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Autoantibody Production in Rabbits

II. ORGAN-SPECIFIC AUTOANTIBODY IN RABBITS INJECTED WITH RAT TISSUES

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Summary. The sera of rabbits injected with rat liver, kidney, heart, muscle, spleen and brain in Freund's complete adjuvant fixed complement with rabbit tissue. This complement-fixing activity was attributed to autoantibodies which were able to fix complement *in vitro* with the tissue of the rabbit in which they occurred. Absorption, gel diffusion and antibody and antigen titrations indicated that some of the anti-liver, anti-kidney, anti-heart, anti-muscle and anti-brain sera contained organ-specific autoantibody. The sera also contained autoantibody reacting with widely distributed antigen(s), which was relatively labile at 65°. The anti-kidney and anti-brain sera reacted with distinct antigens which were extracted from rabbit kidney and brain with a mixture of chloroform and methanol. The natural autoantibody of Kidd and Friedewald was usually labile at 65° and behaved like a macroglobulin on sucrose gradient centrifugation. Sera taken 1 week after immunization with rat tissue contained heat-labile macroglobulin antibody. However, sera taken 1 month after immunization also contained small molecular weight antibody which was stable at 65°.

INTRODUCTION

Antibody against several rabbit tissues has been demonstrated after the injection of various tissues from other species into the rabbit. These include lens, uvea and cornea, brain, heart, adrenal, thyroid and nucleoprotein (see Asherson and Dumonde, 1962). It was therefore of interest to investigate whether the injection of different rat tissues into rabbits led to the formation of different autoantibodies. This paper shows that the injection of rat liver, kidney, brain, heart, muscle and spleen in Freund's complete adjuvant causes autoantibody formation in rabbits, that some of these antibodies are organ-specific and that both macroglobulin and small molecular weight autoantibodies occur.

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MATERIALS AND METHODS

ANIMALS

Dutch rabbits and white adult rats were used.

IMMUNIZATION

Organs from freshly killed rats were homogenized in 0·25 M sucrose and then emulsified with an equal volume of Freund's complete adjuvant (Difco) to give a final concentration of 1 in 4 of wet tissue w/v. Each rabbit received 0·5 ml. of the emulsion into each of the four foot pads. At 10 weeks the rabbits received further footpad injections and at 14 weeks 0·5 g. wet weight of tissue homogenized in 0·25 M sucrose intraperitoneally followed by 1 ml. of 1/10 homogenate intravenously. The animals were bled out and killed at 15 weeks.

SERA

The sera were inactivated at a dilution of 1/4 in veronal-buffered calcium magnesium saline pH 7·2 (Oxoid barbitone CFT diluent tablets) for half an hour at 56° unless otherwise stated. The phrase heat-stable antibody refers to antibody detected in sera inactivated at 65°.

COMPLEMENT FIXATION

The method previously described (Asherson and Dumonde, 1962) was used but 45 minutes were allowed for haemolysis instead of 30 minutes. The results are expressed as the reciprocal of the highest dilution giving 2 plus (50 per cent) haemolysis estimated visually. A rise in titre of two tubes (2 $\log_2$ units) was accepted as significant. The dilutions of the test antigens, in terms of wet weight of tissue, were rabbit liver, rabbit kidney and rat liver 1/80, rabbit spleen, heart and muscle 1/40–1/50, rabbit brain 1/200–1/400, chloroform methanol extracts of liver and kidney 1/10, and of brain 1/20. Difco Kolmer cardiolipin antigen diluted 1/20 was used as Wassermann antigen.

ABSORPTION

A 1/10 homogenate of rabbit tissue in 0·25 M sucrose which had been stored at −18° was mixed with 4 volumes of veronal-buffered calcium magnesium saline and left at 37° for 15 minutes and 4° for 90 minutes. It was centrifuged at 144,700 *g* for 30 minutes and the deposit resuspended in a volume of buffer equal to that of the original homogenate. This suspension was mixed with an equal volume of a 1/4 dilution of serum taken 4 weeks after primary immunization, left at 37° for 30 minutes and 4° for 30 minutes and centrifuged at 44,000 *g* for 30 minutes. The supernatant serum was stored at −18° and inactivated before use. As a control, unabsorbed serum was handled in parallel.

