Effect of Splenectomy on *Trypanosoma lewisi* Infection in Young Rats

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The effect of splenectomy on animals infected with *Trypanosoma lewisi* is unclear, and previous reports are inconclusive or conflicting. We splenectomized rats of different ages after they had been infected with *T. lewisi*. Female Sprague-Dawley rats, *Hemobartonella*-free, were assigned to four groups according to weight: 80, 108, 140, and 170 g. Each group had splenectomized, sham-operated, and nonoperated control subgroups, all infected with *T. lewisi* (0.5 ml of 10⁶ parasites per ml) 90 h before surgery. Before surgery, parasite levels in host blood were similar. At 24 h after splenectomy in all groups, regardless of weight, blood parasite levels were much higher than they were in sham-operated or control animals (*P < 0.001 to P < 0.0001; analysis of variance*). Younger rats (80 and 108 g) had a higher mortality rate after splenectomy than sham-operated and control animals. Older rats (150 and 170 g) had no mortality. These results show the impact of age and the importance of the spleen on parasite-host interactions in rats infected with *T. lewisi*.

At the beginning of this century, Massaglia (15) and independently Laveran and Thiroux (13) reported that the spleen had no trypanolytic activity and that the course of an infection by pathogenic trypanosomes in asplenic animals followed the same evolution as that in intact animals. This contradicted earlier work by Bradford and Plimmer (5), who observed that splenectomized animals with a trypanosome infection died faster than those with intact spleens. In 1929, Brumpt (7) observed that in the weeks after splenectomy in *Trypanosoma lewisi*-infected rats, there was no rise in parasite levels. On the other hand, Kliger (11) reported that rats infected with *T. lewisi* and later splenectomized had more fulminant infection than the controls.

In 1930, Perla and Marmiston-Gottesman (16) attempted to determine the role of the spleen in a *T. lewisi* infection by splenectomizing rats at various intervals before infection and then determining the effect of autoplastic splenic transplants on the course of infection. They concluded that splenectomy performed 48 days before infection in rats known to harbor *Bartonella* organisms produced a much more severe infection that lasted twice as long as that in non-splenectomized animals. In 1931, Taliaferro et al. (21) performed splenectomies on *T. lewisi*-infected rats from day 7 preinfection to day 41 postinfection and reported the absence of significant effect on the reproductive activity of the parasite. The length of the infection, however, was affected.

Thus, confusing and conflicting results are the rule regarding the effect of splenectomy on *T. lewisi* infection. Critical appraisal of the literature suggests several factors which could explain the contradictory results. First, the timing of the splenectomy in relation to infection was seldom taken into account, obviating the complexity and heterogeneity of splenic dynamic populations at different times of infection. Second, the effect of animal weight (and age) was not studied. Third, some of the experiments were performed with rats treated with Neosalvarsan (which could be immunosuppressive) to cure them of *Bartonella* infection. Finally, statistical analysis of the data was seldom offered. For these reasons, it appeared both timely and relevant to study the problem under more controlled experimental circumstances with a more modern outlook.

We have studied the effect of splenectomy performed early (90 h) after *T. lewisi* infection in young rats of various weights.

**MATERIALS AND METHODS**

**Animals.** Female rats of various weights (ages) were purchased from Camm Research Institute, Wayne, N.J. The strains used were Sprague-Dawley (SD), Wistar, and Osborne Mendel. These animals were certified as pathogen-free, including the absence of pathogen-free, including the absence of

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Mycoplasma and Hemobartonella organisms. All animals had a period of acclimation of at least 7 days before experiments were started. They were housed five per cage in a humidity- and temperature-controlled room. A 12-h light cycle was used. Their bedding was changed at least twice a week, and they were fed standard laboratory Chow (Charles River RMH 3000 Oval; Agway Inc., Nazareth, Pa.) and water ad libitum. All rats were weighed upon arrival and monitored thereafter at regular intervals. They were also observed daily for abnormal behavior patterns or external symptoms of disease. All animals were cared for and handled by the guidelines set forth by the National Institutes of Health (10). At the end of the experiment, they were sacrificed by ether inhalation, and the carcasses were incinerated.

