

## FURTHER STUDIES ON *T. LEWISI* INFECTION IN ALBINO RATS\*

### I. THE EFFECT OF SPLENECTOMY ON *T. LEWISI* INFECTION IN ALBINO RATS AND THE PROTECTIVE ACTION OF SPLENIC AUTOTRANSPLANTS

### II. THE EFFECT OF THYMECTOMY AND BILATERAL GONADECCTOMY ON *T. LEWISI* INFECTION IN ALBINO RATS

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#### *The Effect of Splenectomy on the Resistance of Albino Rats to T. lewisi, and the Protective Action of Splenic Autotransplants*

In studying the pathology of *T. lewisi* infection in normal rats it was noted that the spleen undergoes marked hyperplasia within a few days after injection of the organism (1). An effort was made to determine the rôle of the spleen in this infection by splenectomy at various intervals prior to infection with *T. lewisi* and to determine the effect of autoplasmic splenic transplants on the infection in splenectomized rats.

The marked involvement of the reticular and endothelial cells of the spleen in the pathogenic trypanosome infections has led to numerous investigations on the effect of splenectomy on the course of these infections. A review of the literature is given by Taliaferro (2).

Bradford and Plimmer (3) found that rats, dogs, cats and rabbits splenectomized before injection with *T. brucei* died earlier than the controls. Laveran and Mesnil (4) observed that splenectomy did not influence the course of *T. nagana* infection in the rat. Rodet and Vallet (5), as well as Sauerbeck (6), believe the spleen possesses striking trypanolytic power. Roux and Lacomme (7) treated three dogs infected with *T. nagana* with splenic extract of normal cattle and found

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that the parasites decreased in number but this may have been due to trypanolytic crises (Rodet and Vallet (5) ). In a study on 2 splenectomized rats and 5 guinea pigs, Laveran and Thiroux (8) could find no effect of splenectomy on the course of infection with *T. brucei* and found no trypanolytic action of splenic extracts. They believed the spleen merely removed the débris after trypanolytic crises. Mutermilch (9) found that extracts of spleen produced lysis of *T. brucei in vitro*. Bone marrow and liver extracts were also effective but to a less degree, and extracts of other organs had no effect. From this he concluded that the hematopoietic organs are particularly trypanolytic. For a time it was thought that the spleen acted as a refuge for trypanosomes during the crises, and resistant forms were said to exist. But as Laveran and Mesnil (10) showed, such a theory was unwarranted as isolated trypanosomes could be demonstrated in the blood during the crisis by subinoculation methods. Taliaferro, Johnson and Cannon (2) found that splenectomy had no effect on the course of *T. equinum* infection in mice.

Taliaferro describes the formation of a humoral antibody in *T. lewisi* infections in the rat that inhibits the cell division of the parasite (11) but which does not kill them. Regendanz and Kikuth (12) confirmed these observations. They have further shown that in splenectomized rats division of the trypanosomes continued several days longer than in non-splenectomized controls and in some animals the infection ended fatally. They injected these rats with salvarsan prior to splenectomy to sterilize them of the *Bartonella* virus. Taliaferro, Cannon and Goodloe (13) found little effect if splenectomy was performed before injection of *T. lewisi* but if performed after injection the reproduction of the parasites again occurred, the adult forms beginning to divide. Splenectomy was more effective in the presence of *Bartonella* infection.

The rats of our stock are carriers of the *Bartonella* virus and splenectomy in these rats results in *Bartonella* anemia. We therefore had the opportunity of determining the influence of this infection on *Trypanosoma lewisi* infection.

