IMMUNOLOGICAL STUDIES IN RELATION TO THE SUPRARENAL GLAND.

II. Hemolysin Formation in Suprarenalectomized Rats.

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There is a great deal of evidence both from morphological and physiological studies that the suprarenal gland plays a significant rôle in the defensive mechanism of the body against intoxication. During the past 7 years numerous observers have established that the resistance of suprarenalectomized rats and rabbits to trauma and to injections of various poisons and toxins is markedly diminished, particularly during the first weeks following the operation. However, only a few studies on the antibody capacity of animals following bilateral suprarenalectomy have been reported. The literature bearing on the relation of the suprarenal gland to the formation of antibodies has been reviewed by Také and Marine (1). Experiments dealing with the antibody capacity of animals following bilateral suprarenalectomy have been limited because of technical difficulties. The most suitable laboratory animals are the rabbit and the rat since a high percentage of these animals survive bilateral suprarenalectomy. Také and Marine found that bilaterally suprarenalectomized rabbits show hemolysin titers distinctly higher than the control animals. Jaffe and Marine (2) observed that rats repeatedly injected with typhoid vaccine 3 weeks following bilateral suprarenalectomy developed agglutinin titers slightly higher than normal control rats. The evidence presented in the literature, however, is of such a contradictory nature that further studies in this field seemed desirable.

The rat was chosen as the experimental animal as over 85 per cent of rats survive bilateral suprarenalectomy in good condition and these present a state of suprarenal insufficiency for a considerable period of

time. The presence of accessory cortical tissue accounts for the recovery and compensation in these animals.

In a preliminary study on the hemolysin formation in normal rats, it was found that high titers were obtained following a single intraperitoneal injection of a small amount of sheep cells (1 cc. of a 10 per cent suspension). This rapid method of immunization, therefore, is definitely advantageous in studying the early period after bilateral suprarenalectomy. The titer was determined at intervals of 5, 8, 11 and 14 days after injection.

Methods.

The methods used have been described in a previous communication (3). Adult albino rats raised in our laboratory, from Wistar Institute stock, were used. The rats were all about 5 months old and of approximately the same weight. The suprarenal glands were removed at a single operation through the posterior route in the manner described by Jaffe (4).* Over 85 per cent of our rats survived 4 weeks or longer after the operation. All rats were carefully autopsied after the experiments and the operated areas were examined, the presence or absence of gross accessory suprarenal tissue determined and the weight of the thymus recorded. Blood for testing was drawn from the heart by puncture.

In the first group of experiments to be reported, we studied the influence of varying the time interval following suprarenalectomy on the titer, keeping the amount of antigen injected constant. In the second group, the quantity of antigen injected was varied, but the time interval kept constant. In the first part of the work, all rats received a single intraperitoneal injection of 1 cc. of a 10 per cent suspension of sheep cells 2, 7, 14 and 28 days following the operation. A small series of unilaterally nephrectomized rats injected 2 weeks after the operation was tested as a control for operative trauma. In the second group of experiments, two series of suprarenalectomized rats were injected with .05 cc. of a 10 per cent suspension of sheep cells and 1 cc. undiluted sheep cells respectively, 2 weeks after the operation. In preliminary experiments, the range of hemolysin titers in normal rats had been determined with these amounts. Normal rats, and rats in which the perisuprarenal tissue was torn, were tested in both series.

Twenty normal rats were injected with 1 cc. of a 10 per cent suspension of sheep cells and tested 5, 8, 11 and 14 days after the injection. It was found that with this amount, the titer was above 1:2000 in over 70 per cent of the rats. A few rats showed low titers.[1] The average titer of these twenty normals was 1:7000.

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* Ether anesthesia was used in all operative procedures.

Hemolysin Formation in Bilaterally Suprarenalectomized Rats Receiving a Single Intraperitoneal Injection of 1 Cc. of a 10 Per Cent Suspension of Sheep Cells 48 Hours after Operation.

Fourteen rats were used in the first series. Of these, six were bilaterally suprarenalectomized, six were normal and two were traumatized by tearing the perisuprarenal tissue. All were injected

TABLE I.

