5113), and recorded on 35-mm film.

Membrane currents recorded from human atrial cell segments are shown in Fig. 1. With the holding potential (V_H) set at −120 mV, 10-msec-long voltage pulses triggered inward I_{Na} at V_m positive to −70 mV; the current reversed direction at +60 mV (Fig. 1, A and B). In additional experiments, changes in external or internal sodium concentration ($[Na]_o$ or $[Na]_i$) altered I_{Na} and its reversal potential in a manner similar to that observed in studies on nerve axons (*8*).

The steady-state inactivation variable (h_∞) was estimated by measuring the effect of 100-msec-long prepulses (−140 to −60 mV) on test I_{Na} at −40 mV (Fig. 1, C and D). Steady-state inactivation was negligible at potentials negative to −100 mV, reached one-half at about −75 mV, and was complete at about −60 mV.

The final series of experiments showed that I_{Na} in human cardiocytes is sensitive to the external application of sodium channel blocking agents. After 5 μ*M* tetrodotoxin (TTX; Sigma) for 5 minutes, peak I_{Na} was reduced by about 75 percent (Fig. 2A). Similar exposure to 250 μ*M* lidocaine (Astra) blocked about 50 percent of the current (Fig. 2B) (*9*).

It is apparent that the maximum peak I_{Na} in human cardiocytes was much smaller than that recorded in other cardiac preparations (*2, 3, 10–13*). The reason lies in the small area of the test membrane, since our estimates of current densities, 1 to 5 mA/cm^2, agree with those for rat heart cells (*12*). The threshold potential (−60 to −70 mV), V_m of maximum peak I_{Na} (~ −30 mV), and reversal potential (~ +60 mV at $[Na]_o/[Na]_i = 15$) also coincide, but V_m for $h_\infty = 0.5$ (mean ± standard deviation, −77 ± 6 mV; $N = 5$) is about 10 mV more negative than estimates for rat cardiocytes at the same temperature (*12*). The h_∞ discrepancy with the rat cells may be related to the rather short (40 msec) prepulses used in that study, since our h_∞-V_m curves almost coincide with those determined in rabbit Purkinje fibers with prepulses of 200 to 500 msec (*10*). Finally, the degree of I_{Na} block with TTX or lidocaine treatment is in line with that found by others using similar pulsing protocols (*12, 13*).

We conclude that the internally perfused, heart cell segment is a suitable experimental model for the study of membrane ionic channels in the heart.

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Natural Distribution of the *Ixodes dammini* Spirochete

Abstract. *Spirochetes believed to be the cause of Lyme disease were isolated from white-footed mice and white-tailed deer, the preferred natural hosts of* Ixodes dammini, *the tick vector. Evidence suggests that deer act as a reservoir of the disease and provide an overwintering mechanism for both spirochetes and adult ticks. Some tick larvae may acquire the spirochete by transovarial passage and the nymphal stage may transmit the disease to humans.*

Lyme disease, an epidemic inflammatory disorder with a pathognomonic skin lesion that is often followed by joint, neurological, and cardiac manifestations, was first recognized in 1975 in Lyme, Connecticut (*1*). Epidemiologic evidence for the Northeast suggests that nymphal *Ixodes dammini* ticks transmit the causative agent of Lyme disease to humans (*2*). In 1981 spirochetes thought to be the agent were isolated from adult *I. dammini* ticks, which infest a wide variety of avian and mammalian hosts (*3, 4*). Immature ticks or subadults feed on rodents, primarily white-footed mice (*Peromyscus leucopus*), and also on white-tailed deer (*Odocoileus virginianus*) (*5*). Adults feed on white-tailed deer in the spring and fall (*4*). All stages of *I. dammini* have been reported to feed on humans (*6*).