The experiments were divided into three groups. In the first group 7 rats were infected 6 days after splenectomy at the height of the *Bartonella* anemia. A virulent infection resulted with rapid death of the rats within 3 to 7 days after infection. The average number of trypanosomes at the height of the infection reached 1,200,000 per cubic millimeter, about 4 times greater than the number of trypanosomes in the normal controls. The average duration of life was 11.6 days after injection of the trypanosomes. The height of the infection as estimated by the number of trypanosomes was reached at the time of death (see Table I and Fig. 1). We have found (14) that 100 per cent of our stock develop the *Bartonella*



the infection which represents the interval during which reproductive forms are present, averaged 12.6 days. Reproductive forms were present several days longer than in the normal rats. The duration of the infection in the rats was twice as long as the normal group, averaging 60 days.

This experiment indicates that the spleen plays an important rôle in the formation of the reproduction inhibiting factor and also the lytic factor in the infection; 48 days after splenectomy the infection with *T. lewisi* is less severe than in the early period. Apparently a compensatory mechanism has been established but this is less effective than is the normal splenic tissue in combating the *T. lewisi* infection.

In the third group of experiments, six rats in which splenic auto-transplants had been introduced 4 weeks prior to splenectomy were infected with *T. lewisi* 28 days after splenectomy and 8 weeks after transplants had been made. The method of transplantation has been described (15). The transplants at the time the spleen was removed were insufficiently regenerated to prevent the occurrence of *Bartonella* anemia. The results of this experiment are indicated in Table I. The average number of trypanosomes at the height of the infection was 530,000 per cubic millimeter or midway between the splenectomized group infected 48 days after splenectomy and the normal group. The interval from the onset to the height of the infection was 9 days in this group as compared to 7.4 days of the normal rats and 12.6 days of the splenectomized rats without transplants. The duration of the infection in these rats was practically the same as in the normal group. The transplants reduced the severity of the infection of splenectomized rats to *T. lewisi*. Though they did not protect the rat against *Bartonella* anemia 4 weeks after they were implanted, the transplants at the end of 8 weeks, or 4 weeks after splenectomy, had regenerated sufficiently to raise the resistance of the animal to *T. lewisi*. Histological examination of these transplants (15) revealed complete regeneration of all elements of the spleen. They appeared as small spleens in the abdominal wall.

*Reinfection in Splenectomized Rats.*—An attempt was made to determine the rôle of the spleen in the acquired immunity of the rat to reinfection with *T. lewisi*. Ten normal rats were infected with *T. lewisi*. One month after recovery the spleens were removed from these rats.

