ner frequency is less than the measurement frequency (corresponding to a period of 20 seconds for M_s), M_s becomes a very slowly changing measure of earthquake size.

The net result of these two effects is that there is likely to be an upper limit to M_s that will be observed, regardless of the true earthquake size. Clearly, it would be advantageous to measure the size of large earthquakes by some frequency-independent quantity. There is such a quantity, and it is called the seismic moment, M_0. It is related to the zero frequency asymptote of the seismic spectrum, and in geometrical terms it is the product of the fault area multiplied by the fault displacement multiplied by the rigidity modulus (*7*).

There are, as yet, relatively few earthquakes for which we have been able to obtain enough information to calculate M_0. Certainly we have no way to directly plot a frequency-M_0 graph. However, a series of recent papers have provided enough data to permit us to begin to establish the shape of the relationship between M_s and M_0 (*8*, *9*). Figure 2 shows 87 determinations of M_s plotted as a function of M_0, for M_s greater than 5.0. Although the scatter is considerable, the general shape of the relationship seems clear, and a provisional curve has been sketched through the data. A roughly linear trend persists up to an M_s of 7.0 or 7.5, and then the curve bends sharply upward. The highest point (Chilean earthquake of 1960) and the next highest (Alaskan earthquake of 1964) have been studied extensively (*9*) and appear quite reliable. There is a definite suggestion that the curve may become vertical in the vicinity of $M_s = 8.6$ or 8.7, an indication that M_s values greater than this will not be observed, regardless of the value of M_0.

We can use the relationships in Figs. 1 and 2 to make the first attempt at the empirical construction of a frequency-M_0 curve. As shown in Fig. 3, a remarkably linear frequency-M_0 relation is obtained. We have, as yet, no convincing theoretical arguments that predict that this relation should be linear, although a linear relation was postulated by Wyss (*10*). Interestingly, the slope of the line in Fig. 3 (0.61) is identical to the slope of the corresponding relation for the aftershock sequence of the Parkfield earthquake (*10*), although this agreement may be coincidence. Perhaps we should phrase our result as follows: A linear frequency-M_0 relation is entirely consistent with the general shape of frequency-M_s data, given recent observations of M_0 as a function of M_s. It is also consistent with observations of m_b (body wave magnitude measured at a period of 1 second) [a detailed discussion of this point will be presented elsewhere (*11*)].

We may use Fig. 3 to make estimates of the frequencies of earthquakes of various M_0 values. It appears, for example, that events with M_0 of 10^{30} dyne-cm or greater occur, on the average, every 10 years or so, and this is entirely consistent with the observation that two large earthquakes with well-determined M_0 values have occurred within the past 15 years. Apparently, events such as the 1960 Chile earthquake ($M_0 = 2.5 \times 10^{30}$ dyne-cm) and the 1964 Alaska earthquake ($M_0 = 7.5 \times 10^{29}$ dyne-cm) are not at all unusual.

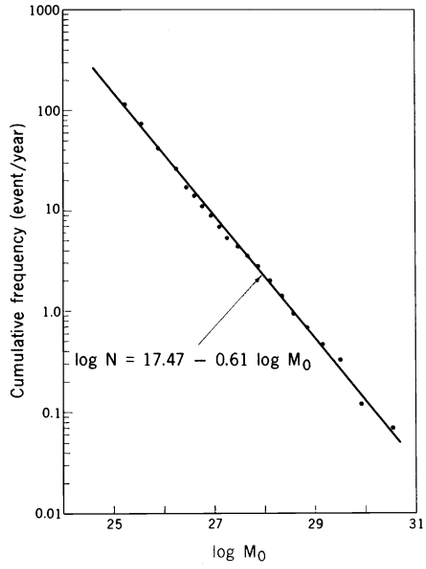

Fig. 3. Cumulative frequency-M_0 graph deduced from the frequency data in Fig. 1 and the M_0-M_s relationship in Fig. 2. The straight line is a least-squares fit to the data.

More importantly, the linear trend in Fig. 3 continues through the points corresponding to the largest values of M_0 so far measured. There are good reasons for believing that there must be an upper bound to earthquake M_0 values, due to the geometry of seismic zones and the strength of crustal material. However, the data presented here show no indication of where this upper bound might be. Also, since we have accurate M_0 data only for very recent earthquakes, it does not seem reasonable to suppose that this linear trend stops immediately beyond the last data point. If the trend is extrapolated beyond the data, an event of $M_0 = 10^{31}$ dyne-cm is predicted to have a mean return period of 50 years. It is not clear that the record of large earthquakes during the last 100 years is sufficiently detailed that the occurrence of such a catastrophic event can be ruled out. The U.S. Environmental Data Service (*12*) has listed 151 earthquakes with M_s greater than or equal to 8.0 during the period 1897 to 1972, and very few of these have been studied in detail.