GEL DIFFUSION

This has been described (Asherson and Dumonde, 1962). In the experiments on sero taken 1 month after primary immunization, the antigen wells contained a 1/3 extract a

rabbit tissue in molar glycine saline. In the later experiments the antigen wells contained rabbit tissue in molar glycine saline buffered to pH 7·2 with sodium barbiturate and 0·01 per cent ethylene diamine tetraacetate was added to the extract and the agar.

SUCROSE GRADIENT CENTRIFUGATION

The method of Charlwood was employed. Ten, 20 and 30 per cent sucrose was layered in a 12 ml. 'lusteroid' tube and left overnight. One millilitre of undiluted serum or serum diluted with an equal volume of saline was mixed with ^{131}I-labelled human γ globulin (for which we are indebted to Dr. P. A. Charlwood) and was carefully layered on top and the tubes centrifuged at 56,500 *g* for 14 hours. The bottom of the tubes was punctured by a needle and successive equal fractions each slightly greater than 1 ml. were collected. The radioactivity was measured and the fluid was dialysed against calcium magnesium saline buffer and tested for antibody activity by complement fixation. The location of the low molecular weight globulin was determined by the ^{131}I-labelled marker, that of the macroglobulin by the distribution of the naturally occurring rabbit sheep cell haemolysin which is a macroglobulin.

CHLOROFORM METHANOL EXTRACTION

One gramme of fresh rabbit tissue was homogenized with saline to give a final volume of 10 ml., and added to 100 ml. of a 1:2 chloroform methanol mixture, left at room temperature for 24 hours, and filtered. The filtrate was evaporated under reduced pressure and the deposit dissolved in 5 ml. of toluene. An aliquot of the toluene solution was taken, the toluene evaporated under reduced pressure and the residue taken up in methanol. The milky fluid produced by adding 9 volumes of buffer was used as antigen. Activity was expressed in terms of the weight of rabbit tissue from which the extract was obtained.

SOLUBLE ANTIGEN

Rabbit tissue was homogenized in 9 volumes of sucrose using a Potter homogenizer and the nuclei removed by centrifugation at 500 *g* for 10 minutes. The deposit obtained by centrifugation at 15,000 *g* for 17 minutes was washed in sucrose, resuspended in M/2 glycine buffered with veronal (pH 7·2) and disintegrated by ultrasonic vibration at 20,000 cycles per second for 2½ minutes.

The supernatant was obtained by centrifugation at 144,700 *g* for 30 minutes.

RESULTS

AUTOANTIBODY FORMATION FOLLOWING A SINGLE INJECTION OF RAT TISSUE

Eighteen rabbits were injected with rat liver, kidney, brain, heart, muscle or spleen in Freund's complete adjuvant. Before immunization, the titre of complement-fixing activity of the sera heated at 56° against rabbit kidney ranged from 16 to $\geqslant$64. When the sera were heated at 65°, the titre fell to $<$4 in seventeen of the eighteen rabbits.

Table 1 shows that after immunization there was a significant rise in the titre of complement-fixing activity against rabbit kidney in at least twelve of the eighteen rabbits when the sera were heated at 56° and in fifteen of the eighteen rabbits when the sera were

TABLE 1

TITRE OF COMPLEMENT-FIXING ACTIVITY AGAINST RABBIT AND RAT TISSUE HOMOGENATES IN THE SERA OF RABBITS AFTER ONE INJECTION OF RAT TISSUE IN FREUND'S COMPLETE ADJUVANT

