IMMUNOLOGICAL STUDIES IN RELATION TO THE SUPRARENAL GLAND

VI a. TRYPANOSOMA LEWISII INFECTION IN NORMAL ALBINO RATS
VI b. TRYPANOSOMA LEWISII INFECTION IN SUPRARENALECTOMIZED ADULT ALBINO RATS

BY J. MARMORSTON-GOTTESMAN, M.D., DAVID PERLA, M.D., AND JEFFERSON VORZIMMER

(From the Laboratory Division, Montefiore Hospital, New York)

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VI a. T. lewisi Infection in Normal Albino Rats

Though much evidence has been accumulating during recent years to indicate the importance of the reticulo-endothelial cells in resistance and antibody formation, little is known of the more fundamental controlling influences of this mechanism. In previous studies (1-5) we have gathered evidence of the importance of the suprarenal gland in antibody formation through the influence of the water balance in the tissues of the body. That the suprarenal glands are essential in the mechanism of natural resistance has been established through the work of several investigators and in previous work in this laboratory (6-9). In an effort to determine what part the suprarenal gland and other glands of internal secretion play in resistance to protozoan diseases, T. lewisi infection, a common disease of rodents, was studied.

T. lewisi infection in the albino rat offers certain advantages for experimental study.* Spontaneous infection is rare, but the disease can be readily transmitted by inoculation of infected blood. Its course can be quantitatively studied. The severity of the infection can be estimated by counts of the trypanosomes in the peripheral blood stream and by estimation of the coefficient of variation in the size of the parasites during the reproductive phase of the infection (12). They produce a mild disease in adult rats and recovery occurs within 4 to 5 weeks, with an acquired immunity that remains permanent.

* For a review of the literature on T. lewisi infection, see Laveran and Menil (10) and Taliaferro (11).
The wide variation in the course and duration of the infection reported by previous workers (11, 13, 14) may be in part due to the fact that the factors of stock, age, weight of the rats, diet and the environmental conditions of the animals were not uniform. We have kept these factors as constant as possible.

The course of *T. lewisi* infection was studied in 40 normal adult albino rats and was made the basis of comparison with infections in suprarenalectomized, nephrectomized, splenectomized, gonadectomized, and thymectomized rats.

**Method**

The rats were all of Wistar Institute stock, raised in our laboratory. Except where otherwise specifically stated, they were 3 months of age, and ranged in weight from 150 to 225 gm. They were kept under constant environmental conditions and maintained on a standard adequate diet.

The strain of the *Trypanosoma lewisi* was obtained through the courtesy of Dr. Linton of Columbia University and was maintained by weekly transfers. 1 cc. of blood from rats infected with *T. lewisi* was drawn from the heart and was diluted with 9 parts of physiological salt solution containing 1 per cent sodium citrate. 1 cc. of this dilution was injected intraperitoneally into a series of rats. Red blood cell counts were made at frequent intervals. Smears of the peripheral blood were made daily during the first week and then at 2 day intervals thereafter until the trypanosomes disappeared from the blood stream. Trypanosome counts were estimated and the curve of the infection determined in each instance.

**The Course of the Experimental Infection in the Normal Adult Albino Rat**

Following intraperitoneal injection the trypanosomes multiplied in the peritoneal cavity for a period of 1 to 3 days. Within 24 to 48 hours trypanosomes appeared in the peripheral blood. They rapidly multiplied and swollen forms became numerous. By the fourth day the count exceeded 100,000 per cubic millimeter. Multiplication forms were present during the first 7 to 9 days and from then on only adult forms were seen. The average interval from the onset to the height of the infection was 7.5 days, the range 5 to 18 days. The number of trypanosomes at the height of infection varied from 115,000 to 800,000 per cubic millimeter. The average number was 336,000 per cubic millimeter. The average duration of the infection was 27.4 days. In two instances the duration of the infection was less than 3
weeks, in sixteen 3 to 4 weeks and in twenty-two 4 to 5 weeks. No instance of infection in our normal rats exceeded 35 days. The average duration of the infection was the same in males and females. In most instances the trypanosomes disappeared from the blood gradually, but in six instances the number of trypanosomes dropped within 24 hours from a high count to zero. In general these findings are consistent with those of previous investigators. The duration of the infection in the rat is not as varied if the factors of stock, age, weight, environment, care and diet are kept uniform.

