cytes have so far been noted in preparations after 2 months in vitro, but sufficiently thorough examination of the slides for this stage has not yet been done. Whether infective gametocytes can be produced in vitro and what are the conditions for their formation are among the many problems now open to experimental attack.

Of more immediate importance is the use of the cultures for the preparation of merozoites, which may be particularly immunogenic (*14*), and for the study of materials produced by merozoites that may function in invasion of erythrocytes (*15*) and may have a role in induction of protective immunity. Of particular interest would be a study of the physiological condition of the erythrocyte, especially with regard to adenosine triphosphate content, in relation to its suitability for development of the parasites (*16*), and for characterization of the requirements of malaria parasites for extracellular development in vitro (*16*).

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17. Supported in part by NIH grant AI 10640 and mainly by AID contract ta-C-1200. We thank R. Klatt for technical assistance.

19 April 1976

Neurons Selective for Orientation and Binocular Disparity in the Visual Wulst of the Barn Owl (*Tyto alba*)

Abstract. *The visual response properties of single neurons in the owl's visual Wulst suggest that this forebrain structure is an analog of the mammalian visual cortex. Features in common with the cat and the monkey visual cortex include a precise topographic organization, a high degree of binocular interaction, and selectivity for orientation, direction of movement, and binocular disparity of straight-line contours.*

The frontal eyes and prey-catching skills of the owl suggest that the bird achieves stereopsis, the highly precise, binocular depth sense (*1*). However, some investigators (*2*) have argued against this possibility because owls lack partial decussation at the optic chiasm; instead, all the fibers from one eye cross to the opposite side of the brain (Fig. 1).

Partial decussation allows information from each eye to be compared by the brain, as Newton first pointed out (*3*), and is a prominent feature of the visual system of all the binocular mammals including man. Since owls lack a partial decussation, it was inferred that they also lack neurons that can be influenced by both eyes and, therefore, the disparity-sensitive binocular neurons thought to mediate the first stages of stereoscopic visual processing in the cat and monkey visual cortex (*4*).

Neuroanatomical studies on the owl (*5*) suggest that such inferences were premature; despite a totally crossed pathway from the eye to the thalamic relay nucleus, the owl has a bilateral projection from the thalamus to the Wulst, a prominent bulge on the surface of the forebrain (Figs. 1 and 2). The finding of a representation of each eye within this structure and other cytoarchitectonic similarities to the visual cortex of mammals, led Karten and co-workers (*5*) to hypothesize that the Wulst might play the role for the owl of the visual cortex in the monkey and the cat.

We have studied the visual response properties of 260 single neurons in the Wulst of the barn owl (*Tyto alba*). Our findings confirm the recent ideas of the neuroanatomists, for the physiological similarities of the Wulst to the visual cortex of the cat and monkey are more striking than the differences. In common with the mammalian cortex are a precise retinotopic organization, a high degree of binocular interaction, and neurons with requirements for stimulus orientation, direction of movement, and binocular disparity.

We used tungsten-in-glass microelectrodes and conventional extracellular recording techniques from a closed chamber. Anesthesia was induced with ketamine (12 mg per kilogram of body weight, injected intramuscularly) and maintained by intermittent injections through a Teflon catheter placed in the pectoral muscle. As the degree of eye