Hemolysin Formation in Bilaterally Suprarenalectomized Rats, Intraperitoneally Injected 48 Hours after the Operation with 1 Cc. of 10 Per Cent Suspension of Washed Sheep Cells and Tested 5, 8, 11 and 14 Days after Injection.

Rat No.	Operation	Interval between operation and injection	Amount injected	Titer				Weight at operation	Weight at death	Weight of thymus
				5th day	8th day	11th day	14th day			
		days						*gm.*	*gm.*	*mg.*
41	Suprarenalectomy	2	1 cc. 1/10	6,000	4,000	800	Dead	160	158	178
42	"	"	"	400	1,600	Dead		150	190	185
43	"	"	"	100	400	80		175	166	326
44	"	"	"	200	400	400	300	165	185	Large
45	"	"	"	16,000	8,000	400	160	200	180	320
46	"	"	"	100	800	20	10	225	220	Small
59	Traumatization	"	"	16,000	10,000	3,000	0?	160	165	162
60	"	"	"	800	800	400	160	150	150	198
61	Control	—	"	6,000	2,000	600	100	183		
62	"	—	"	2,000	600	160	200	193		
63	"	—	"	24,000	8,000	1,600	600	184		
64	"	—	"	2,000	2,000	200	80	170		
65	"	—	"	16,000	32,000	2,000	600	178		
66	"	—	"	3,000	2,000	160	160	185		

intraperitoneally with 1 cc. of a 10 per cent suspension of sheep cells 48 hours after the operation. Tests were made at intervals of 5, 8, 11 and 14 days. As will be noted in Table I the titers of the suprarenalectomized rats were markedly lower than those of the normal rats. 80 per cent of the suprarenalectomized rats showed an initial titer of 1:400 or less. Two suprarenalectomized rats showed a normal

titer. A comparison between the titer obtained in normal rats[1] and that found in rats following suprarenalectomy reveals a marked depression in hemolysin titer 48 hours after suprarenalectomy. If one examines the curve of antibody formation in those rats showing a low titer, it will be noted that there is a subsequent rise in hemolysin titer at the second reading, the peak of the curve being reached on the 8th instead of the 5th day, as in the normal rats. The depression of antibody formation during the 1st week coincides with the marked depression in resistance of suprarenalectomized rats noted by numerous observers (2, 5–7). It was found by one of us (8, 9) that the height of susceptibility of rats to typhoid and to histamine poisoning is reached during the period from 5 to 7 days after bilateral suprarenalectomy.

Hemolysin Formation in Bilaterally Suprarenalectomized Rats Receiving a Single Intraperitoneal Injection of 1 Cc. of a 10 Per Cent Suspension of Sheep Cells 7 Days after Operation.

A second series of thirteen rats, nine of which were suprarenalectomized, two traumatized and two normal, was injected intraperitoneally with 1 cc. of a 10 per cent suspension of sheep cells 7 days after the operation. The titers were determined at intervals of 5, 8, 11 and 14 days after the injection. From Table II it is seen that the suprarenalectomized rats still show a definite depression in antibody formation. In two instances where the titer was 1:2000 and 1:3000 a fragment of suprarenal tissue had been left behind. These two instances are therefore excluded from consideration in the series. In this series, 40 per cent gave initial titers below 1:400, 85 per cent below 1:2000 and 14 per cent gave titers of 1:2000 to 1:3000 or within the low titer range of normal rats. The average titer 7 days after the operation was 1:1070 or approximately one-seventh that of normal rats. These figures are slightly higher than those of Series 1, but they still show a definite depression in the hemolysin titer. It is obvious that the suprarenalectomized rats still yield definitely lower antibody titers when the rats were injected with small amounts of sheep cells 7 days as well as 48 hours after the operation. The titers

[1] See Table I of Paper I (3).

of the traumatized animals are likewise depressed. This observation will be discussed elsewhere in this paper.

TABLE II.

Hemolysin Formation in Bilaterally Suprarenalectomized Rats, Intraperitoneally Injected 7 Days after the Operation with 1 Cc. of 10 Per Cent Suspension of Washed Sheep Cells and Tested 5, 8, 11 and 14 Days after Injection.

