cerned (Table 1). The increase in the number of $y\ B^+$ sons indicates either simultaneous loss of the y^+ and B markers from the Y chromosome or the loss of the entire Y chromosome. The latter could be due to nondisjunction of the X and Y chromosomes at meiosis, but since in some cases the event occurred in mature sperm, some direct action on the Y chromosome, or on its centromere, is indicated [as postulated by Auerbach to account for a similar event after chemical treatment (*5*)]. All of the $y\ B^+$ males were sterile, which would be expected if they lacked the Y chromosome. Many would also be sterile if both markers had been removed by breakage that is followed by rejoining of the broken ends of the Y to form a ring, because the location of the breaks could be such as to exclude from the ring one or more of the genes for fertility. Other studies have shown that less than 5 percent of this class of males with losses of both markers are fertile. Whatever the mechanism, the continued appearance of these males through brood 4 indicates that cells undergoing spermatogenesis (and hence able to produce nondisjunctional sperm at meiosis), as well as mature sperm, are affected. However, after day 12 (broods 4 through 6), the cells would have been dividing mitotically, and nondisjunction does not occur during mitosis without treatment.

A 1:1 dilution of the original solution of this drug when injected into 100 males resulted in 35 survivors, of which 30 were fertile. Among 265 chromosomes tested from brood 1, two lethals were found (0.76 ± 0.53 percent), and none were found among 272 tested chromosomes from brood 2. Among 2910 offspring of the treated males, three $y\ B^+$ sons were found (0.10 ± 0.05 percent). Thus, although mutagenicity at this dilution is indicated, the frequency of mutation has markedly dropped.

The detection of eight lethals among 378 chromosomes in brood 1 gives a mutation frequency of 2.1 ± 0.7 percent. Since the total number of lethals found was small, a calculation based on a Poisson distribution and designed for analysis of small numbers derived from large populations (*6*) shows that this mutation frequency has a lower limit of 0.68 percent ($P = .01$), as compared with the upper limit of 0.32 percent ($P = .01$) of the control rate. Therefore we conclude that LSD-25 has caused a significant increase in the mutation frequency at this dosage.

The fact that mutations were produced only when a highly toxic and partially sterilizing dose was used does not alter the conclusion that the drug can attack the chromosomes and cause mutations. The experience of numerous workers has shown that many chemicals in doses of even higher toxicity have proved to be nonmutagenic (*7*).

The fact that in *Drosophila* LSD-25 produces recessive lethals is significant. At the molecular level, genetic changes are generally considered to be similar among eukaryotes. Penetrability, breakdown products, and reactions with other metabolites are some of the ways in which the two organisms may differ in their reaction to the drug. However, its administration by injection in these experiments makes it somewhat unlikely that breakdown occurred before the chromosomes were reached. Possibly, oxidation or other deterioration of the LSD-25 used in these experiments had occurred, but the sample was stored in the dark and seldom opened. Although negative results regarding mutagenicity of a particular agent are not conclusive proof of its lack of effect, positive results in a well-studied system such as *Drosophila*, even in a small-scale experiment, warrant testing for reproducibility by further investigation.

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Trypanosome Transmitted by Phlebotomus: First Report from the Americas

Abstract. *Transmission studies carried out in the laboratory incriminated* Phlebotomus vexator occidentis *as a vector of a species of trypanosome that infects* Bufo boreas halophilus. *Toads free of parasites contracted the trypanosome after eating infected flies and after intraperitoneal inoculation of flagellates cultured from the hindgut of flies that had fed on infected toads. Discovery of this vector-host-parasite system in the Americas, and the localization of promastigote flagellates (leptomonads) in the hindgut of the vector, should assist in clarifying interpretative problems associated with infection of wild-caught flies in studies on leishmaniasis in the Americas and elsewhere.*

Previous studies of sandflies in California (*1*) revealed small percentages of *Phlebotomus vexator occidentis* infected with a flagellate of unknown origin. Only females that had taken a previous blood meal were infected. Concurrent studies showed that this phlebotomine normally fed only on anurans and reptiles (*1, 2*). An extended search for the source of infection has resulted in this first report of a *Phlebotomus*-transmitted trypanosome from the Americas, the first trypanosome to be reported from a toad in western North America.