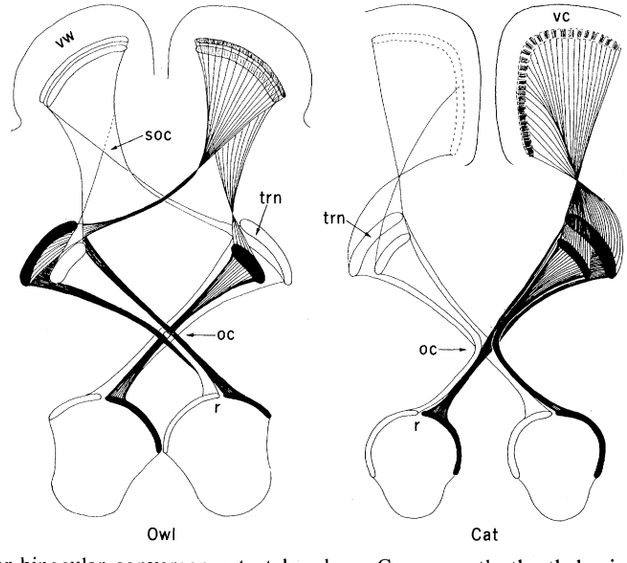

Fig. 1. Schematic representation of the organization of forebrain visual pathways in the cat and owl. Both of these vertebrates demonstrate binocular integration within laminated forebrain structures (visual cortex of the cat and visual Wulst of owl). In the cat (right) binocular convergence occurs at the thalamic level because of partial decussation of fibers from the retina (*r*) at the optic chiasm (*oc*). In contrast, the owl (left) has a total optic decussation, and thalamic fibers representing the temporal retina must recross in the supraopic chiasm (*soc*) for binocular convergence to take place. Consequently the thalamic relay nucleus (*trn*) of the owl represents the whole visual field of the contralateral eye while the corresponding relay nucleus of the cat represents the contralateral hemifield of both eyes. Despite these differences, both cat visual cortex (*vc*) and owl visual Wulst (*vw*) represent the contralateral hemifield, and both have binocular neurons with similar functional characteristics.

