Amplitude Spectrum Representation in the Doppler-Shifted-CF Processing Area of the Auditory Cortex of the Mustache Bat

Abstract. *The mustache bat,* Pteronotus parnellii rubiginosus, *emits orientation sounds containing a long constant-frequency (CF) component that is ideal for echo detection and Doppler shift measurement. About 30 percent of the primary auditory cortex of this bat is chiefly devoted to processing the second harmonic of the CF component in Doppler-shifted echoes. In this Doppler-shifted-CF processing area, single neurons recorded in any electrode penetration perpendicular to the cortical surface have nearly identical best frequencies and best amplitudes (or best pressure levels) at which the neurons show maximum excitation. The best frequency and best amplitude vary systematically with the location of the neurons in the cerebral cortex, so that there are tonotopic and "amplitopic" representation axes, which are radial and eccentric, respectively. In other words, the best-frequency and best-amplitude contours are eccentric and radial, respectively. The amplitude spectrum of a signal is thus represented in the coordinates of amplitude and frequency parallel to the cortical surface. This amplitude spectrum representation is disproportionate according to perceptual significance, so that a signal of 61.5 to 62.0 kilohertz and 30 to 50 decibels SPL (sound pressure level) is projected to a larger area than other signals. Just outside this Doppler-shifted-CF processing area, neurons are found which are specialized for responding to a particular information-bearing element or a particular combination of information-bearing elements in orientation sounds and echoes consisting of CF and frequency-modulated components.*

For echolocation, the mustache bat, *Pteronotus parnellii rubiginosus*, emits orientation sounds, each of which consists of a long constant-frequency (CF) component followed by a short frequency-modulated (FM) component. The second harmonic of the CF component is about 61 khz and is predominant in the sounds (*1–4*). The CF sound is an ideal signal for echo detection and Doppler shift (velocity) measurement, while the FM component is suited for echo localization, echo ranging, and target characterization. The peripheral auditory system of this bat is remarkably specialized for the reception (*3, 5*) and analysis of sounds at about 61 khz (*6*). The primary auditory cortex reflects this specialization of the peripheral auditory system. The area processing CF components (61 to 63 khz) in the orientation sounds and Doppler-shifted echoes is disproportionately large, and within it frequency contours are slightly eccentric. The area processing FM components (50 to 60 khz) is also very large. Except for these two areas, the primary auditory cortex of this bat shows a pattern of tonotopic representation that is shared with other mammals; that is, neurons processing high-frequency sounds are located anteriorly and those processing low-frequency sounds are located posteriorly (*4*). Since the CF component is overwhelmingly intense in orientation sounds and Doppler-shifted echoes, and since the orientation sounds are quite distinct from communication sounds (*7*), this disproportionately larger area tuned to sounds at 61 to 63 khz is undoubtedly the area specialized for processing the CF components of orientation sounds and echoes—in particular, those of Doppler-shifted echoes from targets. Accordingly, this area may be called the Doppler-shifted-CF processing area or simply CF processing area. This does not mean that this area is not concerned at all with processing signals other than CF components of Doppler-shifted echoes. It could be partially involved even in processing nonorientation signals.

The Doppler-shifted-CF processing area is about 2.3 mm^2 and occupies about 30 percent of the primary auditory cortex (Fig. 2A). This area contains many neurons tuned at the same frequency. It would be surprising if all these neurons in the small bat brain were concerned with processing an identical signal. Among neurons with identical best frequencies (BF's) there should be significant differences in terms of some other stimulus parameter, such as intensity or the location of a sound source. In all mammals studied, the primary auditory cortex is tonotopically organized. In cats and dogs, neurons that share identical BF's form a slab oriented in a dorsoventral axis. Thus, the same question may be asked in these species: How do neurons located dorsally differ from those located ventrally? This problem has been studied by Tunturi (*8*). By evoked potential studies, he discovered that a dog's auditory cortex had two coordinates, frequency and intensity. Frequency is represented in the anteroposterior axis and intensity in the dorsoventral axis. Interestingly, the intensity representation is found only for the input from the ipsilateral ear. The dorsal part is less sensitive than the ventral part. Tunturi's finding has, however, been neglected, and I know of no attempt to confirm it. Thus, there is incentive to explore the cortical representation of stimulus parameters other than frequency. Here I report that the Doppler-shifted-CF processing area of the mustache bat has a representation of stimulus (or echo) intensity at right angles to the frequency representation.