Rat No.	Operation	Interval between operation and injection	Amount injected	Titer				Weight at operation	Weight at death	Weight of thymus
				5th day	8th day	11th day	14th day			
		days						*gm.*	*gm.*	*mg.*
40	*Incomplete suprarenal-ectomy	7	1 cc. 1/10	2,000	800	10	10	180	195	370
32	Suprarenalectomy	"	"	1,500	1,000	300	200	170	145	375
33	"	"	"	1,600	80	60	Dead	190	180	365
34	"	"	"	900	160	60	40	160	150	298
35	"	"	"	3,000	Dead			160	145	402
13	"	"	"	200	20	20		228		
14	"	"	"	200	100	20		195		
15	"	"	"	100	100	80		200		
38	*Incomplete suprarenal-ectomy	"	"	3,000	800	Dead		275	255	Large
36	Traumatization	"	"	1,800	3,000	200	40	175	175	273
37	"	"	"	800	800	40	80	190	183	223
27	Control	—	"	4,000	Dead			300	300	230
30	"	—	"	300	160	40		250		

*Small fragment of right left *in situ*.

Hemolysin Formation in Bilaterally Suprarenalectomized Rats Receiving a Single Intraperitoneal Injection of 1 Cc. of a 10 Per Cent Suspension of Sheep Cells 14 Days after Operation.

A third series of twelve rats, eight of which were suprarenalectomized, two traumatized and two normal, was injected intraperitoneally with 1 cc. of a 10 per cent suspension of sheep cells 14 days after the operation and tested 5, 8, 11 and 14 days after the injection. From

Table III it is evident that the antibody titer was higher than in the series injected 7 days after the operation. In only one instance was the initial titer 1:800, and five animals or 60 per cent showed a titer above 1:2000. The average titer was 1:2770 or about one-half the titer of the normal rats. Several of the titers of the operated animals reached the lower normal range. It is interesting that after the 2nd

TABLE III.

Hemolysin Formation in Bilaterally Suprarenalectomized Rats, Intraperitoneally Injected 14 Days after the Operation with 1 Cc. of 10 Per Cent Suspension of Washed Sheep Cells and Tested 5, 8, 11 and 14 Days after Injection.

Rat No.	Operation	Interval between operation and injection	Amount injected	Titer				Weight at operation	Weight at death	Weight of thymus
				5th day	8th day	11th day	14th day			
		days						*gm.*	*gm.*	*mg.*
70	Suprarenalectomy	14	1 cc. 1/10	3,000	Dead			160	150	175
71	"	"	"	1,600	400	300	Dead	168	145	200
72	"	"	"	3,000	Dead			178	178	184
73	"	"			8,000	400		240	160	206
74	"	"	"	1,000	800	800	0	260	260	416
80	*Incomplete suprarenal-ectomy	"	"	6,000	600	Dead		177	170	
81	Suprarenalectomy	"	"	2,000	1,600	600		161	157	
83	"	"	"	800	1,000	600	600	292	232	
75	Traumatization	"	"	1,000	800	1,600	20	260	270	275
76	"	"	"	160	40	20	0	222	185	295
88	Control	—	"	8,000	800	800	300	148	145	275
89 A	"	—	"	12,000	1,000	800	100	200	220	295

*Small fragment left *in situ*.

week, the capacity to form antibodies in response to injections of small amounts of sheep cells definitely rises, but is still somewhat below that of normal rats. As has been established by Jaffe (4) and Marine, there is a marked hyperplasia of the thymus and the lymph nodes 2 weeks after suprarenalectomy. This may be a factor in the explanation of the compensatory rise in antibody formation at this time.

Hemolysin Formation in Bilaterally Suprarenalectomized Rats Receiving a Single Intraperitoneal Injection of 1 Cc. of a 10 Per Cent Suspension of Sheep Cells 4 Weeks after Operation.

A fourth series of nine rats, four of which were suprarenalectomized, two traumatized and three normal, was injected with 1 cc. of a 10 per cent suspension of sheep cells 4 weeks after operation. Two suprarenalectomized rats gave titers below 1:1000. At 4 weeks, the titer of hemolysin formation had not returned to normal but was definitely lowered. No definite comparison can be made from this small series.

