STUDY OF THE DISTRIBUTION AND CATABOLISM OF HOMOLOGOUS ANTIBODIES IN THE ANIMAL ORGANISM

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The immunoprophylaxis and therapy of infectious diseases and, in particular, the use of homologous γ-globulin have become widespread in recent years. It is therefore interesting to study the distribution and metabolism of antibodies introduced into the animal organism.

Very little information on this subject is available in the literature, and it is largely contradictory. Most workers consider that the half-life period of homologous antibodies in the body of a healthy nonimmune rabbit is 4-6 days [4, 6, 12, 13]. However, the distribution of passively injected antibodies in the various organs has received little study. The author knows of only two investigations of this problem [3, 9], but in these the distribution of the antibodies was studied only 4, 8, and 14 days after the injection of homologous immune globulins.

Because of these facts, and also in consideration of data showing the distribution of antibodies between the blood stream and the tissues immediately after injection [13], it was most interesting to study the distribution at catabolism of the antibodies in the body in the early periods after passive immunization, and the present investigation was carried out for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on 50 chinchilla rabbits weighing 2.5-3.5 kg. The donor animals were immunized with two subcutaneous injections a pure fraction of human γ-globulin, at intervals of 10 days, in doses of 0.05 and 0.1 g proteins. The animals were revaccinated 3-6 months later with 0.1 g γ-globulin. On the 7th-8th day after revaccination blood was taken from the rabbits, and the absolute content of antibodies in the serum from this blood was determined by the immunochemical method of Heidelberger and Kendall [8], the protein was estimated by Lowry's method [11], and the titer of the antibodies was determined in the passive hemagglutination reaction (PHR) by Boyden's method [5]. For passive immunization a serum containing antibodies in a dose of 1000 μg/ml

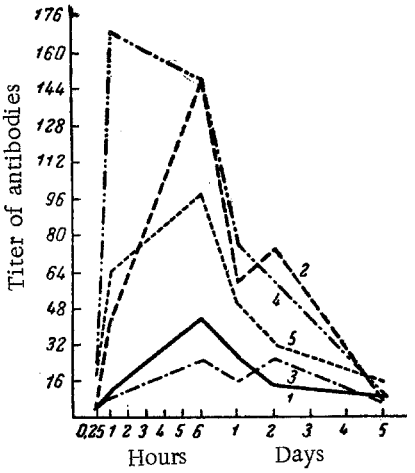

Fig. 1. Titers of antibodies in organs at different times after passive immunization (PHR). 1) Lung; 2) liver; 3) kidney; 4) spleen; 5) lymph gland.

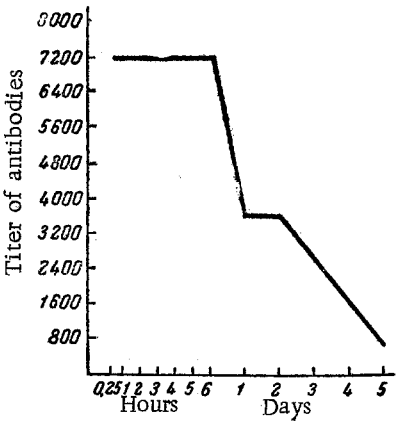

Fig. 2. Titers of antibodies in blood at various times after possessive immunization (PHR).

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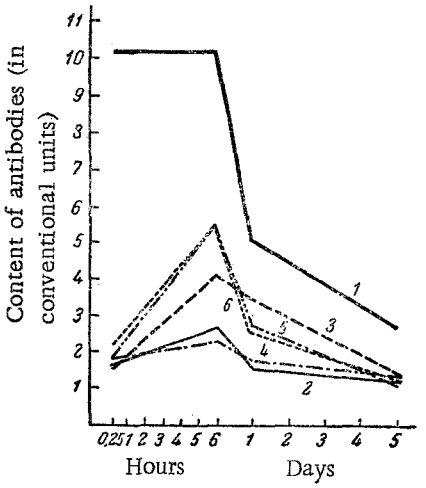

Fig. 3. Content of antibodies in the blood and organs at various times after passive immunization (CFR). 1) Blood serum; 2) lung; 3) liver; 4) kidney; 5) spleen; 6) lymph gland.

was used (the titer in the PHR was 1 : 262,144). The serum was injected intravenously in a dose of 1000 μg antibodies per kg body weight.

At intervals of 15 min and 1, 6, 24, and 48 h, and 5 days after passive immunization, blood was taken from the marginal vein of the rabbits' ear, after which the animals received an intravital perfusion of 0.85% sodium chloride solution at 37° (1 liter of solution per kg body weight) via the inferior vena cava under pentobarbital-sodium anesthesia (pentobarbital sodium in a dose of 40 mg/kg in physiological saline, intraperitoneally) [3]. Shortly after the end of perfusion, the lung, liver, kidney, spleen, and the popliteal and mesenteric lymph glands were removed from the animals. Extracts of the parenchymatous organs were prepared by a method developed in S. Ya. Kaplanskii's laboratory [1], and extracts from the lymph glands by the method of Demling and coworkers [7].