The experiments were performed on 15 specimens of *P. parnellii rubiginosus* from Panama. Under light anesthesia with sodium pentobarbital (23 mg per kilogram body weight) and ether, if necessary, the flat head of a 1.8-cm-long nail was mounted onto the dorsal part of the bat's skull with glue and cement. To immobilize the head, the shank of the nail was locked onto a metal rod with a set screw. The skull covering the left primary auditory cortex was removed. The electrodes (3*M* KCl micropipette or tungsten wire) were inserted normal to the cortical surface and either single-unit or multi-unit activity was recorded at depths not greater than 0.8 mm from the surface. Tone bursts, each with a rise-decay time of 0.5 msec and duration of 40 to 50 msec, were delivered at a rate of 2.0 per second from a condenser loudspeaker. Since the bat flies toward a target to hunt and land, the loudspeaker was placed in front of the animal. The instruments used are described in detail elsewhere (*9, 10*). The responses of a single neuron to identical tone bursts were sampled 50 times and expressed in the form of poststimulus time or cumulative histograms, or both, by a Nicolet computer. The means and the standard deviations of numbers of impulses per stimulus were calculated by a PDP-8 computer. Impulse-count functions were obtained after plotting the mean number of impulses per stimulus against stimulus amplitude (Fig. 1A). Multi-unit activities evoked by identical tone bursts were averaged after full-wave rectification by the computers (Fig. 1B), and magnitudes of responses were examined on the screen of a cathode-ray oscilloscope.

In the initial five bats, it was noticed that neurons encountered during each of the orthogonal penetrations had nearly identical BF's and similar excitatory areas. When an "upper-threshold" neuron with a closed excitatory area (*11*) was first recorded at a superficial depth, for instance, almost all other neurons at increasing depths also had closed excitatory areas. Upper-threshold neurons showed a very nonmonotonic impulse-

count function; that is, the number of impulses per tone burst first increased with stimulus amplitude, then decreased to zero or to a level of spontaneous discharge (for example, *a* to *c* in Fig. 1A). The upper-threshold neurons usually had inhibitory areas on both sides of the excitatory area (*10, 12*). When a neuron with an excitatory area similar to that of a primary auditory neuron was recorded near the surface, on the other hand, almost all other neurons at subsequent depths had similar excitatory areas. Such neurons showed a monotonic impulse-count function, so that the maximum response to a sound appeared at the maximum available stimulus level, 98 db SPL (sound pressure level) (*g* in Fig. 1A).

The minimum threshold (MT) and the impulse-count function measured with the tone burst at a BF were also very similar among almost all neurons encountered in each penetration. Figure 1A represents examples of impulse-count functions of single neurons recorded from different cortical locations. In sharply tuned neurons, the peak of the impulse-count function measured with a tone burst at the BF uniquely indicated the best pressure level or best amplitude (BA) for the maximum excitation of the neurons. When the stimulus frequency slightly differed from the BF, the impulse-count function was less sharply peaked. The BA's of neurons recorded from each penetration were similar. As in the visual and somatosensory cortices (*13*), the auditory cortex is thus organized in columns or slabs. Each column or slab in the Doppler-shifted-CF processing area is characterized by the BF, shape of the excitatory area, MT, and BA.