Parasites. T. lewisi uncloned strain was obtained from P. A. D’Alesandro, Columbia University, New York, N.Y. For all experimental inoculations, we used parasites kept alive by serial passages in young rats of the strains used. Parasite stocks were also frozen by lowering the temperature gradually in whole infected blood and Alsever solution (1:4) (22) with 10% glycerol. The frozen suspension was kept at −70°C.

To count the infected blood, the method of Amrein and Markwalder was used (3) after fixing and staining (1, 6).

Experimental animals were inoculated with 0.5 ml of 10^6 parasites per ml of blood in Alsever solution. The infected blood was obtained from stock donor rats by cardiac puncture with a 21-gauge 1.5 inch (3.81 cm) needle and a heparin-coated syringe.

Splenectomy. All animals undergoing surgery were anesthetized with an intraperitoneal injection of pentobarbital sodium (Fort Dodge Labs., Inc., Fort Dodge, Iowa), 50 mg/kg of body weight (12). The abdomen was cleansed with 70% alcohol, and a midline incision was made down to the peritoneal cavity. The spleen was ligated and gently extracted from its retrogastric position. Gentle finger traction was used, since the spleen was usually so congested that unwanted rupture and subsequent hemorrhage occurred with the use of instruments. The small gastric vessels were ligated, and the wound was closed with 4-0 polyglycolic acid suture (Dexon R; Davis & Heck, Pearl River, N.Y.). Sham-operated animals were treated the same way, except that their spleens were exposed for a few moments but not removed. Operated animals were placed in a lateral recumbent position. They usually awakened in 2 to 3 h, and by 8 h postsurgery, they had resumed their normal activity and diet.

Statistical analysis. Survival of the animals was compared by the Student’s t test (19) or by nonparametric statistics (18). Parasitemia levels were compared with analysis of variance (ANOVA) both intra- and intergroups. Regression analysis was used to correlate spleen size with parasite levels.

Experimental design. Female SD rats were divided into the following groups according to their weights (Table 1), and the discreteness of the groups was established by using ANOVA. Each group consisted of splenectomized, sham-operated, and control subgroups. The 80-, 108-, and 140-g groups were inoculated intraperitoneally with 0.5 ml of 10^6 T. lewisi per ml, and the 170-g group was inoculated with 1.0 ml. Splenectomies and sham operations were performed at 90 h postinfection with the parasites.

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Mean wt ± SEM (g)</th>
<th>P value</th>
<th>Denomination of group (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>79.43 ± 1.75</td>
<td>6.0 × 10^{-15}</td>
<td>80</td>
</tr>
<tr>
<td>23</td>
<td>100.15 ± 1.29</td>
<td>1.0 × 10^{-12}</td>
<td>108</td>
</tr>
<tr>
<td>28</td>
<td>140.15 ± 1.87</td>
<td>1.6 × 10^{-11}</td>
<td>140</td>
</tr>
<tr>
<td>36</td>
<td>169.85 ± 1.25</td>
<td>µ</td>
<td>170</td>
</tr>
</tbody>
</table>

* Only SD female rats were used.

RESULTS

SD rats were chosen over Wistar and Osborne-Mendel rats because they showed significantly higher parasitemia levels. Their peak was on day 7 postinfection. SD rats presented a 2- to 10-fold margin over the other strains, which was statistically significant at all points tested (day 2, \( P = 5.94 \times 10^{-6} \); day 5, \( P = 1.18 \times 10^{-2} \); day 7, \( P = 1.31 \times 10^{-2} \); day 13, \( P = 0.07 \)).

All surgical procedures induced very little bleeding, except for the animals in the 80-g group, which bled profusely during the splenectomy operation. It was noted that infected rats from both splenectomized and sham-operated groups had marked splenomegaly at 90 h postinfection compared to uninfected controls. This phenomenon seemed to correspond with the parasite levels observed just before the operations (linear regression showed a significant correlation between spleen length and parasite levels in two of the groups, the 180- and 170-g groups \( r = 0.90, P = 0.05; r = 0.95, P = 0.05 \)).

The 80-g group. The experimental results for the 80-g group are shown in Table 2. The two subgroups had the same baseline values for parasitemia before splenectomy. At 24 h post-splenectomy a dramatic increase in the splenectomized animals’ blood parasite levels (\( P = 4.8 \times 10^{-3} \), ANOVA) was observed. By day 3 postsplenectomy, half of the experimental animals were dead, and by day 5 postsplenectomy, all operated animals had died (\( P < 0.025 \), Fisher). All control animals remained alive throughout the entire observation period of about 1 month, and they had detectable parasitemia until day 23 postinfection.

