In order to obtain a more objective analysis, each of the 48 AER's were correlated with each other (at zero time lag) to produce a 48 × 48 correlation matrix. From such a matrix one can determine the effect of changing one of the parameters that determines the visual stimulus. For example, in a typical experiment (i) the AER from the first 12 IER's correlated with the AER from the last with an average correlation of 0.89 (standard deviation, 0.014); (ii) the correlations of an AER from a particular visual stimulus and a similar visual stimulus, differing only in the position variable, gave an average value of 0.89 (standard deviation, 0.06); and (iii) the effect of changing other parameters can be similarly computed, as in Table 1. Highly similar patterns of correlations were secured for the other experiments. The correlations were greatly reduced by a change of size, with a medial recording position, or by a change in shape, with a lateral recording position. Information regarding size appears to be contained in the configuration of the AER from medial recording positions, and that regarding shape is contained in the configuration of the AER from lateral recording positions.

Spinelli (*1*) working with monkeys, reported that 8 out of 84 medial positions could produce AER's that contained information differentiating one shape of visual stimuli from another. No points of this type could be found with medial recording positions in the rat, although this may be a sampling error.

On rare occasions a medial position could be found which served as a "nonspecific shape detector." That is, all shapes presented, regardless of size or position, produced a remarkably uniform AER, which differed from the AER from a uniformly lit field, despite appropriate controls of the total energy transmitted.

CRAIG FIELDS

Rockefeller University, New York 10021

References and Notes

1. D. N. Spinelli, *Brain Res.* **5**, 511 (1967).
2. E. R. John, R. N. Herrington, S. Sutton, *Science* **155**, 1439 (1967).
3. N. E. Miller and L. V. DiCara, *J. Comp. Physiol. Psychol.* **63**, 12 (1967).
4. Power spectra were calculated by means of the Welch method [J. W. Cooley, P. A. W. Lewis, P. D. Welch, *IBM Res. Rep. No. RC1743* (1967)].
5. D. Parsons, thesis, Washington University (1963).
6. C. Fields, in preparation.
7. I thank Dr. Neal Miller for his advice and assistance, and Dr. Floyd Ratliff for his critical examination of the experiment. Supported in part by PHS grant MH-13189 to Dr. Neal Miller.

13 June 1969

Growth Pattern of Leishmania in Phlebotomine Sandflies

Anderson and Ayala (*1*) report a trypanosome of the toad *Bufo boreas halophilus* transmitted by *Phlebotomus vexator occidentis*, a sandfly that feeds on reptiles and anurans (*2*). A remarkable feature of these infections is that the flagellates in the sandfly gut and in culture occur only in the leptomonad (promastigote) form, with no indication of their trypanosomal nature until put into the toad.

For many years the leptomonad stages of *Leishmania* in the sandfly gut (infection rate, growth pattern, and so forth) have been used as indices of potential vector species of the various leishmaniases. In the case of Old World species of *Leishmania* affecting man and other mammals, growth of the flagellates is typically at the anterior part of of the midgut (anterior station) with, at times, growth forward into the foregut in the head and mouthparts, whereas the leptomonads of reptilian leishmaniae are typically at the posterior station in the hindgut of the sandfly (*3*).

As Anderson and Ayala point out, the exclusively leptomonad morphology of this toad trypanosome in the sandfly, and its position in the posterior part of the hindgut, will need to be considered in interpreting flagellate infections of wild-caught sandflies in studies of leishmaniasis. With regard to hindgut infections, however, they overlook the fact that the view that the human strains could be expected to adopt only the anterior position is no longer valid for the cutaneous leishmaniases of the New World. They refer specifically to our work (*4*), in which a large number of wild-caught sandflies were found naturally infected with leptomonad flagellates, often limited to the hindgut. They make the sweeping statement that "all evidence so far indicates that posterior station infections in sandflies are not associated with *Leishmania* of man." They ignore the fact that we repeatedly produced similar hindgut infections with various human strains of *Leishmania braziliensis* (s. lat.) by two different methods. (i) Over 700 laboratory-reared phlebotomine sandflies of several species were fed artificially by the micropipette technique on cultures of several different Panamanian strains (*5*). The infection rate was about 80 percent; hindgut infections, together with growth at the anterior station, were characteristic. (ii) Sandflies were fed on hamsters infected with several human strains from Panama and a Peruvian strain from a case of mucocutaneous leishmaniasis (*4*, *6*). The resulting infections, often limited to the hindgut, were quite similar to the natural infections in sandflies.

