THE EFFECT OF BILATERAL SUPRARENALECTOMY IN ADULT ALBINO RATS ON THE NATURAL AND ACQUIRED RESISTANCE TO BARTONELLA MURIS ANEMIA

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By work in this laboratory and that of other investigators, it has been shown that the suprarenal glands are essential in the mechanism of natural resistance (1–12). In the case of suprarenalectomized rats, this resistance can be raised almost to normal by injection of the hormone of the suprarenal cortex (13–15). In the present communication further data are presented on the effect of bilateral suprarenalectomy in the rat on natural and acquired resistance to *Bartonella muris* anemia.

The Effect of Bilateral Suprarenalectomy on the Acquired Resistance to Bartonella muris Anemia

The rat is spontaneously infected with *Bartonella muris* between the 4th and 5th week of life (16). It becomes a carrier and acquires immunity to the infection (17). The spontaneous recurrence of infection following splenectomy with the development of anemia in the adult rat of carrier stock is indicative of a depression in the acquired resistance to *Bartonella muris*.[1]

The rats used in this experiment were bred and raised in our laboratory and were carriers of *Bartonella muris*. Ten albino rats of approximately 3 months of age were suprarenalectomized. On the 6th day after suprarenalectomy each of these was injected intraperitoneally with 2 cc. of whole blood of anemic adult splenectomized rats, drawn at the height of infection. The red cell count and the percentage of hemoglobin were determined and blood smears examined daily.

[1] For a review of the literature on *Bartonella muris* anemia the reader is referred to Marmorston-Gottesman and Perla (17) and to Reitano (18).

In some instances a few *Bartonella* bodies were found in the red cells during 1 or 2 days following the injection of the infected material but no anemia developed in any of the rats. Ford and Eliot (19) have shown that 0.0001 cc. of blood of an anemic rat is sufficient to transmit the anemia to splenectomized rats of non-carrier stock. The suprarenalectomized rats of carrier stock, therefore, withstand a quantity of infecting material 20,000 times as great as is sufficient to produce an anemia in the splenectomized rat of non-carrier stock. The acquired resistance of rats of carrier stock to *Bartonella muris* conferred by a first infection in early life, though strikingly depressed by splenectomy, is uninfluenced by suprarenalectomy.

The Effect of Bilateral Suprarenalectomy on the Natural Resistance of Adult Albino Rats to Bartonella muris Anemia

To determine the effect of suprarenalectomy on the natural resistance to *Bartonella muris* it was necessary to study this infection in rats of non-carrier stock. Rats of the Wistar strain are not carriers of *Bartonella muris* (19). Splenectomy in these rats is not followed by *Bartonella muris* anemia but the anemia will develop if they are subsequently exposed to rats of carrier stock for a few days.

The rats used in the experiments on natural resistance were bought directly from the Wistar Institute and were isolated. Preliminary tests indicated that these rats were not carriers of *Bartonella muris*. Ten suprarenalectomized 3 month old rats and one splenectomized rat of the same age and stock were each injected intraperitoneally on the 6th day following the operation with 1 cc. of whole blood of anemic adult splenectomized rats, drawn at the height of the anemia. Four male and two female normal rats of the same age and stock were each injected intraperitoneally with the same quantity of blood of an anemic rat. The red blood cell count and the percentage of hemoglobin were determined and blood smears were examined daily.

From Table I it is seen that eight of the suprarenalectomized rats died within a period of 3 days following the injection of infecting material and two on the 5th and 6th day. In most instances the rats died before sufficient time had elapsed for the development of a severe anemia. Death was apparently caused by the toxemia of the infection. Rats surviving over a period longer than 2 days after injection developed an anemia. The histological changes character-

istic of acute *Bartonella muris* anemia (17) were found even in those instances in which examination of the blood had failed to reveal the presence of an anemia. Focal necroses in the liver and spleen and erythrophagocytosis by endothelial cells of the spleen with exhaustion destruction of the pulp were present. The splenectomized rat survived.

