

# IMMUNOMORPHOLOGICAL CHANGES IN RATS CAUSED BY IMMUNIZATION WITH AUTOLOGOUS LIVER

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There are reports in the literature that the sera of animals immunized with heterologous tissue react with antigens from the organs of the same animal [1, 2, 4].

Studies have recently been published showing that the tissues of different organs may be antigenic for homologous animals [6, 8].

Some particularly interesting investigations have been carried out on the immunization of animals with antigens from their own tissues. Several authors have obtained positive results in this direction [5, 7, 9].

The object of the present investigations was to study the ability of the sera of rats immunized with their own liver tissue to react with antigens from the tissue of that organ. At the same time, an attempt was made to detect morphological changes arising in the liver of such animals.

## EXPERIMENTAL METHOD

Experiments were carried out on adult female rats of the August line weighing 120-140 g, from which the middle left lobe of the liver was first removed. The resected part accounted for 65-70% of the weight of the liver.

The material for immunization consisted of saline extracts of the liver tissue in a dilution of 1:4. Immunization began one month after hepatectomy, for at this moment the regenerative processes were completely finished and the functional powers of the liver were restored [3].

The rats were immunized with five injections at intervals of 10 days. The antigen was injected intraperitoneally, mixed with Freund's complete stimulant.

Blood was taken from the experimental animals at the time of sacrifice on the 10th day of the last immunization.

The ability of the rats' serum to react positively with the saline extracts of their own liver tissues, and of the tissues of the isologous liver and kidney was studied in the complement fixation reaction (CFR), the gel-precipitation reaction as described by Ouchterlony (PR), and the reaction of immunoelectrophoresis (IER). The CFR was performed by the classical method at 37°, the PR by the usual method, and the IER by the generally adopted method at room temperature. The results of the reactions were read after 24-120 h.

The control consisted of sera taken from hepatectomized rats receiving injections of an antigen prepared from muscle tissue, mixed with the stimulant, from hepatectomized rats receiving injection of stimulant mixed with physiological saline, from hepatectomized rats, and from intact rats not undergoing the operation.

The control animals were immunized at the same time and after the same intervals as the experimental animals.

The amount of antigen injected was equalized in its protein content as shown by Lowry's method with the corresponding experimental group.

At the same time, studies were made of the morphological changes arising in the liver of the experimental and control animals. Histological sections were made by the usual method and stained with hematoxylin-eosin.

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Material injected at immunization	No. of sera	Intensity of reaction	Antigens											
			from autologous liver				from isologous liver				from isologous kidney			
			dilution of serum											
			1:10	1:20	1:40	1:80	1:10	1	1:40	1:80	1:10	1:20	1:40	1:80
Antigens from autologous liver plus Freund's stimulant	15	+	—	—	—	1	—	—	—	3	—	1	—	—
		++	—	1	4	12	—	1	9	8	2	14	—	—
		+++	3	11	11	—	5	14	6	—	13	—	—	—
		++++	12	3	—	—	10	—	—	—	—	—	—	—
Antigens from muscle plus Freund's stim- ulant (control)	15	+	—	—	—	—	—	—	—	—	—	1	—	—
		++	—	6	7	—	—	7	6	—	2	9	—	—
		+++	5	9	3	—	9	8	2	—	10	—	—	—
		++++	10	—	—	—	6	0	—	—	3	—	—	—
Freund's stimulant plus physiologi- cal saline (control)	15	+	1	3	—	—	2	4	—	—	—	4	—	—
		++	2	5	—	—	3	6	—	—	3	5	—	—
		+++	8	7	—	—	7	3	—	—	7	—	—	—
		++++	4	—	—	—	3	—	—	—	—	—	—	—

## EXPERIMENTAL RESULTS

In a preliminary investigation of the serological background in all the animals taking part in the experiment, it was found that in 50% of cases their sera reacted with antigens from isologous liver in a titer of 1:10, and this was taken into consideration when the groups were selected.

The results of the CFR with the sera of the rats after a cycle of immunization of both the experimental and the control animals are shown in the table; they demonstrate that the sera of the experimental group of rats reacted with antigen from their own liver in a titer of 1:80 in 13 of 15 cases.

The sera of the control animals immunized with antigen from muscle tissue in combination with the stimulant, and also with the stimulant combined with physiological saline, gave a reaction with a maximal titer of 1:40. The sera of the animals gave a rather weaker reaction with antigen from isologous liver than with antigen from autologous liver, and a weaker reaction still with antigen from isologous kidney.

The PR in gel of the sera of the rats immunized with extract of autologous liver tissue mixed with the stimulant gave two lines with a saline extract of their own liver. The disappearance of one precipitation line after heating the sera at 60° for 20 min shows that normal antibodies of liver specificity were concerned in the formation (Fig. 1).

