VIABLE LEISHMANIA DONOVANI IN NASAL AND ORAL SECRETIONS OF PATIENTS WITH KALA-AZAR AND THE BEARING OF THIS FINDING ON THE TRANSMISSION OF THE DISEASE

BY CLAUDE E. FORKNER, M.D., AND LILY S. ZIA, M.D.

(From the Department of Medicine, the Peiping Union Medical College, Peiping, China)

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Kala-azar is endemic in large areas of China, India and the Mediterranean basin. The most important unsolved problem in this disease is to find its natural mode or modes of transmission. This subject has been studied for 30 years by numerous workers both independently and as members of various kala-azar commissions. Much useful information has been accumulated, but no solution to the problem has been found.

The most favored theory is that the disease is transmitted by the bite of an insect. Numerous insects have been suspected, among them bed bugs, lice, ticks, mosquitoes and sand-flies. Of these the sand-fly of the genus Phlebotomus seems best to fulfill the requisites as an intermediate host. This insect readily becomes infected when feeding on cultures of Leishmania donovani or on patients or animals suffering from kala-azar; the parasites multiply and develop into flagellates in the intestine of the fly, and eventually there may occur massive infection of the fore gut and pharynx of the phlebotomus. There exist many other facts which suggest that the sand-fly is the transmitting agent of the disease but the practical experience of many workers using hundreds of thousands of sand-flies, many thousands of susceptible animals and not a few human volunteers has failed to confirm this theory of transmission. In only one animal has kala-azar been thought to have been transmitted by the bite of the sand-fly and in this one instance the evidence is not conclusive that the disease was actually transmitted by the bite of the insect (1).

The next most favored theory is that of direct infection through contact with infective material. It has been shown that both the urine and feces of patients suffering from kala-azar may at times contain viable leishmania. This mode of contaminative infection has been investigated by Shortt and his associates. They failed to infect any of thirty-two highly susceptible animals (Chinese hamsters)
by feeding them repeatedly over a long period of time on the deposit from the centri-
 trifuged urine of untreated cases of active kala-azar (2). They succeeded in in-
festing only one of thirty-two hamsters by feeding each animal repeatedly, over a
long period of study, with the feces of hamsters and of patients suffering from
kala-azar (3). These studies suggest that it is possible although not probable
that the disease may be transmitted by contamination with the urine and feces of
certain infected individuals without reference to the presence of an intermediate
host. There exist no data in the medical literature on kala-azar concerning studies
of other external excretions or secretions of the body.

It is common knowledge that the Leishman-Donovan bodies in an
individual suffering from kala-azar exist in many organs of the body,
most abundantly in the mesenchymal macrophages (clasmatocytes, reticulo-endothelial cells, histiocytes) which are found in the adventi-
tial coat of blood vessels, in the common connective tissues, and most
abundantly in the spleen, bone marrow, liver and lymph nodes. The
parasites may at times be found free in the tissues or may be engulfed
by polymorphonuclear or mononuclear leucocytes of the blood. Nu-
merous investigators have shown that a few parasites can be found
in the leucocytes of the blood in a large proportion of patients.

These facts, the known presence of leishmania in lymph nodes and
in blood cells, suggested to us a new approach to the problem of the
transmission of kala-azar. It is a well known fact that the disap-
pearance of leucocytes from the blood stream is accomplished, at
least to a considerable degree, by the pouring out of these cells into
the gastrointestinal tract and onto the surfaces of mucous mem-
branes. It is also known that certain structures, the tonsils and
adenoids, in the upper alimentary and respiratory tracts are lymp-
phoid organs, lymph nodes possessing a more or less specialized func-
tion by virtue of their anatomical position.