For BF, BA, and MT mapping, it was inefficient to obtain an average BF, BA, and MT from a statistically adequate number of neurons in each of many penetrations, so the sampling method was changed in a series of ten bats. In these, a tungsten-wire electrode with a diameter of 10 to 20 μm was inserted to the most active depth, 400 to 500 μm, and multi-unit activity was recorded (*14*). Figure 1B represents an example of such activity. Multi-unit activity was adequate to allow determination of BF, MT, and BA, because it consists of the activities of several neurons with very similar properties in terms of excitatory area and impulse-count function. When a response-magnitude function was not sharply peaked, the range of sound pressure levels that evoked the maximum response was measured and its center was used as the BA. In each of the ten bats used, 10 to 32 penetrations were within the Doppler-shifted-CF processing area and BF, MT, and BA maps were obtained. The data confirm the previous finding (*4*) of a radial tonotopic representation axis (that is, eccentric frequency contours) in this area (Fig. 2, B and C) and show a systematic change in BA (Fig. 2, B to E), but the change in MT was less systematic. In the posterior half of the Doppler-shifted-CF processing area, large BA's are always represented dorsally and small BA's are represented ventrally, with the intermediate BA's between them. In its anterior half, however, there is one or the other of two types of amplitude representation. In six bats, small-to-large BA's were represented in the dorsal-to-ventral direction (Fig. 2B), while in four bats these were represented in the ventral-to-dorsal direction (Fig. 2C). Figure 2, D and E, represent the actual distributions of the BA's around the center of the area showing eccentric frequency contours in these two types of BA representations. In Fig. 2D, the BA increases in a counterclockwise direction with a discontinuity at the anteroventral part. In Fig. 2E, the larger the BA's, the more dorsal their locations are. The overall representation axis is thus V-shaped.

In both types of representation, the BA changes at a rate of 0.23 to 0.42 db per degree (regression coefficient r = 0.77 to 0.87). On the other hand, the MT, not shown in Fig. 2, changes at a rate of 0.11 to 0.21 db per degree (r = 0.49 to 0.64). It is clear that the BA is systematically represented in the Doppler-shifted-CF processing area, but the representation of the MT (or sensitivity) is less systematic. Because the origin of the frequency-amplitude coordinates is off-center in the Doppler-shifted-CF processing area, the BA representation is disproportionate. That is, BA's between 30 and 50 db SPL occupy a much larger area than do those from 80 to 100 db SPL (Fig. 2, B and C). This disproportionate amplitude representation is presumably related to the most commonly encountered echo amplitude in echolocation.

These experiments indicate that individual locations within the Doppler-shifted-CF processing area are specified not only by frequency but also by amplitude of sound (*15*). In other words, amplitude spectra of the CF components in