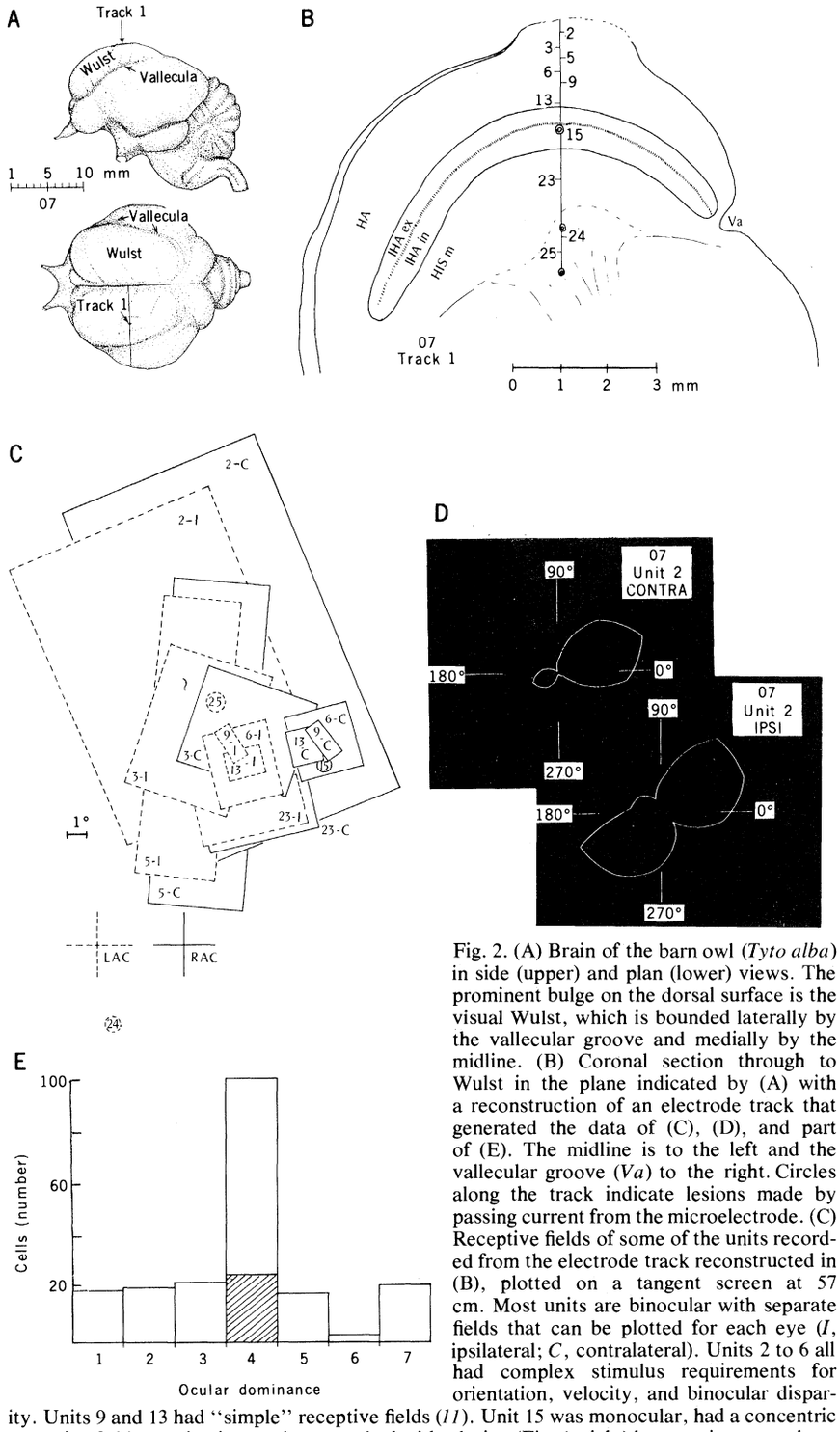

Fig. 2. (A) Brain of the barn owl (*Tyto alba*) in side (upper) and plan (lower) views. The prominent bulge on the dorsal surface is the visual Wulst, which is bounded laterally by the vallecular groove and medially by the midline. (B) Coronal section through to Wulst in the plane indicated by (A) with a reconstruction of an electrode track that generated the data of (C), (D), and part of (E). The midline is to the left and the vallecular groove (*Va*) to the right. Circles along the track indicate lesions made by passing current from the microelectrode. (C) Receptive fields of some of the units recorded from the electrode track reconstructed in (B), plotted on a tangent screen at 57 cm. Most units are binocular with separate fields that can be plotted for each eye (*I*, ipsilateral; *C*, contralateral). Units 2 to 6 all had complex stimulus requirements for orientation, velocity, and binocular disparity. Units 9 and 13 had "simple" receptive fields (*11*). Unit 15 was monocular, had a concentric receptive field organization, and was marked with a lesion (Fig. 1, right) because it occurred at a transition from cells dominated by the ipsilateral eye to cells dominated by the contralateral eye. Units 24 and 25 had properties and waveform suggesting that they were afferent fibers from the thalamic relay nucleus. The projections onto the screen of the left area centralis (*LAC*) and right area centralis (*RAC*) are indicated by broken and solid lines, respectively. Variation in the relative positions of the pairs of receptive fields for each unit is known as receptive field disparity. (D) Computer-generated polar histograms of the mean responses of unit 2 to an oriented slit (0.5° × 10°) moving at 10° per second at 12 different orientations. Full-scale deflection is 36 spikes per sweep. The best response is to a bar oriented at 135° (moving along axis 45° to 225°). A similar pattern of response is independently elicited from each eye. (E) Ocular dominance histogram of 260 neurons recorded from six owls. Classification is based on the scheme of Hubel and Wiesel (*11*), from exclusively contralateral (group 1), through balanced binocular (group 4) to exclusively ipsilateral (group 7). Hatched area indicates cells that could not be driven independently by either eye but only by simultaneous stimulation of both eyes at the appropriate binocular disparity.

movement reported for the owl is very small (*6*), it was not necessary to resort to neuromuscular paralysis. However, since even the smallest movements can confound studies of the slight receptive field disparities of binocular neurons, we monitored and corrected for the presence of eye movements (*7*).

The visual axis was determined by projecting a prominent landmark in the bird eye, the pecten oculi, onto a tangent screen. The spatial relationship between the pecten and area centralis was then determined on retinal whole-mount preparations (*8*).

Receptive field properties were studied with targets displayed on a tangent screen placed 57 cm from the owl's eyes. The position and orientation of the display could be controlled either by a hand-held control stick or by a computer that generated orientation tuning curves (*9*).

Electrode tracks were reconstructed from small lesions produced by passing current through the microelectrode. Sections were stained with cresyl violet or with the Fink-Schneider technique (*10*) and were compared with sections from the opposite Wulst of some preparations that were stained according to the Golgi Rapid technique.

Retinotopic organization was precise, with a large overrepresentation of the area centralis. The anterior, posterior, medial, and lateral margins of one Wulst represented, respectively, the inferior, superior, peripheral, and midline boundaries of the contralateral binocular visual field (Fig. 2, A and C). This pattern of retinotopic organization is identical to that found in cat primary visual cortex (*11*).

