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Immunological Studies on Adrenal Glands
II. IMMUNIZATION WITH ADRENALS OF THE SAME SPECIES

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Summary. Thirty-six guinea pigs were injected with a suspension of pooled guinea-pig adrenals incorporated into Freund adjuvants; twenty-two rabbits were immunized in a similar manner with rabbit adrenal suspensions.

Sera of twenty-six guinea pigs and twelve rabbits reacted in complement fixation and tanned cell haemagglutination tests with adrenal extracts but not with extracts of other organs. Potent antisera also produced one line reaction in double diffusion gel precipitation test with adrenal extracts.

The adrenal-specific antigen was thermolabile, possibly of protein character. Reactions of guinea-pig antisera were limited to guinea-pig adrenal; rabbit antisera reacted with adrenal of all tested mammalian species.

The serological reactions exhibited by antigens prepared from the antibody producer's own adrenal were as strong as those produced by adrenals of other animals of the same species. Additional evidence for auto-specificity of the antibody under investigation was obtained from the experiment in which a guinea pig injected with its own gland removed by left adrenalectomy formed adrenal antibodies.

Some but not all animals developed histological lesions in the adrenal gland. The interpretation of this observation was complicated by occasional positive histological findings in the adrenals of control animals.

INTRODUCTION

In previous investigations (Milgrom and Witebsky, 1962) rabbits were immunized with bovine adrenal suspensions and their antisera used as reagents for analysis of the antigenic composition of the adrenal gland. A thermostable ethanol-insoluble antigen was described which was characteristic of the bovine adrenal gland, apparently of its medullar portion.

In the present paper an attempt was undertaken to elicit antibody production in animals injected with a suspension of adrenals originating from other animals of the same species as well as with suspension of the immunized animal's own adrenal. It was felt that such an experiment might give some information for further investigations on the possible role of immunological phenomena in the etiology of Addison's disease.

MATERIALS AND METHODS

Organs used for the present investigations were obtained from sources described in a previous paper.

In addition, some guinea-pig adrenal glands were surgically removed from living animals. The operation was performed under Veterinary Nembutal anaesthesia. The left adrenal gland was exposed by laparotomy and extirpated after ligature of the adrenal

vessels. The peritoneal cavity was closed with catgut and the skin with metal wound clips, which were removed 8–12 days after the operation.

Organ suspensions and extracts were prepared as described in the preceding paper. The concentration of the suspensions was expressed as previously in percentage, representing the number of grammes of tissue contained in 100 ml. of the suspension. The concentration of the extracts was also expressed in percentage, referring to the original organ suspension from which the extract had been obtained. In addition, the protein concentration of the organ extracts was frequently estimated by the biuret procedure; 10 per cent extracts usually had a protein concentration of 0.6–0.8 per cent.

Ammonium sulphate fractionation of adrenal extracts was performed at a salt concentration of 1.35 M. The thrice-washed precipitate, as well as the supernatant, was dialysed exhaustively against saline. Protein concentrations of the fractions were determined by the biuret method. The precipitate was referred to as adrenal 'globulin' and the supernatant as adrenal 'albumin'.

For immunization, guinea pigs weighing 700–1000 g. and Flemish Giant rabbits weighing 3000–3800 g. were selected. All immunizations were carried out with adrenal suspensions incorporated in Freund adjuvants, by the procedure outlined in the previous paper. All trial and final bleedings were performed 6–12 days after the last injection.

Guinea pigs were subjected to iso- and auto-immunization. The iso-immunized guinea pigs were injected four to nine times with 20 per cent suspensions of pooled adrenal glands originating from other guinea pigs. This group comprised thirty-four normal animals and two adrenalectomized animals.

The auto-immunized guinea pigs were each injected with the respective animal's own gland removed by left adrenalectomy. The immunization of adrenalectomized guinea pigs was started after complete recovery, 4–6 weeks after operation. Because of the scarcity of material this group of guinea pigs, comprising seven animals, was inject only twice and only a 10 per cent adrenal suspension was employed for immunization.

Rabbits were subjected to iso-immunization only; they were injected with pooled adrenal glands of other rabbits. After discouraging results with a less concentrated material, all twenty-two rabbits involved in this experiment were injected with a 50 per cent adrenal suspension. Six to twelve inoculations were carried out.