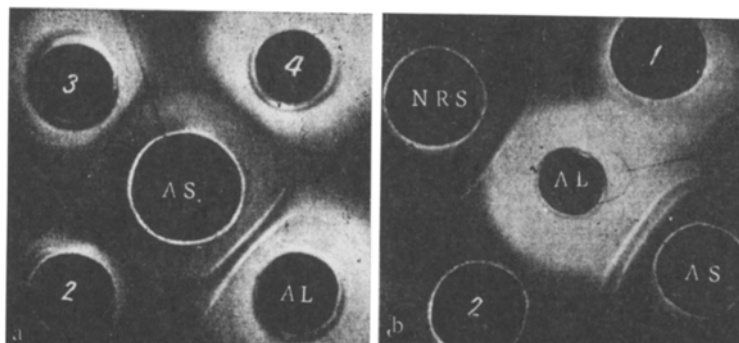


Fig. 1. Precipitation reaction in gel of serum of normal rats (a) and rats immunized with antigens from autologous liver with Freund's stimulant (b). AL) Antigen from autologous liver; AS) immune autologous serum; NRS) normal rat serum; 1) heated normal serum; 2) physiological saline; 3) antigen from guinea pig's liver; 4) antigen from guinea pig's kidney.

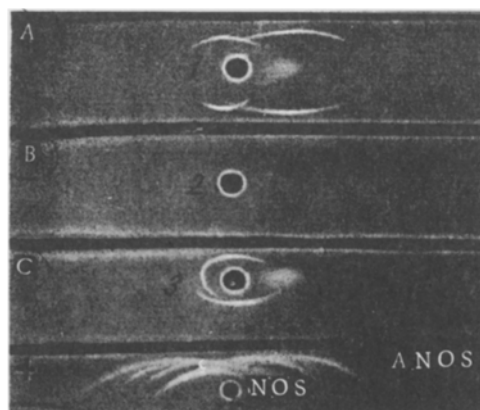


Fig. 2. Reaction of immunoelectrophoresis of the serum of a normal rat and a rat immunized with antigens from autologous liver with Freund's stimulant. 1) Serum of immunized rat; 2) heated normal serum; 3) unheated normal serum; A, B, C) antigens from rat's liver; NOS) normal ox serum; ANOS) antiserum.

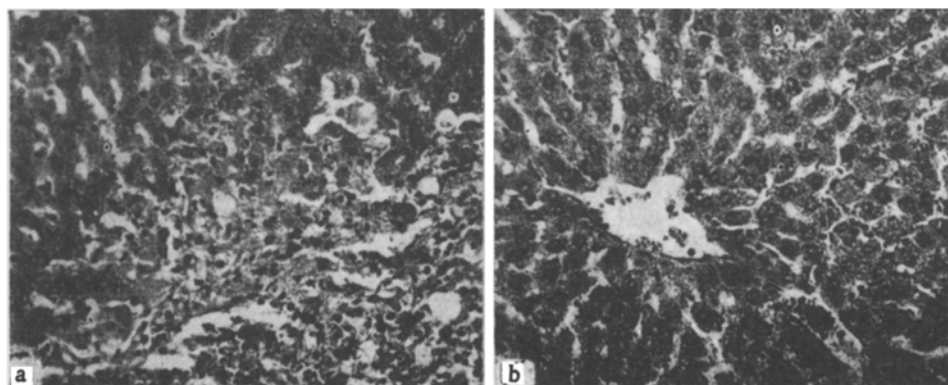


Fig. 3. Normal rat's liver (a) and liver of a rat with a necrotic area (b).

In the IER the sera of normal rats formed precipitation lines with antigens from rat's liver in the zone of the  $\beta$ -globulins, and these lines disappeared after heating to  $60^\circ$ . The sera of the rats immunized with antigens from autologous liver mixed with stimulant formed additional precipitation lines with antigens from rat's liver in the zone of the  $\gamma$ -globulins (Fig. 2).

The study of the morphological picture of the liver from the rats immunized with antigen from autologous liver mixed with stimulant showed disorganization of the columns of liver cells, polymorphism of the nuclei of the liver cells, congestion of the central veins, and moderate leukocyte infiltration, more intensive in some places, with occasional necrotic foci (Fig. 3b). In the rats of the control group, no structural abnormalities were found in the liver (Fig. 3a).

The results thus show that when rats are immunized with antigen from autologous liver mixed with Freund's stimulant, antibodies appear in the blood which reacts with saline extracts from autologous liver tissue. In these circumstances very slight degenerative changes are observed in the liver.

#### SUMMARY

In immunization of rats with autologous liver tissue with Freund's stimulator the blood of these animals shows the appearance of antibodies specifically reacting to water-saline extracts from their own liver. Simultaneously, insignificant destructive processes are noted in the liver of these rats.

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