In the Panamanian infections the flagellates in the hindgut tended to be limited to the short, thin-walled section (the hind triangle) just posterior to the junction with the midgut, with no concentration in or near the rectal ampulla, as with some of the nonmammalian flagellates.

The following are additional data bearing on the relation of sandfly flagellates and human *Leishmania*.

1) Pure cultures were obtained over 100 times from the naturally infected sandflies. Nineteen of these strains were tested in hamsters; five produced lesions indistinguishable from those produced by human strains (*4*, *7*, *8*).

2) Sandflies fed on hamsters infected with wild-sandfly strains acquired the same type of hindgut infection as those fed on human-strain lesions (*4*).

3) Agar gel diffusion tests were made with Panamanian human and sandfly strains (*8*). Two different immunological groups of the human leishmaniae were recognized; five sandfly strains, two of which had failed to infect hamsters, were divided between the two groups; three other sandfly strains were unclassified.

4) Seven of these eight sandfly strains were compared with one of the human strains by electron microscopy (*9*). The ultrastructure of the kinetoplast of the human strain and of six sandfly strains was leishmanial. The other sandfly strain (unique among the 500-odd natural infections and recognized at the original dissection as different morphologically) was probably *Crithidia* sp.

5) Coelho *et al.* (*10*) infected two Brazilian species of sandflies, *P. longipalpis* and *P. renei*, by feeding them on hamster lesions produced by four species of *Leishmania*—*L. braziliensis* (from Brazil), *L. tropica* (Israel), *L. mexicana* (British Honduras), and *L. donovani* (Brazil). The overall infection rate was 57 percent (825 out of 1451). Infections of the anterior portion of the gut predominated, but there were also hindgut infections in 118 (14 percent) of the 825 infections, varying from 8 of 147 (5 percent, *L. tropica*) to 79 of 422 (19 percent, *L. braziliensis*), with an individual high of 43 of 61 (70 percent)

for an espundia strain of *L. braziliensis* in *P. longipalpis*. The flagellate distribution in the hindgut was not described, but it was noted that in 24 cases the infections extended to the rectal ampulla.

From the foregoing it is clear that hindgut infections may represent stages of leishmaniae of man or other mammals. The identification of leptomonad infections of wild-caught phlebotomine sandflies, in both the Old World and New World, requires at least some of the experimental procedures outlined.

MARSHALL HERTIG
Center for Zoonoses Research, University of Illinois, Urbana 61801
PHYLLIS T. JOHNSON
Center for Pathobiology, University of California, Irvine 92664
ELLICOTT MCCONNELL
U.S. Naval Medical Research Unit No. 3, Field Facility, Ethiopia, APO New York 09319

References

1. J. R. Anderson and S. C. Ayala, *Science* **161**, 1023 (1968).
2. B. N. Chaniotis, *J. Med. Entomol.* **4**, 221 (1967).
3. S. Adler, in *Advances in Parasitology*, B. Dawes, Ed. (Academic Press, New York, 1964), p. 35.
4. P. T. Johnson, E. McConnell, M. Hertig, *Exp. Parasitol.* **14**, 107 (1963).
5. M. Hertig and E. McConnell, *ibid.*, p. 92.
6. P. T. Johnson, E. McConnell, M. Hertig, *J. Parasitol.* **48**, 158 (1962).
7. E. McConnell, *Exp. Parasitol.* **14**, 123 (1963).
8. C. R. Schneider and M. Hertig, *ibid.* **18**, 25 (1966).
9. F. G. Wallace and M. Hertig, *J. Parasitol.* **54**, 606 (1968).
10. M. de V. Coelho, A. R. Falcão, A. L. Falcão, *Rev. Inst. Med. Trop. São Paulo* **9**, 177, 192, 299, and 361 (1967).

25 February 1969

Although our differences may be largely semantic, the comments by Hertig, Johnson, and McConnell illustrate one of the problems facing investigators of leishmaniasis. Throughout much of Central and South America, leishmaniasis is almost exclusively a zoontic disease, with numerous animal reservoirs and many strains of *Leishmania*. The view we "overlooked" (that is, "that the human strains could be expected to adopt only the anterior position is no longer valid for the cutaneous leishmaniases of the New World") is precisely why the toad trypanosome, with its massive hindgut concentration and its leishmania-like morphology in the vector, should be considered in interpreting flagellate infections in wild-caught sandflies.