Of 260 neurons, 189 could be activated independently from each eye (Fig. 2, C and E). An additional 26 neurons could be driven only by simultaneous presentation of a target to both eyes (Fig. 2E). And finally, 23 neurons were driven exclusively by the contralateral eye and 22 exclusively by the ipsilateral eye. All 45 of these monocular units had concentrically organized receptive fields (*11*). Three units were difficult to isolate and had concentrically organized receptive fields on both retinas. The remainder of the binocular neurons (212) responded optimally to moving edge targets; they demonstrated different degrees of selectivity for orientation (Fig. 2C), direction (Fig. 2D), velocity, contrast, and size—possibly as a function of the depth of the unit in the Wulst. For example, while recording from the superficial millimeter of Wulst, we never encountered cells with "simple" fields that could be

mapped into ON and OFF areas (*11*). Instead, we commonly encountered neurons that were silent unless we presented the end of an appropriately oriented dark bar to both eyes simultaneously with an appropriate setting on the rotary biprism set before one eye to enable control of binocular disparity.

In a number of cases, by recording from both right and left Wulsts with separate microelectrodes, we could record simultaneously from two different disparity-selective neurons. The responses of three such neurons (two pairs with a reference cell common to each pair) (Fig. 3) show that small changes in prism setting produce large changes in the response elicited by binocular stimulation. In addition, the peak binocular response does not occur at the same prism setting for all three neurons. This is of interest because of disagreement in the literature concerning disparity-selective neurons. While the existence of sharply tuned disparity-selective binocular neurons is well established for the cat (*4*), there is dispute about the magnitude of the neuron-to-neuron variation in preferred disparity (*12*). In our experiment, the neuron-to-neuron variation is significant and independent of any eye movement because the simultaneous measurements made on two neurons show that one may be maximally excited while another is maximally inhibited by the same binocular stimulus [compare units A10 and B8 at 4.5° (Fig. 3)]. Since the prism variations were made in the horizontal plane, these two cells would also be differentially affected by the same target as a function of distance in the natural situation.

In contrast to the more superficial cells, those recorded in the granular layers [IHA externa (ex) and interna (in) (Fig. 2B)] receiving thalamic input had very simple properties. Their receptive fields could be mapped into separate antagonistic regions that were often separated by straight-line boundaries (Fig. 2C), particularly when the cell was binocular (monocular neurons always had concentric boundaries). In a number of penetrations that passed through the granular layers, reconstruction revealed that exclusively ipsilateral units tended to be recorded first, in the IHA externa, whereas exclusively contralateral units were usually recorded in the IHA interna (Fig. 2).

In addition to recording from the Wulst, we also penetrated the main body of the thalamic visual relay nuclei, or opticus principalis thalami (OPT) (*5, 13*), to check the properties of the Wulst's input. Neurons in the OPT were indistinguishable from the neurons of the lateral geniculate nucleus (LGN), which provide the input to the visual cortex of the cat. We found both sustained and transient OPT neurons with either ON center or OFF center, concentrically organized fields; the fields could be plotted only for the contralateral eye. There was precise retinotopic order, but, in contrast to the LGN of the cat, which has a representation of both eyes and a single hemifield, the owl OPT appears to represent both hemifields of the contralateral eye only (Fig. 1). Since thalamic neurons could be excited only from one eye, the Wulst, like the visual cortex of mammals, appears to be the first site of integration of information from both eyes (*14*).

We conclude that, despite its different evolutionary history and its totally crossed primary optic pathway, the owl has a neural basis for binocular depth discrimination directly comparable to that found in cat (*4*), monkey (*4, 12*), and probably man (*15*). It is not yet known whether the binocular neurons of the owl are also like those of cat (*16*), monkey (*17*), and man (*18*) in their sensitivity to early visual experience.