Certainly, if earthquakes with M_0 values much larger than 10^{30} dyne-cm do occur, this could have a significant effect on global estimates of seismic energy release and plate motion due to earthquakes, and such large events may well cause a considerable excitation of the Chandler wobble (*13*).

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Model for Disseminated Cutaneous Leishmaniasis

Abstract. Leishmania *infection of a skin site with interrupted lymphatic drainage results in widespread cutaneous metastases. This model may provide a method for the study of disseminated cutaneous leishmaniasis in man.*

Disseminated cutaneous leishmaniasis is a manifestation of cutaneous leishmaniasis characterized by diffuse skin nodules of parasitized macrophages, anergy to intradermal tests with specific antigen, and resistance to treatment. Although the underlying mechanism responsible for dissemination is largely unknown, it is generally believed to result from specific deficiency of cell-mediated immunity of the host (*1*, *2*). With this in mind, several investigators have tried to reproduce disseminated cuta-

neous leishmaniasis by infecting animals that had been given immunosuppressive agents (*3*) or by injecting a very large dose of parasites into normal animals (*4*). In the present study, we have attempted to produce disseminated cutaneous leishmaniasis in the guinea pig, using *Leishmania enriettii*, by compromising the lymphatic drainage at the site of the lesion.

Skin flaps designed to reduce the lymphatic drainage of an area of skin were made as described by Barker and Billingham (*5*) on 28 outbred Hartley strain guinea pigs. Promastigotes (10^6) of *L. enriettii* were inoculated intradermally on these shaved skin flaps immediately after surgery and into intact skin of a similar region of the flanks of 20 normal guinea pigs. The sites of infection were examined on alternate days after infection.

Promastigotes were obtained from cultures (NNN medium with Grace's insect medium overlay) prepared from lesions of guinea pigs infected with 10^6 promastigotes of *L. enriettii*. Cultures were maintained by serial passage for a maximum of seven passages after which organisms were used to reinfect guinea pigs and new cultures were initiated from the lesions. An infective dose of 10^6 promastigotes from such cultures, when injected intracutaneously into the nose or the dorsal base of the ear of normal guinea pigs, regularly produces a circumscribed lesion which heals spontaneously in 8 to 10 weeks. With such infections, up to 5 percent of animals may show isolated, single metastatic lesions. The strain of *L. enriettii* used in these studies was obtained from D. Dumonde, Kennedy Institute of Rheumatology, London, England.

Lesions due to *L. enriettii* occurred at the injection site in all 28 animals with skin flaps. However, six guinea pigs failed to survive for more than 14 days after the flap was made, probably as a result of the anesthesia, and one of the flaps dried out the day after surgery; it was not possible to follow the outcome of the initial infection in these animals. The remaining skin flaps on 21 guinea pigs remained healthy for 2 to 7 weeks. These animals and 20 guinea pigs infected without flaps were examined for development of metastases and visceral lesions.

Nine (43 percent) of the 21 animals with skin flap infections showed cutaneous metastatic lesions. Two animals had two metastatic lesions each, one had three, one had four, while five others each had at least 40 metastatic lesions. In contrast a single, small, cutaneous metastatic lesion was observed in a single animal (5 percent) of the 20 guinea pigs whose infection was initiated by injection of the organisms into the intact skin. Four animals with extensive metastatic lesions died before the 12th week of the experiment, whereas none of the animals with two to four metastatic lesions or with no such lesions did so.

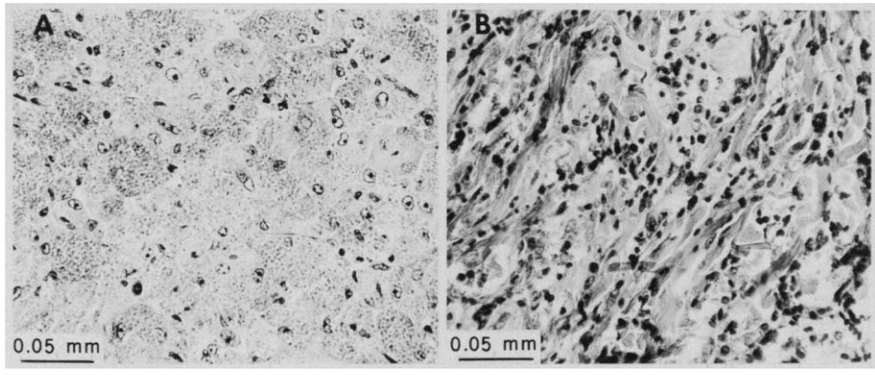

Fig. 1. *Leishmania* lesions in skin flap (A) at 6 weeks and in intact skin (B) at 6 weeks, each produced by 1×10^6 promastigotes of *L. enriettii*. Marked accumulations of heavily parasitized macrophages with few lymphocytes are seen in the skin flap lesion, while the intact skin lesion shows parasitized macrophages with many lymphocytes; hematoxylin and eosin stain ($\times$ 275).

