HOST RESISTANCE TO THE KHARTOUM STRAIN OF *LEISHMANIA DONOVANI*

LESLIE A. STAUBER
Bureau of Biological Research, Rutgers, The State University, New Brunswick, New Jersey

INTRODUCTION

COMPARED with trypanosomiasis and malaria our knowledge of host resistance to leishmaniasis is quite in its infancy. This is also true of other aspects of research in this field and, except for the elucidation of the problem of transmission of *L. donovani* by sandflies, the last decade has seen no great advances in cultivation, serology, chemotherapy or experimental methods for the mammalian pathogenic strains of the parasite.

Evidence does exist, however, for host resistance to this group of organisms. Of the many kinds of animals into which *Leishmania,* from culture, insect gut or mammalian tissue, have been injected some become infected and some do not. Of those infected, there are even some claims for recovery (e.g. Laveran, 1920). Besides, in endemic areas all exposed animals do not become infected. In the human dermal infections, recovery, long known, obviously is a manifestation of acquired resistance. In the human visceral infections, the rarity of second cases, the appearance of post kala-azar dermal leishmanoid and the evidence for spontaneous cure are also considered examples of host resistance. Indeed, Kirk (1944), summarizing the work to that date, believes that known human visceral leishmaniasis may simulate any of the clinical aspects of both the dermal and the visceral infections. He suggests the following course as the basic infection: 1) *Primary stage,* with cutaneous sores at the site of inoculation having a tendency to spontaneous cure. 2) *Secondary stage,* a generalized infection like kala azar, and 3) *Tertiary stage,* the late cutaneous or mucocutaneous involvements.

Kirk points out that all these stages are not likely to be seen in any one individual infection and stresses that some strains of parasites show tendencies for predominant expression of one or the other of the stages listed. He claims the infection "may be terminated at any stage by the defense mechanism of the host gaining the upper hand, with the development of complete immunity and suppression of the parasites," or the parasite may gain the upper hand with the death of the host.

Finally, there is the serological evidence. The reviews of Taliaferro

(1929) and Culbertson (1941) contain numerous references to the presence of and *in vitro* action of antibodies associated with natural infection or produced as a result of the artificial immunization of animals. Some of these, like the formol gel test, are based on non-specific alterations of the plasma proteins; others, like the Witebsky, Klingenstein and Kuhn test, utilize a bacterium as the antigenic source. It appears generally agreed that not one of these antibodies has been proven to play any significant role in host resistance, innate or acquired. Even in *L. tropica* infections, where resistance to reinfection is best established, the nature of antibody action, if any, has not been disclosed. The nearest to the demonstration of true host sensitization in infection is implied by the Montenegro test for American mucocutaneous leishmaniasis.

Although some good work has been done with animal infections, no attempt will be made here to review this large literature. Many of the observations are casual ones; few are at all quantitative. Besides, Kirk's statements bear repetition. He injected dermal and visceral parasites by various routes into white mice and monkeys. "The results were so variable and included so many failures to produce any type of infection that no useful information regarding differentiation of strains has been obtained by this method. Perusal of the literature shows that other workers have had similar experiences. Differentiation of strains by animal inoculation is difficult unless large numbers of suitable animals are available" (see also Harrison and Fulton, 1946). On this basis it is difficult to evaluate even the few claims for recovery from leishmaniasis in susceptible hosts.

The above considerations are not meant to imply that there are no differences among the species or strains of *Leishmania* infecting mammals. However, taken together with the evidence that in most endemic areas leishmaniasis is a zoonosis, the implication is strong that they are a closely related group of parasitic organisms. As Cameron (1956) puts it "The Leishmanias, it would seem, are species which are still in the making."

For these reasons the present discussion will be limited to the study of the recent work on the Khartoum strain of *L. donovani* in experimental animals in our laboratory. While the limitations of such a procedure are obvious, the methodology to be described and the results so far obtained suggest that a beginning has been made toward furnishing an experimental basis for the elucidation of the problems of host resistance to the genus *Leishmania*. Even though only a single strain of one species has been studied, Kirk's requirement has been met in part since the statements made are based on information gleaned from the study of visceral leishmaniasis in more than 5,000 laboratory animals over the past few years.

Materials and Methods

The following animals have been used as hosts: laboratory white rat (Long-Evans and Wistar strains), white mouse (CF_1 strain), guinea pig, rabbit, chinchilla (*Chinchilla lanigera*), cotton rat (*Sigmodon h. hispidus*), golden hamster (*Mesocricetus auratus*) and Mongolian gerbil (*Meriones unguiculatus*).

In the work to be discussed here all inoculations of parasites were made intravascularly and in most cases the unsuitability of superficial veins led to the intracardial route as the routine one. The inoculum was prepared by grinding infected spleen (or occasionally liver) in sterile saline in a Ten Broeck tissue grinder. Dilutions were made in saline or occasionally in serum-saline.

Parasites were counted by two methods:

1) from Giemsa-stained organ impression smears, as parasites per organ-cell nucleus. Counts to 1,000 organ cell nuclei were made except where the ratio was greater than 3 parasites per organ cell nucleus. At such high levels of parasitization, counts were made only to 1,000 parasites.