Bleed	*Test antigen*		*Immunizing rat antigen*																			
			Liver			*Kidney*			*Brain*			*Muscle*			*Heart*		*Spleen*					
			17[f]	18	19	12	13	14	9	10	11	1	2	3	4	5	6	7	8	15
0[a]	Rat liver	56°[b]	5	4	4	6	4	5	5<3		4	5	5	3	6	6	6	5	6	4
1		56°	⩾7	⩾7	⩾7	⩾7	⩾7	⩾7	7⩾7		7	6	5	6	⩾7	⩾7	⩾7	⩾7	7⩾7	
4		56°	⩾8	7	⩾8	8	7	8	7	7	7	6	6	7	⩾7	8	8	8	8⩾8	
0	Rat liver	65°	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	3<2	
1		65°	6	7	7	4	3	3	3	4	3	2	2	2	4	3	4	2	4	2
4		65°	8	6	7	8	6	6	4	6	6	4	3	6	6	7	7	5	7	8
0	Corresponding rabbit	56°	<3	3	<3	5[d]⩾4[d]		4[d]	5<3		⩽3	⩽3	<3		5	5	5	6	6	4
1	organ[c]	56°	7	6	⩾7	6[d]	7[d]	⩾7[d]	⩾7	7	6	4	<3	5	⩾7	7	6⩾7		7	6
4		56°	5	5	5	6[d]	5[d]	6[d]	9	8	⩾8	5	⩽4	6	7	6	6	7	8	6
0	Corresponding rabbit	65°	<2	<2	<2	<2[e]	<2[e]	<2[e]	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	2<2	
1	organ[c]	65°	3	3	3	2[e]	<2[e]	<2[e]	4	2	<2	2	<2	2	<2	2	<2	<2	3<2	
4		65°	5	5	4	2[e]	<2[e]	<2[e]	5	6	4	2	<2	5	<2	2	<2	<2	3<2	
0	Rabbit kidney	56°	5	4	4	6	5	6	⩾6	5	5	6	6	4	6	⩾6	⩾6	⩾6	6	5
1		56°	⩾7	⩾7	⩾7	⩾7	⩾7	⩾7	⩾7	⩾7	⩾7	7	6	6	⩾7	⩾7	⩾7	⩾7	7⩾7	
4		56°	⩾8	6	7	8	8	8	⩾8	7	8	7	7	7	⩾8	8	7	8	8⩾8	
0	Rabbit kidney	65°	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	3<2	
1		65°	3	3	4	4	3	3	3	2	2	2	<2	2	2	3	2	<2	3<2	
4		65°	7	5	6	6	5	6	5	4	3	2	2	3	3	4	3	5	4	8

[a] Time in weeks after immunization.
[b] Temperature of inactivation of serum.
[c] Rabbit organ corresponding to rat organ used for immunization.
[d] Test antigen—rabbit heart.
[e] Test antigen—rabbit liver.
[f] Rabbit No.

The results are expressed as the logarithm to the base 2 of the reciprocal of the highest dilution of serum giving significant complement-fixation.

heated at 65°. A significant rise in the titre of complement-fixing activity against the corresponding rabbit organ (that is the rabbit organ corresponding to the rat organ used for immunization) occurred in all rabbits injected with rat liver, kidney, brain and heart, in two of the rabbits injected with rat muscle and in two of the rabbits injected with rat spleen.

AUTOANTIBODY FORMATION FOLLOWING REPEATED INJECTIONS OF RAT TISSUE

After the fourth injection of rat tissue in Freund's complete adjuvant there was a significant rise in titre of heat-stable complement-fixing activity against rabbit kidney in

ten of the eleven rabbits tested. Table 2 shows that in eight rabbits there was a significant rise in titre of the heat-stable complement-fixing antibody against the corresponding rabbit organ.

TABLE 2

TITRE OF COMPLEMENT-FIXING AUTOANTIBODY IN RABBITS BEFORE AND AFTER FOURTH INJECTION OF RAT ORGANS
(Serum inactivated at 65°)