It is an interesting fact, indeed an almost unbelievable one, that the
nasal and pharyngeal passages have not been searched for the presence
of leishmania. There exist no records in the medical literature on
kala-azar of the examination of the tonsils or adenoids or of the exu-
date from these organs, for the presence of leishmania. The reason
for the lack of interest in this phase of the subject becomes apparent
as one reads the papers of and talks with the workers in this field.
There have existed certain axioms regarding the parasite and its trans-
mission which have militated against the theory that the parasites,
### TABLE I

Data concerning Patients with Kala-Azar in Whom the Nasal Contents Were Examined for Presence of Leishmanias

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs.)</th>
<th>Sex (M/F)</th>
<th>Duration of symptoms (mos.)</th>
<th>Duration of symptoms (cm.)</th>
<th>Edge of spleen below left costal margin (cm.)</th>
<th>R.B.C. per c.mm.</th>
<th>Hemo-globin per 100 cc.</th>
<th>W.B.C. per c.mm.</th>
<th>Leishman-Donovan bodies in nasores from Spleen or liver</th>
<th>Globulin test on blood</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>P94</td>
<td>24</td>
<td>M</td>
<td>1.5</td>
<td>11.0</td>
<td>3.71</td>
<td>10.0</td>
<td>4,850</td>
<td></td>
<td>Moderate number</td>
<td>Pos.</td>
<td>+++</td>
</tr>
<tr>
<td>P95</td>
<td>26</td>
<td>M</td>
<td>1.5</td>
<td>9.0</td>
<td>3.05</td>
<td>9.2</td>
<td>2,500</td>
<td></td>
<td>Moderate number</td>
<td>Neg.</td>
<td>+++</td>
</tr>
<tr>
<td>P96</td>
<td>61</td>
<td>F</td>
<td>2</td>
<td>11.0</td>
<td>2.40</td>
<td>7.6</td>
<td>1,350</td>
<td></td>
<td>Moderate number</td>
<td>Neg.</td>
<td>+++</td>
</tr>
<tr>
<td>P97</td>
<td>11</td>
<td>M</td>
<td>1.5</td>
<td>6.0</td>
<td>2.24</td>
<td>7.8</td>
<td>1,500</td>
<td></td>
<td>Many</td>
<td>Pos.</td>
<td>+++</td>
</tr>
<tr>
<td>P98</td>
<td>39</td>
<td>M</td>
<td>1.5</td>
<td>11.0</td>
<td>4.03</td>
<td>8.2</td>
<td>2,200</td>
<td></td>
<td>Few</td>
<td>Neg.</td>
<td>+++</td>
</tr>
<tr>
<td>P78</td>
<td>20</td>
<td>M</td>
<td>1.5</td>
<td>23.0</td>
<td>3.95</td>
<td>8.8</td>
<td>2,100</td>
<td></td>
<td>Many</td>
<td>Pos.</td>
<td>+++</td>
</tr>
<tr>
<td>P84</td>
<td>8</td>
<td>M</td>
<td>10</td>
<td>4.5</td>
<td>4.15</td>
<td>9.4</td>
<td>4,950</td>
<td></td>
<td>Few</td>
<td>Neg.</td>
<td>+++</td>
</tr>
<tr>
<td>P99</td>
<td>20</td>
<td>M</td>
<td>3</td>
<td>8.0</td>
<td>3.24</td>
<td>7.4</td>
<td>9,100</td>
<td></td>
<td>Moderate number</td>
<td>Pos.</td>
<td>+++</td>
</tr>
<tr>
<td>P87</td>
<td>8</td>
<td>F</td>
<td>7</td>
<td>16.5</td>
<td>2.77</td>
<td>7.5</td>
<td>3,500</td>
<td></td>
<td>Few</td>
<td>Pos.</td>
<td>+++</td>
</tr>
<tr>
<td>P89</td>
<td>4</td>
<td>F</td>
<td>17</td>
<td>16.5</td>
<td>3.58</td>
<td>7.9</td>
<td>4,400</td>
<td></td>
<td>Moderate number</td>
<td>Neg.</td>
<td>+++</td>
</tr>
<tr>
<td>P102</td>
<td>38</td>
<td>M</td>
<td>12</td>
<td>7.0</td>
<td>2.22</td>
<td>6.8</td>
<td>4,500</td>
<td></td>
<td>Many</td>
<td>Pos.</td>
<td>+++</td>
</tr>
<tr>
<td>P104</td>
<td>4</td>
<td>M</td>
<td>6</td>
<td>6.5</td>
<td>3.50</td>
<td>9.5</td>
<td>2,800</td>
<td></td>
<td>Moderate number</td>
<td>Pos.</td>
<td>+</td>
</tr>
<tr>
<td>P105</td>
<td>14</td>
<td>M</td>
<td>10.5</td>
<td>22.0</td>
<td>2.40</td>
<td>6.0</td>
<td>1,800</td>
<td></td>
<td>Many</td>
<td>Pos.</td>
<td>+++</td>
</tr>
<tr>
<td>P107</td>
<td>26</td>
<td>M</td>
<td>14</td>
<td>10.0</td>
<td>2.84</td>
<td>8.7</td>
<td>2,260</td>
<td></td>
<td>Few</td>
<td>Neg.</td>
<td>+++</td>
</tr>
<tr>
<td>P108</td>
<td>27</td>
<td>M</td>
<td>18</td>
<td>17.0</td>
<td>3.10</td>
<td>9.8</td>
<td>1,900</td>
<td></td>
<td>Many</td>
<td>Pos.</td>
<td>++</td>
</tr>
</tbody>
</table>