2) from smears made from suspensions prepared by grinding in saline approximately 100 mg. of organ in the tissue grinder and spreading 0.005 ml. of this suspension on a slide over an area of approximately 1 cm. square. After staining with Giemsa, the parasites were counted in a sample of the material spread on the slide and an estimate obtained of the number of parasites per milligram of infected organ. On multiplying either the ratio obtained with method 1 or the density per mg. obtained with method 2 by the weight in milligrams of the infected organ, a figure is obtained which is indicative of the number of parasites in the whole organ. There is a high degree of correlation between the counts obtained by the two methods and the relationship between them suggests that each unit obtained from the organ impression smear (method 1) represents approximately 200,000 parasites.

We accept and confirm the statistical evaluation of such counting procedures as described by Goodwin (1944) and Fulton, Joyner and Chandler (1950). We have also considered the influence of such factors as organ hyperplasia on the counts obtained. The high correlation between the counts obtained by the two methods, one independent of organ cell complexity, has given assurance that no further corrections are necessary for the data as used in this study.

Since method 1 is the easier to perform, it is the one which has been usually followed in this work. Previous work (Stauber, 1955) has shown that in infections produced by intravascular inoculation better than 95% of the parasites injected are found in the liver within an hour. This pro-

portion remains true for the early part of the infection but because the rate of increase in the spleen remains higher than in the liver, the total number of parasites in the spleen finally approaches that in the liver if the animal lives long enough. No other organ examined in these infections regularly contains appreciable numbers of parasites, except the bone marrow. Furthermore, since we do not yet have a method of satisfactorily determining the total numbers of parasites in the bone marrow, the parasites in liver and spleen are considered adequate expressions of the total parasite burden of the host.

The threshold of patency by method 1 is one parasite per thousand organ-cell nuclei. Calculated as described, this amounts to approximately 200 parasites per milligram of organ being sampled. Consequently, not finding a parasite under the procedure outlined means merely that less than 200 parasites per mg. were present. Applied to whole organs of some of the animal species studied these thresholds are approximately 25,000 parasites for mouse spleen, 19,000 for hamster spleen, 200,000 for rabbit spleen, 450,000 for gerbil liver, 700,000 for hamster liver and 2,000,000 for chinchilla liver. We have not resorted to cultivation or subinoculation procedures to establish densities lower than this, though the culture method of Germuth, Eagle and Oyama (1950) appears nearly able to detect a single organism and we believe we can do this by intracardial subinoculation in the hamster. Because of the failure to obtain values below the thresholds indicated, certain assumptions are made for a few of the points in Tables II and III and Figs. 1 and 3, but these are obvious.

Results

The primary infection in the hamster. The principal aspects of this infection as we see it in our laboratory have already been published and the variability of the responses has been noted (Stauber, 1955; Stauber, Franchino and Grun, 1958). After intracardial inoculation of a few million parasites, the number of organisms increases initially, then less and less sharply, but continue to rise until the death of the host 35-45 days later. With such large inocula, as mentioned previously, the number of parasites in the spleen at death does not equal that in the liver. These differences in rates of accumulation of parasites in spleen and liver are not yet understood but are made evident in the data in Table I. In this experiment tenfold dilutions of inoculum to contain from 5,000,000 to 0.05 parasites per 0.1 ml. when injected, resulted in infections whose duration varied inversely with the number of parasites in the inoculum. It appears that as little as one parasite may produce a fatal infection in the hamster though it may take from 169 to more than 251 days to reach the fatal consequence. Such being the case, it appears that the hamster

shows neither innate nor acquired resistance to the parasite. It should be noted, however, that if the injection of parasites is made intraperitoneally evidence of what might be called one aspect of innate resistance can be obtained. It requires ten times as many parasites intraperitoneally as intracardially to produce a median time to death comparable to group I in Table I. It requires 2500 times as many parasites intraperitoneally to duplicate a median time to death comparable to group IV in the same

TABLE I

TERMINAL FINDINGS IN HAMSTERS AFTER INTRACARDIAL INJECTION OF DIFFERENT NUMBERS OF *L. donovani*

Group No.	Inoculum Size*	Median time to Death (Days)	Total parasites Spleen (in Millions)	Total parasites Liver (in Millions)	Ratio Parasites-Liver Parasites-Spleen
I	1×10^{0}	37	311	2,182	7.0
II	1×10^{-1}	47	246	1,101	4.5
III	1×10^{-2}	64	475	1,438	3.0
IV	1×10^{-3}	82	561	439	0.8
V	1×10^{-4}	116	301	643	2.1
VI	1×10^{-5}	123	671	861	1.3
VII	1×10^{-6}	161	102	176	1.7
VIII	1×10^{-7}	169†	288	82	0.3‡
IX	1×10^{-8}	251†	—	—	—§

* Expressed as dilutions of initial suspension. The undiluted ($=10^{0}$) contained 5,000,000 leishmania per inoculum of 0.1 ml.