Serum	*Immunizing rat antigen*	*Test rabbit antigen*													
		*Corresponding autologous organ**		*Homologous organ*											
				Liver		*Kidney*		*Brain*		*Muscle*		*Heart*		*Spleen*	
		Pre†	*Post*‡	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
2	Muscle	<4	8	<4	<4	<4	8	<4	8	<4	8	<4	4	<4	<4
4	Heart	<4	16	<4	<4	<4	16	<4	<4	<4	4	<4	16	<4	<4
5	Heart	<4	16	<4	<4	<4	16	<4	<4	<4	<4	<4	8	<4	<4
6	Spleen	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4
7	Spleen	<4	8	<4	4	<4	16	<4	8	<4	<4	<4	<4	<4	8
8	Spleen	<4	32	<4	16	<4	32	<4	16	<4	<4	<4	8	<4	16
15	Spleen	<4	<4	<4	<4	<4	16	16	8	<4	<4	<4	<4	<4	<4
11	Brain	8	32	<4	<4	<4	8	8	32	<4	<4	<4	<4	<4	<4
12	Kidney	8	128	<4	16	4	64	<4	8	<4	<4	<4	4	<4	16
17	Liver	<4	⩽4	<4	⩽4	<4	8	<4	<4	<4	<4	<4	<4	<4	⩽4
18	Liver	<4	32	<4	32	<4	128	<4	4	<4	<4	<4	<4	<4	4

* Rabbit organ corresponding to rat organ used for immunization.
† Serum taken 10 weeks after primary immunization.
‡ Serum taken 7 days after the fourth immunization.
The results are expressed as the reciprocal of the highest dilution of serum giving significant complement-fixation.

AUTO-REACTIVE NATURE OF ANTIBODY

Table 2 also shows that the sera of these eleven rabbits reacted in comparable titres with autologous and homologous rabbit tissues. Table 4 shows that the highest dilutions of autologous and homologous rabbit tissue giving significant complement fixation with a fixed quantity of antibody were comparable.

DISTRIBUTION OF ANTIGEN ACTIVITY IN RABBIT TISSUES

Table 3 records the highest dilution of adult and foetal rabbit liver, kidney, brain, heart and muscle giving significant complement fixation with eleven sera heated at 65°, obtained 1 month after a single injection of rat tissue.

TABLE 3

ANTIGEN TITRATION. MINIMUM CONCENTRATION OF RABBIT ORGAN HOMOGENATE REACTING WITH IMMUNE SERA*

Rabbit serum	*Concentration*	*Immunizing rat antigen*	*Test rabbit antigen*									
			Liver		*Kidney*		*Brain*		*Heart*		*Muscle*	
			Adult	*Foetal*	*Adult*	*Foetal*	*Adult*	*Foetal*	*Adult*	*Foetal*	*Adult*	*Foetal*
3	1/8	Muscle 65°†	<80	<80	320	⩽20	<160	<40	<10	<20	160	80
5	1/32	Heart 56°	640	640	1280	320	10240	320	320	320	160	160
	1/8	65°	<80	<80	160	⩽20	<160	<40	<10	<20	<20	<10
9	1/64	Brain 56°	80	160	2560	320	10240	160	20	<20	⩽20	40
	1/8	65°	<80	<80	640	40	10240	40	<10	<20	⩽20	⩽10
10	1/32	Brain 56°	320	160	1280	320	20480	1280	40	40	40	80
	1/8	65°	320	160	320	40	20480	80	<10	<20	⩽20	⩽10
11	1/64	Brain 56°	<80	<80	1280	160	20480	640	40	40	40	40
	1/8	65°	<80	<80	160	⩽20	10240	<40	<10	<20	20	⩽10
12	1/64	Kidney 56°	160	160	1280	160	5120	80	20	<20	⩽20	40
	1/16	65°	<80	<80	640	80	⩽160	<40	<10	<20	<20	<10
13	1/64	Kidney 56°	<80	<80	640	80	2560	<40	20	<20	⩽20	20
	1/8	65°	<80	<80	320	⩽20	<160	<40	<10	<20	<20	<10
14	1/64	Kidney 56°	160	160	1280	160	5120	<40	20	40	⩽20	40
	1/16	65°	<80	<80	640	160	⩽320	<40	<10	<20	<20	<10
17	1/64	Liver 56°	160	320	640	80	5120	<40	20	⩽20	⩽20	20
	1/8	65°	1280	160	320	40	⩽320	<40	<10	<20	<20	<10
18	1/16	Liver 56°	640	320	320	80	5120	40	20	40	⩽20	20
	1/8	65°	1280	160	160	<20	<160	<40	<10	<20	<20	<10
19	1/32	Liver 56°	2560	320	640	160	10240	80	<10	⩽20	⩽20	20
	1/16	65°	1280	160	160	<20	<160	<40	<10	<20	<20	<10
C7	1/64	56° Infection with *Eimeria stiedae*	2560	1280	2560	640	2560	640	640	320	640	320

* Sera taken 28 days after primary immunization.