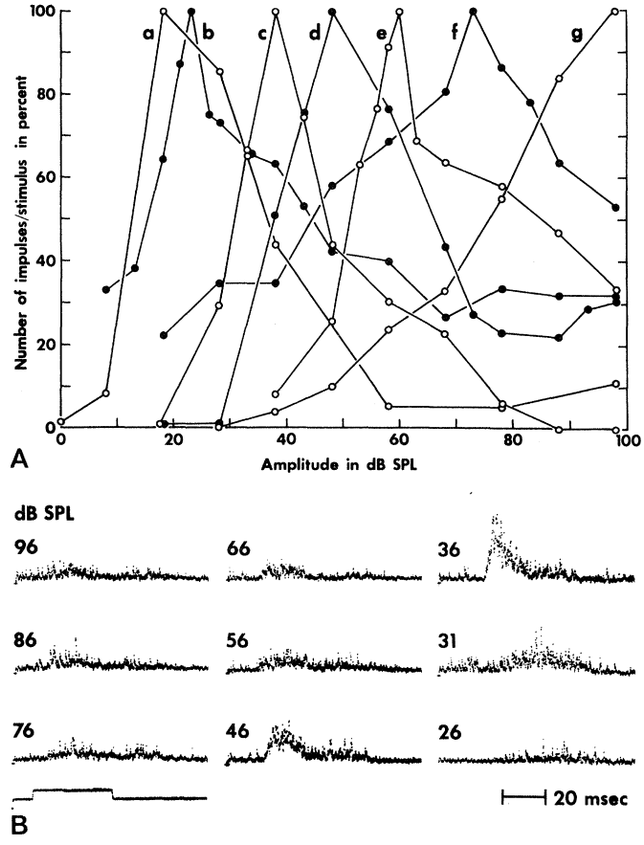

Fig. 1. (A) Impulse-count functions of seven single neurons recorded in seven different penetrations within the Doppler-shifted-CF processing area. The ordinate and abscissa respectively represent the number of impulses per 50-msec-long tone burst as percentage and stimulus level in decibels SPL (that is, decibels in pressure ratio referred to 0.0002 dyne/cm^2 root-mean-square). At 100 percent, these neurons discharged less than five impulses at the onset of or during each stimulus. The frequency of the tone burst was at the best frequency of each neuron, which ranged from 61.25 to 63.97 khz. (B) Multi-unit responses after full-wave rectification and averaging. The frequency of the stimulus is 61.48 khz, which is the best frequency of a cluster of neurons. The amplitude of the tone burst is indicated by the figures on the left of each of the averaged responses. The minimum threshold (MT) and best amplitude (BA) are 22 and 36 db SPL, respectively.

echoes are given by the coordinates in which amplitude is represented at right angles to the frequency representation axis. This does not mean that the amount of Doppler shift (relative velocity of the target) and echo amplitude (subtended angle of the target) are uniquely represented only by the excitation of a group of neurons in a single column, because their impulse-count functions are not extremely sharply peaked and because their tuning curves are not necessarily extremely sharp. A spatial pattern of neural activity in the cortex reflects amplitude spectra of incoming signals. At the periphery of the bat auditory system, the frequency of a signal is represented by the location of activated neurons and its amplitude by their discharge rate. In the auditory cortex, the frequency and the amplitude of a signal are each represented by the location of activated neurons.

Auditory nerve fibers commonly show more discharges to higher levels of tone bursts (*6*). Thus the different BA's found in the auditory cortex must be due to a neural interaction intervening between primary auditory neurons and cortical neurons. The mechanisms that produce different MT's themselves cannot be responsible for the various BA's, although the MT's show some tendency to vary together with the BA's. It has been found that lateral inhibition plays an essential role in the production of various BA's (*12*). The degree of overlap of inhibitory areas on the excitatory area determines the BA. That is, the larger the overlap, the lower the BA. In the little brown bat (*Myotis lucifugus*), about half of the inferior collicular neurons studied show a nonmonotonic impulse-count function (*10, 12, 16*). It is thus very likely that the majority of the various BA's observed in the cortex are due to neural interactions at subcortical levels.

In this and previous experiments (*10, 12*) it has been found that lateral inhibition in the auditory system plays a role in producing various BA's, "level-tolerant" bandwidths (or sharper tuning curves), a sharper amplitude spectrum representation, and feature detectors, and that such roles of lateral inhibition depend on the systematic tonotopic organization of the auditory system. If the auditory system were not tonotopically organized, formation of the neural circuit to take these roles would be very difficult. At the auditory nerve, all tuning curves of single fibers are triangular, so that their bandwidths increase with stimulus level; that is, they are level-intolerant. This is true even in unusually sharply tuned neurons with a BF between 60.76 and 61.25 khz. On the average they responded to a sound from 55.7 to 64.4 khz at 60 db above their minimum thresholds (*6*). In the auditory cortex, however, the bandwidths of the tuning curves of some neurons are narrow and nearly identical regardless of stimulus level; that is, they are level-tolerant. For example, in the ventral part of the Doppler-shifted-CF processing area where the BA is low, neurons usually showed an upper threshold. Their excitatory areas were sandwiched in between inhibitory areas and were often very narrow, and the bandwidth was level-tolerant. In the narrowest tuning curve measured, the best frequency was 61.51 khz and the bandwidth was about 0.3 khz regardless of stimulus level. Thus some of the upper-threshold neurons were really specialized for responding to a sound occurring within a remarkably narrow frequency range. Furthermore, such upper-threshold neurons failed to respond to FM sounds and noise bursts and showed the properties of CF-specialized neurons, which comprise one of several types of feature detectors (*10, 17*).