For years Dr. Hertig and his associates at the Gorgas Memorial Laboratory have made notable contributions concerning the epidemiology of American cutaneous leishmaniasis. They have experimentally shown that, in Panama at least, strains of *Leishmania* species from infected humans localize in the anterior hindgut (the hind-triangle) of laboratory-fed sandflies. In this sense we were incorrect in saying that hindgut infection of sandflies had not been associated with *Leishmania* of man (*1*).

However, our original comment on hindgut infections referred to wild-caught sandflies. As we hoped our cited references would indicate, the statement, "all evidence so far indicates that posterior station infections in sandflies are not associated with *Leishmania* of man," was made with reference to the development of various *Leishmania* species in known vector species of *Phlebotomus*. We did not ignore the results of the experimental laboratory work of the authors (*2–4*), but in the above context we did not interpret experimentally induced mid- and hindgut infections (in sandflies fed on cultures of *Leishmania* or on infected hamsters) as constituting evidence that these species were vectors, particularly since the infection rates were, "based on dissections of sandflies found dead or in distress from the second to the 16th day after feeding" (*2*). To our knowledge, no flagellates from natural or experimentally induced uncomplicated hindgut infections have as yet produced *Leishmania* infections in mammals.

Because of our reported results (*1*) and those of our originally cited references (*5*), we did not feel that hindgut infections in wild-caught Panamanian sandflies should be equated with hindgut infections resulting from flies fed experimentally. Among other things, the presence of flagellates in the hindgut of a sandfly can be interpreted as representing (i) infective forms of a *Leishmania* or *Trypanosoma*; (ii) developmental stages of infective forms; or (iii) "dead end" or dying infections in a nonvector species. Concerning the latter interpretation, it was only in sandfly species not demonstrated to be vectors, that *Leishmania* species localized in the midgut, hindgut, or both (*6*). Conversely, in species incriminated as vectors of mammalian *Leishmania* (*5–7*), the parasites invariably occurred anteriorly (that is, in the foregut, pharynx, and so forth), and experimental transmission to hamsters and humans was achieved. Also, of the more than 100 pure cultures isolated from naturally infected Panamanian sandflies, the five that produced *Leishmania* lesions in hamsters all came from flies having anterior (foregut) infections (*2*, *4*). It should be noted that the few hindgut infections with American strains of *Leishmania* reported by Coelho *et al.* (*6*), to which the authors refer, were always accompanied by simultaneous infections of the midgut, foregut, or both.

The authors themselves suspected that only strains that produced heavy anterior infections in sandflies would infect hamsters and thus identify the strain as a *Leishmania* (*4*). Human and wild-caught strains of this type were tested by serological (*8*) and electron microscopic techniques (*9*) and confirmed as *Leishmania*. Strains from uncomplicated hindgut infections apparently were not so tested. The unidentified hindgut infections of wild flies could be distinguished easily from infections in laboratory-reared sandflies fed on cultures of *L. braziliensis* (*2*, *3*). We cannot find a single reported instance of a strain from Panama, which originated from a wild-caught sandfly with an uncomplicated hindgut infection, that has been shown to be *Leishmania* by infecting hamsters, serological evidence, or study of the morphology of the kinetoplast. It is our feeling that many of the hindgut infections of wild flies in the Americas may prove to be (i) a *Trypanosoma* rather than a *Leishmania*; (ii) *Leishmania* of insectivores such as are known for lizards in the Old World (*10*); or (iii) "dead end" development of *Leishmania* species in nonvector sandflies. [Such speculation was omitted from the final edited copy of our report (*1*).] As the authors point out in their letter, hindgut infections also "may represent stages of leishmaniae of man or other mammals," but we feel that until the nature of uncomplicated hindgut infections in wild-caught flies is clarified, several areas of speculation should remain open.