† Two of eight animals in group VIII and 7 of 8 animals in group IX showed no parasites at 251 days after inoculation. This approximates the chances that, at such dilution, inoculum did not contain a parasite.

‡ Based on only one animal dead of the infection.

§ One animal infected—not dead at 251 days when experiment was ended.

table. These represent enormous losses of parasites upon intraperitoneal injection. Similar results are obtained by subcutaneous injection of parasites. Although we have made no extensive study of this, the results, viewed in the light of Table I, would seem to indicate that only about 500 of the 40 million parasites injected survived. This information may explain in part the greater variability of results obtained by various investigators using other than intravascular inoculation (Kirk, 1944; Harrison and Fulton, 1946).

The primary infection in the gerbil. The course of infection in the gerbil also depends on the size of the inoculum injected. This is shown in Fig. 1 where each point represents the median parasite count in a group

of seven animals. When large numbers of organisms (40,000,000 and 2,000,000 parasites) are injected, a 2½- to 3½-fold rise to a peak occurs in the liver followed by a decline in numbers to levels below those at two hours after inoculation. The decline begins earliest for the group receiving the largest inoculum. When smaller numbers of parasites are injected (200,000), although the data are less significant since in the early period

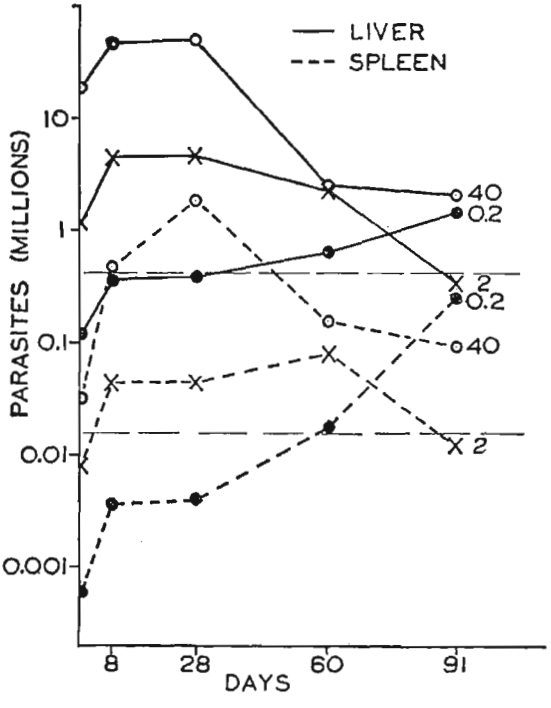

FIG. 1. Course of infection with *L. donovani* in the gerbil determined by parasite counts in liver and spleen after intracardial injection of 40 million, 2 million or 0.2 million parasites. (indicated by numbers at right). Each point on a curve is the median value for a group of seven animals except for the 91st day (only 3 animals). The two horizontal lines of dashes represent the thresholds of patency for liver (upper) and spleen (lower) respectively.

of the infection a number of the animals in each group never reach a condition of patency, the rise to the peak takes a much longer time. Similar number curves are seen in the spleens of the same animals. The spleen counts also show, as in the hamster, less than 5% of the number of parasites found in the liver of the gerbil at 2 hours and 8 days after inoculation.

Figure 2 shows the mean spleen weight/body weight ratios for the animals in this experiment and, together with Fig. 1, shows the correlation between the numbers of parasites injected, the course of the infection, and the amount of splenic hypertrophy induced.

These data indicate that although the gerbil is susceptible to infection and is a host suitable for multiplication of the parasite within it, true

acquired resistance occurs which leads to the virtual, but not necessarily complete, elimination of the parasites from the gerbil.

Comparative host resistance. As indicated above, the methods employed are adequate to disclose differences in the responses of different hosts to infection with *L. donovani.* Furthermore, the reproducibility of

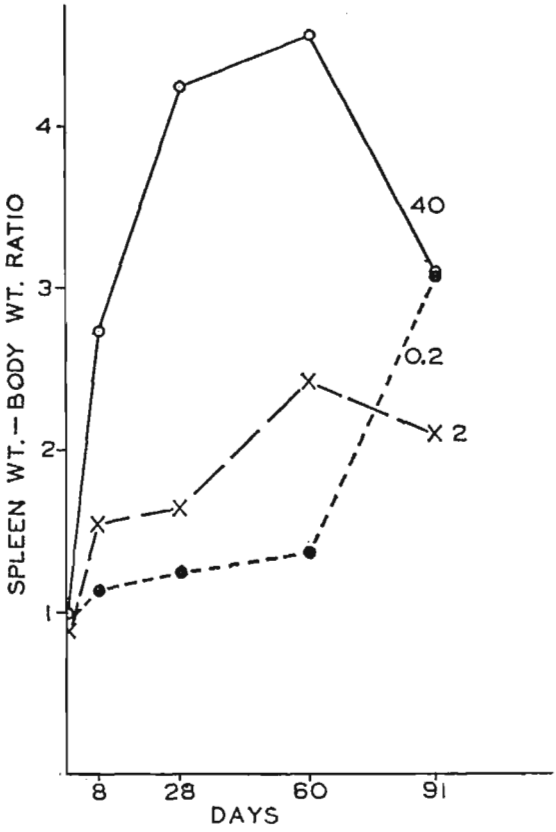

FIG. 2. Changes in group median spleen wt./body wt. ratio for the same gerbils seen in Fig. 1 at various times after inoculation with 40, 2 or 0.2 million parasites (indicated by numbers at right).

the results of an experiment is of a high order. For example, in an experiment similar to that described in Table I but performed three years previous, almost identical results were obtained.