In the serological tests procedures outlined in the previous paper were followed. In most complement fixation tests guinea-pig antisera diluted 1/5 and rabbit antisera diluted 1/5 were used.

RESULTS

FREQUENCY AND TIME OF THE APPEARANCE OF ADRENAL ANTIBODIES IN ISO-IMMUNIZED ANIMALS

Out of thirty-six guinea pigs injected with pooled guinea-pig adrenal suspension, positive serological reactions were obtained in twenty-six, or 72 per cent. Of twenty-two rabbits injected with pooled rabbit adrenal suspension, twelve, or 55 per cent, were positive.

The adrenal antibodies to be described appeared always to be an effect of immunization. No traces of these antibodies were ever found in the serum of the test animals before immunization. The trial bleedings were not performed frequently enough to establish a precise relationship between the number of injections performed and the appearance of antibodies. We may state that none of five guinea pigs tested which later turned out to be positive showed any antibody in the serum obtained from the bleeding performed after one

TABLE 1

APPEARANCE OF ADRENAL ANTIBODIES IN RABBIT AS A FUNCTION OF TIME OF IMMUNIZATION AND NUMBER OF INJECTIONS. COMPLEMENT FIXATION TEST WITH RABBIT ANTI-RABBIT ADRENAL SERUM R #1021 AT 1 : 15 DILUTION AND RABBIT ADRENAL EXTRACT. READINGS TAKEN AFTER (*a*) 25- AND (*b*) 75-MINUTE INCUBATION

At the time of bleeding			*Dilutions of* 10 *per cent extract* 1 *to*													
No. of injections	*Days after first injection*	*Days after last injection*	1		3		9		27		81		243		729	
			a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
0	0	0	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3	22	8	++++	+++	++++	++++	++++	+	+++	+	+	−	−	−	−	−
5	39	9	++++	++	++++	++	++++	+++	++++	+++	++++	+++	++++	+++	++	−
8	89	7	+++	−	+++	−	+++	−	+++	−	+++	−	+++	−	++	−
10	117	6	++	−	++	−	++	−	+	−	+	−	−	−	−	−
12	133	7	++	−	−	−	−	−	−	−	−	−	−	−	−	−

injection. On the other hand, all guinea pigs and rabbits that produced antibodies showed positive reactions after three to five injections. In animals which failed to produce antibodies after five injections, additional immunization never elicited antibody production. The maximum titre in antibody-producers was reached after three to five injections and it was never increased by further immunization; on the contrary, in some cases the further immunization caused a decrease in titre, or even the disappearance of antibodies (Table 1).

SEROLOGICAL REACTIONS OBTAINED WITH ADRENAL ANTIBODIES

In the first stage of this investigation guinea-pig antisera were tested against antigenic preparations of pooled guinea-pig adrenals and rabbit antisera against similar preparations of rabbit origin. Complement fixation proved to be the most sensitive technique and easily reproducible results were obtained by this method with both suspensions and saline extracts of the adrenal gland. The guinea-pig antisera when tested at 1 : 5 dilutions were positive with antigens diluted up to five hundred times more than their anti-complementary titre. On the other hand, even potent guinea-pig antisera gave negative results if diluted more than 1/40. Reactions of rabbit antisera were stronger in both antigen and antiserum titrations. Table 2 presents an example of a titration of a potent antiserum of rabbit origin.

TABLE 2

COMPLEMENT FIXATION TEST WITH RABBIT ANTI-RABBIT ADRENAL SERUM R #1016 AND RABBIT ADRENAL EXTRACT. READINGS TAKEN AFTER (*a*) 25- AND (*b*) 60-MINUTE INCUBATION

Dilutions of 10 per cent rabbit adrenal extract	*Serum dilutions*							
	1 : 15		1 : 30		1 : 60		1 : 120	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
1 : 1	++++	++++	++++	++	+	−	−	−
1 : 3	++++	++++	++++	+++	++	−	−	−
1 : 9	++++	++++	++++	++++	++	+	−	−
1 : 27	++++	++++	++++	++++	++++	++++	±	−
1 : 81	++++	++++	++++	++++	++++	++++	++++	+
1 : 243	++++	+++	++++	+++	+++	+++	++++	++
1 : 729	+++	++	+++	+	++	+	+	−

The second successfully applied serological method was tanned cell haemagglutination. For coating the erythrocytes, adrenal extracts with protein concentration of 0.01 to 0.1 per cent were used. The antisera tested agglutinated the coated red cells up to a 1 : 2500 dilution. Quite a good agreement in the results of the tanned cell haemagglutination and complement fixation tests was observed. However, the tanned cell haemagglutination test, especially with guinea-pig sera, was not as easily reproducible as the complement fixation test.