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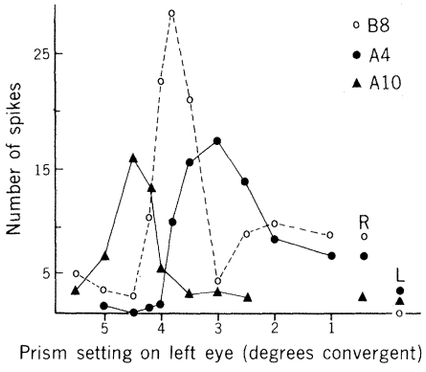

Fig. 3. Disparity-tuning curves for three binocular neurons recorded with the reference cell technique (*7*). Unit B8, the reference cell, was recorded (from the right visual Wulst) simultaneously with unit A4 (from the left visual Wulst). The right electrode was then advanced so that B8 and A10 could be recorded simultaneously. Each point gives the unit's response to a 10° × 0.5° vertical slit swept from left to right (amplitude 10°) at 20° per second on a screen at 57 cm. The points at right show the response of each unit to monocular presentation of the stimulus (*R*, right eye alone; *L*, left eye alone). The connected points show the response to binocular presentation of the stimulus at each of a number of settings on the prism which changes the horizontal alignment of the left eye (and therefore changes the relative retinal alignment, or retinal disparity, between the left and right images of the binocular target). Because the visual axes are divergent (Fig. 2C), binocular response is best when there is a convergent setting on the prism (that is, in which the base of the prism is toward the midline), but each cell has a distinct preference for a different disparity. For example, at a prism setting of 4.5°, A10 is maximally active while simultaneously recorded B8 is inhibited below the monocular level. Successively recorded A4 and A10 have virtually nonoverlapping curves which can be compared directly by means of the reference cell B8. Preferences for stimulus disparity were sharply defined since misalignments of a fraction of the receptive field size resulted in marked changes of responsiveness. Receptive field size measured at right angles to the preferred orientation was around 6° for units B8 and A4 and 4° for unit A10.

References and Notes

1. Stereopsis is remarkable not only for its precision (stereoscopic acuity in man is 10 seconds of arc—a fraction of the photoreceptor separation) but also for its ability to "break" camouflage, because prior monocular form recognition is not required. In other words a pattern (or food object) may be invisible monocularly but stand out with stereopsis [B. Julesz, *Foundations of Cyclopean Perception* (Univ. of Chicago Press, Chicago, 1971); J. Pettigrew, *Sci. Am.* **227**, 84 (August 1972)]. Stereopsis has been demonstrated unequivocally in the monkey with random-dot stereograms [E. W. Bough, *Nature (London)* **225**, 42 (1970)], and there is some behavioral evidence for stereopsis in the cat [R. Fox and R. Blake, *ibid.* **233**, 55 (1971); R. Blake and H. V. B. Hirsch, *Science* **190**, 1114 (1975)]. Preliminary experiments on owls with random-dot stereograms are promising (M. Konishi, unpublished results).
2. G. L. Walls, *The Vertebrate Eye and Its Adaptive Radiation* (Hafner, New York, 1942); C. B. Blakemore, thesis, University of California at Berkeley (1968).
3. I. Newton, *Opticks*, Query 15 (reprinted by Dover, New York, 1952).
4. A neural basis for stereopsis requires binocular neurons sensitive to horizontal retinal disparity, since this is the sole cue for stereopsis (*1*). Disparity-selective binocular neurons were first described in area 17 of the cat [J. D. Pettigrew, thesis, University of Sydney (1965); _____, T. Nikara, P. O. Bishop, *Aust. J. Exp. Biol. Med. Sci.* **45**, 49 (1967); H. B. Barlow, C. B. Blakemore, J. D. Pettigrew, *J. Physiol. (London)* **193**, 327 (1967); J. D. Pettigrew, T. Nikara, P. O. Bishop, *Exp. Brain Res.* **6**, 391 (1968); D. E. Joshua and P. O. Bishop, *ibid.* **10**, 389 (1970)], but have also been found in the prestriate cortex of the monkey [D. H. Hubel and T. N. Wiesel, *Nature (London)* **225**, 41 (1970)] and area 18 of the cat [J. D. Pettigrew, *Nature (London) New Biol.* **241**, 123 (1973)].
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7. The methods we used to follow eye movements included (i) direct ophthalmoscopic observation of the movement of the pecten across a reference grid of light projected into the eye, (ii) a light-lever method with two laser beams reflected to the screen from small mirrors on the contact lenses, and (iii) the reference-cell technique, in which a second microelectrode is used to record from a binocular neuron whose small receptive fields can be replotted whenever necessary [D. H. Hubel and T. N. Wiesel, *J. Neurophysiol.* **30**, 156 (1969)]. The largest eye movement ever recorded during about 25 hours of observation was a 3.5° lateral movement that was part of a startle response to a large, rapidly approaching target. Such movements were accompanied by wing movements and blinking of the nictitating membrane. The majority of eye movements were flicks less than 1° in amplitude and 1° to 2° drifts (approximately 0.5° per second). There appeared to be little correlation between movements of each eye.
8. The pecten is a pleated, pigmented organ proj-