To test for differences in susceptibility and resistance, groups of animals of the eight species mentioned previously were infected under roughly standard conditions of route and size of inoculum (part of this work has already been reported by Grun and Stauber, 1957). The inoculum size was approximately of the order of 20,000-500,000 parasites per gram of body weight. Groups of animals so infected were sacrificed at

intervals after inoculation; typical times were 1-2 hours, 1 day, 4, 8, 16, 28, 60, 120 and more days, depending on the characteristics of the infection. Usually 4-8 animals constituted a group. Not all these samplings were obtained in a single experiment but subsequent experiments filled in the gaps of information sought. On necropsy, body, spleen and liver weights and spleen and liver impression smears were obtained. Homogenate smears (method 2) were made only early in each series to furnish the necessary comparison material. This was true also for blood and bone marrow (femur) smears.

Because the final ranges of parasite numbers in the combined data extend over so many logs of numbers (1×10^0 to 1×10^{10}) and the important points on the time scale extend from a few days to more than 6 months, the data in Tables II and III have been expressed in the form of multiples of parasite increase for a few specific periods of time. These periods of time are somewhat arbitrarily chosen since, except perhaps for the first eight-day period for many of the species, no other points of time are correspondingly significant for more than one or two species. Besides, the points of time used for the liver do not always characterize entirely similar parts of the course of infection occurring in the spleen. Finally, all the brackets of time are not included for each species because of the death or recovery of the host. A log-log plot of the data (Fig. 3), although it, too, has limitations, is also helpful in comparing the results.

In spite of these limitations a number of interesting points concerning host resistance are evident from Tables II and III and Fig. 3. A great range from high susceptibility to almost complete resistance is shown with further variations within the categories. If innate resistance is defined as the unsuitability of the host as a culture medium for the parasite, its capacity to destroy the introduced parasite before the production and mobilization of antibodies and special cellular response, or both combined, then the changes in parasite numbers over the first 8 days may be an adequate relative expression of this. On this basis the animals shown in Tables II and III and Fig. 3 fall into 3 groups. (Since the time brackets in the tables are different, comparisons should only be made in the vertical columns.) The hamster and cotton rat are excellent hosts, there being usually about 50 times as many parasites at 8 days as there were at 1 hour after inoculation. The chinchilla, mouse, gerbil and guinea pig are moderately good hosts and the rabbit and rat are quite unsuitable. There is a possible error of interpretation here, applicable to any of the species, since some of the parasites observed at 1-2 hours may already be dead. This would falsely lower the fold increase for the 8-day period, though it would be an expression of a kind of host resistance. In the experiments

TABLE II

THE COURSE OF INFECTION WITH *Leishmania donovani* IN THE LIVERS OF A SERIES OF EXPERIMENTAL ANIMALS AFTER INTRAVASCULAR INOCULATION OF PARASITES

Animal Host	Changes in Parasite Densities with Time			
	1 Hour to 8 Days	8 Days to 62 Days	62 Days to End of Exp.	1 Hour to Peak Day
Cotton Rat	44	61	2.9 (196)	7,784 (196)
Hamster	50	100 (62)	>1.0	>5,000 (>62)
Chinchilla	3	50	1.9 (120)	285 (120)
Mouse	5	0.5	0.9 (90)	5 (8)
Gerbil	3	0.5	0.1 (143)	6 (28)
Guinea Pig	7	<0.04	—	13 (16)
Rabbit	0.8	0.07	—	1.2 (4)
Rat	0.4	<0.02	—	0

Numbers in parentheses are days to end of experiment or to peak of infection. All other numbers are expressed as multiples of the value at the beginning of the time interval. For the cotton rat, as expressions of rates of reproduction, the multiples in the 3 time intervals divided by the number of days in the interval, give values of 5.5, 1.2 and 0.02, which therefore represent the mean change in parasite density per day in the respective periods. The data enclosed in the block represent moderate susceptibility, those above it relatively high susceptibility, and those below it relatively low susceptibility. It will be seen that these degrees of susceptibility are not the same in all three time intervals.