We isolated and identified spirochetes believed to be the agent of Lyme disease from the blood of feral *P. leucopus* and *O. virginianus* and from subadult *I. dammini* ticks removed from these mammals. Beginning in mid-June 1982, white-footed mice were collected weekly from the same endemic focus on Shelter Island, New York, that yielded the spirochete-infected adult *I. dammini* in 1981 (*3*). Of 77 *P. leucopus* trapped during an 8-week period, five had spirochetemia (Table 1). Dark-field examination of blood obtained aseptically by cardiac puncture failed to disclose spirochetes; it was only by culturing the whole blood samples in BSK medium (*7*) that spirochetes could be detected. The spirochetes were detected 10 to 31 days (mean, 19 days) after the blood (0.1 ml) was added to the medium (7 ml) (Table 2). It is not known whether the longer time required for growth in certain cultures was due to the presence of only small numbers of spirochetes at sampling time or to the presence of inhibitory substances in the rodents' blood. The rodent spirochetes have been routinely subcultured (*8*), and their identity as the previously reported *I. dammini* spirochete (*3*) was confirmed by direct immunofluorescence (*9*) and monoclonal antibody tests (*10*). The low frequency of blood spirochetes in the mice could indicate that *P. leucopus* is an inefficient reservoir or that the spirochetemia is transient and that timing factors are crucial for recovering the organisms from blood.

Of 306 *I. dammini* subadults, 113 (101 larvae and 12 nymphs) were infected with spirochetes (Table 1). The infected ticks were removed from 33 (43 percent) of the tick-infested *P. leucopus*. Since 23 of the infected larvae were unengorged, that is, without blood in their midgut, the larvae may have acquired the spirochete through transovarial passage. The occurrence of transovarial transmission of the

Lyme disease spirochete was recently established for naturally infected *Ixodes pacificus* and *Ixodes ricinus* (*11*). Our findings suggest that this phenomenon may occur in *I. dammini*.

Twenty-seven *I. dammini* (22 larvae and 5 nymphs) were removed from the five spirochetemic *P. leucopus*. Two engorged larvae and one unengorged larva and two engorged nymphs from four mice were positive for spirochetes under dark-field examination (Table 2). The white-footed mice were also infested with 490 larval and nymphal *Dermacentor variabilis* (Table 1). None, including 15 *D. variabilis* from spirochetemic mice, contained spirochetes (Table 2). Detection of infected engorged larvae and infected unengorged nymphs confirms the hypothesis (*2*) that nymphal ticks can serve as vectors of the spirochete and therefore can transmit the disease to humans.

Spirochetes were isolated 2 days after aortic blood from a 2-month-old female *O. virginianus* (*12*) was added to BSK medium (*7*). The isolate was identified by its reactivity with fluorescein isothiocyanate–conjugated rabbit antiserum to the *I. dammini* spirochete. (The culture then became contaminated, precluding further tests.) Seventeen unengorged larvae, five engorged larvae, one unengorged nymph, and two engorged nymphs—all *I. dammini*—were recovered from this deer. Of these, one engorged larva and one engorged nymph contained spirochetes, as determined by dark-field examination. In addition, methanol-fixed smears of aortic blood drawn from one male fawn in November 1981 (*13*) and from 8 of 11 deer harvested on Shelter Island in mid-January 1982 were shown by direct fluorescence to contain the spirochete. These deer had numerous *I. dammini* males and fewer engorged females. The females were all copulating with males.

Since deer host all stages of *I. dammini*, we suggest that they are a reservoir of the spirochete (*14*). The known distribution of *I. dammini* spirochetes indicates an arthropod-borne natural cycle of low mammalian host specificity. Further investigations could show the causative agent of Lyme disease to be widely distributed (*15*).