Symptoms of the Infection.—Laveran and Mesnil (10) observed that very young rats failed to gain weight during the first few days of the infection. A definite clinical syndrome in the adult rat was noted when T. lewisi infection was first introduced into our stock. During the first 48 hours, the rat was quiet, his appetite was poor, the eyelids were edematous and sticky, and edema of the penis was present. These symptoms rapidly subsided and the rats appeared normal during the rest of the infection. In two instances during the second week of the infection partial paralysis of the hind limbs was observed which disappeared within 3 weeks. After the first two or three transfers of the strain of T. lewisi, symptoms of edema of the lids and penis were no longer noted and no further instance of paralysis was observed. The infection was associated with fewer symptoms when the Trypanosoma lewisi strain had undergone several passages.

A moderate anemia developed during the first few days of the infection which was most severe at the height of the infection. The red cell count rarely dropped below 4,500,000. The anemia was associated with the appearance of Bartonella muris bodies in the red cells. From experimental studies reported in a previous communication (15) it was found that the virus of Bartonella muris anemia could be separated from the trypanosome infected blood by passage through young rabbits. The concomitant anemia was more severe in 3 week old rats than in mature rats and the T. lewisi infection ran a more prolonged and severe course in the young rats. Death from trypanosome infection in very young rats may occur with a severe anemia, the red cell count dropping to 1,000,000 or less. Bartonella muris bodies are present in large numbers in the red cells. Autopsy of the fatal cases reveals icteric tint to the subcutaneous fat, enlargement of the spleen and fatty changes of the liver, heart and kidneys.
Pathology of the Trypanosoma lewisi Infection in the Normal Adult Rat

A series of 19 rats were inoculated with blood from a rat injected with Trypanosoma lewisi and killed at intervals of 2 days from the first day of infection to the 38th day after inoculation. Macroscopically the only pathological changes were noted in the spleen. It enlarged progressively until at the height of the infection it was 6 to 7 times the normal size, amounting to 1.55 per cent of the body weight as compared with 0.27 per cent normally. It diminished in weight after the 10th day but at the end of the infection it was still larger than normal. Histological studies show a progressive enlargement of the follicles and marked congestion of the pulp. The reticular and endothelial elements became engorged with red blood cells, were greatly increased in size and many underwent disintegration. Some of the Kupffer cells of the liver show erythrophagocytosis. In the later stages of the infection the follicles of the spleen diminish in size, the congestion disappears and there is some increase in the connective tissue of the pulp. No trypanosomes were found in the tissues of rats killed at the height of the infection when several hundred thousand trypanosomes per cubic millimeter were present in the peripheral blood stream.

In mice infected with pathogenic trypanosomes the spleen may hypertrophy to 60 times the normal size (16). Taliaferro (11) states that splenic enlargement is more marked in the severe trypanosome infections of mouse, rat, guinea pig and dog,—which die with large numbers of parasites in their blood,—than in the goat, sheep, rabbit and cattle in which trypanosomes are only rarely found.

T. lewisi infection in the rat results in severe injury to the reticular and endothelial elements of the spleen. Evidence of such injury is afforded by the concomitant occurrence in many rats of the Bartonella anemia at the height of the trypanosome infection. T. lewisi infections produce the same effect as splenectomy. Cannon and McClelland (17) found that repeated injections of large amounts of India ink over a long period of time in normal rats is followed by the appearance of Bartonella anemia. As much as 60 to 80 cc. of India ink (4 per cent suspension) was used per rat. They attribute this
anemia to physiological "blockade" of the reticulo-endothelial elements.

_Complement Fixation in Trypanosoma lewisi Infection_

Although complement fixation in diseases due to the pathogenic trypanosomes has been extensively investigated (11), no studies on complement fixation in _T. lewisi_ infection in the rat have been reported to our knowledge.

Manteufel (18) used aqueous and alcoholic extracts of organs of rats infected with _T. lewisi_ in _T. equiperdum_ studies in horses with contradictory results. Bessemann and Leynen (19) in studying methods of obtaining sensitive antigens for use in diagnosis in _T. equiperdum_ (dourine) used among other things _T. lewisi_ as a source of the test antigen and found it unsatisfactory.