TABLE III

THE COURSE OF INFECTION WITH *Leishmania donovani* IN THE SPLEENS OF A SERIES OF EXPERIMENTAL ANIMALS AFTER INTRAVASCULAR INOCULATION OF PARASITES

Animal Host	Changes in Parasite Densities with Time			
	1 Hour to 8 Days	8 Days to 62 Days	62 Days to End of Exp.	1 Hour to Peak Day
Cotton Rat	17	1,863	10.6 (196)	335,000 (196)
Hamster	100	1,000 (62)	>1.0	>10,000 (>62)
Chinchilla	3	300	20 (120)	17,500 (120)
Mouse	3	15	1.0 (90)	46 (36)
Gerbil	15	7	0.1 (143)	172 (44)
Guinea Pig	4	0.2 (38)	—	4 (8)
Rat	1.8	<0.5 (23)	—	4 (6)
Rabbit	1.5	<1.1 (22)	—	5 (4)

Numbers in parentheses are days to end of experiment or to peak of infection. All other numbers are expressed as multiples of the value at the beginning of the time interval. For meaning of blocks see legend for Table II.

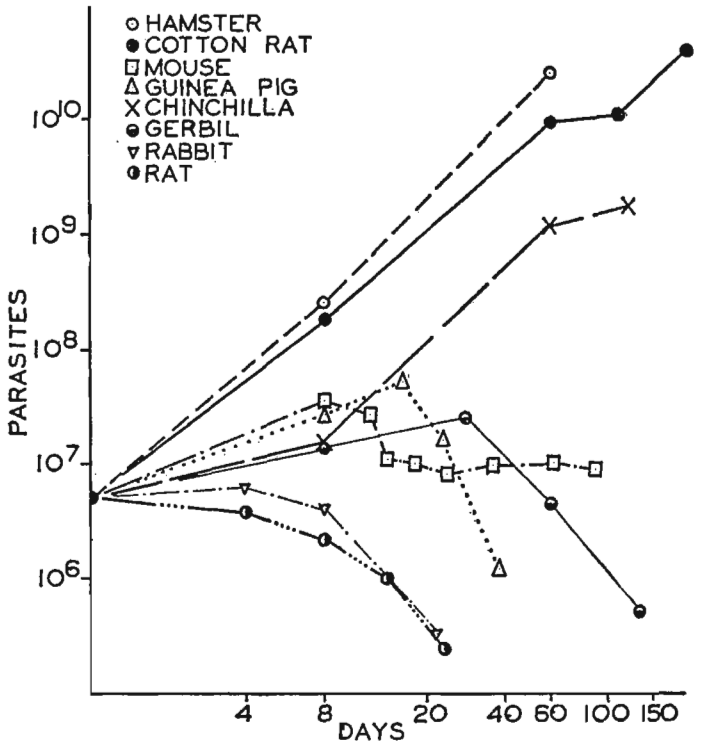

Fig. 3. Representative courses of infection in eight species of small mammals as determined from parasite counts in the liver on various days after inoculation with *L. donovani*. All data adjusted to approximately the same initial density of parasites in the liver at 1 to 2 hours after inoculation. The threshold of patency for these conditions lies close to the axis for 1×10^6 parasites.

cited, however, this factor does not seem to be important (1) because the per cent recovery of parasites at 1-2 hours seems to be similar (60-75%) for all species and (2) no significant decrease in parasite numbers occurs within the first 24 hours to suggest relatively quick digestion of parasites dead when phagocytized. In fact, in all susceptible species, there is evidence for an increase in parasite numbers within the 24-hour period.

In the second period of time (8 to 62 days), it is clear that the chinchilla now approaches the cotton rat and the hamster in susceptibility and is very unlike the mouse, gerbil and guinea pig which it first resembled. It should be noted that the higher numbers in this period of time for these 3 animal species, as seen in the tables, and the steeper slope in Fig. 3 for the chinchilla, do not mean increased rates of multiplication over those of the 1-hour to 8-day period. Actually these rates are less in the 8 to 62 day period, except possibly for the chinchilla, since the total

increases shown are for only 8 days in the first period, and for 54 days in the second period. The guinea pig in the 8 to 62 day period shows a number peak and a sharp decline. Its over-all response for this period is very much like that of the resistant rat and rabbit though in the case of the guinea pig this must be acquired resistance since appreciable multiplication occurs up to day 16. The mouse remains similar to the gerbil in this period, reaching a low peak and declining slightly.

In the final period (beyond 62 days), the number of parasites continues to increase in the cotton rat and chinchilla, though the rate of increase is greatly lessened. A hamster surviving into this period also continues to show an increasing parasite burden but when inoculated with the number of parasites used here (approximately 120,000 parasites per gram body weight) it rarely survives the midpoint of this time bracket. In order to obtain infections which will allow survival as long as this (like group III of Table I) much smaller numbers of parasites must be injected. Since the hamster dying of leishmaniasis has approximately the same number of parasites in its organs regardless of size of inoculum (Table I), the number for the fold increase in this period will thus depend somewhat upon the number of parasites injected. The figures in Tables II and III for the hamster are, then, neither exact determinations nor exactly comparable to the other data but are the best compromises that could be obtained.

In this final period the mouse shows more resemblance to the cotton rat and chinchilla than to the gerbil, which it resembled earlier. It continues to show significant numbers of parasites and to remain patently infected as long as examined (up to 90 days). However, since the level of parasitization does not increase, an acquired resistance is manifest, although we are yet unable to say whether only destructive forces are producing this effect or whether inhibition of reproduction of the parasite occurs as well.