Of all antisera under examination, only two guinea-pig and six rabbit sera gave

positive precipitation reactions when tested against adrenal extracts. In the double diffusion gel precipitation technique the reactions were more easily seen when the more sensitive micro-procedure was used rather than the macro-procedure. One line of precipitation was observed as exemplified in Fig. 1.

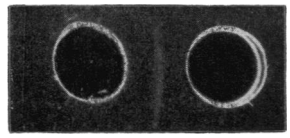

FIG. 1. Double diffusion gel precipitation (micro-procedure). Left well: undiluted rabbit anti-rabbit-adrenal serum R #1261. Right well: rabbit adrenal extract at 1.1 per cent protein concentration.

PHYSICOCHEMICAL PROPERTIES OF ADRENAL ANTIGEN

As already mentioned, positive reactions with both adrenal suspensions and saline extracts were obtained. In contrast, no reactions with alcoholic extracts of adrenal gland could be demonstrated.

The adrenal antigen was thermolabile. The antigenic activity of both adrenal suspensions and adrenal extracts was markedly affected by heating for 15 minutes at 56°; temperatures higher than 70° completely destroyed the activity (Table 3). The heated

TABLE 3

COMPLEMENT FIXATION TEST WITH RABBIT ANTI-RABBIT ADRENAL SERUM R #1016 AT 1 : 15 DILUTION AND UNALTERED AND HEATED RABBIT ADRENAL EXTRACT. READINGS TAKEN AFTER (*a*) 20- AND (*b*) 60-MINUTE INCUBATION

Dilutions of 10 per cent extract	*Rabbit adrenal extract*							
	Unaltered		*Heated for 15 minutes at*					
			56°		70°		80°	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
1 : 1	++++	+++	++++	+++	+++	±	−	−
1 : 3	++++	+++	++++	++	+	−	−	−
1 : 9	++++	++++	+	−	−	−	−	−
1 : 27	++++	++++	−	−	−	−	−	−
1 : 81	++++	++++	−	−	−	−	−	−
1 : 243	++++	++	−	−	−	−	−	−
1 : 729	+++	+	−	−	−	−	−	−

extracts not only failed to react in complement fixation, tanned cell haemagglutination and precipitation tests, but they also failed to inhibit the reaction between the anti-adrenal sera and red cells coated by unaltered extracts.

The adrenal 'globulin' re-dissolved from the ammonium sulphate precipitate readily coated tanned red blood cells for the reaction with adrenal antisera. Like the whole extract, the globulin preparation was thermolabile (Table 4). The adrenal 'globulin' very strongly inhibited the reaction between the anti-adrenal sera and the red cells coated by whole adrenal extract, demonstrating that most, if not all, adrenal extract activity was

contained in the 'globulin' fraction. In contrast, red cells coated by the supernatant from ammonium sulphate precipitation — adrenal 'albumin' — were not agglutinated by the anti-adrenal sera under investigation; adrenal 'albumin' was also inactive in the inhibition experiments.

TABLE 4

TANNED CELL HAEMAGGLUTINATION. REACTION OF GUINEA-PIG ANTI-GUINEA-PIG ADRENAL SERUM GP #448 WITH HUMAN RED CELLS COATED BY UNALTERED AND HEATED GUINEA-PIG ADRENAL 'GLOBULIN'

Dilution of antiserum	*Agglutination of red cells coated by adrenal 'globulin' at 0.012 per cent protein concentration*		
	Unaltered	*Heated for 5 minutes at*	
		56°	70°
1 : 10	++	+	±
1 : 30	++	++	−
1 : 90	++	++	−
1 : 270	++	++	−
1 : 810	++	+	−
1 : 2430	+	−	−

In the double diffusion gel precipitation procedure reaction patterns as exemplified in Fig. 2 were observed. In the experiment presented in Fig. 2 rabbit anti-rabbit adrenal serum was tested simultaneously against rabbit adrenal extract and adrenal 'globulin'.

