homologous lung tissue, while heated antilung serum continued to react with antigens from homologous lung tissue, but did not always react with antigens from homologous liver tissue.

From the data presented, it is seen that tissue antibodies contained in normal rabbit serum are broken down at 65°, while antibodies from immunized rabbits which react in a complement fixation reaction with antigens from their own organs are more stable to heating and behave similarly in this respect to immune antibodies.

In the gel precipitation reaction the sera of immune rabbits gave clear lines of precipitation with heterogeneous tissues. For example, the serum of rabbit No. 42, immunized with antigens from mouse lung tissue, gave 5 lines of precipitation with an extract from this organ. In the GP, immune serum reacted mainly with antigens from homologous kidney tissue (up to 2 lines of precipitation were produced) and did not always react with antigens from homologous lung and liver tissues (see figure).

In view of the fact that in immune rabbit serum there are antibodies which most characteristically react both in the CFR and in the GP in gel with antigens from homologous kidney tissue, we decided to test the cytotoxicity of this serum in tissue culture with respect to the latter. Heterogeneous kidney tissues of the same animal with whose antigens the rabbits were immunized served as the control. Here it was established (Table 2) that in normal rabbit serum the tissue cultures of rat and rabbit kidney grow in approximately the same way. On the 3rd day of explantation growth was found in 91-100% of the cases. In the first 24 h of culturing the growth zones of the experimental cultures were in the form of a single-layer of the epithelial type. Connective tissue elements appeared somewhat later. In culturing rat kidney tissue on the serum of rabbits immunized with rat kidney tissue, in the majority of cases there is observed strong growth inhibition of the experimental cultures. When the experimental cultures in the first hours of culturing showed some growth, their proliferating cells then underwent destruction and breakdown.

TABLE 2. Nature of the Growth of 3-Day Tissue Cultures of Rat and Rabbit Kidneys on Serum of Normal Rabbits Immunized with Water-Salt Extracts of Rat Kidney Tissue

Serum	Titer of serum in CFR to antigens from kidney tissue of the animal		Culture of kidney tissue of the animal	Character of explantate growth		
	rats	rabbits		Growth of cultures (in %)	Mean growth coefficient	Ratio of epithelial and connective tissue growth
Normal:						
No. 213	1:40++	1:40+++	Rat	$\frac{34}{91.8}$	2.85	$\frac{31}{0}$
No. 205	1:10+++	1:10+++	"	$\frac{27}{100.0}$	3.68	$\frac{27}{0}$
No. 265	1:20++	1:20++	"	$\frac{26}{100.0}$	3.21	$\frac{24}{2}$
No. 288	1:80+++	1:80+++	"	$\frac{25}{75.0}$	1.94	$\frac{4}{11}$
Immune:						
No. 213	1:1280++	1:320++	Rat	$\frac{26}{0}$	0	0
No. 213	Same	Same	Rabbit	$\frac{27}{100.0}$	3.14	$\frac{9}{18}$
No. 2402	1:1280++	1:160++	Rat	$\frac{44}{0}$	0	0
No. 2402	Same	Same	Rabbit	$\frac{55}{100.0}$	3.18	$\frac{30}{25}$
No. 1	1:160++	Not done	Rat	$\frac{25}{36.0}$	0.45	$\frac{7}{5}$
No. 205	1:160+++	" "	"	$\frac{27}{14.7}$	0.50	$\frac{4}{1}$

Note. In the column "Growth of Culture (in %)" in the numerator—total number of cultures, in the denominator—number of growing cultures. In the column "Ratio of epithelial and connective tissue growth" in the numerator—number of cultures with epithelial growth, in the denominator—with connective tissue.

The cells were round and decreased in size, the protoplasm acquired a coarse-grained structure. The cells of the growth zones of the kidney tissue cultures growing on serum of normal rabbits were polymorphic, had large bright nuclei and fine-grained cytoplasm. From explantation of rabbit kidney tissue on antikidney serum relatively good growth of the cultures was observed: the number of growing cultures reached 100%, with an average growth coefficient of 3.14-3.18.

Thus, the serum of rabbits immunized with an extract of rat kidney tissue (antibody titer to heterogeneous tissue ++ and +++ 1:160, ++ 1:1280, antibody titer to homologous kidney tissue ++ 1:160, ++ 1:320) inhibits the growth of cultures of rat kidney tissue and causes destructive changes in the cells in the growth zone.

These same sera in comparison with the control (normal rabbit serum) did not have any important effect on the growth of rabbit kidney tissue cultures. In other words, kidney antibodies of rabbit serum are biologically active with respect to heterogeneous kidney tissue and inactive to the same degree with respect to homologous kidney tissue.

Therefore, the data obtained show that tissue antibodies contained in normal rabbit serum are destroyed if they are heated to 65° for 30 min; immune heterogeneous antibodies are stable to this temperature. Antibodies to homologous organs which develop in the serum of rabbits immunized with antigens from heterogeneous tissue differ from the corresponding tissue antibodies contained in the serum of normal rabbits. This difference is expressed in the fact that the antibodies to antigens from homologous kidney tissue are not destroyed at 65° for 30 min, and behave similarly to immune antibodies. In antikidney serum antibodies to antigens from homologous liver tissue are not destroyed at this temperature. Antibodies contained in antilung serum continue to react with antigens from homologous lung tissue at the temperature stated above. With antigens from tissue of homologous kidneys, immune serum

gives clear lines of precipitation in gel. This serum does not have a cytotoxic effect on cultures of their own (rabbit) kidney and are cytotoxic for cultures of heterogeneous kidney tissue.

#### SUMMARY

Normal tissue antibodies contained in the sera of normal rabbits and reacting to extracts from their own organs are destroyed when heated to 65° during 30 min; antibodies detected in the sera of immune rabbits and reacting to antigens from the tissue of their own organs are resistant to this temperature. The sera of rabbits immunized with heterogeneous organ tissue exhibit clear-cut precipitation lines (up to 2 lines) in gel with antigens from the tissue of the rabbit's own kidney.

The sera of rabbits immunized with antigens from rat kidney tissue and reacting in the complement fixation test and the precipitation in gel reaction to antigens from the tissue of the rabbit's own kidney are cytotoxic for a rat kidney tissue culture and nontoxic for rabbit kidney tissue.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

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