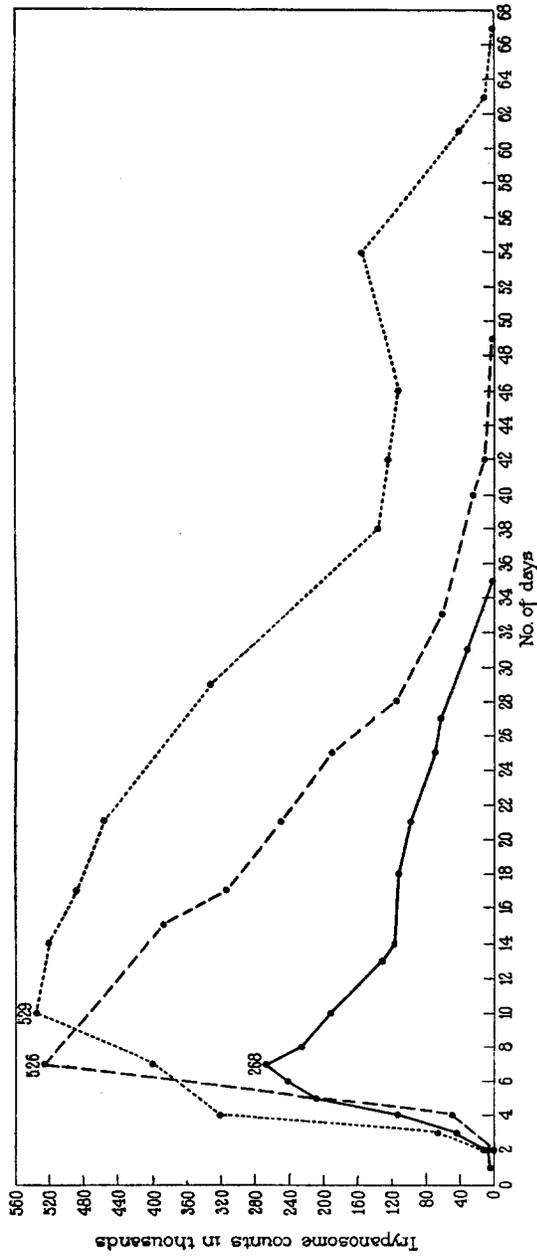


Fig. 1. The effect of splenectomy, and of splenectomy in rats with autotransplants of splenic tissue, on the course of *T. lewisi* infection in adult albino rats.

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

Normal—

Splenectomized rats infected 48 days after operation . . . . .

Splenectomized rats with splenic autotransplants performed 4 weeks prior to splenectomy and infected 24 days after

splenectomy—

Six days later all were reinjected with *T. lewisi*. In only one instance did they persist in the blood for a period of 48 hours. No developmental forms appeared. Repeated injections of *T. lewisi* were made, but no reinfection occurred.

#### DISCUSSION

Our observations are consistent with the findings of Regendanz and Kikuth (12), who studied the effect of splenectomy on the course of *Trypanosoma lewisi* infection in rats. Prior to splenectomy, their rats were injected with salvarsan to free them from the *Bartonella* virus. They found that the infection in the splenectomized rats was more severe than in the normal rats. They did not, however, make quantitative estimates of the course of the infection. The experiments reported in our communication indicate that the spleen plays a very important rôle in the defense mechanism to *T. lewisi*. The capacity of the rat to produce both the immune substance which inhibits the reproduction of the trypanosomes (reproduction-inhibiting immune factor) and the trypanolytic immune substance are markedly reduced by the removal of the spleen, even though the rats are not infected with *T. lewisi* until 7 weeks after the operation. This supports the contention of Taliaferro (2) that the reticulo-endothelial elements of the spleen are probably the main source of these immune substances.

The fact, however, that removal of the spleen does not influence the acquired immunity to *T. lewisi* following a first infection suggests that the immune defense mechanism may be a humoral one. In *Bartonella* infection on the other hand immunity may be a cellular one, dependent on the reticular and the endothelial elements of the spleen. In this disease an acquired immunity is broken down by splenectomy, but not by other procedures such as thymectomy, supra-renalectomy or gonadectomy. In trypanosomiasis the acquired immunity is a humoral one and hence is difficult to destroy. Complement fixing antibodies are present in the blood of rats for months after recovery from *T. lewisi* but no protective immune substances have thus far been demonstrated in the blood of rats either during the infection with *Bartonella* anemia or following it (Ford).

The conflicting results of investigations on the effect of splenectomy

on the pathogenic trypanosome infections is due in part to the difference in the species of experimental animal used. The spleen of the rat or mouse is considerably larger in proportion to the body weight than that of the rabbit or guinea pig. Taliaferro (2) suggests that the quantity of the reticular and endothelial cells in the spleen of the rabbit and guinea pig represents only a small part of the reticulo-endothelial tissue of the body. He believes that splenectomy in these animals therefore has little effect on the course of the trypanosome infections. This assumption however is based on insufficient evidence. It is possible that the relative quantity of hemolymph tissue in these different animals may bear some relation to the severity of the effects of splenectomy. Though it is still a debated question the true hemolymph nodes can probably be considered as accessory splenic tissue. The quantity of this tissue in the rat is extremely small. Macmillan (16) was able to find only 4 hemolymph nodes in the rat, 2 just above the kidney near the midline and 2 at the apex of the lungs in the posterior mediastinum. The rabbit and guinea pig, the sheep and the goat have relatively much more hemolymph tissue. The domestic pig on the other hand is said to have no true hemolymph nodes as determined by lymphatic injection, though many "red" nodes (Meyer (17)). Warthin (18) has demonstrated in the sheep and the goat a marked hypertrophy of the hemolymph nodes within the first few weeks following splenectomy, and considers these nodes as accessory erythropoietic tissue. It is possible that the hemolymph nodes in splenectomized animals assume a specific protective action which is normally a function of splenic tissue.