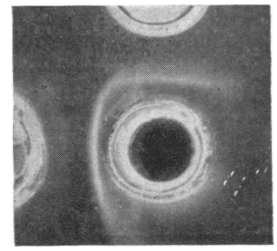

FIG. 2. Double diffusion gel precipitation (micro-procedure). Lower right well: undiluted rabbit-anti-rabbit-adrenal serum R #F41. Lower left well: rabbit adrenal extract at 1.1 per cent protein concentration. Upper well: rabbit adrenal globulin at 1.1 per cent protein concentration.

As is to be seen, the two lines of precipitation fused in a perfect identity reaction. Apparently the active principle of adrenal extract was contained in its 'globulin' fraction. In contrast with adrenal 'globulin', the adrenal 'albumin' failed to give precipitation.

ORGAN AND SPECIES SPECIFICITY

In the experiment presented in Table 5 guinea-pig anti-guinea-pig adrenal sera were tested against extracts of various guinea-pig organs and rabbit anti-rabbit adrenal sera against extracts of various rabbit organs. As can be seen, only adrenal extracts gave definite reactions; extracts of other organs gave negative results or at best very weak reactions. The tanned cell haemagglutination and precipitation reactions were even more

TABLE 5

A. COMPLEMENT FIXATION TEST WITH GUINEA PIG ANTI-GUINEA-PIG ADRENAL SERA AND GUINEA-PIG ORGAN EXTRACTS

Antisera at 1 : 5 dilution	*Highest dilution of 10 per cent extract giving +++ or ++++ reaction after 60-minute incubation*					
	Adrenal	*Brain*	*Thyroid*	*Heart*	*Liver*	*Spleen*
GP #248	243	<1	<1	<1	3	<1
GP #81	243	<1	<1	<1	1	<1

B. COMPLEMENT FIXATION TEST WITH RABBIT ANTI-RABBIT ADRENAL SERA AND RABBIT ORGAN EXTRACTS

Antisera at 1 : 15 dilution	*Highest dilution of 10 per cent extract giving +++ or ++++ reaction after 60-minute incubation*							
	Adrenal	*Brain*	*Thyroid*	*Lung*	*Liver*	*Spleen*	*Testicle*	*Ovary*
R #1016	729	3	<1	<1	9	9	9	nd
R #1261	243	<1	<1	<1	<1	<1	<1	3

adrenal-specific, and with extracts of other than adrenal organs no reactions at all were observed. Similarly, extracts of other organs when added to the antisera under investigation did not inhibit their reaction with adrenal extracts.

Table 6 illustrates an experiment in which guinea-pig and rabbit anti-adrenal sera

TABLE 6

COMPLEMENT FIXATION TEST WITH ANTI-ADRENAL SERA AND ADRENAL EXTRACTS OF VARIOUS SPECIES ORIGIN

	Highest dilution of 10 per cent extract giving +++ or ++++ reaction after 60-minute incubation					
	Guinea pig	*Rabbit*	*Man*	*Ox*	*Rat*	*Dogfish*
Guinea pig anti-guinea-pig adrenal serum #448 at 1 : 5 dilution	243	<1	<1	<1	<1	<1
Rabbit anti-rabbit adrenal serum #1016 at 1 : 15 dilution	243	729	243	243	729	<1

were tested against adrenal extracts of various species. As can be seen, the reactions of guinea-pig antisera were species restricted, limited to guinea-pig adrenal only. In contrast, rabbit antisera reacted not only with rabbit adrenal extract but also with adrenal extracts of other mammalian species (note negative results with dogfish adrenal extract). Very similar results were obtained by means of tanned cell haemagglutination and precipitation tests.