The control unoperated Wistar rats injected with the same amount of infecting material developed a severe anemia. All of these recovered. The red cell count and the percentage of hemoglobin began to drop within 5 days and rapidly reached a level below 2 million red cells per cubic millimeter and 10 per cent hemoglobin. The females developed an anemia less severe and more transient than the males. This sex variation in the severity of the anemia has been repeatedly observed in our studies on splenectomized rats of carrier stock.

These observations are at variance with those of Ford and Eliot who were unable to transmit the anemia to normal rats of non-carrier stock. Adler (20, 21) succeeded in infecting normal mice by repeated passage of *Bartonella muris ratti*. After several transfers the virulence of the organism was enhanced so that the virus produced an anemia in mice. Mice that had recovered from a previously induced infection could not be reinfected with this strain.

The natural resistance to *Bartonella muris* infection is lowered by suprarenalectomy. The rapidity with which the anemia developed was no greater nor was the anemia more severe in the suprarenalectomized rats than in the normal rats, but all the suprarenalectomized rats died of the toxemia. The mortality from suprarenalectomy alone in rats during the first 3 weeks is less than 10 per cent in our laboratory. The mortality from *Bartonella muris* anemia in splenectomized rats of carrier stock is 30 per cent.[2]

In a second experiment on the effect of suprarenalectomy on the natural resistance of rats to *Bartonella muris* the effect of the injec-

[2] Adler (21) has pointed out that there are variations in virulence in different strains of *Bartonella muris*. The mortality of splenectomized rats in Jerusalem is 96 per cent, in Hamburg it is 30 per cent and in Sumatra it is 70 per cent (Kurschner and Timmerman (22)). The high mortality in these rats may be due to climatic conditions and not to any marked variation in the potency of a strain. We have noted a higher mortality rate in summer than in winter.

TABLE I

*The Effect of Bilateral Suprarenalectomy on the Natural Resistance of Adult Albino Rats to B. muris (B. muris-Free Stock Used in the Experiment). Effect of Injections of 1 Cc. of Whole Blood of Anemic Rat**

Rat No.	Sex	Operation	Amount of blood* injected	Days after operation	Red blood cell count and hemoglobin — No. of days after injection								
					1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	Remarks
			cc.										
1953	M	Bilat. Suprar.	1	7	9,500 T 110%	8,000 T 90%	7,500 T 90%	9,500 T 85%	Died				
1954	M	"	1	7	9,300 T 105%	6,000 T 95%	7,500 T 85%	Died					
1955	M	"	1	7	9,300 T 110%	9,500 T 110%	Died						
1956	M	"	1	7	10,200 T 110%	5,900 T 60%	6,100 T 85%	"					
1957	M	"	1	7	10,100 T 110%	8,300 T 100%	9,500 T 110%	"					
2041	M	"	1	7	10,200 T 110%	Died							
2042	M	"	1	7	10,200 T 115%	9,700 T 100%	7,400 T 90%	"					
2043	M	"	1	7	10,000 T 118%	Died							

2044	M	Bilat. Suprar.	1	7	11,000 T 118%	Died							
2045	M	"	1	7	9,750 T 120%	"							
2040	M	Spleen	1	7	10,100 T 105%	8,500 T 105%	9,500 T 110%	4,400 T 60%	2,200 T 35%	2,000 T 10%	4,000 T 20%	Recovered	
2039	M	"	0	7	10,000 T 115%	9,800 T 110%	10,500 T 115%	10,500 T 108%	9,800 T 100%	9,500 T 105%		"	
2048	M	Control	1		11,000 T 110%	10,200 T 100%	6,500 T 60%	2,000 T 40%	2,200 T 30%	1,800 T 35%	4,000 T 60%	"	
2049	M	"	1		11,000 T 90%	9,700 T 110%	4,500 T 65%	3,000 T 45%	2,400 T 40%	3,000 T 32%	4,500 T 65%	"	
2106	M	"	1		11,500 T 110%	11,400 T 80%	6,900 T 80%	7,000 T 80%	6,500 T 70%	3,800 T 58%	5,500 T 70%	7,000 T 90%	Recovered
2107	M	"	1		10,000 T 105%	10,500 T 105%	7,500 T 90%	5,500 T 78%	6,000 T 50%	5,600 T 70%	6,000 T 80%	8,500 T 100%	"
2110	F	"	1		9,500 T 110%	9,000 T 90%	7,000 T 70%	5,500 T 58%	5,200 T 55%	6,500 T 80%	6,500 T 95%	9,000 T 110%	"
2111	F	"	1		10,500 T 105%	9,500 T 100%	6,000 T 80%	5,000 T 45%	4,500 T 40%	4,900 T 80%	6,500 T 95%	7,500 T 110%	"

* Blood obtained from splenectomized rat at height of anemia.