The 108-g group. To simplify our results, we analyzed the data for the 108-g group in two parts: the values of all the animals in the group and the values of all animals except those that had zero baseline parasite levels. When the whole group was studied, there was no difference between the parasite levels of the three subgroups before splenectomy. Up to day 3 postsplenectomy, the differences were not significant (ANOVA). By days 6 and 8 postsplenectomy, significantly different parasite levels were
encountered among the subgroups. By day 11 postsplenectomy, the levels were no longer significantly different.

Table 3 summarizes the parasitemia values for the animals in the 108-g group, excluding those that started with zero baseline parasite levels. We feel that these exclusions (two in each subgroup) distort our results because of their abnormal behavior in terms of acquiring the infection. Baseline values are comparable for these, and results at 24 h postsplenectomy show that the differences among the groups are statistically significant ($P = 8.7 \times 10^{-4}$, ANOVA). This difference is evident until day 8 postsplenectomy, when it disappears. After this time, comparison was made only between the sham-operated and control subgroups, since all the experimental animals had died by day 4 postsplenectomy.

The 140-g group. For the 140-g group (Table 4), we observed similar presplenectomy parasitemia baseline values. A statistically significant difference was observed 24 h postsplenectomy which disappeared rather promptly. There were enormous standard deviations in both splenectomized and sham-operated subgroups from days 7 to 12 postinfection.

The 170-g group. The 170-g group had comparable baseline values for parasite levels (Table 5). At 24 h postinfection ($P = 2.1 \times 10^{-2}$, ANOVA) and lasting until day 16 postsplenectomy ($P = 1.0 \times 10^{-2}$, ANOVA), the subgroups had statistically significant differences in parasite levels. On days 3, 5, and 8 postsplenectomy, a difference was evident between the splenectomized and sham-operated groups (student’s $t$ test). On the same days, there were differences in parasite levels between the sham-operated and control subgroups.

This group received a rechallenge of $T. lewisi$ (1.0 ml of $10^5$ $T. lewisi$ per ml) approximately 3 months after the first infection. No parasites were ever observed (data not shown) in the peripheral blood of any of the animals after daily examinations during an observation period of 10 days.

Return of parasitemia to baseline values. We determined whether there was a difference between the different subgroups of the same group on the day the animals returned to their baseline parasite levels. The 80- and 108-g splenectomized animals all died before returning to their parasitemia baseline values. The sham-operated and control subgroups of the 108-g group showed differences on the day they returned to baseline values (17.25 ± 0.65 days versus 12.0 ± 0.57 days; $P = 10^{-3}$, ANOVA). The 170-g group also showed differences on the day the animals returned to parasitemia baseline values when the three subgroups were compared ($P = 8.97 \times 10^{-4}$, Fisher test).

### Table 3. Summary of the results of splenectomies performed in the 108-g group excluding animals that had a baseline of zero

<table>
<thead>
<tr>
<th>Group</th>
<th>Parasites on the following days postinfection (postsplenectomy)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 (-2)</td>
</tr>
<tr>
<td>Splenectomized (n = 3)</td>
<td>7.62 ± 0.46</td>
</tr>
<tr>
<td>Sham operated (n = 3)</td>
<td>8.96 ± 0.30</td>
</tr>
<tr>
<td>Control (n = 3)</td>
<td>9.6 ± 3.34</td>
</tr>
<tr>
<td>F ratio</td>
<td>0.1808</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Results are expressed as mean $T. lewisi \times 10^{2}$ per ml of rat (SD female) blood ± SEM.

*On day 5, one splenectomized rat was dead; on day 7, two splenectomized rats were dead; on day 10, three splenectomized rats were dead. These data are not significant by the Fisher test, since the rats that started with a zero baseline (excluded here) had a late-blooming infection.