The trypanosome found in the blood of the toad *Bufo boreas halophilus* proved infective for all of 38 *P. vexator occidentis* females fed on infected toads. As with most previously reported sandfly-transmitted trypanosomes (*3*), flagellates multiplied rapidly in the insect's midgut and then migrated to the hindgut, producing a "posterior station" infection. Trypanosome infections developed in noninfected *B. boreas* following (i) ingestion of infected sandflies and (ii) intraperitoneal inoculation of flagellates cultured from the hindgut of flies fed on infected toads. In the laboratory, unrestrained toads readily eat sandflies confined in the same cage. Both the western toad and *P. vexator occidentis* are nocturnally active and use burrows of the California ground squirrel, *Citellus beecheyi*, as diurnal resting sites.

All sandflies and infected toads were collected at a 10-hectare study area in Solano County, where 5 of 32 toads found between July and October 1967 were infected. Natural and experimental infections were diagnosed by wet mounts of peripheral blood and physiological saline; 0.05-ml samples of blood

centrifuged in hematocrit tubes (*4*); and by xenodiagnosis (that is, by feeding wild-caught, apparently uninfected *P. vexator occidentis* on toads and later examining these sandflies for infection) whenever flies were available.

We found no natural infections in 29 gravid or parous wild-caught female sandflies; an additional 37 dissected flies were nulliparous females that had not yet fed on blood. All of 42 females used for xenodiagnosis of uninfected toads remained uninfected. Thus it appears evident that most (or very likely all) of the 38 infections in wild-caught sandflies, fed on infected toads in the laboratory and examined at 1 to 14 days after feeding, were contracted from the toads.

Except for the more central position of the nucleus (Fig. 1, a and b), this trypanosome resembles *T. bocagei* Franca as reported from *Bufo bufo* near Peking (*3*) and *B. melanostictus* from Tonkin, Vietnam (*5*). Trypanosomes stained with Wright's or Giemsa stain show a darkly stained, rectangular kinetoplast located immediately posterior to the lightly stained nucleus.

Development of infection in the sandfly was studied at ambient temperature in the laboratory. Only round amastigote (*6*) forms measuring 5 to 7 μ were seen in the sandfly stomachs examined 24 hours after feeding on an infected toad. Flagellates undergoing binary fission were occasionally seen. By 48 hours the rounded forms had become elongate (10 by 1.5 μ) with 20-μ flagella. On the 3rd day a dense infection of promastigote forms (Fig. 1c) extended from the midgut through the rectal pouch, and rosettes of flagellates were seen throughout.

Once the blood meal was fully digested (by the 4th or 5th day) flagellates were no longer found in the stomach, but promastigote forms lined the hindgut in a palisade formation with flagella toward the intestinal wall. Epimastigote forms [or crithidia, with flagellum emerging at the side of the body and running along a short membrane (*6*)] were not seen at any stage of the extrinsic development in sandflies. By the 6th day, flagellates were so numerous and compressed in the posterior hindgut that the intestine was distended beyond its normal diameter; the anterior hindgut was now usually devoid of flagellates. Stained flagellates from a 6-day infection are elongate (8 to 10 by 2.0 μ) with long flagella (11 to 25 μ). Experimental laboratory infections persisted throughout the ensuing life (6 to 14 days) of the sandflies.

The parasite, from the hindgut of a sandfly fed on an infected toad, is being cultured on *Leishmania* agar (*7*) with an overlay of Locke's solution containing 500 units of penicillin and 500 μg of streptomycin per milliliter of solution. Rosettes and amastigote and promastigote forms (Fig. 1d) are found in the culture. Elongate forms over 65 μ including the flagellum (10 to 15 μ) can occasionally be seen in old cultures.

In laboratory transmission experiments, two toads from outside the study area diagnosed as uninfected by examination of wet smears and xenodiagnosis were force-fed six and nine sandflies, respectively. These flies had fed on an infected toad 10 days previously. Four other control flies fed on the same host were heavily infected. Positive xenodiagnosis demonstrated that parasites were present in the peripheral blood of both toads 14 days after ingesting the flies. The finding of trypanosomes in blood centrifuged in hematocrit capillary tubes confirmed these xenodiagnoses, but parasitemias were low and parasites still had not been seen in wet mounts of blood and saline of one of the toads when last examined at 13 weeks after infection. The sensitivity of sandfly xenodiagnosis in early or cryptic infections may be related to the long time (more than an hour) required for these flies to engorge (*2*). Ten toads caught at the same time as the two laboratory infected toads have remained negative for trypanosomes and serve as controls.