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Table 1. Prevalence of spirochetes in *P. leucopus* and in attached ticks. The mice were live-trapped over an 8-week period from 28 June to 20 August. All ticks were allowed to drop off after their hosts were exsanguinated by cardiac puncture under CO_2 anesthesia. Ticks from each mouse were separated according to species, developmental stage, and degree of blood engorgement. Each tick was immersed in aqueous Merthiolate (1:1000), rinsed in distilled water, and immediately triturated on a slide with one drop of sterile phosphate-buffered saline (*p*H 7.4) supplemented with $MgCl_2$. When spirochetes were sighted (by dark-field microscopy), the contents of the slide were smeared, air-dried, fixed in methanol, stained with rabbit antiserum to *I. dammini* spirochetes (*9*), and examined under an ultraviolet microscope. Subadult *D. variabilis* were processed in the same manner as *I. dammini*. Values in parentheses are percentages.

Week of collection	*P. leucopus* collected	*I. dammini* subadults infected	*D. variabilis* subadults*	*P. leucopus* with spirochetemia
1	11	2 of 6 (33)	86	1 (9)
2	11	2 of 4 (50)	76	
3	8	4 of 9 (44)	44	1 (13)
4	12	11 of 27 (41)	56	2 (17)
5	9	13 of 32 (41)	34	
6	6	6 of 30 (20)	17	
7	12	41 of 92 (45)	76	
8	8	34 of 106 (32)	101	1 (13)
Total	77	113 of 306 (37)	490	5 (6.5)

*None were infected.

Table 2. Tick burdens of the five *P. leucopus* with spirochetemia and status of the ticks with respect to blood engorgement and developmental stage. Abbreviations: N, nymphs; L, larvae.

Date mouse collected	Age and sex	*I. dammini*	*D. variabilis*	*I. dammini* with spirochetes	Days in culture*
29 June	Adult female	1 engorged L	3 unengorged L, 2 engorged N	None	31
14 July	Young adult female	1 engorged N	1 engorged L, 1 engorged N	1 engorged N	14
21 July	Adult male	10 unengorged L, 4 engorged N	1 unengorged L	1 engorged N	10
21 July	Adult male	1 engorged L	1 engorged L, 1 unengorged L, 1 engorged N, 2 unengorged N	1 engorged L	10
18 August	Young adult male	7 engorged L, 3 unengorged L	2 unengorged L	1 engorged L, 1 unengorged L	31

*Days between inoculation of medium and observation of spirochetes.

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8. Active culture (0.2 ml) was transferred into 7.8 ml of fresh BSK medium every 5 to 7 days. All cultures were maintained at 35° to 35.5°C.
9. The immunofluorescence test was conducted with fluorescein isothiocyanate–conjugated rabbit antiserum to the *I. dammini* spirochete (obtained from W. Burgdorfer).
10. The monoclonal antibody used reacted in an indirect immunofluorescence assay with the *I. dammini* spirochete but not with *Leptospira interrogans, Borrelia hermsii, Borrelia recurrentis, Treponema pallidum*, or *Treponema phagedenis* (A. G. Barbour, S. L. Tessier, W. J. Todd, in preparation). The method for the assay was described by A. G. Barbour, S. L. Tessier, and H. G. Stoenner (*J. Exp. Med.*, in press).
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12. The fawn was killed by a car on 3 August 1982 and had been dead for 4 to 6 hours before its blood was collected. One milliliter of blood was immediately added to 7 ml of BSK medium.
13. This fawn was killed by a car on 5 November 1981 and had been dead no longer than 30 minutes before smears of aortic blood were made.
14. Heat-inactivated (1 hour at 56°C) deer serum added to BSK medium at a final concentration of 6.6 percent produced noticeably greater spirochete growth than BSK medium with an equal volume of heat-inactivated rabbit serum.
15. Since this report was submitted the *I. dammini* spirochete was isolated from cultures of the blood of one meadow vole, *Microtus pennsylvanicus*.
16. We thank the Nature Conservancy for permission to conduct this research at the Mashomack Preserve on Shelter Island, N.Y. Special personal thanks go to J. M. Laspia for his support and consideration and to H. Knoch and M. Scheibel for assistance with harvested deer.

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