† Temperature of inactivation of serum.

The results are expressed as the reciprocal of the minimum concentration of rabbit tissue giving significant complement-fixation.

The serum of a rabbit experimentally infected with *Eimeria stiedae*, the cause of hepatic coccidiosis, was also used (Asherson and Rose, 1963). The nine sera from rabbits that received rat liver, kidney and brain revealed the highest complement-fixing antigen activity in the corresponding rabbit organ. The complement-fixing antigen activity of foetal tissue was less than that of adult tissue.

After the sera had been heated at 56° the activity of the antigens fell in the order brain, kidney and liver whether anti-brain or anti-kidney sera were used for testing. The anti-liver sera revealed more complement-fixing antigen activity in brain than in liver.

Table 4 presents results with nine sera inactivated at 65° obtained after repeated injections of rat tissue. The sera of rabbits which received rat liver, kidney, brain and heart revealed the greatest complement-fixing antigen activity in the corresponding rabbit organ and rabbit kidney.

TABLE 4

ANTIGEN TITRATION. MINIMUM CONCENTRATION OF RABBIT ORGAN HOMOGENATE REACTING WITH SERA OF RABBIT IMMUNIZED WITH FOUR INJECTIONS OF RAT ORGANS

(Serum inactivated at 65°)

Serum	*Immunizing rat antigen*	*Test rabbit antigen*						
		*Corresponding autologous organ**	*Homologous organ*					
			Liver	*Kidney*	*Brain*	*Muscle*	*Heart*	*Spleen*
2†	Muscle	160	<20	320	640	640	<20	<20
4	Heart	640	20	320	<40	<20	320	<20
5	Heart	640	20	320	<40	<20	320	<20
7	Spleen	40	160	640	640	<20	<20	80
8	Spleen	160	320	1280	1280	<20	80	160
11	Brain	2560	<20	320	⩾5120	<20	<20	<20
12	Kidney	640	1280	1280	640	<20	40	80
17	Liver	320	640	320	160	<20	<20	<20
18	Liver	2560	2560	640	160	<20	<20	80

* Rabbit organ corresponding to rat organ used for immunization.
† The sera were used at a dilution of 1/4.

ABSORPTION STUDIES

Sera taken 1 month after primary immunization were absorbed with rabbit kidney and the corresponding rabbit organ homogenates. Selective absorption was obtained with three out of three anti-liver sera, two out of two anti-kidney sera, three out of three anti-brain sera, one out of two anti-muscle sera and two out of three anti-spleen sera. Typical results are given in Table 5. The serum of rabbit No. 8, which had received rat spleen, had a titre of 64 against the Wassermann antigen. This was removed by absorption with rabbit kidney and spleen. These results show that the sera reacted with antigens with much higher activity in some rabbit organs than in others.

GEL-DIFFUSION STUDIES

Two of the three anti-muscle sera and three of the three anti-brain sera taken 1 month after immunization gave precipitation lines with the corresponding rabbit organ. A doubtful line was obtained with the three anti-liver sera. One of the anti-spleen sera gave a line with rabbit serum. The other sera and sera taken before immunization were uniformly negative. This made it unlikely that the lines were artefacts of the type described by

TABLE 5

ABSORPTION OF RABBIT SERA BY RABBIT KIDNEY AND OTHER RABBIT ORGANS

Rabbit serum	*Immunizing rat antigen*	*Absorbed with rabbit*	*Test antigen*		
			Rat liver	*Rabbit kidney*	*Corresponding rabbit organ*
19	Liver	Nil	128	64	32
		Kidney	128	⩽8	⩽8
		Liver	128	64	⩽8
14	Kidney	Nil	256	256	64*
		Kidney	128	64	⩽16*
		Liver	128	256	<8*
10	Brain	Nil	64	128	256
		Kidney	64	⩽16	256
		Brain	64	64	⩽16
3	Muscle	Nil	128	128	128
		Kidney	128	32	64
		Muscle	128	256	32
5	Heart	Nil	256	256	128
		Kidney	256	16	16
15	Spleen	Nil	256	256	64
		Kidney	256	32	⩽16
		Spleen	256	256	⩽8

* Rabbit liver used as antigen.