*The Effect of Thymectomy in Young and Adult Rats on *T. lewisi* Infection*

The effect of thymectomy on the course of *T. lewisi* infection in young and adult rats was studied. The rats were divided into two groups. In the first group of experiments 20 6-week old albino rats were used. Ten of these were thymectomized and 10 were kept as normal controls. In the second group, 10 adult, 3-month old rats were thymectomized. One week after operation, the rats of both groups were infected with *T. lewisi*.

*Method.*—Ether anaesthesia was used. The skin over the sternum was incised vertically and retracted. A purse string suture was put through the pectoral muscles, laterally and superiorly through the loose areolar tissue of the neck and through the submaxillary gland on one side. A similar purse string suture was put through the skin. The sternum was cut with small dull pointed scissors in the midline from above downward for a distance of 1.5 cm., care being taken to cut exactly in the midline to avoid the great vessels. The thymus was exposed, rapidly removed with fine mouse tooth forceps from below upward, and the purse string sutures were rapidly tied. If the chest is allowed to remain open longer than a few seconds the animal may succumb. If the rat survives the operation, it makes a rapid recovery.

The results of this experiment are tabulated in Table II and in the curves of the average daily counts of the trypanosomes in the circulating blood (Fig. 2). The course of the infection in 6-week old rats was slightly different from that of normal rats. The average number of trypanosomes at the height of infection was 316,000 per cubic millimeter as compared with 337,000 in the adult normal rats (see Paper VI, same issue of the journal (1)). The height of the infection was reached, however, somewhat earlier. In the adult normals the interval between the onset and the height of infection is 7.5 days, whereas in the younger group the interval between the onset and the height of infection is 6.4 days. The duration of the infection in the young rats is somewhat less than in the adult rats—22 days average duration in the young as compared to 27.4 days in the adult group. Thymectomy in the young rat has a decidedly beneficial effect on the course of the infection. The average number of trypanosomes at the height of infection is 176,500 per cubic millimeter and the interval between onset and the height of infection is 5 days. In the curve of the daily average counts the height of infection is reached on the 4th day and the average count on this day is 137,000. In the curve of the daily counts of the control young rats, the height of infection is on the 7th day and the average count on this day is double that of the thymectomy group, 273,000. The duration of the infection of the thymectomized rats is decidedly shorter than in the normal young controls. The average duration in the young controls is 22 days, and in the thymectomized young rats, 16.6 days.

The course and duration of the *T. lewisi* infection in adult thymectomized rats was likewise somewhat shorter than in adult normal rats.

The average number of trypanosomes at the height of infection was 241,000 and the interval between the onset and the height was 6.6 days. In the controls the average number of trypanosomes at the height of the infection was 337,000 and the interval from the onset to the height of infection was 7.5 days. The duration of the infection in the thymectomized adult rats is 19.1 days as compared with an average of 27.4 days in the control group.

Thymectomy has a favorable effect on the course of a subsequent infection with the *Trypanosoma lewisi* infection in both young and adult rats, but especially in young rats. The duration of the infection is decidedly less and the intensity of the infection is diminished. Developmental forms are observed during a shorter period and abortive infections are more frequently observed. The removal of the thymus, particularly in the young, has a stimulating effect on the formation of immune substances that inhibit the reproduction of the trypanosomes and have a stimulating effect on the formation of trypanolytic substances of the serum.

#### *Effect of Unilateral and Bilateral Gonadectomy on T. lewisi Infection*

Unilateral gonadectomy was performed on six adult albino rats and bilateral gonadectomy on six rats. One week after operation the rats were infected with *T. lewisi*.

