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5. Supported by AF-AFOSR 877-65. I thank B. Hamilton for his help in designing and for constructing the assay apparatus, and R. Reynolds, NSF undergraduate research participant, for his preliminary studies that led to this work.

6 January 1969; revised 17 March 1969

Leishmania braziliensis Isolated from Sloths in Panama

Abstract. *Two edentates, the two-toed sloth* Choloepus hoffmanni *and the three-toed sloth* Bradypus infuscatus, *infected with* Leishmania *were found in Panama. The rates of infection were 14.1 and 1.3 percent in* Choloepus *and* Bradypus, *respectively.* Leishmania braziliensis sensu lato *was cultured from skin, blood, spleen, liver, or bone marrow of 13 sloths often from two or more tissues from the same animal. This strain is indistinguishable from* Leishmania *strains isolated from humans in Panama.*

As part of a study of human cutaneous leishmaniasis in the Republic of Panama, the local mammalian fauna has been investigated in the search for reservoir hosts. Until now natural infections of leishmaniasis have been reported (*1*, *2*) in six different genera of forest mammals belonging to the orders Rodentia, Carnivora, and Primates. Natural leishmanial infections have been demonstrated with relative frequency in sloths (order Edentata).

The first case found was a three-toed sloth, *Bradypus infuscatus* Wagler, collected in an area from which human cutaneous leishmaniasis has not been reported for several years. A promastigote (leptomonad) flagellate was cultured from the heart blood, liver, spleen, and from one out of three samples of skin. All four cultures, both original isolations and transfers at the third passage, were intradermally inoculated on the noses of 18 golden hamsters. One died within a few days; the others became infected. Within 2 to 3 weeks after inoculation, a swelling typical of experimental cutaneous leishmaniasis in these animals appeared at the site of inoculation (Fig. 1), from which the tissue stage or amastigote form of the parasite (Leishman-Donovan body) was demonstrated in great numbers. Both swelling of the hamsters' noses and morphology of the parasite in stained smears were indistinguishable from those obtained under similar experimental conditions with Panamanian strains of *Leishmania braziliensis sensu lato* isolated from humans.

From November 1967 to June 1968, 162 wild sloths were examined. This includes 77 (38 females, 39 males) *B. infuscatus* and 85 (31 females, 54 males) two-toed sloths *Choloepus hoffmanni* Peters from central Panama.

Usually within a week of their capture, sloths were bled from the heart in order to make blood cultures; at this time thick and thin blood smears were made in most cases; part of the blood was kept without anticoagulant in order to obtain clot cultures later. After bleeding, 2 or 3 samples of skin were obtained by biopsy and cultured (*2*). The nose, jaw, and ears were the main sites of skin biopsies; ear biopsy involved the edge of the pinna as well as skin. During autopsy, cultures were made from liver and spleen, and in certain cases, also from bone marrow. Every strain of hemoflagellate cultured was examined with phase-contrast illumination with attention given especially to the morphology and motility of parasites. At least two hamsters were inoculated on the nose with about 15×10^6 cultural forms of each strain, or substrain, in cases where more than one positive culture was obtained. After they were inoculated, hamsters were observed during a period of a few weeks for swelling on the nose. Skin smears were made from animals with such swellings about day 20, and later in those hamsters that showed no gross indications of infection; in these cases, several skin smears were usually made. No animals with negative skin smears were considered to be uninfected until one or two skin cultures were examined from the site of inoculation.

From 24 *C. hoffmanni* and three *B. infuscatus*, promastigote flagellates were cultured in 13, 11, 9, 8, and 2 animals from blood, spleen, liver, skin, and bone marrow, respectively. Positive cultures made from different tissues in the same animal were frequent. Among the 27 isolates we can distinguish the following: (i) A rather polymorphic promastigote flagellate that infects hamsters when it is inoculated intradermally on the nose was isolated from 13 sloths, 12 out of 85 *C. hoffmanni* (14.1 percent) and one out of 77 *B. infuscatus* (1.3 percent). This parasite is considered to be *L. braziliensis s. lat.*, since all 13 isolates are indistinguishable, both morphologically and by their infectivity in the hamster, from the etiological agent of human cutaneous leishmaniasis in Panama. The other 14 isolates, which failed to infect hamsters, and which seem to include at least two different parasites, are still under study. (ii) One of them, isolated from 11 sloths (10 *C. hoffmanni*, 1 *B. infuscatus*) and mostly from blood, is a promastigote somewhat larger than *L. braziliensis*. Some strains frequently show in culture individuals with a long slender elongation of the posterior end. A similar feature was described by McConnell (*3*) in connection with promastigotes cultured from the gut of Panamanian phlebotomine sandflies. (iii) The three remaining strains were cultured only from blood clots. One, from *C. hoffmanni*, is morphologically similar to *Leishmania*; another flagellate from a specimen of each sloth species is markedly different morphologically from any of the other promastigotes isolated from sloths.

The sloths found naturally infected with *Leishmania* were of both sexes and different ages. Although in no case was there any gross sign of infection, the skin of 8 out of 13 sloths yielded positive cultures. In one case the parasite was isolated not only from liver and spleen, but also from all three samples of skin that were cultured; similarly, in another animal results were positive in two skin cultures, as well as in the spleen culture. In two more animals, two out of three skin cultures were positive.

Fig. 1. Characteristic swelling of hamster's nose as a result of intradermal inoculation with a sloth strain of *Leishmania braziliensis s. lat.*

Infected animals came from several localities in the provinces of Panama and Colon on both sides of the Isthmus, and from the Canal Zone. In some of these localities human leishmaniasis has almost disappeared during the last few years, mainly because of deforestation, while in other areas the forest is still untouched and human infections are frequent when man enters the jungle.