In experiments presented in Table 7 and Fig. 3 the inter-species cross reactions of rabbit antisera were submitted to more precise investigation. Inhibition of tanned cell haemagglutination (Table 7) revealed that the reaction of rabbit anti-rabbit adrenal sera with red

TABLE 7

TANNED CELL HAEMAGGLUTINATION. INHIBITION OF REACTION BETWEEN RABBIT ANTI-RABBIT ADRENAL SERUM R #F41 AND HUMAN RED CELLS COATED BY (A) RABBIT ADRENAL 'GLOBULIN' AND (B) BOVINE ADRENAL 'GLOBULIN'*

		Haemagglutination at a dilution of the inhibiting specimen listed in the first column of 1 *to*						
		1	3	9	27	81	243	729
Rabbit adrenal extract, protein concentration 1 per cent	A	−	−	−	−	−	++	++
	B	−	−	−	−	−	−	++
Bovine adrenal extract, protein concentration 1 per cent	A	++	++	++	++	++	++	++
	B	−	−	−	−	−	−	++

* 0.1 ml. of the inhibiting specimen at increasing dilutions +0.1 ml. of serum R #F41 at 1 : 40 dilution +0.1 ml. of tanned and coated red cells.

cells coated by bovine adrenal antigen may be readily inhibited by both rabbit and bovine adrenal extracts. However, the homologous reaction — with red cells coated by rabbit adrenal antigen — could be inhibited only by rabbit adrenal extract.

In Fig. 3 an experiment was presented in which rabbit anti-rabbit adrenal serum was tested simultaneously against rabbit and beef adrenal extract in double diffusion gel precipitation. The lines of precipitation fused in partial identity reaction with a spur

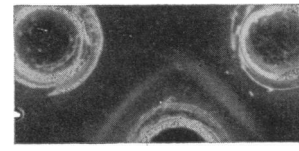

FIG. 3. Double diffusion gel precipitation (micro-procedure). Lower well: undiluted rabbit anti-rabbit adrenal serum R #F41. Upper left well: bovine adrenal extract at 0.3 per cent concentration. Upper right well: rabbit adrenal extract at 0.3 per cent protein concentration.

formation from the site of the rabbit adrenal extract. In order words, both experiments — inhibition of tanned cell haemagglutination and precipitation tests — showed that the homologous rabbit adrenal extract had more active antigenic sites than the heterologous bovine extract.

It was very important to find out whether the active antigen originated from the cortex or medulla since these two parts of adrenal glands are distinctly different both from the embryo-genetical and functional point of view. Guinea-pig and rabbit adrenals are too small to separate the medulla without heavy contamination by the cortex; but, by thorough preparation of frozen adrenal slices, it is possible to obtain relatively pure cortex material. The serological reactions produced by cortex suspensions and extracts with anti-adrenal sera were apparently as strong as those produced by the preparations of the whole

glands (Table 8). This demonstrated that the antigen in question is present in the cortex, but information was not obtained as to whether it also is present in the medulla.

TABLE 8

COMPLEMENT FIXATION TEST WITH GUINEA-PIG ANTI-GUINEA-PIG ADRENAL SERUM GP #141 AT 1 : 5 DILUTION AND SUSPENSIONS OF GUINEA-PIG WHOLE ADRENAL AND ADRENAL CORTEX. READINGS TAKEN AFTER (*a*) 15-, (*b*) 30- AND (*c*) 60-MINUTE INCUBATION

Dilutions of 7 per cent adrenal suspension	*Whole adrenal*			*Adrenal cortex*		
	a	*b*	*c*	*a*	*b*	*c*
Undiluted	++++	++++	++++	++++	++++	++++
1 : 3	++++	++++	++++	++++	++++	++++
1 : 9	++++	++++	++++	++++	++++	++++
1 : 27	++++	++++	++++	++++	++++	++++
1 : 81	++++	++++	+++	++++	+++	+++
1 : 243	++++	+++	+++	+++	++	+
1 : 729	+++	−	−	+	−	−

AUTO- V. ISO-ANTIBODIES

In the described experimental series guinea pigs were immunized with guinea pig, and rabbits with rabbit adrenal suspensions. Antibodies elicited by such a procedure should be tested to find out whether they react with antigens of all animals of the given species including the antibody producer itself (auto-antibodies) or whether they react with antigens contained only in some proportion of individuals of the given species excluding the antibody producer (iso-antibodies). To answer this question anti-adrenal sera were tested against suspensions and extracts of the adrenal glands removed from the antibody-producing animal after final bleeding, and these results were compared with the results obtained by testing the same antiserum against preparations obtained from adrenals of other animals. No significant titre differences were ever observed in experiments of this type (Table 9), pointing to the auto-antibody character of the antibodies under investigation.