*Method.*—Under ether, a median incision was made in the abdominal wall. The testes were gently drawn into the abdominal cavity by gentle traction on the spermatic cord. The pedicle was ligated and the testes were removed. Care was taken to avoid unnecessary manipulation of the spermatic cords. The abdominal wound was sutured. The rats recovered rapidly from the operation.

The results of this experiment are given in Table II and in Fig. 2. Unilateral gonadectomy in the adult rat has no effect on the trypanosome infection. The average number of trypanosomes at the height of infection is 276,000, reached in an average interval of 6.6 days as compared with the average number of trypanosomes at the height of the infection 337,000 in an average interval of 7.4 days. In the curve of the daily average counts the height is reached on the 7th day with 236,000 per cubic millimeter as compared with the normal 268,000 on the 7th day. Unilateral gonadectomy does not influence the course of a subsequent *Trypanosoma lewisi* infection.

TABLE II

*Effect of Thymectomy and Gonadectomy on Trypanosoma lewisi Infection*

Operation	No. of rats	Days between operation and infection	Duration of infection in days				Interval in days from onset to height of infection				No. of trypanosomes per cubic mm. at height of infection				Per cent surv.	Per cent died
			Aver.	Min.	Max.	Mean	Aver.	Min.	Max.	Mean	Aver.	Min.	Max.	Mean		
			(Expressed in thousands)													
Thymect. in adults.....	10	6	19.1	7	27	22	6.6	4	8	8	241	60	570	196	100	0
6 week thymect.....	10	—	16.5	6	24	20	5	4	6	5	176	60	315	160	100	0
Unilat. gonadect.....	6	6	28	17	32	27.5	6.6	5	9	6	276	140	428	290	100	0
Bilat. gonadect.....	6	6	26.5	25	31	25.5	7.2	7	9	7	889	550	1186	930	100	0
Unoperated adult normals.....	40	—	27.4	6	35	28	7.5	5	18	7	337	113	800	285	100	0
Unoperated 6 week normals.....	10	—	22	17	27	22	6.4	4	8	7	316	60	570	225	100	0

TABLE III

Operation	No. of rats	Days between operation and infection	Duration of infection in days				Interval in days from onset to height of infection				No. of trypanosomes per cubic mm. at height of infection				Per cent surv.	Per cent died
			Aver.	Min.	Max.	Mean	Aver.	Min.	Max.	Mean	Aver.	Min.	Max.	Mean		
			(Expressed in thousands)													
Bilat.*** supraren.....	12	6	5.8	2	19	5	4.2	2	8	4	220	10	640	212	—	—
Bilat. supraren.....	6	6	25	12	31	26.5	8.6	5	11	9	338	160	640	305	33	67
Splenectomy**.....	7	6	11.6	3	18	13	11.6	3	18	13	1200	349	2695	1000	0	100
Splenectomy.....	6	48	60	50	68	61	12.6	4	21	12	705	411	1125	600	90	10
Spl. trans.* and splen.....	6	28	34.7	28	41	35	9	7	15	7	530	224	787	589	100	0
Unil. nephrect.....	8	6	26.7	6	45	32	8	6	10	8	330	100	890	290	100	0
Thymect. in adults....	10	6	19.1	7	27	22	6.6	4	8	8	241	60	570	196	100	0
6 week thymect.....	10	6	16.5	6	24	20	5	4	6	5	176	60	315	160	100	0
Unil. gonadect.....	6	6	28	17	32	27.5	6.6	5	9	6	276	140	428	290	100	0
Bilat. gonadect.....	6	6	26.5	25	31	25.5	7.2	7	9	7	889	550	1186	930	100	0
Trauma to supr. area.	5	6	24	16	28	25	6.4	5	8	6	380	166	700	300	100	0
Unoperated adult normals.....	40	—	27.4	6	35	28	7.5	5	18	7	337	113	800	285	100	0
Unoperated 6 week normals.....	10	—	22	17	27	22	6.4	4	8	7	316	60	570	225	100	0

\*\*\* Died during infection.