*NS, Not significant.
10^{-5}, ANOVA). Splenectomized and sham-operated subgroups did not show a difference (P = 0.56, student’s t test) on the day of return. When both were compared with the controls, there was a statistically significant difference (20.12 ± 1.11 days versus 18.14 ± 0.71 days versus 13.33 ± 0.72 days; P = 8.97 × 10^{-3}, ANOVA). In the 140-g group, we were not able to detect any difference as to the day of return to parasitemia baseline values among the three subgroups when analyzed with ANOVA. When the results were analyzed with a contingency table analysis, a difference (P = 1.8 × 10^{-2}) was evident. Most of the sham-operated and control animals returned to baseline values on or before day 7 postinfection, whereas splenectomized animals usually returned to baseline values around day 9 postinfection.

Peak parasitemia values of the groups. When the data were analyzed for differences in the day the peak infection occurred, no differences were observed in the 80-, 140-, and 170-g groups (contingency table analysis; data not shown). The 108-g group showed a difference on the day on which peak infections occurred, when the group as a whole was analyzed (P = 1.3 × 10^{-3}; data not shown).

Analysis of the maximum parasite levels reached by the animals (Table 6) consistently showed that all splenectomized groups presented higher parasitemia levels than the two control groups. Results also showed that there was no statistical difference between peak values of parasitemia from sham-operated and control subgroups.

**DISCUSSION**

Several considerations are relevant to the proper analysis and interpretation of the results obtained.

First, the rat strain used (SD) for this work consistently yielded blood parasite levels of 10^6 parasites per ml. These levels are in accordance to those reported by Perla and Marmoston-Gottesman (16), who reported ranges from 10^6 to 10^7 parasites per ml in their studies. However, levels reported in more recent literature (personal communication, P. A. D’Alesandro, 1981 and 1982) are between 10^6 to 10^7 parasites per ml. One explanation for this could be that we used infected whole blood for animal inoculation. This may have resulted in the transfer of immune serum factors which may have prevented parasitemia levels from reaching higher values.

**TABLE 4. Summary of results of splenectomies performed on the 140-g group**

<table>
<thead>
<tr>
<th>Group</th>
<th>Parasites on the following days postinfection (postsplenectomy)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 (-)</td>
</tr>
<tr>
<td>Splenectomized (n = 8)</td>
<td>1.2 ± 0.35</td>
</tr>
<tr>
<td>Sham operated (n = 8)</td>
<td>1.4 ± 0.35</td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>0.8 ± 0.38</td>
</tr>
<tr>
<td>F ratio</td>
<td>0.59</td>
</tr>
<tr>
<td>P value</td>
<td>NS⁵</td>
</tr>
</tbody>
</table>

*a All values are expressed as mean T. lewisi × 10^5 per ml of rat (SD female) blood ± SEM.

**TABLE 5. Summary of splenectomies performed in the 170-g group**

<table>
<thead>
<tr>
<th>Group</th>
<th>Parasites on the following days postinfection (postsplenectomy)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 (0)</td>
</tr>
<tr>
<td>Splenectomized (n = 9)</td>
<td>43.75 ± 3.02</td>
</tr>
<tr>
<td>Sham operated (n = 8)</td>
<td>21.5 ± 7.02</td>
</tr>
<tr>
<td>Control (n = 9)</td>
<td>35.1 ± 10.16</td>
</tr>
<tr>
<td>F ratio</td>
<td>1.5644</td>
</tr>
<tr>
<td>P value</td>
<td>NS⁵</td>
</tr>
</tbody>
</table>

*a Values are expressed as mean T. lewisi × 10^5 per ml of rat (SD female) blood ± SEM.

b Day 7, splenectomized versus sham-operated rats, P = 7.4 × 10^{-4} (Student’s t test).

c Day 9, splenectomized versus sham-operated rats, P = 2.6 × 10^{-2} (Student’s t test).

d Day 7, sham-operated versus control rats, P = 3.0 × 10^{-3} (Student’s t test).

e Day 9, sham-operated versus control rats, P = 1.0 × 10^{-5} (Student’s t test).

⁵ NS, Not significant.
and may also have caused a shift of the parasitemia curves to the left; our peak day was 4, whereas in other reports it was 8 to 10 (20). Another explanation could be the genetic, major histocompatibility complex-mediated differences among the rat strains we used and those used by other investigators. This explanation, however, seems unlikely, since there are recent reports (2, 14) implying that differences in animal resistance to trypanosome infections are probably not associated with the major histocompatibility complex, but are variations in immune responsiveness to parasite antigens in conjunction with variations in nonimmunological mechanisms not presently known. Sex could be a factor, since in some allograft systems, males may be weaker responders than females (Z. González and E. A. Santiago-Delpín, unpublished observations). Still, the first explanation appears to us to be the more likely one.