Berenbaum, Kitch and Cope (1962). The one anti-muscle serum, the two anti-heart sera and the two anti-liver sera prepared by repeated injections of rat tissue reacted with the corresponding rabbit organ and otherwise failed to react with rabbit liver, kidney, brain, spleen or a 1/20 dilution of the serum of the rabbit from which the organs were taken. The serum prepared by the repeated injection of rat kidney gave a faint line with rabbit heart and kidney and one of the four sera prepared by the injection of rat spleen gave lines with rabbit liver, kidney and heart. The lines produced by the anti-liver and anti-kidney sera did not stain for protein.

NATURE OF ANTIBODY

The sedimentation behaviour of the complement-fixing activity in six sera was studied by sucrose gradient centrifugation. The highest concentration of macroglobulin, as shown by the titration of the natural rabbit sheep cell haemolysin, occurred in fractions 3 and 4. The highest concentration of the ^{131}I-labelled human γ globulin occurred in fractions 7 and 8. Fraction 1, taken from the bottom of the tube, showed no complement-fixing activity. The complement-fixing activity of two sera from unimmunized rabbits, containing natural antibody, and two sera from rabbit No. 18, taken 7 and 28 days after immunization with rat liver, appeared in fractions 2–5. The highest concentration of activity appeared in fractions 3 and 4. These fractions could be diluted 1/2 to 1/4 before losing activity. The activity in two sera from rabbit No. 11, taken 7 and 28 days after

immunization with rat brain appeared in fractions 3–6 and 2–7. The highest concentration against rabbit brain and kidney appeared in fractions 4 and 5. These fractions could be diluted to 1/8 to 1/16 without losing activity. It was concluded that some of the complement-fixing activity of these sera had the sedimentation behaviour of macroglobulin.

Some of the complement-fixing activity in the two sera taken 28 days after the injection of rat liver and rat brain appeared in fractions 7 and 8. This activity was lost on dilution. It was concluded that the complement-fixing activity of sera taken 28 days after immunization had the sedimentation behaviour of both 7S and macroglobulin.

The complement-fixing activity in the 7S globulin fractions was unchanged by heating at 65°; that of the macroglobulin fraction was destroyed in most sera. The activity in the macroglobulin fraction of serum taken 7 days after immunization showed a drop of two to three tubes, but could be detected in the undiluted fractions. These results showed that complement-fixing activity associated with the macroglobulin fraction was heat labile at 65° while activity associated with the small molecular weight γ globulin was heat stable.

NATURE OF ANTIGEN

Chloroform methanol extracts were prepared from rabbit liver, kidney and brain and tested for complement-fixing antigen activity with anti-liver, anti-kidney and anti-brain sera. The liver extract was inactive. Table 6 shows that two anti-brain and two anti-

TABLE 6

CHLOROFORM METHANOL EXTRACTS OF RABBIT TISSUE. ANTIBODY AND ANTIGEN TITRES

Rabbit serum	*Immunizing rat antigen*	*Test (rabbit) antigen*			
		Extract of rabbit kidney		*Extract of rabbit brain*	
		Antibody titre	*Antigen titre*	*Antibody titre*	*Antigen titre*
10	Rat brain	16	80	64	2048
11	Rat brain	8	10	64	1024
12	Rat kidney	32	160	32	<20
14	Rat kidney	32	80	16	20

The antibody titre is the reciprocal of the highest dilution of serum giving significant complement fixation.

The antigen titre is the reciprocal of the highest dilution of rabbit organ homogenate giving significant complement fixation with a 1/8 dilution of serum.

kidney sera fixed complement with kidney and brain extracts and that these sera revealed the highest complement-fixing antigen activity in the extract of the corresponding rabbit organ. The serum of rabbit No. 9 taken 1 month after immunization with rat brain was inactive. One of the three anti-rat liver sera reacted with the rabbit brain extract. Two of the anti-brain sera reacted in low titre with the Wassermann antigen.