*These measurements were from the mid-clavicular line at the left costal margin to the tip of the spleen.
LEISHMANIA DONOVANI IN KALA-AZAR

particularly viable ones, could exist in the secretions or on the surfaces
of mucous membranes. Because the parasite is in one of the stages of
its life cycle a flagellate, and is closely related to the parasite of Afri-
can trypanosomiasis, known to be transmitted by the tsetse fly, it
has been assumed by many workers, notwithstanding some evidence
to the contrary, that an insect intermediate host is essential for trans-
mision. Another general conception, and in our opinion a false one,
is that the parasite of kala-azar is a delicate organism not capable
of surviving in the presence of bacilli and cocci and unable to resist
much change in its chemical or physical environment.

EXPERIMENTAL

During the last 5 months we have examined material obtained by
gently passing ordinary culture swabs into the nasal passages of fifteen
patients suffering from proved kala-azar and then smearing slides
with the material so obtained on the swab. In nine of these fifteen
cases we have found in the smears typical Leishman-Donovan bodies
in small numbers. Some of the preparations revealed the leishmania
readily after a few minutes of search whereas others required very
careful study over periods of from 20 minutes to 2 hours before organ-
isms were demonstrated. The parasites were in all respects identical
with those obtained by means of puncture of the spleen or liver of the
patients, which procedure was carried out in each case. The Leishman-
Donovan bodies usually were extracellular although occasional ones
were demonstrated within polymorphonuclear neutrophiles. The
parasites existed in the presence of many contaminating bacilli, cocci
and in some instances spirochetes. The Leishman-Donovan bodies
were present also in the ordinary mucus or semipurulent material
blown from the nose by the patients and existed independent of any
bleeding or demonstrable mucous membrane lesions. Table I gives
some of the data concerning the fifteen patients examined.

In one instance (Case P78) a swab lightly passed over the surface of
the tonsil and then onto a slide demonstrated that the surface of the
tonsil was the residence of significant numbers of typical Leishman-
Donovan bodies. The saliva of this patient also contained a few leish-
mania. The patient received 2.3 gm. of neostibosan in a period of 29
days but died of complicating pyogenic infection. At autopsy the
tonsils were examined. Smears from them revealed massive infection. In some areas the smears contained many hundreds of leishmania in each oil immersion field. The section of the tonsil confirmed the impression gained from the smear. Many macrophages laden with parasites were in and under the mucous membrane. A small ulcerated area of the mucous membrane contained innumerable bacteria in polymorphonuclear cells and macrophages and in addition many leishmania both free and intracellular were in the exudate of the ulcer. Many parasites were scattered throughout the whole tonsil chiefly concentrated in macrophages in the lymph cords and in the germinal centers of the follicles.

Having proved that the parasites were commonly present in the nasal and oral secretions the next step in our investigation was to ascertain whether or not these leishmania were viable. The proof of this point in two cases was accomplished by injecting intraperitoneally into hamsters emulsions, in normal salt solution, of the exudate blown by the patients from the nose into sterile Petri dishes.