In addition, an experiment was performed in which the antigen was prepared from the

TABLE 9

COMPLEMENT FIXATION TEST WITH GUINEA-PIG ANTI-GUINEA-PIG ADRENAL SERUM GP #280 AT 1 : 5 DILUTION AND SUSPENSION OF ADRENAL GLANDS ORIGINATING FROM VARIOUS INDIVIDUAL GUINEA PIGS INCLUDING THE ANTIBODY PRODUCER. READINGS TAKEN AFTER 60-MINUTE INCUBATION

Dilutions of 7 per cent adrenal suspension	*Adrenals originating from guinea pig*				
	#*A*	#*B*	#*C*	#*D*	#280
1 : 3	++++	++++	++++	++++	++++
1 : 9	++++	++++	++++	++++	++++
1 : 27	++++	++++	++++	++++	++++
1 : 81	++++	++++	++++	++++	++++
1 : 243	+++	+++	+++	+++	++
1 : 729	±	±	±	±	±

adrenal gland removed before immunization. In two guinea pigs, left adrenalectomy was performed and the organs were preserved. After recovery, the animals were immunized in the routine way with a pooled guinea-pig adrenal suspension; one of them produced adrenal antibodies. The serum of this animal was tested in complement fixation test against five individual guinea-pig adrenal suspensions, a pooled guinea-pig adrenal suspension, and a suspension of the animal's own adrenal which had been removed before immunization. The titres of all seven antigens were identical.

In performing experiments of the type presented in this paper, it is always tempting and desirable to use for immunization organ material originating from the animal used for immunization. This was successfully accomplished in the work on thyroid serology performed in this department, and we tried to fulfil this in the present experimental series as well. Accordingly, seven guinea pigs were submitted to left adrenalectomy, and their organs were preserved frozen. After recovery each animal was immunized with a suspension of its own gland. Because of the limited amount of material only two injections could be made, and only a 10 per cent suspension was used. Of seven animals injected, only one, GP #10, produced adrenal antibodies giving definite reactions. Table 10 presents the

TABLE 10

REACTIONS OF ANTISERUM GP #10 ORIGINATING FROM A GUINEA PIG INJECTED WITH A SUSPENSION OF ITS OWN ADRENAL

Complement fixation test with antiserum at 1 : 5 dilution and suspensions of adrenal glands originating from various individual guinea pigs including the antibody producer. Readings taken after 60-minute incubation

Dilutions of 7 per cent adrenal suspension	*Adrenals originating from guinea pig*					
	#*A*	#*B*	#*C*	#*D*	#*E*	#10
1 : 3	++++	++++	++++	++++	++++	++++
1 : 9	++++	++++	++++	++++	++++	++++
1 : 27	++++	+++	+++	++++	++++	++++
1 : 81	−	±	−	−	++	−

titration of various individual guinea-pig adrenal suspensions against this antiserum. The suspension of the animal's own gland was prepared from the right adrenal removed after the final bleeding. Here again practically equal titres were observed.

HISTOPATHOLOGY

Adrenal glands of twenty-four guinea pigs and all eleven rabbits from the iso-immunization series above presented were tested for pathological lesions by Dr. Kornel Terplan of the University of Buffalo Department of Pathology. In nine guinea pigs and three rabbits definite pathological changes were found. We present three typical protocols:

Guinea pig #1247 injected five times with pooled guinea-pig adrenal suspension. Bled 48 days after the first and 12 days after the last injection. Accumulation of lymphocytes throughout the cortex, very massive lymphocytic infiltration at corticomedullary border zone. In the areas of infiltration closer to medulla a few polymorphonuclear leucocytes. Numerous pericapillary infiltrations, predominantly with lymphocytes, but also with a few polymorphonuclear leucocytes.

Guinea pig #448 injected five times with pooled guinea-pig adrenal suspension. Bled out

67 days after the first and 9 days after the last injection. Distinct changes in medulla and reticular zone of cortex. Rather massive leucocytic infiltration, also some lymphocytes, especially in the medulla.

Rabbit #1016 injected six times with pooled rabbit adrenal suspension. Bled out 70 days after the first and 7 days after the last injection. Infiltration with lymphocytes and some polymorphonuclear leucocytes, particularly distinct at corticomedullary border extending into inner medulla. Also very minute predominantly polymorphonuclear infiltration in the outer and midcortical zone.