\*\* Died at height of infection.

\* Transplants performed 4 weeks prior to splenectomy.

Bilateral gonadectomy decidedly depresses the resistance to *T. lewisi*. Though the average duration of the infection is essentially

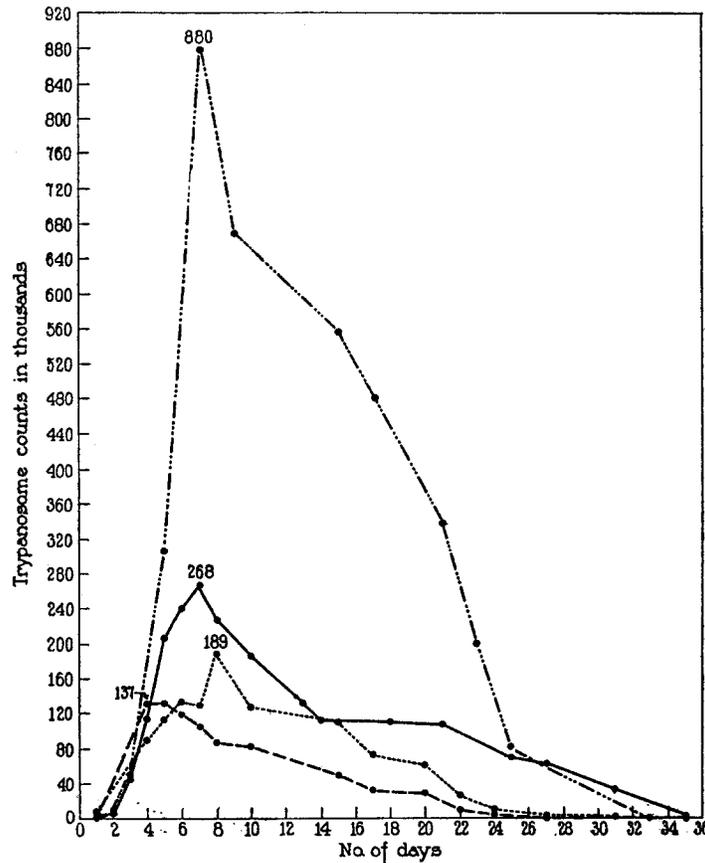


FIG. 2. The effect of bilateral gonadectomy and of thymectomy in young and adult albino rats on the course of *T. lewisi* infection.

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

- Normal —————
- Thymectomy in adult rat . . . . .
- Thymectomy in young rats - - - - -
- Bilateral gonadectomy . . - . - . .

the same as in the normal group, 26.5 days, the severity of the infection is markedly increased. The average number of trypanosomes