A second consideration is the peculiarity of the 140-g group. This group was maintained under circumstances different from those of the other experimental groups. Animals in this group were exposed to fluctuating temperatures in the environment due to circumstances beyond our control. This group showed large standard errors of the mean (SEM) in parasitemia levels; spleen length values did not correlate with parasite levels as in other groups, and their mean day of return to baseline parasite levels was much earlier than that observed in the other groups. Also, they had a milder infection than the other groups. Nevertheless, the general behavior of the experimental versus the control animals follows the same pattern and trend as the other groups.

Our last preliminary consideration concerns the 108-g group. This group was analyzed in two parts: one group included all the animals, and the second group excluded those animals which started with zero baseline parasitemia levels presplenectomy. This was done because we felt that zero baseline parasitemia values could be misleading for mean and SEM values. The 108-g group had two distinct populations of rats in regard to susceptibility to *T. lewisi*; one group took the infection readily, whereas the other lagged until after splenectomy was performed. The question arose (but remains unanswered) as to what would have been the parasitemia values obtained for the zero baseline animals if they had not been splenectomized. We feel, based on the acquired experience with stock animals, that these animals probably lie within the values accepted for methodological probabilistic error and that they would have failed to develop any measurable level of parasites (at least 10⁴ parasite per ml).

The mortality observed in the younger experimental groups (80 and 108 g) was not caused by a concomitant *Hemobartonella* infection, as ruled out by careful examination of peripheral blood smears and stains at three time points: when they arrived, just before splenectomy, and just before they died. Perla and Marmoston-Gottesman report (16, 17) that *Hemobartonella* anemia follows splenectomy by 4 to 10 days, persisting for 2 to 3 weeks, with mortality from 20 to 30%, although morbidity was 100%. If at 7 days postsplenectomy, at the height of the anemia, the animals are infected with *T. lewisi*, mortality rises from 30 to 100%. Nonetheless, *T. lewisi* (9) has been reported to produce a secondary anemia of its own which is only transient and not fatal. Mortality was not secondary to anesthesia or surgery, the appropriate controls of which had no mortality.

The 80-g group of rats not only had a dramatic increase in blood parasite levels at 24 h postsplenectomy, but also early death of all splenectomized animals was seen by day 5 postsplenectomy (two died on day 1, one died on day 2, and four died on day 4). Control animals remained alive throughout the observation period. The days in which both splenectomized and control animals reached their peak infections were similar. The days on which the animals returned to baseline parasitemia values cannot be compared, since the splenectomized animals all died before returning to baseline values, whereas controls returned to baseline values by day 15. When the splenectomized and control groups were compared for maximal parasitemia values,
spleenectomized animals had nearly a fourfold increase in parasite counts. It is concluded from this 80-g group that splenectomy 90 h postinfection with *T. lewisi* has a fulminating effect resulting in the death of the animals.

The 108-g group was divided into two subgroups. When all the animals were analyzed together, the nonsignificant increase in parasitemia levels of the splenectomized animals was primarily caused by the large variance. Sham-operated and control animals behaved the same way until day 3 postsplenectomy, when the effect of the surgical trauma was seen. Days 6 and 8 postsplenectomy were the only days on which a significant effect of splenectomy was seen, and that was due to the reduced variance in the splenectomized group. By day 9 postsplenectomy, all splenectomized animals were dead, whereas sham-operated, control, and surgical control animals were all alive. There was a significant difference in the day of return to baseline values between sham-operated and control animals, reflecting the effect of the surgical trauma. There was also a significant difference between peak parasitemia levels; sham-operated and control animals had comparable values, implying that the surgical trauma had an effect on hindering infection but not on peak parasite values. This is reinforced by the fact that the peak day of infection was also different from controls. However, when the animals that started with zero baseline parasitemia values were eliminated, we obtained numerically different results, although the general pattern was still valid. By day 4 postsplenectomy, all splenectomized animals were dead. The significant differences seen between sham-operated and control animals through day 6 postsplenectomy was expected (great variance and surgical trauma); nevertheless, when sham-operated and control values are compared at their peak parasitemia levels, they are comparable.