The gerbil's response in this final period is that of an animal which has acquired a significant level of resistance (see also Fig. 1). The parasite burden is reduced almost to zero and thus it comes finally to resemble the guinea pig. Concerning the latter, as well as the rabbit and the rat, no further statements are possible since the parasite level is reduced below the threshhold of patency long before the beginning of this period.

The final column is the total fold increase to peak day. For hamster, chinchilla and cotton rat no cessation of parasite reproduction occurs, so there appears to be little evidence of any effective host resistance at all. The rate of accumulation of the parasites does, however, decrease at each successive interval after 8 days; whether this is a partial manifes-

tation of acquired immunity or an athrepsis cannot be decided as yet. It may be that the host fails to supply the requirements for the parasites at a rate equal to the parasite's potential for reproduction. The subtle difference between the findings in spleen and liver suggest that the problem is more complex than implied by such an hypothesis.

Since the hamster and chinchilla are susceptible to the "toxic" or debilitating influences of *L. donovani*, they eventually die. The cotton rat, tolerant or indifferent to these influences, remains alive, without symptoms, presumably as long as it lives (Fulton and Niven, 1951), a response very much like that described years ago for the Chinese hamster (Meleney, 1925).

A few added comments are necessary regarding some of the differences between the results recorded for spleen and liver in Tables II and III. In the first time period the high multiple of 15 for the gerbil spleen seems appreciably different from the figures for others in the moderately resistant group, but no explanation is available.

In the period from 8 to 62 days in the spleen of the susceptible cotton rat, hamster and chinchilla, the rates of increase of parasites were about 10 times as great as in the liver during the same period. They were still not as great, however, as the rates of increase during the period from 1 hour to 8 days in the spleen or in the liver.

The very large numbers shown in the final column of Table III (1 hour to peak day) for cotton rat, hamster and chinchilla bear out the statements made previously concerning the initial and final totals of parasites observed in the spleens of these animals. This would indicate that in some unknown manner the spleen is a more hospitable organ for the multiplication and accumulation of parasites even though (or because?) it initially receives many times fewer organisms.

Superinfection. In the chinchilla repeated superinfection (as many as 6 times) during the course of an infection, with numbers of parasites small in comparison with those already accumulating in its organs, has been tried. No alteration of the course of the initial infection could be detected.

In the hamster the experiments were run differently. A small initial infecting dose of parasites was given so that, at a later date, superinfection could be obtained with such a significantly higher number of parasites that the two infections could be distinguished. For the initial infection the number of parasites given was below the threshold of patency until 8 or 16 days later, at the time of superinfection. This gave the host an appreciable time of contact with the parasite, presumably time in which to respond with any available mechanisms for resistance.

A typical experiment is shown in Fig. 4. The initial infection was 300,000 parasites and the superinfection 47,000,000 parasites. Some control groups of animals were given only the superinfecting dose, other controls were not superinfected at all, and a final control group, pre-

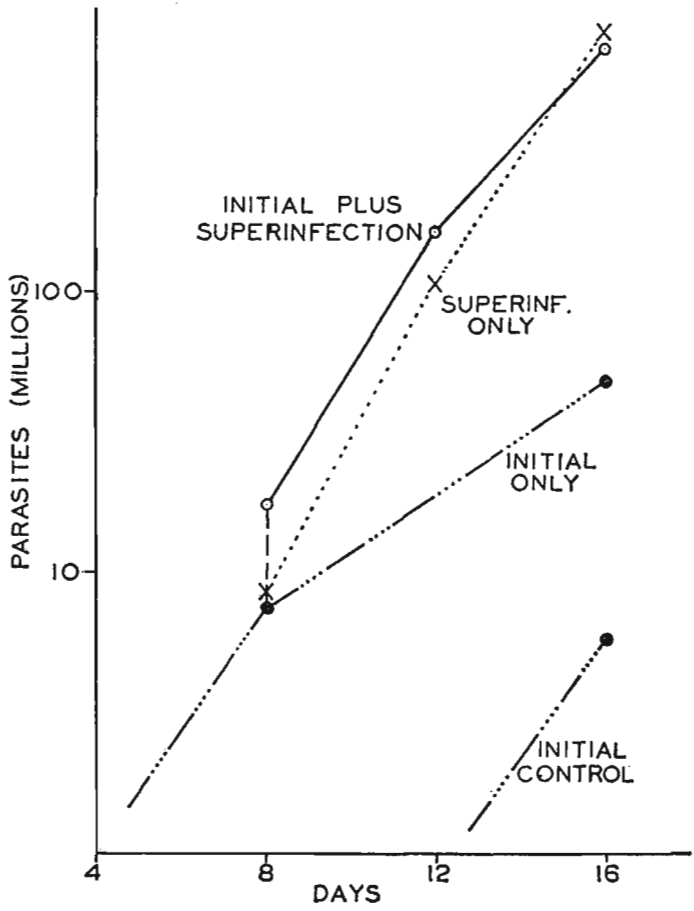

FIG. 4. Superinfection of hamsters with *L. donovani*. Superinfection performed on 8th day after beginning of the experiment.

viously uninfected, received at the time of the superinfection the same number of parasites as the animals initially infected (namely 300,000). It is obvious from Fig. 4 that the rate of parasite increase in the superinfected animals was about as great as in those animals receiving such a large number of parasites for the first time. On the other hand, the rate of increase of parasites in the non-superinfected control group showed

the decrease in rate expected for the period beyond 8 days, as already discussed in connection with Table II.

