

RELAPSE PHENOMENA IN RATS INFECTED WITH SINGLE
SPIROCHETES (*BORRELIA RECURRENTIS* VAR.
TURICATAE)

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Antigenic variation by the spirochete is generally believed to be responsible for the relapse phenomena in spirochetel relapsing fever. Schuhardt (1942) has reviewed the literature prior to 1942 on this subject, and little if any evidence has been presented subsequently to alter or extend this concept. Among the unanswered questions in spirochetel relapse phenomena are: (a) the antigenic variation capacity of a single spirochete, and (b) the capacity of an antigenic variety to recur in a series of relapses in a given animal. Although Cunningham, Theodore, and Fraser (1934) believe that antigenic varieties do not recur, other workers are not convinced that this possibility has been ruled out. Consequently we undertook a study of single spirochete infections in white rats in an effort to answer these two and possibly other questions related to the relapse phenomenon in spirochetel relapsing fever.

METHODS

A modification of the method developed by Hansen (1926), for the isolation of single fungus spores, was used for isolating single spirochetes for inoculation into rats. Microcapillaries approximately 1 cm long and 10 μ in diameter were filled with serum containing motile spirochetes. Two or three of these filled capillaries were mounted on a micro slide in saline and covered with a micro cover glass. Each capillary was examined microscopically from end to end using dark-field illumination and the 4 mm objective. A capillary containing a single spirochete was rechecked and then placed in the lumen of an 18 gauge needle, which previously had been partially plugged with 2 per cent agar, and mounted on a syringe containing approximately 1 ml of sterile saline. The contents of the syringe and needle, including the microcapillary, then were injected intraperitoneally into a white rat.

The rats were checked daily for evidence of infection by microscopic examination of uniform dark-field preparations of tail blood. The preparations consisted of 0.01 ml of a 1:20 dilution of the blood covered with a 1 inch square cover glass. At least 100 fields were examined before considering a preparation microscopically negative. Rats which became infected were bled from the heart on the second day of negativity following each attack or relapse and at intervals up to 30 days after inoculation. The blood thus obtained was allowed to clot overnight in the refrigerator, and the serum was removed and stored at 4 to 8 C for use in serological tests.

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The spirocheticidal activity of the serum was determined by the capillary tube technique described by Schuhardt (1940) using mixtures of approximately 0.01 ml of the test serum dilution and 0.01 ml of a serum suspension of first attack spirochetes obtained from freshly defibrinated heart blood. When relapse spirochetes were desired for test purposes, these were passed in 0.01 ml of tail blood to fresh rats and were used as the first attack spirochetes in the serum of the passage rat. Test and control (using pooled normal rat serum) preparations were incubated for 2 hours at 37 C, after which they were examined by comparing the number of motile spirochetes in 20 fields of both the test and control preparations.

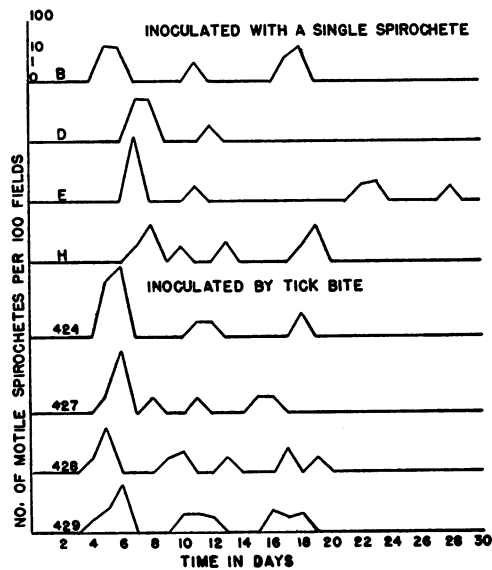


Figure 1. Microscopic relapses in rats infected with *Borrelia recurrentis* var. *turicatae*.

EXPERIMENTAL RESULTS

Four of 11 rats inoculated with single spirochetes developed infections which were followed by means of the dark-field microscope. The microscopic sequences of these infections, as indicated by the daily number of spirochetes found per 100 dark-fields examined, are compared graphically in figure 1 with the infection sequences of 4 rats infected by the bite of ticks. The incubation periods in the rats inoculated with single spirochetes ranged from 5 to 7 days, whereas the tick infected rats presented 4 to 5 day incubation periods. Otherwise the relapsing tendency in the two groups of rats was essentially similar.

Sera, which were collected from each of the 4 single cell infected rats 30 days after the inoculation of the spirochete, were tested against all available first attack, relapse passage and late brain passage spirochetes from both tick-bite and single cell infected rats. These spirochetes were demonstrated to be antigenically diverse by testing them against antisera collected at various intervals

between first attacks and relapses of the spirochetes in different animals. In every instance the antisera, collected 30 days after infection from the rats infected by single cell inoculation, showed complete immobilization of all test spirochetes.

These results convince us that each spirochete possesses the inherent capacity for antigenic variation characteristic of the strain of relapsing fever spirochetes involved in this study. Furthermore it is apparent that each single spirochete infection, regardless of the number of microscopic relapses, stimulates antibody production to all of the antigenic varieties demonstrable for the strain. Since it is an established fact that the blood of rats infected with these spirochetes frequently remains infective for a week or more after the last microscopically observable relapse, the antigenic variation apparently continues beyond the observed relapses and therefore could help to account for the broad antibody coverage of the 30 day antisera.