During the experiments reported here, other types of specialized neurons were found in the area dorsal to the Doppler-shifted-CF processing area: two FM-specialized neurons, six CF-FM-specialized neurons, and two neurons sensitive to harmonically related sounds. The CF-FM-specialized neurons responded to neither CF nor FM sounds alone, regardless of stimulus amplitude, but responded remarkably to CF-FM sounds similar to the second harmonic in orientation sounds and their Doppler-shifted echoes. In three of the six, the CF tones alone inhibited their background discharges and caused very faint subthreshold off-responses. (Responses at threshold are defined as 0.1 to 0.2 impulse per stimulus on the average.) The FM sounds alone evoked very faint subthreshold responses, if any. The prominent responses to CF-FM sounds could be due to a facilitation of the subthreshold responses to the FM sounds by a subthreshold rebound from the inhibition

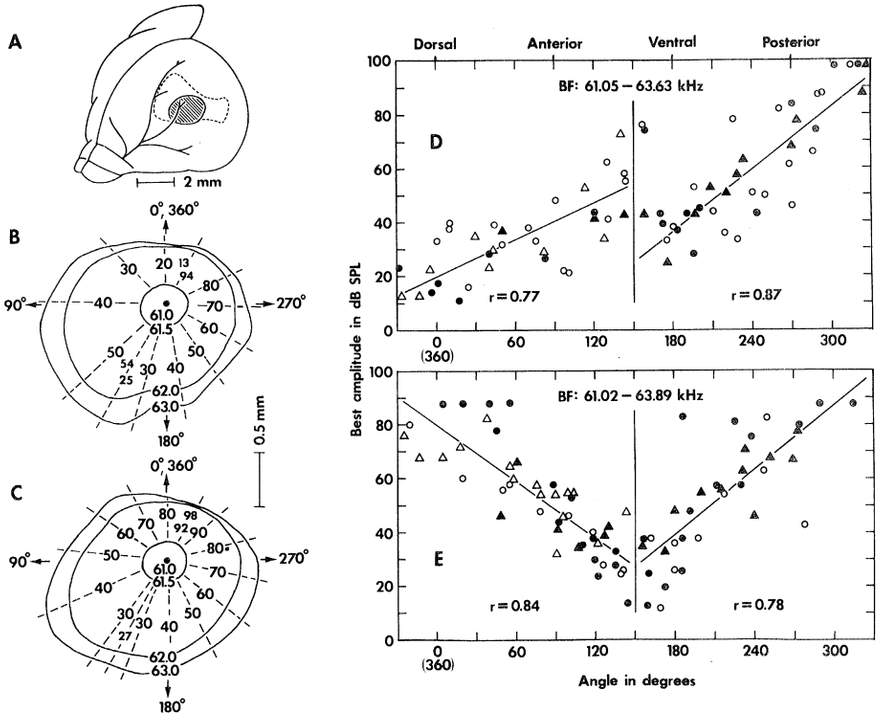

Fig. 2. (A) Dorsolateral view of the left cerebrum. The primary auditory cortex and the Doppler-shifted-CF processing area are indicated by the dashed line and the shaded area, respectively. (B and C) The BF and BA contours are shown by solid and dashed lines, respectively. The BF in kilohertz and BA in decibels SPL are respectively shown by three-digit and two-digit numbers. The BA contour maps were composed from the data shown in (D) and (E). The BF contour maps were obtained in the same way as described in (*4*). The contour for 64.0 khz, not shown, is just outside the 63.0-khz contour. (It should be noted that the BA contour maps of the individual bats are not as smooth as the averaged ones.) (D and E) Each of the graphs represents the data obtained from four bats, indicated by four different symbols. The ordinates and abscissas represent BA's in decibels SPL and angles in degrees, respectively. The angle is expressed counterclockwise, starting from the dorsal part of the Doppler-shifted-CF processing area. The regression coefficient (*r*) is shown below each regression line. The range of BF's of neurons sampled is also shown in each graph.