Most of the Panamanian "anterior station infections" were in the cardia (that is, the anterior portion of the midgut), with very few laboratory-infected flies having flagellates in the foregut (pharynx, and so forth) (*2*). The use of the term "anterior station infections" for such midgut infections seems unfortunate. In vector studies the terms anterior and posterior station generally refer to the method of transmission—anterior station transmission occurring via the bite and posterior station transmission by contamination with infective fecal material from the vector. Although we initially used the above terminology, we now feel it is misleading to refer to infections localized (perhaps only temporarily) in the cardia or foregut

or the hindgut as anterior and posterior station infections. This terminology also permits no consideration for the possible lack of infectivity of such organisms for vertebrate hosts.

JOHN R. ANDERSON
STEPHEN C. AYALA
Department of Entomology and Parasitology, University of California, Berkeley 94720

References

1. J. R. Anderson and S. C. Ayala, *Science* **161**, 1023 (1968).
2. M. Hertig and E. McConnell, *Exp. Parasitol.* **14**, 92 (1963).
3. P. T. Johnson, E. McConnell, M. Hertig, *J. Parasitol.* **48**, 158 (1962).
4. E. McConnell, *Exp. Parasitol.* **14**, 123 (1963).
5. S. Adler and O. Theodor, *Annu. Rev. Entomol.* **2**, 203 (1957); V. Safiyanova and A. Alekseev, *Med. Parazitol. Parazit. Bolez.* **36**, 580 (1967); ———, *Parasitology* **1**, 191 (1967).
6. M. de V. Coelho, A. R. Falcão, A. L. Falcão, *Rev. Inst. Med. Trop. São Paulo* **9**, 177, 192, 299, and 361 (1967).
7. J. Strangways-Dixon and R. Lainson, *Trans. Roy. Soc. Trop. Med. Hyg.* **60**, 192 (1966).
8. C. R. Schneider and M. Hertig, *Exp. Parasitol.* **18**, 25 (1966).
9. F. G. Wallace and M. Hertig, *J. Parasitol.* **54**, 606 (1968).
10. S. Adler, in *Advances in Parasitology*, B. Dawes, Ed. (Academic Press, New York, 1964), p. 35.

28 April 1969

Experimental Herpes Encephalitis: Crystalline Arrays in Endoplasmic Reticulum

In an electron microscopic study of herpes simplex encephalitis in rabbits (*1*), we encountered an unusual cytoplasmic crystalline array. Typical herpes simplex virus particles were seen in nuclei of neurons and astrocytes. The virus was not observed in macrophages and endothelial cells; however, in the cytoplasm of these cells, crystalline arrays of round osmiophilic particles were frequently seen. The particles, appearing as spheres with centers of low electron density, measured 22 to 24 nm in diameter. No tubular arrays were observed. They were always enclosed within membranes (Fig. 1, arrow) of the endoplasmic reticulum, and were never seen free in the cytoplasm.

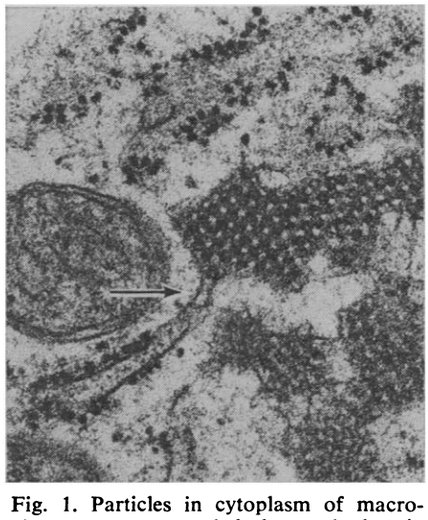

Fig. 1. Particles in cytoplasm of macrophage are surrounded by endoplasmic reticulum membrane (arrow) (× 44,200).

Although described by others (*2–14*), the nature of these unique aggregates has not been satisfactorily elucidated. In a recent report they were considered to represent polio virus within the endoplasmic reticulum on the basis of their size (*12*). However, the same aggregates have been found within the endoplasmic reticulum of experimental herpes simplex and rubella (*4*) infections where the morphology of the infecting viruses is altogether different from that of these particles. Furthermore, such crystals have been found in several virus-associated diseases [porcine polio (*3*), Rous sarcoma virus-induced tumors (*2, 5*), infectious mononucleosis (*8, 10*), Burkitt's lymphoma (*7*), and Aleutian disease of mink (*14*)], in a variety of conditions less clearly associated with viruses [transmissible canine tumors (*6, 11*), leukemia (*7, 10, 13*), reticulum cell sarcoma (*7*), and Dego's disease (*9*)], and in cultured cells from presumably normal human tissue (*7*). While it is possible that these crystals represent a virus common to all these states, their occurrence predominantly in mononuclear, lymphoid, and endothelial cells over such a wide range of conditions makes this unlikely. These data, and the consistent localization within the endoplasmic reticulum of endothelial cells and macrophages suggest that these aggregates represent a host cell response to virus disease or cellular proliferative states.