If the normal decrease in rate of accumulation of parasites as the infection progresses is a measure of a partially effective acquired resistance, these results might be explained by hypothesizing that the superinfection desensitizes the hamster back to its original susceptibility. At any rate, as in the chinchilla, there is no evidence that the ultimate outcome of the infection (namely death) is altered by the superinfection or by this supposed partial resistance.

In a series of experiments where successful treatment (with stibogluconate) of a heavy infection was followed by reinfection, ultimate death from leishmaniasis occurred in about the time expected on the basis of the size of the reinfecting dose. Also no kind of non-specific (ground celluflour, tuberculin, foreign red cells, foreign serum, india ink, ground uninfected liver and spleen suspensions, Newcastle virus vaccine) or specific (suspensions of killed leishmania in ground infected spleen or liver, killed leptomonads) stimulation of the hamster has afforded any significant measure of protection against the subsequent introduction of live parasites. The only conclusion from these experiments is that the hamster acquires little resistance to *L. donovani.*

It seems logical that an animal with such high susceptibility as the hamster or such high resistance as the rat would be unsuited to demonstrate appreciable heightened resistance to re-exposure. On the other hand, the gerbil would appear well suited for the demonstration. It is susceptible enough to allow parasite multiplication for a number of days (Figs. 1 and 3; Tables II and III) yet it ultimately eliminates nearly all the parasites which have so accumulated. Up to now only a single trial has been completed but the results of that superinfection experiment are found in Figs. 5 and 6. As controls, the pooled data obtained in other experiments with primary infections in the gerbil are presented. There are obvious objections to this procedure but it is the best we have to offer at this time. The inocula are all prorated to the same infecting dose to aid visual comparison.

In the experiment as conducted, 94 gerbils were infected with 10 million parasites and the course of this infection was followed by sampling at 2 hours, 8, 29, 60, and 120 days after inoculation. On the 60th day, when the sample counted already showed marked reduction of the parasites from the peak which occurred on or about the 28th day, 70 animals were superinfected with 30 million parasites. Sample counts were made from groups of seven each of the superinfected animals at 2 hours, 4, 8, 16, 24, 31 and 60 days after this second injection. It appears that a previous infection does increase the gerbil's capacity to destroy more rapidly para-

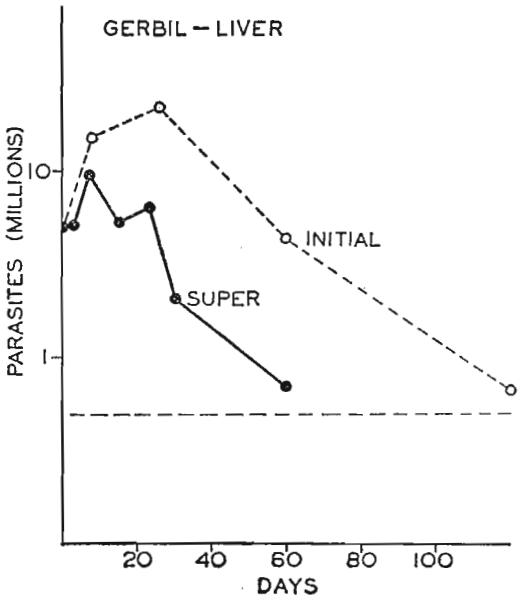

FIG. 5. Initial and superinfection of the gerbil with *L. donovani* as determined from parasite counts of liver impression smears. Curve for the initial infection from pooled data. Curve for the superinfection from a single experiment only (8 animals per group). Initial infection: 10 million parasites; superinfection: 30 million parasites. Curves adjusted to same density of parasites at 1 hour after inoculation. Horizontal line of dashes is the threshold of patency for the gerbil liver.

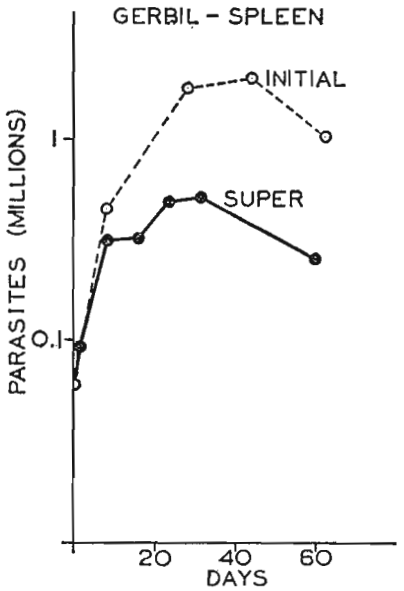

FIG. 6. Same as Fig. 5, initial and superinfection of the gerbil as determined by parasite counts of spleen impression smears. Base line of figure is the threshold of patency for the gerbil spleen.

sites which are introduced at a later time. Since parasite multiplication occurs at almost the rate seen in the primary infection, at least for a few days, it is obvious also that the degree of resistance afforded by a single previous infection is not very high. Repeated superinfections have not yet been tried. It is not yet known whether the apparent acquired resistance is an infection-type immunity based on the continued presence of the parasite, or whether true residual immunity occurs.