TABLE IV.

Hemolysin Formation in Bilaterally Suprarenalectomied Rats, Intraperitoneally Injected 28 Days after the Operation with 1 Cc. of 10 Per Cent Suspension of Washed Sheep Cells and Tested 5, 8 and 11 Days after Injection.

Rat No.	Operation	Interval between operation and injection	Amount injected	Titer			Weight at operation	Weight at death	Weight of thymus
				5th day	8th day	11th day			
		days					*gm.*	*gm.*	*mg.*
51	Suprarenalectomy	28	1 cc. 1/10	2,000	4,000	Dead	175	160	
52	"	"	"	200	Dead		175	170	552
53	"	"	"	300	200	160	175	145	
55	"	"	"	1,000	Dead		140	130	313
57	Traumatization	"	"	160	200	80	220	280	185
58	"	"	"	3,000	800	160	170	—	—
103 A	Control	—	"	1,600	Dead		250	260	223
104 B	"	—	"	400	1,000	80	245	250	186
105 C	"	—	"	8,000	2,000	200	290	300	261

From these four series it may be concluded that in biliaterally suprarenalectomized rats, the hemolysin formation resulting from a single intraperitoneal injection of 1 cc. of a 10 per cent suspension of sheep cells is depressed in all periods up to and including 4 weeks following the operation. The depression is greatest during the 1st week, lessens slightly during the 2nd week and diminishes considerably during the 3rd week. After 4 weeks, the hemolysin titer is still below the normal reading.

Hemolysin Formation in Bilaterally Suprarenalectomized Rats Receiving a Single Intraperitoneal Injection of .05 Cc. of a 10 per Cent Suspension of Sheep Cells 2 Weeks after Operation.

The second group of experiments was carried out to determine the effect of varying amounts of antigen on the antibody formation.

TABLE V.

Hemolysin Formation in Bilaterally Suprarenalectomized Rats, Intraperitoneally Injected 14 Days after the Operation with .05 Cc. of 10 Per Cent Suspension of Washed Sheep Cells and Tested 5, 8 and 11 Days after Injection.

Rat No.	Operation	Interval between operation and injection	Amount injected	Titer			Weight at operation	Weight at death	Weight of thymus
				5th day	8th day	11th day			
		days					*gm.*	*gm.*	*mg.*
103	Suprarenalectomy	14	0.05 cc. 1/10	300	300	40	180	170	175
107	"	"	"	300	200	20	195	180	200
108	"	"	"	200	300	200	140	110	160
109	"	"	"	200	200	40	200	185	310
110	"	"	"	400	200	10	190	170	271
111	"	"	"	40	40	Dead	260		
112	Traumatization	"	"	200	80	"	275	300	176
113	"	"	"	40	0	"	300	305	196
114	"	"	"	20	20	10	260	260	140
136	Control	—	"	400	400	30	184		
137	"	—	"	400	400	20	225		
138	"	—	"	200	400	160	166		
92	"	—	"	200					
93	"	—	"	800	0	0			
98	"	—	"	1,000	400		155	155	

In the first series of this group, fifteen rats were used, six of which were suprarenalectomized, six normal and three traumatized by tearing the perisuprarenal tissue. All were injected 2 weeks after the operation intraperitoneally with .05 cc. of a 10 per cent suspension of sheep cells (brought up to a volume of .5 cc.). This quantity of antigen represents one-twentieth the amount used for injection

throughout the first group of experiments. It will be noted from Table V that the range of hemolysin titers in normal rats was from 1:200 to 1:1000, the average titer being 1:500. In the suprarenalectomized group, the titers ranged from 1:20 to 1:400, the average titer being 1:240 or one-half the normal. Here as in the first group of experiments, the hemolysin formation in rats injected 2 weeks after bilateral suprarenalectomy is less than that of the control rats. It is significant that as in the first group, the traumatized controls gave lower titers than either the normal or suprarenalectomized rats of this period.

Hemolysin Formation in Bilaterally Suprarenalectomized Rats Receiving a Single Intraperitoneal Injection of 1 Cc. of Undiluted Sheep Cells 2 Weeks after Operation.