Studies on the complement fixing antibodies of rats recovered from _T. lewisi_ were made in an effort to obtain a comparative standard for antibody formation under different conditions. Aqueous and alcoholic antigens were used, prepared from the liver and spleen of rats infected with _T. lewisi_. Saline washings of heated and unheated cultures of _T. lewisi_ grown on Novy and McNeal medium were also tested.

Methods.—The saline extracts were made as follows: The spleens of infected rats were macerated and ground in a mortar and shaken with 30 cc. of physiological salt solution per spleen during 30 minutes. The mixture was rapidly filtered and the filtrate preserved in 0.5 per cent phenol. Only freshly prepared extracts were used. The livers of infected rats were macerated with saline and the volume was brought up to 15 cc. per gram of liver. The suspension was shaken thoroughly for 30 minutes, left over night in the ice chest, again shaken and filtered. The anticomplementary titer of the antigens was determined and one-fourth the anticomplementary unit was used in each test. The tests were carried out as follows: 0.1 cc. of inactivated serum, 0.1 cc. of antigen in the dilution estimated by previous titration and 0.1 cc. of guinea pig serum diluted as indicated by previous complement titration (2½ units). The total volume was brought up to 1 cc. and incubated during 1 hour at 37°. The hemolytic system, consisting of 0.1 cc. of 5 per cent suspension of washed sheep cells and 0.1 cc. of amboceptor in dilution to equal 2½ units was then added.

We have found that during the course of the infection the serum of the infected rat is anticomplementary in very high dilutions, some-
times higher than 1/1000, owing to the fact that both antigen and antibody are present in the serum of the rat during the infection.

Complement fixation in rats recovered from the infection for several weeks were tested with the antigens described. All the antigens gave positive results with positive sera but the strongest reactions were obtained with saline extracts of spleen and liver and saline washings of trypanosome cultures. A sample protocol is given below.

<table>
<thead>
<tr>
<th>Sera</th>
<th>Saline extract spleen</th>
<th>Saline extract liver</th>
<th>Saline washings trypanosome cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled positive*</td>
<td>1/400</td>
<td>1/160</td>
<td>1/600</td>
</tr>
<tr>
<td>Positive Rat 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Positive Rat 3</td>
<td>1/700</td>
<td>1/80</td>
<td>1/1000</td>
</tr>
<tr>
<td>Positive Rat 4</td>
<td>1/100</td>
<td>1/200</td>
<td>1/400</td>
</tr>
<tr>
<td>Positive Rat 5</td>
<td>1/200</td>
<td>1/20</td>
<td>1/200</td>
</tr>
<tr>
<td>Pooled negative**</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
</tr>
<tr>
<td>Negative Rat 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Positive sera were obtained from rats 2 weeks after they had recovered from *T. lewisi* infection.

** Negative sera were obtained from normal uninfected rats.

Further studies are in progress to determine the course of antibody formation during the life of the rat following infection.

Since the original observations of Rabinowitch and Kempner (20) it is known that a rat which has recovered from an infection with *T. lewisi* cannot be reinfected. The mechanism of this permanent immunity is not understood but studies on antibody formation suggest that the immunity is primarily a humoral one.

Laveran and Mesnil had observed the phagocytosis of *T. lewisi* in the peritoneal cavity of immune rats reinjected with trypanosomes. That phagocytosis by monocytes and circulating leucocytes plays a part in the immune reaction is evident, but many observers have failed to note phagocytosis of trypanosomes.

**Summary**

*T. lewisi* infection was studied in a large series of normal adult and young albino rats. The importance of using rats of the same stock, age, and weight for comparative studies with this infection is empha-
sized. The infection produced by intraperitoneal inoculation of blood of an infected rat lasts about 1 month. The height of the infection is reached at about the seventh day. *T. lewisi*, though never fatal in the adult rat, produces a definite disease entity with a progressive splenomegaly during the first 10 days of the disease. Histologically there is hyperplasia of the follicles and congestion of the pulp with marked erythrophagocytosis by reticular and endothelial elements. The spleen diminishes in size with the recovery of the animal. A moderate anemia develops, due to the concomitant infection with *Bartonella muris* viris. During the course of the infection the best index of the resistance of the animal is afforded by the daily trypanosome counts, by the interval during which developmental forms are present and by the duration of the infection. The serum of the rat during this period is highly anticomplementary. After recovery complement fixing antibodies can be detected for a long period of time. The best antigens for the detection of complement fixing antibodies are saline extracts of rat spleens removed at the height of *T. lewisi* infection and saline washings of unkill cultures of trypanosomes.