during the CF tones. The two neurons strongly responded to simultaneous delivery of two sounds corresponding to the second and third harmonics in the CF component of the orientation sound and Doppler-shifted echoes, but poorly responded to each of them alone. One of them did not respond to noise bursts with broad bands. These two types of neurons, which responded better or selectively to a certain combination of two information-bearing elements in biologically significant sounds, have, to the best of my knowledge, not yet been reported in any other animals (*18*).

It is probably a common feature that amplitude spectra of acoustic signals, which would change with time, are represented by spatiotemporal patterns of activities of cortical auditory neurons, but it should be noted that the auditory cortices contain the neurons that are specialized for responding to a particular acoustic signal frequently used by the animal.

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References and Notes

1. The frequency of the CF component can be slightly different among *Pteronotus parnellii rubiginosus* living in different parts of Central America [H.-U. Schnitzler, *Z. Vgl. Physiol.* **68**, 25 (1970); Grinnell (*2*)].
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3. N. Suga, J. A. Simmons, T. Shimozawa, *J. Exp. Biol.* **61**, 379 (1974). See also the studies on the lateral lemniscal evoked potential by Grinnell (*2*).
4. N. Suga and P. H.-S. Jen, *Science* **194**, 542 (1976).
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6. N. Suga, J. A. Simmons, P. H.-S. Jen, *J. Exp. Biol.* **63**, 161 (1975); N. Suga and P. H.-S. Jen, *ibid.*, in press.
7. A. Gordon and N. Suga, unpublished results.
8. A. R. Tunturi, *Am. J. Physiol.* **168**, 712 (1952).
9. N. Suga, *J. Physiol. (London)* **198**, 51 (1968); *ibid.* **200**, 555 (1969).
10. ———, in *Basic Mechanisms in Hearing*, A. R. Møller, Ed. (Academic Press, New York, 1973), pp. 675–742.
11. Unlike auditory nerve fibers, a majority of cortical auditory neurons show nonmonotonic impulse-count functions. In extremely nonmonotonic functions, excitatory responses disappear when a stimulus amplitude increases beyond a certain level or upper threshold. The upper threshold is due to lateral inhibition (*10, 12*). In some neurons an upper threshold appeared at all frequencies of excitatory sounds, so that their excitatory areas (that is, the area above an excitatory threshold curve) were closed at the top. It should be noted that upper-threshold neurons have specific amplitude selectivity. They are therefore different from auditory nerve fibers which show impulse-count functions that fit the Weber-Fechner function or power function.
12. N. Suga, *J. Physiol. (London)* **181**, 671 (1965).
13. D. H. Hubel and T. N. Wiesel, *ibid.* **160**, 106 (1962); V. B. Mountcastle, *J. Neurophysiol.* **20**, 408 (1957).
14. E. G. Jones histologically examined the auditory cortex of the mustache bat. The auditory cortex is 0.7 to 0.9 mm thick at the Doppler-shifted-CF processing area.
15. Oblique penetrations to the cortical surface gave rise to data that further confirmed the tonotopic and amplitopic representation in the Doppler-shifted-CF processing area (N. Suga and T. Manabe, unpublished results).
16. A. D. Grinnell, *J. Physiol. (London)* **167**, 38 (1963).
17. Specialization of auditory neurons of *Myotis lucifugus* has been studied in terms of responses to the three basic types of acoustic signals used by various types of animals: CF tones, FM sounds, and noise bursts. In these studies CF-, FM-, and noise-specialized neurons have been found, each of which selectively responds to one of the above three types of signals. Such specificity of the neurons to the signals does not change with stimulus level (it is level-tolerant) and the lack of response of these neurons to the other two types of signals is due to inhibition—that is, the "rejection mode" operating in the nervous system (*10*). These two things are the minimum requirements for neurons to be called specialized neurons or feature detectors. The term specialized neurons implies that the neurons express the output of a neural circuit operating as a filter.
18. Neurons that selectively responded to a particular type of communication sound have been found in primates [Z. Wollberg and J. D. Newman, *Science* **175**, 212 (1972)]. However, it has not yet been clarified whether these neurons responded only to a particular combination or combinations of components in their communication sounds.
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Babesia rodhaini: Requirement of Complement for Penetration of Human Erythrocytes