It should be emphasized, however, that the lesions were observed also, though apparently less frequently, in adrenals of guinea pigs and rabbits exposed to Freund adjuvant immunization with organs, of animal's own species, other than the adrenal gland. This control series comprised thirty-one guinea pigs and twenty-three rabbits; in the adrenal sections of three guinea pigs and one rabbit, lesions have been found very similar to those observed in the experimental series.

DISCUSSION

The experimental data presented in this paper showed that a great proportion of both guinea pigs and rabbits injected with adrenal suspensions originating from their own species and incorporated into Freund adjuvants produced antibodies specific for adrenal. The adrenal specificity of the antisera in question was so high that they could be successfully applied as reagents to distinguish suspensions and extracts of adrenals from the corresponding preparations of other organs. The reactions of guinea-pig antisera were species restricted, being practically limited to the guinea-pig adrenal. In contrast, antibodies produced by rabbits reacted with antigenic preparations of all mammalian species tested. However, both inhibition of tanned cell haemagglutination and double diffusion gel precipitation reactions revealed that the homologous reaction (with rabbit adrenal) involved more antigenic groups than the heterologous (with adrenals of other species).

In using pooled organ extracts for immunization injections, one must always remember that the antibody produced may be directed against antigens present in the donor animals but absent in the antibody producer. It is now known that the group specific iso-antigens are not limited to red blood cells (blood groups) but they are to be found also in leucocytes (leucocyte groups) and in serum γ globulins (serum groups). In a previous paper of this department (Rose, Metzgar and Witebsky, 1960) it has been shown that rabbits injected with pooled pancreas of other rabbits produced antibodies against antigens contained in pancreas of some but not all rabbits. These antibodies never reacted with the extract prepared from the pancreas of the antibody producer itself; they revealed organ (pancreas) and group specificity and they certainly should be considered as iso- but not as auto-antibodies. In contrast, adrenal antibodies reacted with adrenal preparations of all tested animals of the given species including the preparations originating from the antibody producer itself, and consequently they may be called *auto*-antibodies. Additional evidence for the *auto* character of the adrenal antibody in question has been obtained from an experiment in which such an antibody was formed by a guinea pig injected with a suspension of its own adrenal gland. Only one out of seven animals immunized by such a procedure produced adrenal antibodies. The lower frequency of successful immunizations in this experimental series than in the immunization by pooled guinea-pig adrenal should not be explained by an immunogenic weakness of the animal's

own organ but by the scantiness of the material; considerably less adrenal tissue was injected into auto-immunized animals than into those immunized with pooled adrenal.

The antigen responsible for the serological reactions observed was found to be connected with thermolabile proteins precipitable by ammonium sulphate. These findings pointed out that besides thyroglobulin, 'globulin' of other organs may be of importance in the organ specificity. (In this connection see the discussion presented by one of us (E. W.) as early as 1929.)

In the previous paper quite a different adrenal-specific antigen was described: this was a thermostable ethanol-insoluble antigen found in bovine adrenal extracts with rabbit anti-bovine adrenal sera used as reagents. Animals submitted to immunization with adrenal suspensions of their own species did not produce antibodies against the thermostable antigen. (Neither was any antibody response observed in a preliminary study in which rabbits were immunized with extracts of boiled rabbit adrenal.) This might be explained by the assumption that the thermostable adrenal-specific antigen is active in hetero- but not in the iso-immunization procedure. But the question still remains open whether rabbit and guinea-pig adrenals contain the thermostable adrenal-specific antigen at all — for the time being such an antigen has been shown in bovine and sheep adrenals only.

On the basis of experiments on allergic encephalomyelitis as well as on experimental thyroiditis one could expect that the immunization described in this paper might be accompanied by lesions in adrenal glands. Colover and Glynn (1958), as well as Steiner, Langer, Schatz and Volpe (1960) injected guinea pigs with guinea-pig adrenals incorporated into Freund adjuvants and they observed definite histological lesions in adrenal glands of experimental, but not of control animals. In our material definite changes were found in some of the experimental animals — but similar changes have been observed in guinea pigs and rabbits exposed to the Freund type of immunization using organs other than the adrenals of the animal's own species. In other words, no final evidence was produced showing that adrenal antibody, or other immune mechanisms (cell mediated) stimulated by adrenal injections, was responsible for the described lesions of the adrenals. Pathological findings obtained by iso-immunization will be published later in more detail in collaboration with Dr. Terplan.

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