In addition to laboratory confirmation of sandfly transmission, there is strong circumstantial evidence that the trypanosome is transmitted only by sandflies in nature. Although *B. boreas* is common in California, infected toads are being reported now for the first time. These toads were found in a rural Upper Sonoran woodland where both the California ground squirrel and *P. vexator occidentis* were present. Like all known western North American phlebotomines, this sandfly occurs only in association with relatively undisturbed rodent colonies (*1*, *8*). It is apparently very susceptible to encroaching civilization, especially when ground squirrel populations are controlled or eliminated. The resulting paucity of an insect vector in areas where toads are most likely to be collected would ex-

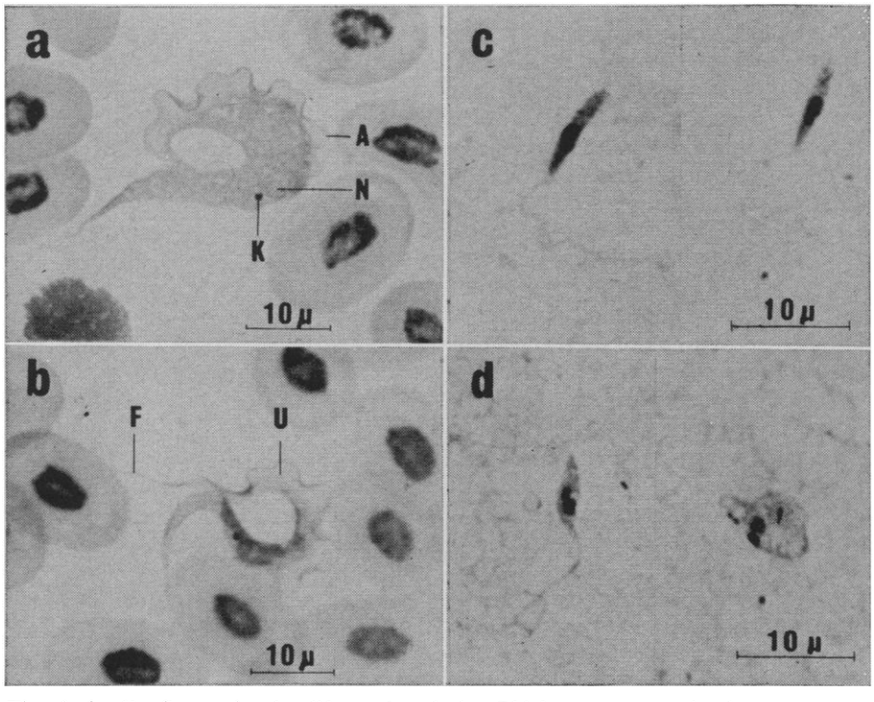

Fig. 1 (a–d). Stages in the life cycle of the *Phlebotomus*-transmitted trypanosome of *Bufo boreas*. (a) Broad trypomastigote form, the rectangular kineoplast (*K*) and the nucleus (*N*) are always juxtaposed in the middle third of the body; (*A*) double appearing axoneme. (b) A narrow trypanosome from the same toad; (*F*) free anterior flagellum; (*U*) undulating membrane. Average measurements of 30 trypanosomes include: total length, 65.3 μ; posterior of body to nucleus, 25.2 μ; nucleus to anterior of body, 25.7 μ; free flagellum, 14.4 μ; and width at nucleus, 5.1 μ. (c) Promastigote (leptomonad) flagellates from the hindgut of *P. vexator occidentis* 3 days after feeding on the same toad as in (a). (d) Forms from a 2-week-old culture.

plain the trypanosome not having been found previously.

The trypanosome found in *B. boreas* appears specific for toads. Intraperitoneal inoculations of 0.5 ml of culture overlay produced infections in all of five uninfected toads from outside the study area. Trypanosomes were not seen in wet smear mounts until 3 weeks after inoculation and parasitemias remained low. Similar inoculations failed to infect one *Sceloporus occidentalis*, two *Rana aurora*, two *R. catesbiena*, three *R. pipiens*, and two *Hyla regilla*.

Preliminary studies suggest that the incidence of infection of toads increases with age (*9*). The overall low incidence of infection in toads parallels the previously determined low infection rates in wild-caught sandflies (*1*).