Abstract. *A system has been developed in vitro in which human red cells, in the presence of fresh human (or rat) serum, are parasitized by the hemosporidian protozoan* Babesia rodhaini. *The ability of* B. rodhaini *to penetrate red cells depends on factors of the alternative complement pathway (properdin and factor B) as well as ionic magnesium and the third (C3) and the fifth (C5) components of complement. These data indicate a novel mechanism by which a parasite is able to utilize the complement system. The data are in accord with and further amplify earlier observations that demonstrated a requirement for complement in the development of babesial infection in rats.*

Species of *Babesia* parasitize a wide variety of mammals (*1*). Scattered cases of human infection have been reported (*2*), and the most recent outbreak involved seven patients on Nantucket Island (*3, 4*). Clinically, the disease is similar to malaria; patients usually experience headache, chills, fever, and myalgia (*4, 5*). Like other protozoa such as plasmodia, *B. rodhaini* causes numerous pathophysiologic alterations in the host, including a transient glomerulonephritis with glomerular deposits of immunoglobulin G (IgG) and the third component of complement (C3), hypocomplementemia, proteinuria, hepatosplenomegaly, severe anemia, and thrombocytopenia (*6, 7*). Babesiosis differs from human malaria in that the organism causing the former shows no evidence of sexual reproduction (*8*), and primary infection induces complement depression which correlates linearly with the degree of parasitemia. In humans with relapsing malaria (caused by *Plasmodium vivax*), the cyclic paroxysmal release of parasites is associated with a transient drop in complement levels; however, primary infection only occasionally shows this pattern (*7, 9*).

Previous studies have demonstrated that rats maintained on a magnesium-deficient diet were afforded some degree of protection against *B. rodhaini* (*10*). Furthermore, rats were less susceptible to red cell parasitization after treatment with cobra venom factor, an agent known to cause inactivation of the complement system. Sodium flufenemate, which irreversibly inactivates C3, also blocked development of the infection (*11*). Finally, mice deficient in both C3 and the fifth (C5) component of complement failed to develop babesial infections (*11*). The evidence suggested that an intact complement system was essential for development of babesial parasitemia. Using a newly developed system of babesial infection in vitro, we investigated the role of alternative pathway and terminal complement components in the development of the infection. As will be demonstrated, penetration of human erythrocytes by the parasite requires the presence in serum of properdin, factor B, C3, C5, and magnesium ions.

Complete removal of factor B of the alternative (properdin) complement pathway from 10 ml of fresh normal human serum was accomplished by affinity column chromatography on 3 g of Sepharose 4B (with activated CNBr; Pharmacia Fine Chemicals, Uppsala) covalently linked to 8.5 mg of antibody to human factor B. The factor B antibodies were separated from 10 ml of whole rabbit serum containing specific antibody by affinity column chromatography on 3 g of CNBr-activated Sepharose 4B containing 4 mg of purified factor B. Antiserums had been prepared by a general immunization regimen described elsewhere (*12*); the factor B antigen (C3 proactivator) was purified as described previously (*13*). To prevent inadvertent immune ac-