The anti-brain sera reacted with a soluble antigen present in the supernatant prepared by the ultrasonic disintegration of low speed deposits of rabbit kidney and brain. The anti-kidney sera only reacted with the kidney supernatant. Liver supernatant was inactive.

DISCUSSION

These results show that the injection of several rat organs in Freund's complete adjuvant caused the formation of organ-specific complement-fixing activity in the rabbit. The complement-fixing activity in the sera was attributed to antibody because it behaved on diethylaminoethyl cellulose chromatography like a mixture of macroglobulin and small molecular weight γ globulin (Asherson and Dumonde, 1962) and on sucrose gradient centrifugation like macroglobulin or a mixture of macroglobulin and small molecular weight γ globulin.

The titres of antibody activity at constant antigen concentration and of antigen activity at constant antibody concentration, measured with sera inactivated at 65°, suggested that the anti-liver, kidney, brain, muscle and heart sera showed relative organ specificity. The absorption studies showed that some of the anti-liver, kidney, brain, muscle and spleen sera reacted with at least two rabbit antigens, while the Ouchterlony plate provided evidence for organ-specific antibodies in sera prepared against liver, brain, muscle and heart. The titration of the complement-fixing activity of chloroform methanol extracts of rabbit brain and kidney suggested that the anti-brain and anti-kidney sera reacted with two distinct antigens soluble in fat solvents, one occurring in high concentration in rabbit brain and the other in rabbit kidney. Brain antigen of this type was described by Witebsky and Steinfeld (1928).

The titration of antigen activity using sera inactivated at 65° provided evidence of organ-specific autoantibody. However, when the sera were inactivated at 56°, the antigen activity of rabbit brain was greater than rabbit liver or rabbit kidney whether the serum was prepared by the injection of rat liver, kidney, brain or heart. It was concluded that these sera contained a relatively heat labile autoantibody which reacted with a widely distributed antigen and a heat-stable antibody with an organ-specific component. The similarity of the titres obtained with autologous and homologous rabbit tissue showed that the sera contained true autoantibodies capable of fixing complement with the tissues of the rabbit in which they occurred. These results do not, however, exclude the possibility that the sera also contained isoantibodies resembling those described by Rose, Metzgar and Witebsky (1960). The finding of autoantibodies able to react with the tissue of the rabbit in which they occurred indicated that at least some of the autoantigens were not freely accessible to circulating autoantibody.

The autoantibody found in the sera before immunization resembled natural autoantibody. This has been shown to be destroyed at 65° and to lack organ specificity (Kidd and Friedewald, 1942). However, a group of eight unimmunized animals purchased from a dealer contained complement-fixing autoantibodies which were heat stable at 65°. The titres against rabbit kidney after heating the sera at 56° ranged from 32 to 128. After heating at 65° the titres ranged from 8 to 32. The mean drop was 2·25 $\log_2$ units. Nevertheless the finding of heat-stable and organ-specific autoantibodies following the injection of rat tissue shows that the rise in autoantibody titres was not merely due to an increase in the level of natural autoantibody.

Asherson and Dumonde (1962) showed that certain autoantibodies labile at 65° behaved chromatographically like macroglobulins. We have now shown by sucrose gradient centrifugation that macroglobulin antibody is heat labile, while small molecular weight antibody is heat stable. Because of the low antibody titres in the fractions obtained by gradient centrifugation the presence of small amounts of low molecular weight

antibody in sera could not be excluded. With this proviso the natural autoantibody of Kidd and Friedewald and the autoantibodies formed 1 week after the injection of rat tissue were apparently macroglobulins while those antibodies formed 1 month after immunization contained both 7S and macroglobulin antibody. This is in keeping with the finding of Bauer and Stavitsky (1961) and Benedict, Brown and Ayengar (1962) that the early antibody response to protein antigens contains a larger macroglobulin component than the later response.

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