In the second series of the second group eighteen rats were used, six of which were bilaterally suprarenalectomized, nine normal and three traumatized in the perisuprarenal area. All were injected 2 weeks after the operation with 1 cc. of undiluted sheep cells. In the normal rats of this series, hemolysin titers in no instance exceeded 1:4000. 65 per cent showed titers below 1:2000. These titers are strikingly lower than those found in the normal rats injected with 1 cc. of a 10 per cent suspension (one-tenth the amount).[1] The average titer in the series of rats injected with 1 cc. of undiluted sheep cells was 1:2000 as compared with 1:7000, the average titer in the normal rats receiving one-tenth the amount of antigen.

Coca (10) found that small quantities of sheep cells are more effective in the production of hemolysin in rabbits than larger amounts. There is a definite optimum antigen amount for the production of hemolysin in rats. Larger or smaller amounts are less effective.

In contrast with this drop in hemolysin titer in *normal rats* injected with 1 cc. of undiluted sheep cells, the *suprarenalectomized* series injected 2 weeks after the operation with the same amount of antigen shows a definitely higher titer than that given by the normal rats. 100 per cent gave a titer of 1:2000 or above, 90 per cent gave a titer above 1:4000 and 50 per cent, a titer above 1:8000! In the normal rats, only 40 per cent gave titers above 1:2000 and none gave titers above 1:4000. 60 per cent gave titers of 1:1600 or less. The average

titer in the suprarenalectomized animals injected with large amounts of antigen was 1:7330. Quantities of 1 cc. of a 10 per cent suspension produced slightly lower titers in suprarenalectomized rats when injected 2 weeks after the operation than in normal rats. Quantities

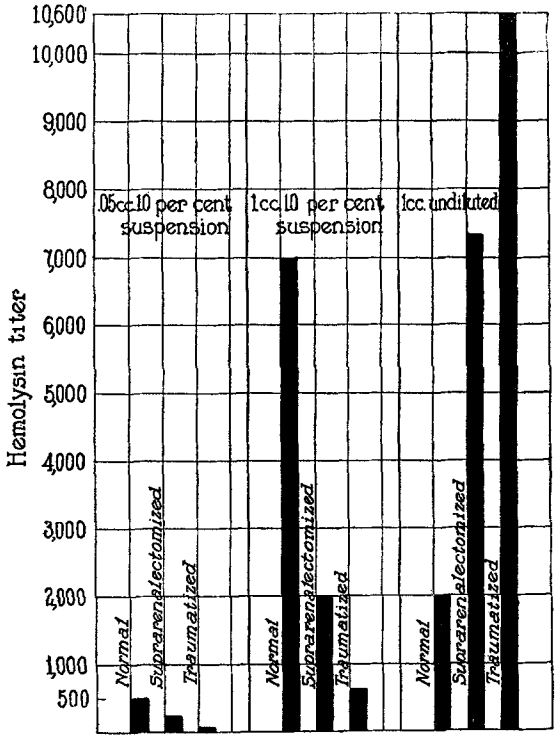

FIG. 1. The relation of hemolysin formation to the quantity of antigen injected in normal rats, in suprarenalectomized rats and in rats in which the perisuprarenal tissue had been traumatized. All rats were injected intraperitoneally 2 weeks after the operation and tested 5 days after the injection. The hemolysin titer in each group is the average optimum titer obtained in the series of rats with .05 cc. of a 10 per cent suspension, 1 cc. of a 10 per cent suspension and 1 cc. of undiluted sheep cells, respectively.

ten times this amount, however, produced titers definitely higher in the suprarenalectomized rats than in the normal rats. The optimum antigen amount necessary to produce the highest titer in suprarenalectomized rats injected 2 weeks after the operation, therefore, is

evidently ten times the optimum necessary to produce the highest titer in normal rats.

The difference in curves illustrating the antigen-antibody ratio can be readily observed in Fig. 1. The titers obtained in suprarenalecto-

TABLE VI.

Hemolysin Formation in Bilaterally Suprarenalectomied Rats, Intraperitoneally Injected 14 Days after the Operation with 1 Cc. of Undiluted Washed Sheep Cells and Tested 5, 8, 11 and 14 Days after Injection.