The letter "T" is used in place of the last three zeros in the red cell count. The hemoglobin is expressed in percentages as calculated from readings with the Dare hemoglobinometer.

TABLE I—*Concluded*

Rat No.	Interval between injection of *B.muris* and death	Pathology
	days	
1953	5	Typical changes characteristic of *B. muris* anemia
1954	4	" " " " " " "
1955	3	" " " " " " "
1956	4	" " " " " " "
1957	4	" " " " " " "
2041	1	No pathological change characteristic of *B. muris* anemia
2042	4	Typical changes characteristic of *B. muris* anemia
2043	1	Congestion of pulp of spleen with enlarged follicles and erythrophagocytosis but no other changes characteristic of *B. muris* anemia
2044	1	No pathological change characteristic of *B. muris* anemia
2045	1	" " " " " " " "

TABLE II

*The Effect of Bilateral Suprarenalectomy on the Natural Resistance of Adult Albino Rats to B. muris (B. muris-Free Stock Used in the Experiment). Effect of Injections of 0.2 Cc. of Whole Blood of Anemic Rat**

Rat No.	Sex	Operation	Days after operation	Red blood cell count and hemoglobin									
				No. of days after injection									
				1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
2100	M	Bilat. Suprar.	7	8,500 T 105%	8,900 T 95%	8,250 T 100%	10,000 T 90%	8,000 T 80%	8,000 T 70%	6,000 T 80%	90%	7,000 T 90%	8,000 T 95%
2101	M	"	7	11,000 T 110%	10,000 T 110%	6,200 T 95%	8,000 T 90%	7,200 T 82%	7,600 T 68%	6,200 T 80%	80%	6,800 T 85%	8,500 T 100%
2102	M	"	7	10,000 T 110%	11,500 T 85%	9,000 T 90%	8,000 T 100%	8,600 T 89%	7,900 T 75%	8,600 T 80%		6,800 T 85%	10,500 T 100%
2103	M	"	7	9,150 T 110%	11,000 T 110%	10,000 T 100%	8,200 T 90%	8,200 T 95%	8,800 T 85%	7,500 T 60%	5,200 T 70%	7,400 T 85%	8,000 T 90%
2104	M	"	7	10,000 T 110%	8,500 T 110%	9,000 T 100%	8,900 T 90%	6,200 T 68%	8,000 T 70%	8,000 T 80%	9,000 T 82%	9,000 T 85%	9,500 T 90%
2105	M	"	7	10,500 T 105%	9,000 T 95%	8,000 T 95%	8,500 T 80%	8,000 T 80%	7,800 T 70%	7,500 T 80%	8,500 T 100%	8,500 T 110%	
2108	M	Normal		10,500 T 110%	8,800 T 105%	8,750 T 95%	6,500 T 75%	6,000 T 60%	6,900 T 70%	7,000 T 95%	7,000 T 100%	9,000 T 110%	
2109	M	"		9,800 T 110%	9,000 T 90%	9,500 T 100%	7,000 T 70%	6,500 T 70%	6,400 T 75%	8,000 T 85%	7,500 T 100%	9,000 T 110%	

* Blood obtained from splenectomized rat at height of anemia.

The letter "T" is used in place of the last three zeros in the red cell count. The hemoglobin is expressed in percentages as calculated from readings with the Dare hemoglobinometer.

tion of smaller amounts of infecting material was determined. Six suprarenalectomized adult albino rats of Wistar stock and two adult male control rats of the same stock were each injected intraperitoneally with 0.2 cc. of blood of anemic rats on the 7th day following operation. Within 5 days after the injection, all the rats developed a moderate anemia and within 11 days they recovered. There was no essential difference in the course of the disease between the operated and the control rats.