The hamsters were followed and, at the time of death or when sacrificed, smears were made from the spleen, liver and in some instances from lymph nodes. These smears were subsequently stained with Wright's stain and examined microscopically. Pieces of liver, spleen, lymph nodes, bone marrow, lung, kidney and occasionally of other organs were fixed in Zenker-formol (formalin 10 per cent) and after sectioning, stained with hematoxylin and eosin. The results of these studies are shown in Table II.

For the first case (No. P94) twelve normal hamsters of one lot were selected. Five of the animals were inoculated with 1.0 cc. of the emulsion. Seven were held as control animals. Of the five injected animals two died 14 days after injection and at autopsy showed no demonstrable cause of death and no leishmania in smears or sections of the spleen, liver and lymph nodes. These two animals did not live long enough for the experiment to be satisfactory. The remaining three injected animals were sacrificed at the end of 79, 93 and 93 days respectively. In two of these animals the spleen was slightly enlarged. Other than this no gross abnormalities were found. Two of the three inoculated animals which lived long enough for a satisfactory experiment showed in the smears and in sections of the liver and spleen at autopsy numerous leishmania. In one of these animals the peripheral lymph nodes were also infected, whereas the lymph nodes of the other animal were not examined.

Four of the seven control animals died spontaneously 10, 19, 21 and 21 days respectively after the beginning of the experiment. In one of these postmortem changes were marked and no tissues were examined. The tissues of the other three
### TABLE II

Data concerning Intraperitoneal Inoculations into Chinese Hamsters of Emulsions in Normal Saline of Nasal Discharge from Two Patients with Kala-Azar

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Animal No.</th>
<th>Inoculated or control</th>
<th>Amount of emulsion injected</th>
<th>Duration of experiment</th>
<th>History of animal</th>
<th>Presence or absence of leishmaniasis in smears of spleen</th>
<th>Presence or absence of leishmaniasis in sections of spleen</th>
<th>Presence or absence of leishmaniasis in liver</th>
<th>Presence or absence of leishmaniasis in sections of liver</th>
<th>Presence or absence of leishmaniasis in lymph nodes</th>
<th>Presence or absence of leishmaniasis in sections of lymph nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A23-H3</td>
<td></td>
<td>Control</td>
<td>None</td>
<td>19</td>
<td>Found dead</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A25-H5</td>
<td></td>
<td>Control</td>
<td>None</td>
<td>21</td>
<td>Found dead</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A28-H8</td>
<td></td>
<td>Inoculated</td>
<td>1</td>
<td>93</td>
<td>Killed</td>
<td>Pos.</td>
<td>Pos.</td>
<td>—</td>
<td>Pos.</td>
<td>Pos.</td>
<td>—</td>
</tr>
<tr>
<td>A32-H12</td>
<td></td>
<td>Control</td>
<td>None</td>
<td>10</td>
<td>Found dead</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A33-H13</td>
<td></td>
<td>Inoculated</td>
<td>1</td>
<td>1</td>
<td>Found dead</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A34-H14</td>
<td></td>
<td>Inoculated</td>
<td>1</td>
<td>45</td>
<td>Killed</td>
<td>Neg.</td>
<td>Neg.</td>
<td>Pos.</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A35-H15</td>
<td></td>
<td>Inoculated</td>
<td>1</td>
<td>Incomplete</td>
<td>Living</td>
<td>Incomplete</td>
<td>Incomplete</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A36-H16</td>
<td></td>
<td>Inoculated</td>
<td>1</td>
<td>Incomplete</td>
<td>Living</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A37-H17</td>
<td></td>
<td>Inoculated</td>
<td>1</td>
<td>Incomplete</td>
<td>Living</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A39-H19</td>
<td></td>
<td>Control</td>
<td>None</td>
<td>Incomplete</td>
<td>Living</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A40-H20</td>
<td></td>
<td>Control</td>
<td>None</td>
<td>Incomplete</td>
<td>Living</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A41-H21</td>
<td></td>
<td>Control</td>
<td>None</td>
<td>Incomplete</td>
<td>Living</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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</tr>
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</table>
showed no leishmania when either smears or sections were examined. The three remaining control animals were sacrificed at the end of 82, 93 and 93 days respectively and no leishmania were demonstrable in the tissues.