**VI b. T. lewisi Infection in Suprarenalectomized Adult Albino Rats**

It has been established that suprarenalectomy in albino rats lowers their resistance to toxins, chemical poisons and bacterial infections and diminishes their capacity for antibody formation (6–9). In an effort to determine the rôle of the suprarenal gland in resistance to protozoan infections, *T. lewisi*, a common protozoan of rats, was studied. In the first part of this communication the course of the infection and its pathology was described. Experiments were next carried out to determine the effect, if any, of suprarenalectomy on the acquired immunity to *T. lewisi* of rats recovered from a first infection.

The experiments were divided into two groups. In the first group eighteen adult rats were suprarenalectomized and in five the suprarenal areas were traumatized. 6 days after the operation all the rats were injected intraperitoneally with 1 cc. of a 10 per cent dilution of whole blood drawn from a rat infected with *T. lewisi*. Smears and counts of the peripheral blood were made at frequent intervals.

The results of this experiment are given in Table I and Fig. 1. It is to be noted that of the 18 rats, 12 or 67 per cent, died within 2 to 19
days. The average duration of life of these rats with a fatal infection was 5.8 days after injection with *T. lewisi*, and the average interval in days between the onset and the height of infection was 4.2 days. The trypanosome count was not greater in the suprarenalectomized rats than in normal rats. Apparently, however, toward the height of the infection, the toxic effect of the trypanosomes was sufficient to kill a large percentage of the suprarenalectomized rats. The average num-

TABLE I

The Effect of Bilateral Suprarenalectomy and Unilateral Nephrectomy on Trypanosoma lewisi Infection

<table>
<thead>
<tr>
<th>Operation</th>
<th>No. of rats</th>
<th>Days between operation and infection</th>
<th>Duration of infection in days</th>
<th>Interval in days from onset to height of infection</th>
<th>No. of trypanosomes per cubic mm at height of infection</th>
<th>Per cent surv.</th>
<th>Per cent died</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bil. supra. fatal instances</td>
<td>12</td>
<td>6</td>
<td>5.8</td>
<td>219 5 4.2 2 8 4 220 10</td>
<td>640 212</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bil. supra. recovered instances</td>
<td>6</td>
<td>6</td>
<td>25</td>
<td>12 3126.5 8.6 5 11 9 338 160 640 305</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bil. supra. recovered and fatal instances</td>
<td>18</td>
<td>6</td>
<td>12.5</td>
<td>231 6 5.9 2 11 5 229 10</td>
<td>640 212 33 67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trauma to supra. area</td>
<td>5</td>
<td>6</td>
<td>24</td>
<td>16 28 6.4 5 8 6 380 166 700 300 100</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unil. nephrect.</td>
<td>8</td>
<td>6</td>
<td>26.7</td>
<td>645 32 8 10 8 330 100 890 200 100</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unoperat. adult normals</td>
<td>40</td>
<td>—</td>
<td>27.4</td>
<td>635 28 7.5 5 18 7 337 113 800 285 100</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Animal died 24 hours after infection.

ber of trypanosomes in the suprarenalectomized rats at the height of infection was 338,000 per cubic millimeter as compared with 337,000 for those which had been normal. The average interval between the onset and the height of the infection in those rats which survived the infection was 8.6 days. The average duration of the infection in the
surviving suprarenalectomized rats was 25 days as compared to 27.4 days of the normal group.

During the same period 20 rats were suprarenalectomized as controls. Of these rats, 80 per cent survived more than 1 month. In experiments with several hundred suprarenalectomized rats in this laboratory during the past 5 years it has been found that about 80 per cent of suprarenalectomized rats survive longer than 1 month. The mor-

![Graph](image)

**Fig. 1.** The effect of bilateral suprarenalectomy, unilateral nephrectomy and traumatization of the suprarenal area on the course of *Trypanosoma lewisi* infection in albino rats.