The two-toed sloth has a higher rate of infection with *L. braziliensis* (14.1 percent) than any forest mammal so far found infected in Panama. Moreover, infection has been demonstrated consistently throughout the period of study, which indicates the potential importance of this edentate in the epidemiology of cutaneous leishmaniasis.

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28 February 1969; revised 27 March 1969

Acetylcholine Receptor: Covalent Attachment of Depolarizing Groups at the Active Site

Abstract. *Following reduction of the acetylcholine receptor in the electroplax with dithiothreitol, the quaternary ammonium compounds bromoacetylcholine bromide and the* p-*nitrophenyl ester of* (p-*carboxyphenyl*)*trimethylammonium iodide react near the active site probably with a sulfhydryl group. The covalently attached quaternary ammonium moieties additionally interact with the active site noncovalently to activate the receptor and cause depolarization of the cell.*

The acetylcholine receptor transduces the binding of acetylcholine into a permeability change. Although the detailed mechanism is unknown, the phenomenology has been extensively studied. One class of ligands, acetylcholine and its congeners, reversibly activate the receptor, while another class, including compounds such as *d*-tubocurarine, competitively inhibit activation. The electroplax of the electric eel, *Electrophorus electricus*, has been a useful preparation for quantitating these phenomena (*1–4*). It has been possible, using this same preparation, to modify the receptor chemically *in situ* and in this way to infer some of its properties (*5*). The receptor appears to contain both sulfhydryl and disulfide groups (*5*), which supports the early suggestion that the receptor is a protein (*6*).

The presence of a disulfide group on the receptor is indicated by the following evidence. Brief application of a low concentration of the reducing agent dithiothreitol to the innervated membrane of the electroplax causes subsequent inhibition of depolarization by receptor activators. This inhibition is completely reversed by application of an oxidizing agent such as 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB); if, however, an alkylating agent such as *N*-ethylmaleimide is applied after reduction, the inhibition can no longer be reversed by DTNB. The demonstration that a quaternary ammonium maleimide derivative, 4-(*N*-maleimido)phenyltrimethylammonium iodide (MPTA), alkylates the sulfhydryls formed by reduction, three orders of magnitude faster than either its tertiary amine analog or *N*-ethylmaleimide, is strong evidence for the location of the reducible disulfide bond on the receptor and in fact close to the active site (*7*). This large enhancement of rate, characteristic of *affinity labels* (*8*), is presumably due to the quaternary ammonium group binding reversibly to the negative subsite of the active site bringing one of the reactive ethylenic carbons of the maleimide group in approximate juxtaposition to one of the sulfhydryls of the receptor. This increases manyfold the probability of a successful collision. Additional evidence that MPTA is covalently bound near the active site is that upon reaction MPTA causes a small depolarization (approximately 1 mv) which is not reversed when the unreacted MPTA is washed out (*7*). Of three other closely related and approximately equally reactive quaternary ammonium maleimide derivatives, one depolarizes upon reaction slightly more than MPTA (approximately 2 mv) and two cause no depolarization (*9*). A compound of another type, *p*-(trimethylammonium)benzenediazonium fluoborate, has been reported to be an affinity label of the acetylcholine-receptor in the electroplax and not to cause any depolarization upon reaction (*10*). We report here two quaternary ammonium compounds which, like the maleimide derivatives, react covalently only with the reduced receptor. The resultant covalently attached quaternary ammonium groups interact with the active site causing in both cases a considerably greater depolarization than that obtained with the other compounds.

Bromoacetylcholine bromide (BAC) is an analog of acetylcholine which will react with nucleophiles substituting for bromide on the α-carbon of the acetyl group. BAC is also a substrate for acetylcholinesterase, as was previously reported (*11*), and in the following experiments BAC was applied to the electroplax in the presence of 50 μM eserine to inhibit the endogenous cholinesterase. Added to the innervated side of the electroplax, BAC acts as a receptor activator (Fig. 1). Repetitive application of BAC seems to have no irreversible effect on the electroplax. Following reduction of the receptor by dithiothreitol, application of BAC results in a response somewhat smaller than the response before dithiothreitol; however, the response is only partially reversed after BAC is washed out (Fig. 1). Furthermore, if the dithiothreitol-treated electroplax is treated with *N*-ethylmaleimide to alkylate the sulfhydryls formed by reduction, then BAC added subsequently still depolarizes, but in this case completely reversibly. Finally, the portion of the depolarization caused by the application of BAC to the dithiothreitol-treated electroplax which is not reversed by prolonged washing is reversed by $10^{-4}M$ *d*-tubocurarine (Fig. 1). With the removal of the *d*-tubocurarine the cell depolarizes again. We conclude that unreacted BAC is an activator of the receptor, that BAC reacts in the vicinity of the active site of the reduced receptor probably with a sulfhydryl group, and that the quaternary ammonium group of the covalently bound moiety interacts reversibly with the negative subsite activating the receptor and causing depolarization of the membrane. The reversible portion of the depolarization following BAC addition to the dithiothreitol-treated electroplax is then presumably due to BAC reversibly binding to unreduced receptors.

The second compound, the *p*-nitrophenyl ester of (*p*-carboxyphenyl)trimethylammonium iodide (NPTMB), is an active ester which can acylate a nucleophile such as a sulfhydryl group, releasing *p*-nitrophenol. Added to the innervated membrane of the electroplax, $10^{-4}M$ NPTMB (Fig. 2) elicits a barely detectable depolarization of about