ecting forward from the elongated optic nerve head of the bird's eye [see K. G. Wingstrand and O. Munk, *Biol. Skr. K. Dan. Vidensk. Selsk.* **14**, 1 (1965) for a comprehensive introduction]. Retinal whole mounts were stained with cresyl violet to define the area of highest concentration of ganglion cells [A. Hughes, *J. Comp. Neurol.* **163**, 197 (1975)]. Since the angular subtense of the base of the pecten was determined with a reversible ophthalmoscope during each experiment, it was possible to establish the angular separation of the pecten and area centralis by comparing their linear separation with the length of the pecten's base on the retinal whole mount. This separation, together with the asymmetrical shape of the pecten's projection, gave the position of the area centralis on the tangent screen.

9. Stepping motors rotated the variable-slit aperture on the projector and a Risley variable biprism in front of one eye. Tuning curves were obtained by interleaving single sweeps of the stimulus slit at a variety of slit orientations or prism settings in quasi-random order until the mean response at each setting could be estimated.
10. G. E. Schneider, *Science* **163**, 895 (1969).
11. Simple cells have receptive fields that can be easily mapped by flashing a small spot into adjacent antagonistic regions with straight-line boundaries; fields in the thalamic relay can also be mapped into antagonistic regions but have concentric boundaries [D. H. Hubel and T. N. Wiesel, *J. Physiol. (London)* **160**, 106 (1962)].
12. Since the retinal disparities involved in binocular depth discrimination are small (*1*), one could predict technical problems in the study of disparity-selective binocular neurons in a paralyzed preparation. Of crucial importance are residual eye movements that cannot be eliminated completely because of the slight pulsations accompanying respiration and heartbeat [R. W. Rodieck, J. D. Pettigrew, P. O. Bishop, T. Nikara, *Vision Res.* 7, 107 (1966)]. In the monkey, in which disparity detection is accurate to tens of seconds of arc (J. Lott-Brown, personal communication), optical stabilization of the image may be necessary to overcome the noise introduced by these residual pulsations. In the cat, the range of neuron-to-neuron variation in preferred disparity appears to be large enough to be useful in comparison to the narrow disparity-tuning curves, particularly those recorded at some distance from the area centralis, where the variation is greater (*4*). This is disputed for area 17 [D. H. Hubel and T. N. Wiesel, *J. Physiol. (London)* **232**, 29P (1973); but note also H. B. Barlow, C. Blakemore, R. C. Van Sluyters, *ibid.* **242**, 38P (1974)]. These technical difficulties introduced by residual eye movement can be largely overcome by monitoring two binocular neurons simultaneously [see (*7*) and Fig. 3].
13. The OPT of the owl is a large and complex group of cell nuclei; we recorded from the two external laminae.
14. This picture is complicated by the presence in the cat LGN of subtle, binocular inhibitory effects [K. J. Sanderson, P. O. Bishop, I. Darian-Smith, *Exp. Brain Res.* **13**, 178 (1971)] for which we did not test.
15. The existence of disparity-selective binocular neurons in humans has been inferred from psychophysical studies [for example, D. E. Mitchell and C. Ware, *J. Physiol. (London)* **236**, 707 (1974)].
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19. Supported by National Science Foundation grant BMS 75-19180 (M.K.), Public Health Service grant MH-25852 (J.D.P.), and the Spencer Foundation. G. Blasdel and L. Crowder provided technical assistance with the optical and computing apparatus, and M. Cooper helped with the histology.