Conclusions and Summary

A whole range of resistance to *L. donovani* has been found within the series of animal species studied. It extends from the complete susceptibility of the hamster which eventually succumbs to the intracardial introduction of a single parasite, to the innately resistant rat and rabbit which soon dispose of even as many as several million parasites.

The chinchilla and cotton rat are highly susceptible also. The chinchilla dies of the infection, but the cotton rat is able to bear an enormous burden of parasites with no apparent ill effects.

In terms of acquired resistance, the mouse, gerbil and guinea pig are the most interesting species because each is susceptible enough to permit some increase in parasites for a number of days after inoculation, but each later checks this increase. The mouse apparently does not successfully eliminate the leishmania from its body, the parasite density remaining above the threshold of patency for many days.

The gerbil and the guinea pig do effect virtual removal of the parasites; the guinea pig accomplishes this result in about one-fourth the time that the gerbil requires.

Superinfection of the hamster and chinchilla show no detectable increase in resistance resulting from the prior infection. The gerbil, however, shows a measurably heightened resistance to a second infection.

The mechanisms responsible for the tolerance of the cotton rat to the damaging effects of large numbers of parasites, for the innate resistance of the rabbit and rat, or for the acquired resistance of the gerbil and guinea pig are still unknown.

Acknowledgments

This investigation was supported by a research grant (E-92) from the National Institute of Allergy and Infections Diseases, Public Health Service.

The author is greatly indebted to a number of his students for their assistance in this work. Special credit is due to John Grun, since a portion of this material will appear in thesis form under his name (see also

reference: Grun and Stauber, 1957). Others who merit mention are Paul Actor, Elizabeth M. Franchino and Richard N. Rossan. Thanks are due the West Foundation, Brant Lake, N.Y., for generosity in supplying most of the gerbils used in the work reported here.

REFERENCES

Cameron, T. W. M. 1956. *Parasites and parasitism.* Methuen & Co., Ltd., London.

Culbertson, J. T. 1941. *Immunity against animal parasites.* Columbia Univ. Press, New York.

Fulton, J. D., Joyner, L. P., and Chandler, R. L. 1950. Studies on protozoa. II. The golden hamster *(Cricetus auratus)* and cotton rat *(Sigmodon hispidus)* as experimental hosts for *Leishmania donovani. Trans. Roy. Soc. Trop. Med. & Hyg.* 44, 105-112.

Fulton, J. D., and Niven, J. S. F. 1951. Studies on protozoa. III. Visceral leishmaniasis in the cotton rat *(Sigmodon hispidus). Trans. Roy. Soc. Trop. Med. & Hyg.* 44, 717-728.

Germuth, F. G., Jr., Eagle, H., and Oyama, V. 1950. An evaluation of the criteria of cure in experimental leishmaniasis of the golden hamster. *Amer. J. Trop. Med.* 30, 377-385.

Goodwin, L. G. 1944. The chemotherapy of experimental leishmaniasis. I. The spleen as an index of infection in the Syrian hamster. *Trans. Roy. Soc. Trop. Med. & Hyg.* 38, 151-160.

Grun, J., and Stauber, L. A. 1957. A comparative study of experimental leishmaniasis in the mouse, Mongolian gerbil, hamster, white rat, cotton rat and chinchilla. *J. Parasitol.* 43 (Suppl.), 16.

Harrison, C. V., and Fulton, J. D. 1946. The effect of treatment on the spleen of the golden hamster *(Cricetus auratus)* infected with *Leishmania donovani. Brit. J. Exp. Pathol.* 27, 1-8.

Kirk, R. 1944. Studies in leishmaniasis of the Anglo-Egyptian Sudan. VI. The evolution of the Leishmania infections in man. *Trans. Roy. Soc. Trop. Med. & Hyg.* 38, 61-70.

Laveran, A. 1920. La leishmaniase canine chez la souris. *Bull. Soc. Pathol. Exot.* 13, 680-687.

Meleney, H. E. 1925. The histopathology of kala azar in the hamster, monkey and man. *Am. J. Pathol.* 1, 147-168.

Stauber, L. A. 1955. Leishmaniasis in the hamster. *In* Cole, W. H. *Some Physiological Aspects and Consequences of Parasitism.* Rutgers Univ. Press, New Brunswick, N.J.

Stauber, L. A., Franchino, E. M., and Grun, J. 1958. An eight day method for screening compounds against *Leishmania donovani* in the golden hamster. *J. Protozool.* 5, (in press).

Taliaferro, W. H. 1929. *The immunology of parasitic infections.* The Century Co., New York.