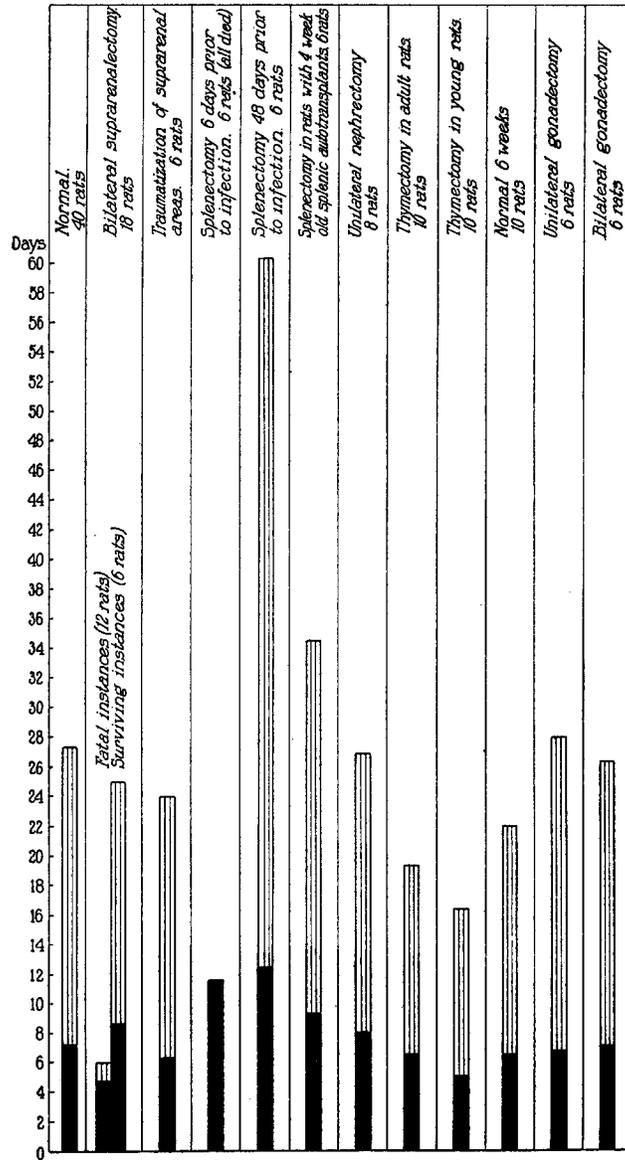


FIG. 3. The duration of infection with *T. lewisi* in normal rats, rats with bilateral suprarenalectomy, traumatization of the suprarenal area, unilateral nephrectomy, unilateral gonadectomy, bilateral gonadectomy, young and adult rats with thymectomy, rats in which splenectomy was performed 6 days prior to infection, rats in which splenectomy was performed 48 days prior to infection, rats with splenectomy in which splenic autotransplants were performed 4 weeks prior to splenectomy.

The solid portion of the bars represents the interval in days from the onset of the infection to the height of the infection.

The entire column represents the average duration in days of the infection.