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Multiplication of a Human Parasite (*Leishmania donovani*) in Phagolysosomes of Hamster Macrophages in vitro

Abstract. Leishmania donovani, *the etiological agent of human visceral leishmaniasis, was grown in hamster peritoneal macrophages in vitro. By electron microscopy, using a lysosomal marker, these parasitic protozoa were seen to multiply within host cell phagolysosomes. The survival mechanism of this intracellular parasite is based apparently upon resistance to macrophage lysosomal enzymic digestion.*

Most refractory to host immunity and chemotherapy are perhaps some intracellular parasites whose survival often depends on a delicate balance of host-parasite interplay (*1*). These parasites frequently live in different ecological niches within the host. For instance, nowhere else but in a living red blood cell can the erythrocytic stage of malaria grow well (*1*), whereas *Microsporidia* (*2*) and *Trichinella* (*3*) often invade muscle cells. Still others even parasitize the mononuclear cell series of the host reticuloendothelial system, phagocytic cells that constitute the first line of host cellular defense against invading microorganisms. The success of this type of intracellular parasitism hinges on subtle mechanisms whereby the parasites plainly resist lysosomal enzymes (*4*) or avoid exposure to lysosomes by residing in separate vacuoles (*5*, *6*). One typical example of such a parasite is *Leishmania donovani*, a trypanosomatid protozoan that is the causative agent of kala azar, a debilitating and often fatal disease of man. Although it has long been recognized that the amastigote stage of this parasite infects host macrophages, the mechanism of its survival within these cells remains open to speculation (*1*). This mechanism undoubtedly bears directly on the pathogenicity of this parasite and the failure of susceptible hosts to develop effective immunity against it. In this report, we present evidence indicating that, despite the fusion of lysosomes with parasite-containing or parasitophorous vacuoles, the *L. donovani* amastigotes not only persist but multiply within the phagolysosomes of cultured macrophages.

We used an in vitro system consisting of hamster peritoneal macrophages and amastigotes of *L. donovani* (*7*). Amastigotes of *L. donovani* were isolated from infected hamster spleen by differential centrifugation. Macrophages were collected from Syrian golden hamsters and cultured with 5 percent CO_2 in air, at 37°C, in an enriched medium 199 with the flying cover slip method. Details of the culture technique will be described elsewhere (*8*). It suffices to mention here that relatively homogeneous populations of macrophages can be maintained under satisfactory conditions regarding their adhesion, spreading, and survival on a long-term basis, by coating cover slips with polylysine and the use of lactobumin hydrolysate.

To study the *Leishmania*-macrophage interactions, 1- to 3-day-old cell monolayers on cover slips were covered with 0.2 to 0.3 ml of medium containing appropriate numbers of parasites to make a parasite to cell ratio of 2 to 1. Infection was allowed to occur for 4 hours, after which time samples were washed thoroughly to remove free parasites and then incubated for up to 7 days; experimental conditions were as those for the maintenance of macrophages. At different intervals, samples were removed and Giemsa stained for light microscopy to evaluate the infectivity and multiplication of the parasites within the macrophages. Infectivity and multiplication were assessed by counting the numbers of parasitized cells per 200 macrophages, and the number of parasites per 100 infected cells, respectively. The results from more than ten experiments clearly showed that the parasites attained a severalfold increase in number in 1 week. A typical example is presented in Table 1. Using an identical parasite–host cell system but handled in a different way, Herman (*9*) obtained somewhat similar, but much less consistent results. During the entire period of our study, no extensive cell detachment from cover slips and no cell lysis due to infection were apparent. Moreover, the increase in parasite numbers was well correlated with the frequency of dividing amastigotes observed, while the percentage of infected cells remained constant (Table 1). These results thus provide clear evidence indicating intracellular growth of *L. donovani* amas-

Table 1. Infectivity and multiplication of *Leishmania donovani* amastigotes in in vitro cultured hamster peritoneal macrophages.

Days after infection	Infected cells (%)	Number of parasites per cell
1/6	50*	1.9†
1	45	1.7
3	50	3.3
7	52	7.7

*Obtained from examining at least 200 cells per sample. †Obtained by counting the number of parasites in at least 100 infected macrophages per sample.