Rat No.	Operation	Interval between operation and injection	Amount injected	Titer				Weight at operation	Weight at death	Weight of thymus
				5th day	8th day	11th day	14th day			
		days						*gm.*	*gm.*	*mg.*
115	Suprarenalectomy	14	1 cc. undiluted	6,000	1,000	Dead		250	195	313
116	"	"	"	16,000	3,000	1,800	200	250	210	—
117	"	"	"	8,000	4,000	2,000	200	200	180	214
118	"	"	"	8,000	3,000	Dead		230	215	516
119	"	"	"	2,000	800	400		265	210	375
121	"	"	"	4,000	3,000	1,000		270	250	320
122	Traumatization	"	"	8,000	2,000	800	160	270	300	210
123	"	"	"	18,000	4,000	Dead		168	160	155
124	"	"	"	6,000	3,000	160		225	220	410
139	Control	—	"	4,000	600	400	30			
140	"	—	"	4,000	2,000	800	80			
141	"	—	"	1,600	400	160				
142	"	—	"	1,600	1,600	800	200			
143	"	—	"	800	2,000				200	170
144	"	—	"	1,600	200	200			200	110
151	"	—	"	1,000	1,000	1,000	400			
152	"	—	"	1,600	1,600					
153	"	—	"	2,000	600	400	200		505	115

mized rats injected with 1 cc. undiluted sheep cells correspond to the titers obtained in normal rats injected with one-tenth this quantity, but exceed titers obtained in normal rats injected with the same quantity.

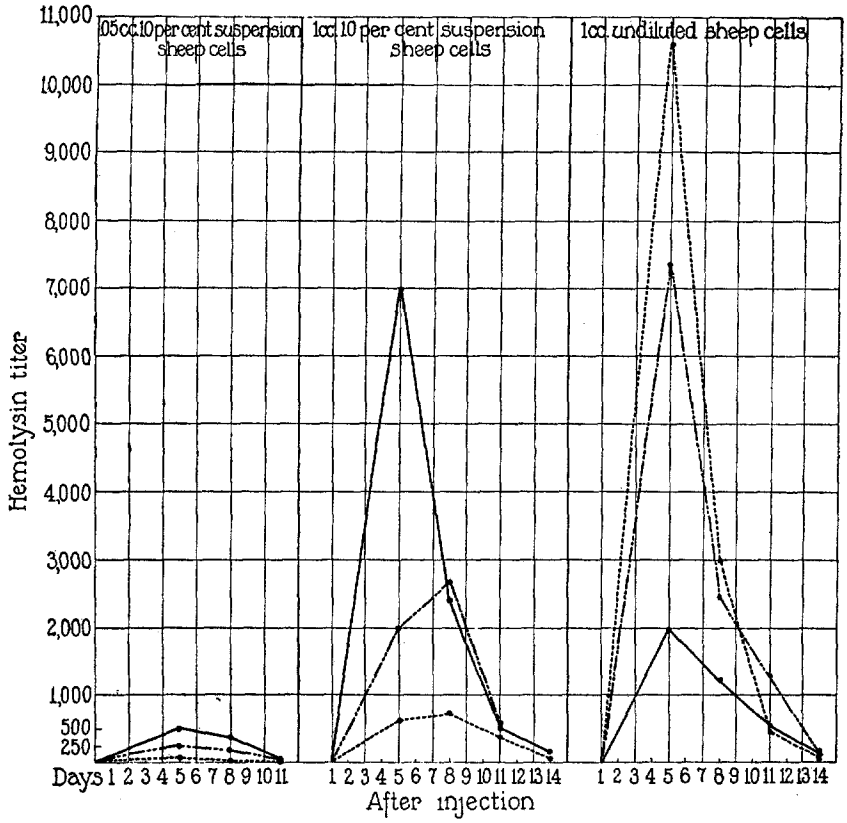

FIG. 2. Hemolysin formation with varying quantities of antigen in normal, suprarenalectomized and traumatized rats. The curves indicate the course of the average hemolysin formation following a single intraperitoneal injection of .05 cc. of a 10 per cent suspension, 1 cc. of a 10 per cent suspension and 1 cc. of undiluted sheep cells respectively, 2 weeks after the operation.