The antibody content in the blood serum and extracts of the organs was determined by the PHR by Boyden's method [5] and the complement fixation reaction (CFR) at 50% hemolysis in the cold [2]. In addition, the antibody content was determined in the individual protein fractions of the blood serum and of the organ extracts by a combination of electrophoresis of the proteins on paper with the PHR [10]. A statistical analysis of the numerical results was made by Student's method. The differences between the indices were taken as significant when $P < 0.05$.

EXPERIMENTAL RESULTS

The maximal content of antibodies in the organs was observed 6 h after intravenous injection of the antibodies, after which the antibody titers fell to regain their original level on the 5th day (15 min after passive immunization).

Among the investigated organs the largest accumulation of antibodies was found in the spleen ($1 : 147 \overset{\times}{:} 1.15$), in the liver ($1 : 146 \overset{\times}{:} 1.15$), and in the lymph glands ($1.84 \overset{\times}{:} 1.18$) (Fig. 1). These results are statistically significant, except for the titer of the antibodies found in the spleen 1 h after passive immunization by comparison with the results obtained 6 h after ($P > 0.5$).

The content of antibodies in the serum remained unchanged until the 6th hour after passive immunization ($1 : 7131 \overset{\times}{:} 1.15$). The antibody titer after 24-48 h had fallen to one-half ($1 : 3566 \overset{\times}{:} 1.15$), and after 5 days to one-eleventh ($1 : 676 \overset{\times}{:} 1.05$) (Fig. 2).

To confirm the results obtained, similar investigations were carried out by another serological method. A highly sensitive quantitative modification of the CFR, working at 50% hemolysis in the cold, was used for this purpose, enabling the objective estimation of the fixation of complement by antibodies in conventional units (CFU) [2]. The results obtained (Fig. 3) were similar in principle to those obtained by the PHR.

Comparison of the dynamics of the changes in the content of antibodies in the blood serum and organs at various times after passive injection of antibodies showed that in the first 6 h a dynamic equilibrium was established between the blood and the tissues, after which steady destruction of the antibodies took place in the course of metabolism.

The results of these investigations suggest that the dynamic equilibrium in the distribution of the antibodies between the blood serum and the tissues was always strongly displaced to the side of the serum. Consequently, the presence of antibodies in a particular organ in higher titers than in the blood is convincing evidence that this organ is concerned in the synthesis of immune proteins.

The selective accumulation of antibodies in the spleen, liver, and lymph glands after passive injection points to the active participation of these organs in the catabolism of the antibodies.

The results of the study of the localization of antibodies in individual fractions of the serum and tissue proteins show that in the spleen and, in particular, in the liver, in contrast to the other organs and the blood serum, the highest titer of antibodies was found in the fraction possessing mobility close to that of the serum β-globulins (see table). In the statistical analysis of the data given in the table, the titers of the antibodies with mobility of the

Localization of Antibodies in Globulin Fractions of Serum and Tissue Proteins

Tissue	Titers of antibodies in eluates of individual protein fractions (mean data)		
	γ-globulins	β-globulins	α-globulins
Donor's blood serum	1:32 768	1:1 024	1:64
Test serum.	$\frac{\times}{1:47:1,40}$	$\frac{\times}{1:24:1,30}$	$\frac{\times}{1:4:1,30}$
Extract of lung	$\frac{\times}{1:4,6:1,14}$	$\frac{\times}{1:4,8:1,29}$	$\frac{\times}{1:3:1,30}$
Extract of liver	$\frac{\times}{1:3,5:1,14}$	$\frac{\times}{1:10,5:1,42}$	$\frac{\times}{1:3:1,17}$
Extract of kidney. . .	$\frac{\times}{1:4,6:1,29}$	$\frac{\times}{1:2,3:1,14}$	$\frac{\times}{1:3:1,18}$
Extract of spleen . . .	$\frac{\times}{1:5,3:1,18}$	$\frac{\times}{1:8:1,00}$	$\frac{\times}{1:2,3:1,14}$
Extract of lymph gland	$\frac{\times}{1:7:1,14}$	$\frac{\times}{1:6:1,18}$	$\frac{\times}{1:31:1,18}$

Note. The localization of the antibodies was determined 6 h after passive immunization.

β-globulins are compared with those of the antibodies moving in the electrical field with the γ-globulins. For the liver and spleen, P was less than 0.02 and less than 0.05 respectively.

It may be concluded from the results described above that antibodies with electrophoretic mobility of the β-globulins are fixed selectively by the liver and spleen.

SUMMARY

The passive hemagglutination reaction after Boyden and the complement fixation reaction in 50% hemolysis on cold were used to study the distribution and catabolism of homologic antibodies in the body of a healthy, non-immune rabbit in an early period after passive immunization. It has been found that the maximum level of antibodies of the organs under investigation is observed 6 h after an intravenous injection of antibodies, the largest number of antibodies being discovered in the spleen, liver, and lymphatic glands, which is evidence of an active part taken by these organs in the catabolism of immune globulins.

It has been found that in the liver and spleen, as distinct from other organs and blood serum, the greatest titer of antibodies is determinable in a fraction which is close in mobility to the β-globulins of serum. This gives one reason to suppose the existence of a selective fixation by the above-mentioned organs of antibodies with the electrophoretic mobility of β-globulins.

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