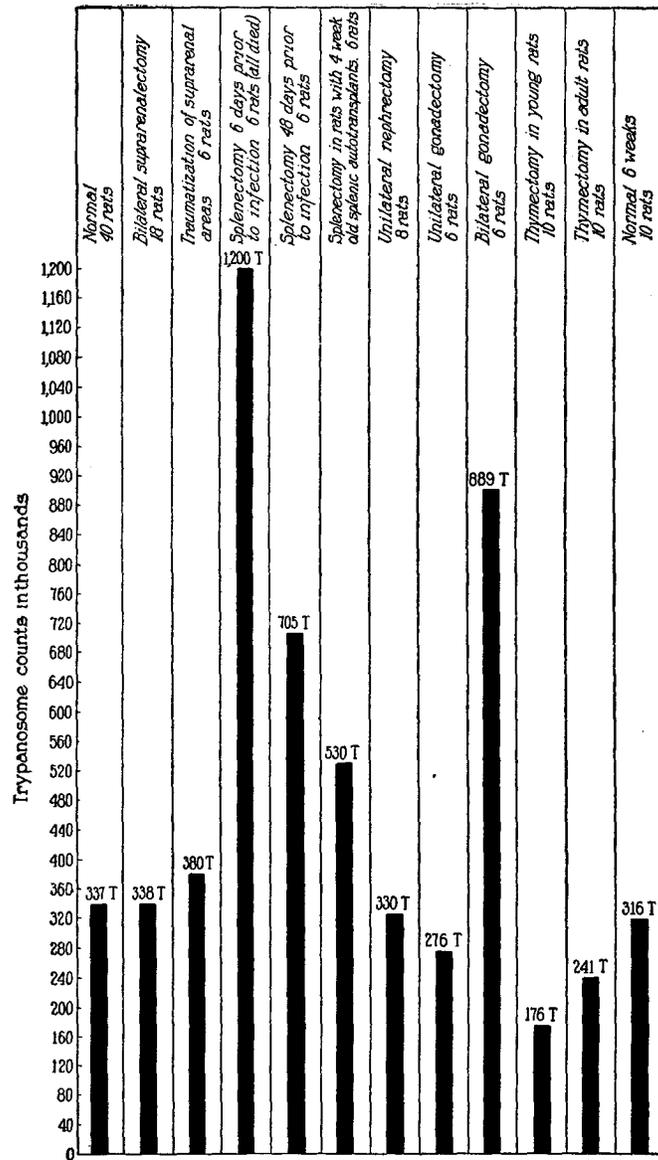


FIG. 4. The number of trypanosomes at the height of infection in rats with bilateral suprarenalectomy, traumatization of suprarenal area, unilateral gonadectomy and bilateral gonadectomy, thymectomy in young rats, thymectomy in adult rats, splenectomy in rats infected 6 days after operation, splenectomy in rats infected 48 days after operation, splenectomy in rats in which splenic autotransplants had been performed 4 weeks prior to splenectomy.

The bars represent the average number of trypanosomes at the height of infection with *T. lewisi*.

at the height of infection is 889,000 or almost three times as many as the normal group. The curve of the daily average counts reached a peak of 880,000 on the 7th day as compared with 268,000 on the 7th day in the normals. In 3 of the rats with bilateral gonadectomy the count exceeded 1,000,000 per cubic millimeter. Splenectomy resulted in similarly high counts. However, the disease in the bilaterally gonadectomized rats did not last longer than in the normals nor were there any fatalities. We must conclude that removal of the gonads interferes with the formation of the immune substance which inhibits reproduction of the trypanosomes, but does not interfere with the formation of the trypanolytic antibodies. In 4 instances in this group the disease terminated sharply by crisis, which is less frequently the case in normal rats.

Thymectomy in young rats diminishes the severity of the infection and shortens its course. Bilateral gonadectomy in the adult increases the severity of the infection but does not influence the duration of the infection.

#### SUMMARY

*T. lewisi* infection in normal adult 3 month old albino rats raised from a single stock and maintained under identical conditions was studied. Daily quantitative estimates of the trypanosomes in the circulating blood were made and the course of the infection was studied. Bilateral suprarenalectomy in rats lowers the resistance to a subsequent infection with *T. lewisi*. About 70 per cent of these rats die in an average period of 5.8 days after injection. The multiplication of the parasites, in the circulating stream, however, is not more considerable in the suprarenalectomized than in the previously normal rats, nor is the duration of the disease in the surviving rats any longer than in the normal group. The removal of the suprarenal glands does not alter the immune reaction to the parasite, but lowers the natural resistance of the animal to the toxic effects of the protozoan infection. Bilateral suprarenalectomy does not lessen the immunity of rats recovered from *T. lewisi* infection to subsequent infection. Unilateral nephrectomy does not influence the course of a subsequent infection with *T. lewisi* infection. The mortality of splenectomized rats from *Bartonella muris* anemia increases from 30 to 100 per cent following

the injection of *T. lewisi* at the height of the anemia 7 days after splenectomy. *T. lewisi* infection 48 days after splenectomy that is to say at a time when the *Bartonella* anemia is no longer present produces a more severe infection than in normal rats. The number of trypanosomes at the height of infection averages 3 times the ordinary and the infection endures twice as long. Both the immune substance that inhibits the reproduction of the parasite and the lytic factor are markedly depressed. Splenic autotransplantation performed 4 weeks prior to splenectomy raises the resistance of rats to a subsequent *T. lewisi* infection. Thymectomy in 6 week old rats diminishes the severity of a subsequent trypanosome infection and shortens its course. Both the formation of the immune substance which inhibits reproduction of the trypanosomes and formation of trypanolytic antibodies are stimulated by this procedure. In the adult rat thymectomy shortens the course of the infection but the severity is only slightly diminished. Bilateral gonadectomy in the adult increases the severity of the infection. The number of trypanosomes at the height of the infection is almost three times the normal. However, the duration of the infection is the same as in the normal rats. The reproduction-inhibiting factor is depressed by bilateral gonadectomy but not the trypanocidal factor. Unilateral gonadectomy does not influence the infection.

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