If the infecting dose is small suprarenalectomy does not influence the course of the anemia subsequently induced nor is the toxemia of the milder infection sufficient to cause death of the rat.

DISCUSSION

Normal adult albino rats of non-carrier stock possess a high degree of natural resistance to spontaneous infection with *Bartonella muris* anemia, but may be readily infected by the injection of whole blood of an anemic splenectomized rat. Though the morbidity of the disease under these conditions is high, the mortality is extremely low. The removal of the spleen in rats of non-carrier stock causes a marked increase in the susceptibility to spontaneous infection with *Bartonella muris*, but the mortality is considerably lower (19) than in suprarenalectomized rats of non-carrier stock similarly infected.

Suprarenalectomized rats of non-carrier stock injected with large amounts of blood of anemic rats are killed by the toxemia of the infection before the anemia becomes severe. The nature of the rôle of the suprarenal gland in natural resistance to this infection is of a general character. There is no change in the type of tissue reaction following infection in suprarenalectomized rats but an increased susceptibility to toxic substances produced in the course of a first infection.

The acquired resistance to *Bartonella muris* is unaffected by subsequent suprarenalectomy. This procedure in the rat does not alter the acquired resistance to *Trypanosoma lewisi* established by a first infection (6). Suprarenalectomy does not influence the acquired resistance to typhoid vaccine (23). These observations indicate that once a cellular or humoral immunity is established to an infection or

an antigenic substance, this acquired resistance cannot be broken down by subsequent suprarenalectomy.

The spleen plays a more specific rôle in both the natural and acquired resistance to infection with *Bartonella muris*. In infection with *Trypanosoma lewisi* in the rat the spleen is important in natural resistance but an acquired resistance established by a first infection is uninfluenced by subsequent splenectomy. In those instances in which the acquired resistance to an infection is dependent on circulating antibodies, as in *Trypanosoma lewisi* infection, removal of the spleen has no effect on the resistance to reinfection. In *Bartonella muris* anemia such circulating antibodies are not demonstrable. The resistance is dependent on the activity of cells of the splenic pulp (24). It may be that a hormonal substance is secreted by these cells as suggested by previous work of the authors (24) and by the recent experiments of Lauda and Flaum (25). They joined rats by parabiosis. Splenectomy in one did not result in anemia but subsequent removal of the spleen in the second rat was followed by anemia in both rats.

Recently McCarrison (26) and Willis and Mahta (27) reported the spontaneous occurrence of *Bartonella muris* anemia in rats of carrier stock fed on a diet deficient in vitamins A and C.

Apparently deficiency in vitamin content of diet was followed by a depression of the acquired resistance to this infection. Disturbances in cellular metabolism in rats suffering with vitamin A deficiency have been observed in various epithelial tissues of the body, such as the cornea and respiratory tract (28, 29). It is not improbable that disturbances of a similar nature in the function of the cells of the splenic pulp may occur. Further, rats fed for long periods of time on a diet deficient in vitamin A acquire severe infections of the accessory nasal sinuses, respiratory tract and urinary tract (30). Such infections resulting in acute inflammatory changes in the spleen, as is commonly observed in *Trypanosoma lewisi* infection in the rat or in other severe infections (24), may depress the protective function of the cells of the spleen to *Bartonella muris* anemia.

SUMMARY AND CONCLUSIONS

Normal adult Wistar rats (non-carrier stock) are readily infected with *Bartonella muris* and develop a severe anemia if large amounts of

infecting material are used. The normal adult rat of Wistar stock possesses a relatively high natural resistance to spontaneous infection with this organism.

Bilateral suprarenalectomy in Wistar rats lowers the natural resistance to a subsequent infection with *Bartonella muris*. This procedure does not alter the type of tissue response to the virus but lowers the natural resistance of the rat to toxic effects of the infection. The acquired immunity to *Bartonella muris* conferred by a first infection is not broken down by subsequent suprarenalectomy.

The mechanisms of acquired and natural resistance are dependent on different physiological processes in the organism and are not merely quantitative variations of the same process as is generally assumed.

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