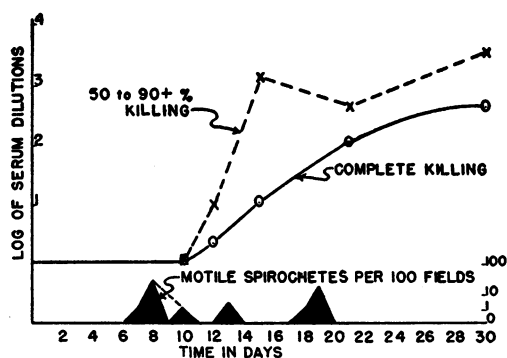


Figure 2. Correlation of the spirocheticidal antibody titer with the microscopic relapses of rat H after infection with a single spirochete.

Frequently a rat, after receiving late infection blood, will develop mixed infections of spirochetes, some susceptible and others resistant to the antiserum collected from the original rat at the time of passage of the infection. We believe that the presence of the susceptible spirochetes in the blood of the passage rat is evidence of a reverse antigenic variation tendency by these organisms. In the original rat the survival of the reverse antigenic variants is suppressed by the presence of serum antibodies, whereas in the passage rat these antibodies are absent. This reverse variation tendency might account for the relatively smaller numbers of spirochetes observed in most relapses when compared with the first attack of these spirochetes.

Figure 2 presents graphically the type of results obtained when sera from rats infected with single spirochetes were tested for spirocheticidal titers against first attack spirochetes. In this instance heart blood was collected from rat H on the 10th, 12th, 15th, 21st, and 30th days after inoculation of the single spirochete. Dilutions of these sera were tested for spirocheticidal activity against first attack spirochetes from a tick-bite infected rat. It is apparent that no antibodies

against these spirochetes were demonstrable in the serum collected on the 10th day. However, a single spirochete was found in the tail blood of the rat on this day. Therefore we are inclined to believe that the first attack was still in progress, and that the broken line (figure 2) between the 8th and 10th days represents this progress better than the two black areas. By the 12th day all spirochetes in the test preparation were killed by the 1:2 dilution of the serum, and 50 to 90 per cent were killed at a serum dilution of 1:10. The complete killing antibody curve shows a continuously rising titer to 1:400 at the end of 30 days after inoculation of the single spirochete. This convinces us that none of the test spirochetes could have recurred as the cause of a relapse in rat H after the 12th day of the infection. In other rats, in which relapses have been prevented by chemotherapy, we have failed to obtain complete killing titers above 1:10 to 1:50. Consequently we are inclined to believe that the high titer of complete killing (1:400) obtained when the infection was allowed to run its course is evidence of continued antigenic stimulation against first attack varieties after the termination of the first attack. We attribute this continued antigenic stimulation to the reverse antigenic mutants which, although destroyed by the antibody already present, exert additional antigenic stimuli.

The 50 to 90 per cent killing curve of the spirocheticidal activity of the serum of rat H is significant in at least two respects. First the 160 fold difference between the complete killing titer (1:10) and the 50 to 90 per cent killing titer (1:1,600) in serum collected on the 15th day of infection indicates that the antibodies against the different spirochetes were diluted out at different serum concentrations. We interpret this to mean that the test suspension contained more than one antigenic variety of the spirochetes, and the different antibody titers to these varieties would imply that either some spirochetes are better antigens than others or that they occurred in larger numbers than the others in the first attack and relapse of rat H. Probably the latter concept is adequate.

The dip from 1:1,600 to 1:400 in the 50 to 90 per cent killing titer between the 15th and 21st days of the infection and the subsequent rise in this titer to 1:3,200 by the 30th day are interesting because of the fact that this dip correlates with the relatively heavy relapse observed microscopically in this animal on the 18th and 19th days of the infection. We believe that this dip in antibody titer is an instance of a tendency toward a temporary negative phase caused by the binding of circulating antibody by the antigens liberated from the spirochetes involved in the last relapse. We can only speculate relative to the source of the antigens necessary for this binding of antibody, since it is obvious that none of the test spirochetes could have been the cause of the last relapse (which took place in the presence of a complete killing titer of approximately 1:100). Possibly this is another manifestation of the tendency of these organisms to mutate to previously experienced antigenic varieties, and that these mutants were predominantly those which accounted for the 160 fold difference between the two curves on the 15th day of the infection.

SUMMARY

Four white rats infected with single spirochetes gave relapsing infections essentially similar to rats infected by the bite of single ticks.

Antisera, collected from each of the four single cell infected rats 30 days after inoculation, immobilized all antigenic varieties of the spirochetes tested. Therefore it appears that each relapsing fever spirochete possesses the inherent antigenic variation capacity of the strain of spirochete used in this study.

Single cell infected rats tend to show a constantly rising spirocheticidal titer against first attack spirochetes which would prohibit these antigenic varieties from recurring as the cause of relapses.

There is considerable evidence that these spirochetes in addition to the progressive antigenic variation capacity which accounts for the relapsing tendency of this infection, show a decided reverse antigenic variation tendency.

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