——— Normal rats.

— — — — Suprarenalectomized rats.

——— Traumatized rats.

Hemolysin Formation in Rats Receiving a Single Intraperitoneal Injection of 1 Cc. of a 10 Per Cent Suspension of Sheep Cells 2 Weeks after Traumatization of the Perisuprarenal Tissue.

Throughout the experiments reported in this paper, it had been observed that the traumatized control rats in all postoperative periods

TABLE VII.

Hemolysin Formation in Bilaterally Traumatized Rats, Intraperitoneally Injected at Varying Days after the Operation with 1 Cc. of 10 Per Cent Suspension of Washed Sheep Cells and Tested 5, 8, 11 and 14 Days after the Injection.

Rat No.	Operation	Interval between operation and injection	Amount injected	Titer				Weight at operation	Weight at death	Weight of thymus
				5th day	8th day	11th day	14th day			
		days						*gm.*	*gm.*	*mg.*
59	Traumatization	2	1 cc. 1/10	16,000	20,000	3,000		160	165	162
60	"	"	"	800	800	400	160	150	155	198
36	"	7	"	1,800	3,000	800	40	175	175	273
37	"	"	"	800	800	40	80	190	183	223
75	"	14	"	1,000	800	1,600	80	260	270	275
76	"	"	"	160	40	20	0	222	185	295
57	"	28	"	180	200	80		220	280	185
58	"	"	"	3,000	800	160		170	280	185
125	"	14	"	20	40	40	100	210	280	190
127	"	"	"	800	160	100	150	205	265	171
128	"	"	"	20	200	20	100	216	280	155
129	"	"	"	800	600	200		235	250	
130	"	"	"	400	800			210	280	140
131	"	"	"	800	1,600	200	100	165	185	75
132	"	"	"	300	1,600	400	100	190	210	80
133	"	"	"	2,000	1,600	400	100	155	195	95

including 4 weeks gave, with few exceptions, strikingly low titers. This finding was so striking that in a series of rats the perisuprarenal tissue was traumatized, and the rats were subsequently injected with 1 cc. of a 10 per cent suspension of sheep cells 2 weeks after the operation. The titers were tested on the 5th, 8th, 11th and 14th days after injection. Rats 125 to 133 tabulated in Table VII record the results

of this series. One of the nine rats gave a titer of 1:2000; all the others were below 1:800. These titers corresponded in general with those given by the suprarenalectomized rats during the 1st week. This profound depression was still observed in Rat 57, 4 weeks after the operation.

In four rats, one kidney was removed as an operative control. These rats were injected 2 weeks after the operation with 1 cc. of a 10 per cent suspension of sheep cells. As indicated in Table VIII the titers were the same as in the normal unoperated rats.

TABLE VIII.

Hemolysin Formation in Unilaterally Nephrectomized Rats, Intraperitoneally Injected 14 Days after Operation with 1 Cc. of 10 Per Cent Suspension of Washed Sheep Cells and Tested 5, 8, 11 and 14 Days after the Injection.

Rat No.	Operation	Interval between operation and injection	Amount injected	Titer				Weight at operation	Weight at death	Weight of thymus
				5th day	8th day	11th day	14th day			
		days						*gm.*	*gm.*	*mg.*
99	R. removed	14	1 cc. 1/10	8,000	400	400		140	145	110
100	"	"	"	12,000	200	600		145	145	233
101	"	"	"	1,600	200	200	80	230	235	230
102	"	"	"	32,000				200	205	209

It is justifiable to conclude from these observations that traumatization of the perisuprarenal tissue in some way results in a prolonged depression in antibody-forming capacity. This depression persists at the same low level from a few days after the operation to at least the 5th week. Traumatization of the perisuprarenal tissue in rats results in a depression of hemolysin formation which is even more marked than in a corresponding suprarenalectomized series at the same postoperative interval. It is suggested that traumatization of the perisuprarenal area probably results in severing or injuring the sympathetic or parasympathetic nerves innervating the suprarenals. This injury may cause a depression of function of cortex as well as medulla.