Each point on the curve of the infection of the suprarenalectomized rats indicates the average count of the surviving animals on that day.

The curves represent the daily average counts expressed in thousands per cubic millimeter.

- Normal
- Bilateral suprarenalectomy
- Unilateral nephrectomy

Mortality of 70 per cent to *T. lewisi* infection in the suprarenalectomized rats, therefore, indicates a severe drop in resistance.

The curve in Fig. 1 indicates the average daily trypanosome counts. Each point on the chart represents the average count of all the rats living on that day. The curve is practically the same as the curve of the composite average counts of the normal infected group.

An effort was made to reinfect the surviving rats but without success.
Pathology of the T. lewisi Infection in Suprarenectomized Rats

A comparative study of the pathological changes in the suprarenelectomized group dying of T. lewisi infection and the group that had been normal prior to infection did not reveal any striking differences in cellular reaction.

**TABLE II**

*Effect of Trypanosoma lewisi on the Weight of the Spleen in Normal and Suprarenectomized Rats*

<table>
<thead>
<tr>
<th>Normal adult rats</th>
<th>Adult rats infected with Trypanosoma lewisi</th>
<th>Suprarenectomized adult rats infected with Trypanosoma lewisi</th>
<th>Suprarenectomized uninfected adult rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>1001, 1002, 1003, 1004, 1005, 1006, 1007, 1008, 1009, 1010, 1011, 1012, 1013, 1014, 1038, 1039</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aver. wt.</td>
<td>.269%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. wt.</td>
<td>.448%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. wt.</td>
<td>.104%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean wt.</td>
<td>.268%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per cent weight of spleen to body weight</td>
<td>1001, 1002, 1003, 1004, 1005, 1006, 1007, 1008, 1009, 1010, 1011, 1012, 1013, 1014, 1038, 1039</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>1454, 1462, 1460, 1463, 1464, 1465, 1466, 1467, 1468, 1469, 1470, 1471, 1472, 1473, 1474, 1475, 1476</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per cent weight of spleen to body weight</td>
<td>1001, 1002, 1003, 1004, 1005, 1006, 1007, 1008, 1009, 1010, 1011, 1012, 1013, 1014, 1038, 1039</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>1458, 1466, 1469, 1470, 1471, 1472, 1473, 1474, 1475, 1476, 1477, 1478, 1479, 1480, 1481, 1482, 1483</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per cent weight of spleen to body weight</td>
<td>1001, 1002, 1003, 1004, 1005, 1006, 1007, 1008, 1009, 1010, 1011, 1012, 1013, 1014, 1038, 1039</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>10, 11, 12, 14, 16, 17, 18, 19, 20, 22, 25, 28, 32, 36, 38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per cent weight of spleen to body weight</td>
<td>1001, 1002, 1003, 1004, 1005, 1006, 1007, 1008, 1009, 1010, 1011, 1012, 1013, 1014, 1038, 1039</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>Died</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Twelve rats were suprarenelectomized and six of these were infected with T. lewisi after 6 days. The other six were kept as suprarenelectomized controls. One rat in each group was killed every other day and the pathological changes were noted and compared with those in normal rats infected with T. lewisi. The ratio of the spleen weight to the body weight was noted in both groups and compared with the normal. Suprarenelectomy without infection does not cause an increase in the weight of the spleen (Table II), but histological studies made at frequent
intervals reveal a progressive hyperplasia of the follicles with no increase in the total volume. The hyperplasia of the follicles is part of a generalized lymph tissue and thymus hyperplasia evident throughout the body after suprarenalctomy, as pointed out by Marine (21) and Jaffe (20).

The degree of splenomegaly in the suprarenalctomized and infected group was definitely less than in the unoperated infected group. On the tenth day after infection the spleen weighed 0.832 per cent of the body weight as compared with 1.554 per cent in the unoperated infected group (Table II). Though the degree of hypertrophy of the spleen is less, the character of the cellular response is the same in both groups. The reticular and endothelial elements are markedly distended with engulfed red blood cells and hemosiderin pigment. The pulp is congested and the follicles prominent.