For the second case (No. P78) nine normal hamsters of the same lot were divided into two groups, one of five which were inoculated and one of four held as controls. The five inoculated animals each received 1 cc. of emulsion of the nasal discharge of the second patient. Of these five, one died within 24 hours after inoculation. At autopsy diffuse peritonitis was found. The tissues were not examined microscopically. One of the remaining four inoculated animals was killed 45 days after the beginning of the experiment. Smears of tissue from the spleen and liver showed no leishmania but the peripheral lymph nodes contained numerous typical leishmania. The remaining three inoculated animals are still living and will be examined later at a more satisfactory time for terminating the experiment. One of the four control animals was killed 48 days after the beginning of the experiment but no leishmania could be found in smears of the spleen, liver or lymph nodes. The remaining control animals are still living.

The results of our investigations have proved for the first time that leishmania can be demonstrated in the secretions of the nose or oral cavity or both in a large proportion of patients suffering from kala-azar, and that in two such patients, the only ones in which studies are complete, these leishmania were viable and capable of producing infection in susceptible animals.

The literature on experimental kala-azar contains numerous reports of the disease having been experimentally produced in hamsters, monkeys, mice, dogs and other animals by feeding with the infected tissues of man or animals or with feeding of cultures of the parasite. Indeed in one instance in man infection was believed to have been produced by the accidental sucking into the mouth of infected material (4).

There exists, therefore, a series of facts which strongly support the theory of the transmission of the disease from person to person by way of the upper respiratory and alimentary tracts. This evidence consists of the facts enumerated above and is recapitulated as follows: (1) A rich source of infective material is present in the discharges from the nose and mouth of patients suffering from kala-azar. (2) Animals and presumably man can be infected readily by the ingestion of infective material.

The only point which now needs to be demonstrated to confirm the finding that one of the natural modes, possibly the most important
natural mode, for the transmission of kala-azar from person to person, by way of the upper respiratory and alimentary tracts, is actually to transmit the disease from an infected to a normal individual by these routes.

This experiment has been approached in two ways, by transferring infected material from the nasal cavities of patients to the nasal and mouth cavities of hamsters and to the nasal cavities of two human volunteers. It is still too early to report the results of these experiments.

The concept of the transmission of kala-azar from man to man by way of the upper respiratory and alimentary tracts suffices to explain many of the epidemiological problems in the disease. There is no serious objection to this concept either from the protozoological or the epidemiological point of view. A thorough consideration of these aspects of the problem will be given elsewhere.

**SUMMARY AND CONCLUSIONS**

1. Smears from the nasal cavities of fifteen patients suffering from kala-azar have been examined and in nine of these typical Leishman-Donovan bodies have been found.

2. Smears from the surface of the tonsil and from the saliva in one of the above nine cases showed the presence of leishmania. The tonsils of this patient, who died as the result of kala-azar and secondary infection, at autopsy were shown to be massively infected with Leishman-Donovan bodies.

3. Leishmania in the nasal discharge of two patients were shown by inoculation into susceptible animals to be viable and capable of producing infection. Sufficient time has not elapsed to determine the viability of the organisms from the remaining cases.

4. These experiments show for the first time that a rich source of infective material from a large proportion of patients with kala-azar is available for direct transmission of the disease.

5. Strong evidence is presented as a basis for the concept that one of the natural modes, perhaps the most important natural mode, of transmission of kala-azar is from person to person by way of the upper respiratory and alimentary tracts.

6. Two normal human volunteers and numerous normal experimen-
tal animals have been inoculated into the nasal and oral cavities with the nasal discharge, known to contain leishmania, from patients with kala-azar. The results of these experiments will be reported at a subsequent date.

BIBLIOGRAPHY