Discovery of this host-parasite-vector system in California raises the possibility that similar systems involving *Leishmania* species may also be present here, and it also should assist in clarifying interpretative problems associated with infections of wild-caught sandflies in the Americas and elsewhere. For example, the high rates of infection with flagellates in wild-caught sandflies in Panama, especially the large proportion with hindgut infections (*10*), may be attributable to infection with nonmammalian parasites (species of either *Leishmania* or *Trypanosoma*). Previous explanations implied that these sandflies were infected with mammalian *Leishmania* (*11*). However, all evidence so far indicates that posterior station infections in sandflies are not associated with *Leishmania* of man (*12*). As the hindgut forms of the *B. boreas* trypanosome are morphologically similar to those of various *Leishmania* species in their vectors, the relationships of anuran, reptilian, and mammalian flagellates should be considered in epidemiological studies of leishmaniasis.

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Neuronal Perikarya of Rat Brain Isolated by Zonal Centrifugation

Abstract. *Cerebral cortex and hippocampus of rat were disrupted in a specially designed tissue-press and prepared as a 2 percent suspension in 10 percent buffered Ficoll medium and fractionated by density-gradient centrifugation in the B XIV zonal rotor. The suspension was injected into a buffered 30 percent Ficoll, 58 percent sucrose discontinuous gradient previously loaded in the B XIV zonal rotor spinning at 3500 revolutions per minute. Intact neuronal perikarya were recovered as a discrete band in the dense sucrose zone of the gradient after centrifugation at 35,000 revolutions per minute for 45 minutes.*

Among the many techniques used to isolate nerve cells from glial cells (*1*) the microdissection methods of Hyden (*2*) and Lowry (*3*) and the sieving-fishing method by Roots and Johnston (*4*) can provide pure neuronal perikarya, but not in numbers sufficient for macroscale chemical characterization. More recently methods have been described in which density-gradient and swinging-tube ultracentrifugation techniques are used (*5*). We now report a technique by which brain tissue is disrupted with a tissue-press. Intact neurons and glial cells can be separated in the B XIV zonal ultracentrifuge head

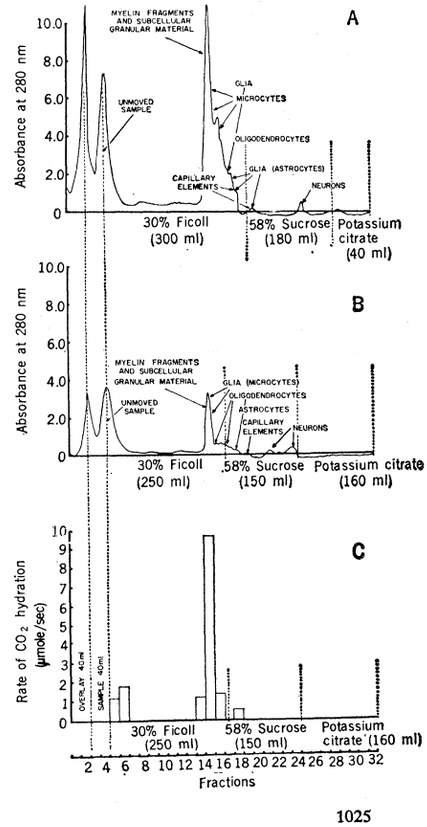

Fig. 1. Rate-zonal separation of cerebral cortex and hippocampus tissue of rat. (A) Separation of cerebral cortex after centrifugation at 53,840 × 10^6 $\omega^2 t$, including acceleration and deceleration. (B) Separation of hippocampus tissue after centrifugation at 50,002 × 10^6 $\omega^2 t$, including acceleration and deceleration. (C) Occurrence of net carbonic anhydrase activity (nonenzymatic hydration of CO_2, 1.85 μmole sec^{-1}, is subtracted) in fractions of separated rat hippocampus tissue of Fig. 1B. The serially displaced gradient 20-ml fractions monitored at 280 nm were recorded on linear scale chart paper with a recorder sensitivity setting of 0 to 12.5 units (that is, 0 to 100 percent absorbance at 280 nm was equivalent to 0 to 12.5 optical density units on the chart). Peak 1 contains fragmented myelin and amorphous subcellular constituents of remaining unmoved sample. Peak 2 contains glial constituents (microcytes, oligodendrocytes, and astrocytes) separating centrifugally in the 30 percent Ficoll zone with the larger astrocytes and capillary elements of brain penetrating into the 58 percent sucrose zone. Peak 3 contains intact neuronal perikarya banding discretely from peak 2, in the 58 percent sucrose zone.