In the second group of experiments 15 normal adult rats were infected with *T. lewisi* as described above and 1 month after all the trypanosomes had disappeared from the peripheral blood stream, the suprarenal glands were removed. After 5 days all were reinjected with *T. lewisi*. Reinfection did not occur in a single instance. It was not possible to overcome the acquired resistance by suprarenalctomy, regardless of the quantity of material used for reinjection.

*Effect of Unilateral Nephrectomy and Traumatization of the Suprarenal Area on the Course of a Subsequent T. lewisi Infection*

In an effort to control the factor of the operative procedure the left kidney was removed from eight adult rats by the posterior route and in five others the tissue about the suprarenal areas was injured. Six days after operation the rats were injected intraperitoneally with *T. lewisi* as described above. From Table I and Fig. 1 it is seen that the infection in these animals did not differ from that of normal rats. The average duration from the onset to the height of infection in the nephrectomized group is 8 days as compared with 7.5 of the normal group. The average number of trypanosomes at the height of infection was 330,000 per cubic millimeter, the range 100,000 to 890,000. All the rats survived the infection. The average duration of infection in the nephrectomized group was 26.7 as compared to 27.4 in the normal. The longest infection lasted 32 days.

One month after recovery all the rats were reinjected with *T. lewisi* but no reinfection was observed.
DISCUSSION

Bilateral suprarenalectomy lowers the resistance of adult albino rats to *T. lewisi* infection. In these rats the disease is fatal in almost 70 per cent of instances whereas of animals previously normal none died. Despite this high mortality the infection as characterized by the rate of growth of the trypanosomes does not differ essentially from the infection in normal rats. Neither the reproduction-inhibiting immune factor (Taliaferro) nor the trypanolytic immune factor is diminished by suprarenalectomy. Apparently the toxic effect of the infection is lethal in these animals. Suprarenalectomy further diminishes the degree of splenic response as estimated by the size of the spleen, but does not alter the reaction of the reticular and endothelial elements of the spleen or of the lymphoid tissue to the *T. lewisi* infection.

One infection with *T. lewisi* confers a permanent immunity in normal rats. It is of significance that suprarenalectomy does not break down the permanent immunity to *T. lewisi* infection acquired as a result of a first infection. This fact emphasizes an essential difference in the mechanisms of natural resistance and acquired resistance. The natural susceptibility of the rat to various toxins and poisons and infections is markedly increased by suprarenalectomy. But the acquired immunity established as a result of a first infection is unaffected by suprarenalectomy.

We have found in other studies that the *Bartonella muris* anemia of splenectomized rats cannot be transmitted to suprarenalectomized rats. It has been demonstrated in a previous communication that *Bartonella muris* anemia in the adult splenectomized rat represents a flaring up of a latent infection with the *Bartonella muris* virus and the development of the anemia is indicative of a depression in the acquired immunity of the rat to the virus (15). The failure of the suprarenalectomized rat to develop the anemia either spontaneously or after injection of anemic blood of splenectomized rats indicates that the suprarenal gland does not influence the mechanism of acquired immunity to this infection. This observation with the *Bartonella muris* infection and the fact that the permanent immunity to *T. lewisi*, acquired as a result of a previous infection, is unaffected by suprarenalec-
tomy are of significance. Once a humoral or cellular immunity is established to an infection this acquired resistance cannot be sufficiently depressed to permit of a second infection. Acquired resistance 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 supposed.

SUMMARY AND CONCLUSIONS

Bilateral suprarenalectomy in rats lowers the resistance to a subsequent infection with *T. lewisi*. Almost 70 per cent of these rats die within an average period of 5.8 days after infection. The multiplication of the parasites in the circulating stream is not more considerable than in rats previously normal nor is the duration of the disease in the surviving rats any longer than in the normal group. Bilateral suprarenalectomy does not prevent the formation of immune substances to the parasites but appears to lower the natural resistance of the rat to the toxic effects of the protozoan infection. The acquired immunity to *T. lewisi* of normal rats as a result of infection is not broken down by subsequent suprarenalectomy. Unilateral nephrectomy does not affect the course of a subsequent infection with *T. lewisi*.

BIBLIOGRAPHY