Note added in proof: Since the submission of this comment, deMartino (*15*) has demonstrated similar structures with occasional tubular profiles in the endothelial endoplasmic reticulum in glomerular capillaries of rhesus monkey and nephritic man. This finding, confirming Chandra's (*7*) observation, makes it unlikely that the structure represents viral particles.

J. RICHARD BARINGER*
JOHN F. GRIFFITH
Massachusetts General Hospital, Boston, Massachusetts 02114

References and Notes

1. J. R. Baringer and J. F. Griffith, *J. Neuropathol. Exp. Neurol.*, in press.
2. J. S. Munroe, F. Shipkey, R. A. Erlandson, W. F. Windle, *Nat. Cancer Inst. Monogr., No. 17* (1964), p. 365.
3. A. Koestner, L. Kasza, J. E. Holman, *Amer. J. Pathol.* **49**, 325 (1966).
4. K. S. W. Kim and E. S. Boatman, *J. Virol.* **1**, 205 (1967).
5. E. Bucciarelli, G. F. Rabotti, A. J. Dalton, *J. Nat. Cancer Inst.* **38**, 359 (1967).
6. C. Lombard, P. Cabanie, J. Izard, *J. Microscop.* **6**, 81 (1967).
7. S. Chandra, *Lab. Invest.* **18**, 422 (1968).
8. H. L. Moses, P. R. Glade, J. A. Kasel, A. S. Rosenthal, Y. Hirshaut, L. N. Chessin, *Proc. Nat. Acad. Sci. U.S.* **60**, 489 (1968).
9. S. Neshida and R. O. Howard, *Lancet* **1968-I**, 1200 (1968).
10. B. G. Uzman, personal communication.
11. T. J. Kakuk, R. W. Hinz, R. F. Langham, G. H. Conner, *Cancer Res.* **28**, 716 (1968).
12. K. Blinzinger, J. Simon, D. Magrath, L. Boulger, *Science* **163**, 1336 (1969).
13. L. Recher, J. G. Sinkovics, J. A. Sykes, J. Whitescarver, *Cancer Res.* **29**, 271 (1969).
14. K. S. Tsai, I. Grinyer, I. C. Pan, L. Karstad, *Can. J. Microbiol.* **15**, 138 (1969).
15. C. deMartino, *Z. Zellforsch.* **97**, 502 (1969).
16. The virus was isolated in the laboratory of Dr. J. Enders and obtained through the courtesy of Dr. S. Kibrick.
17. Supported by special fellowship 2 F 11 NB-1664-03 NSRB (J.R.B.) from the National Institute of Neurological Diseases and Stroke.

* Requests for reprints should be addressed to Dr. Baringer, Veterans Administration Hospital, 42nd Avenue and Clement Street, San Francisco, California 94121.

12 May 1969

In the meantime, the spinal cords of five additional cynomolgus monkeys that had been infected experimentally with type 3 poliovirus were examined by electron microscopy and by a fluorescent antibody technique. It seems remarkable that only in those animals that had become severely paralytic (4 to 5 days after inoculation) could we observe crystalline aggregates of round dense particles within the cytoplasm of endothelial cells and of mononuclear inflammatory cells. Poliovirus antigen was demonstrated once again by immunohistochemical examinations within the same cell types. It should be emphasized that the crystalline arrays were not always enclosed within cisternae of the endoplasmic reticulum but were most frequently seen to be embedded in the ground cytoplasm proper. Furthermore, the individual particles usually did not exhibit an electron-translucent center as did those encountered by Baringer and Griffith in experimental herpes simplex encephalitis and by others in a variety of pathological conditions. We are, therefore, still of the opinion that the crystalline formations recently described by us represent indeed aggregates of newly synthesized poliovirus particles.

K. BLINZINGER
Max Planck Institute for Psychiatry, Munich 23, West Germany

7 July 1969