In the 140-g group, peak parasitemia values among splenectomized, sham-operated, and control animals were different (*P* = 2.0 × 10⁻², ANOVA) even though their overall values were very low. No mortality was observed in this group.

In the 170-g group, we observed significant differences in parasite levels 24 h postsplenectomy which continued until day 16 postsplenectomy. The significant differences observed between sham-operated and control animals on days 3, 5, and 8 postsplenectomy are probably due to surgical trauma. By days 12 and 16 postsplenectomy, splenectomized and sham-operated animals had similar parasitemia levels; the difference was observed when compared with controls. No mortality was observed in this group.

Unfortunately, there is very little literature with which to compare our experiments. First, Taliaferro et al. (21) used one age group of young (110 to 125-g) *Bartonella*-free rats; sham operations in those experiments consisted of extirpating other organs (castration in 75- to 125-g rats) which could have immunological repercussions; Taliaferro et al. measured the reproductive forms of the parasites as their parameter. Perla and Marmostán-Gottesman (16, 17) were primarily interested in the effect of splenic autotransplant; they used *Bartonella*-infected rats which were subjected to Neosalvarsan treatment before the experiments. Taliaferro et al. (21) were not able to conclude that age affected the effect of splenectomy in *T. lewisi*-infected rats; when old, 200- to 300-g rats were used, they were infected with *Bartonella* organisms in addition to *T. lewisi*. We report a correlation between spleen length (centimeters) and parasite counts; Taliaferro et al. (21) reported no correlation between spleen weight to body weight ratio with length of infection or between spleen size and effect of splenectomy. Both authors reported infections that lasted much longer than ours. It is interesting to note that Taliaferro et al. (21) injected whole blood in 0.7% saline intraperitoneally into rats to establish the infections, and, although they reported reproduction forms and not levels of parasite, they had longer times of infection than we were able to obtain. Their infections lasted 40.6 ± 1.7 days.

Several conclusions emerge from our data (in SD outbred, pathogen-free female rats): (i) parasitemia levels were significantly increased from those of controls 24 h after splenectomy; (ii) this difference in parasite levels continued for several days; (iii) younger rats (80 and 108 g) were killed by the combination of splenectomy and *T. lewisi* infection; (iv) peak levels were always much more severe in splenectomized animals than in various controls; and (v) the presence of the spleen is crucial in the defense against *T. lewisi* in young rats.

The fact that the length of infection was affected after splenectomy suggests that the production of the trypanocidal antibody was in some way altered. We did not measure reproductive forms, so we cannot conclude what effect, if any, splenectomy may have had on the formation of the reproduction-inhibiting antibody, ablastin.

Not only is the weight of the rats relevant in determining the fatality or the severity or both of the infection by *T. lewisi*, but also the time of splenectomy vis a vis the time of infection could be crucial to the effects observed. Our splenectomy protocol, 90 h postinfection, has not been reported before in this model, although it is widely known that spleen cells are at their best
for the fusion protocols of hybridomas (in vitro) 4 to 5 days postinoculation with antigen. In this period, immunoglobulin M plaque-forming activity is maximal (8). Removal of the spleen at a time of such great activity (which is evidenced by the splenomegaly observed) must undoubtedly perturb the delicate balance inherent in all immune interactions. The question remains, however, whether this is the crucial time or whether an even earlier time has equal or opposite effects on the clearance rate of T. lewisi from these animals. Further considerations would therefore involve different times of splenectomy in a fixed-weight group, preferably the 108-g group, so as to determine whether changing the time of splenectomy would protect the rats that died at 90 h postinfection splenectomy, and the 170-g group, so as to determine whether rats that survived at 90 h postinfection splenectomy would die.

Although it has been reported (4) that if splenectomy is followed by exposure to a polysaccharide antigen memory of the antigen upon a second exposure is eliminated, this does not seem to be the case with T. lewisi, since rechallenge with T. lewisi performed on the 170-g group resulted in no observable parasitemia in peripheral blood of any of the subgroups for a period of 10 days.

We have presented data that sustain the importance of the spleen in mounting an immune response in young rats against a blood-borne nonpathogenic parasite, and we have suggested further experiments to expand our results. The full comprehension of all cellular interactions in immunity will eventually unravel present enigmas.

ACKNOWLEDGMENTS

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LITERATURE CITED