It has been thoroughly established by the work of Dreyer (11), Tscheboksaroff (12), Stewart and Rogoff (13) and others that the output of epinephrine from the suprarenal gland is controlled by a nervous mechanism and that interruption of the nervous pathway results in a diminution of the epinephrine output. The extent of the nervous control of the cortex, however, is entirely unknown. Depression of antibody-forming capacity to small amounts of antigen may be an expression of impaired suprarenal function. With the removal of suprarenal glands in rats, regeneration of thymus and lymphoid tissue occurs. This hyperplasia does not occur following traumatization of perisuprarenal tissue. This lymphoid hyperplasia may be in some way responsible for the partial recovery in suprarenalectomized rats of the antibody-forming capacity 2 weeks after the operation.

DISCUSSION.

It has been thoroughly established that the resistance of suprarenalectomized rats and rabbits to injections of various toxins is markedly diminished particularly during the first few weeks following the operation. As has been pointed out by Marine (14) and others, it is not established that antibody formation is necessarily an indication of resistance. However, it would appear *a priori* that during the height of diminished resistance, some depression in antibody reaction capacity may be expected. Our studies indicate that certainly in the 1st week following suprarenalectomy, a marked hemolysin depression does occur and is most marked at the height of susceptibility. The significant chemical changes in the blood plasma known to follow suprarenalectomy may play a rôle in this depression. Physicochemical changes such as change in the dispersion of the proteins in the serum, slight variations in the pH, variations in the concentration of metallic ions in the serum may all play a part in the depression in hemolysin during the early period following suprarenalectomy. However, the serum of suprarenalectomized rats when added in varying concentrations to sera of known hemolytic titer did not produce any depression of hemolysis. This adds further evidence that the depression in antibody formation in the early period after suprarenalectomy may be due to a disturbance in the antibody-forming mechanism of the body. This depression coincides with the di-

minished resistance of rats to toxins and poisons during the first 2 weeks and may be intimately related to it.

Marine and his coworkers (1, 4) found an increase in antibody formation following suprarenalectomy. They employed sublethal amounts of antigen. Their results are comparable with those in our work in which large amounts of antigen were used. Periods earlier than 3 weeks were not tested by these investigators. The effects of comparably small amounts of sheep cells were not tried. The rise in antibody production following the use of large amounts of sheep cells 2 weeks after suprarenalectomy may be indirectly dependent on loss of suprarenal function and on the consequent lymphoid hyperplasia following suprarenalectomy.

Traumatization of the perisuprarenal tissue had an effect similar to that of suprarenalectomy in depressing hemolysin formation particularly during the 1st week following the operation. This depression persisted in all periods up to 4 weeks. It is interesting to note, however, that with ten times the normal optimum antigen amount, the titers in the traumatized as well as in the suprarenalectomized rats were higher than in normal rats.

SUMMARY.

In a large series of suprarenalectomized rats, hemolysin formation, to a fixed amount of sheep cells injected intraperitoneally (1 cc. of a 10 per cent suspension), was studied 48 hours, 7, 14 and 28 days after operation. The hemolysin formation was studied in suprarenalectomized rats injected 2 weeks following the operation, with one-twentieth and ten times this amount. Hemolysin formation in rats traumatized by tearing the perisuprarenal tissue and injected 2 weeks after the operation with 1 cc. of a 10 per cent suspension of sheep cells was studied and the results compared with those in the normal control rats. The effect of the operative procedure was controlled by removing one kidney in a series of rats and determining the antibody response to sheep cells.

1. Bilateral suprarenalectomy in rats subsequently injected intraperitoneally with 1 cc. of a 10 per cent suspension of sheep cells resulted in a depression of hemolysin titer during 5 weeks following the operation, the depression being most marked during the 1st week.

2. Bilaterally suprarenalectomized rats injected intraperitoneally 2 weeks after operation with 1 cc. of undiluted sheep cells gave hemolysin titers higher than did normal rats.

3. The quantity of antigen necessary to yield the maximum titer in suprarenalectomized rats 2 weeks after operation is ten times the quantity necessary to yield the same titer in normal rats.

4. Traumatization of the perisuprarenal tissue in rats produced the same effect on the antibody-forming capacity as suprarenalectomy.

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