Although maintenance of skin flaps for the 8- to 12-week course of the disease was not possible, histological examination of lesions in nine skin flaps, made 2 to 7 weeks after the flaps were constructed, showed striking morphologic differences compared to lesions created in normal skin. In lesions in normal skin there is, initially, a concentration of parasitized macrophages, followed at 4 weeks after infection by a marked accumulation of lymphocytes which increases progressively with a corresponding decrease in the number of parasitized macrophages until resolution of the lesion occurs (8 to 10 weeks).

In contrast, in four infected flaps that had remained viable for four or more weeks, heavily parasitized macrophages replaced as much as two-thirds of the thickness of the skin flap (Fig. 1A). These lesions were accompanied by small numbers of lymphocytes, which were more concentrated about neurovascular bundles. In contrast, the lesions in normal skin followed their expected course and at 6 weeks contained large numbers of lymphocytes and relatively small numbers of parasitized macrophages as compared with the flap lesions (Fig. 1B).

Five animals with flap infections that developed extensive metastases (more than 40 lesions) were autopsied. Striking morphologic changes were evident in the lymph nodes of all animals and in three of the spleens. The lymph nodes and spleens showed increased prominence of the germinal centers with abundant plasma cells, and the lymph nodes showed minimal paracortical activity. Abundant parasitized macrophages appeared singly or in aggregates, mainly in the cortical and the paracortical zones of the lymph nodes (Fig. 2).

Furthermore, in the livers of two of the animals with extensive metastatic lesions, there were multiple small granulomatous lesions, consisting of foci of macrophages containing amastigotes surrounded by lymphocytes. A third animal showed numerous foci in the liver consisting of lym-

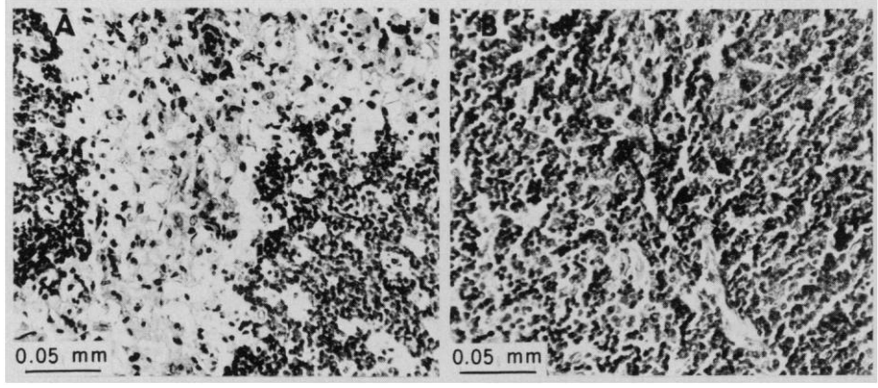

Fig. 2. Morphology of the draining lymph nodes (A) from a guinea pig that was infected on skin flap and developed widespread metastases, and (B) from a guinea pig that was infected on intact skin with no metastases. In each case the initial lesion was produced by 1×10^6 promastigotes of *L. enriettii*. In (A), parasitized macrophages are seen in the cortical and paracortical areas, which in (B) are occupied by lymphocytes; hematoxylin and eosin stain ($\times$ 275).

phocytes surrounding groups of Kupffer cells, but no amastigotes were seen in the cytoplasm of the cells.

The cell-mediated and humoral antibody responses of the guinea pigs with infected flaps which later developed metastases were quite different from those of intact animals that were free of metastases. Thus the nine guinea pigs that developed metastases after infection of flaps were nonresponsive to an intradermal injection of 50 μg of crude soluble leishmanial antigen when tested 9 weeks after infection, whereas all guinea pigs infected on intact skin showed a delayed-type response at that time. However, at a later stage in the infection, the four guinea pigs with two to four metastases converted to a positive delayed skin reaction. The peripheral blood lymphocytes of two of the guinea pigs with more than 40 metastases, when examined in vitro for antigen-induced blastogenesis as assessed by the uptake of [^{3}H]thymidine (*6*), were unresponsive. In contrast, the peripheral blood lymphocytes of four animals infected on intact skin and of the four animals with two to four metastases responded to antigen when cultured in vitro.

Antibody was measured in guinea pigs with and without metastases by direct agglutination of promastigotes (*7*) and indirect hemagglutination of sheep red blood cells coated with crude soluble leishmanial antigen (*8*). Substantial antibody titers were detected by both methods in all animals having metastatic lesions. In addition, three animals with extensive metastatic lesions also had high levels of serum γ-globulins as measured by microzone cell electrophoresis, although their antibody titers were not different from those of other guinea pigs in the metastatic group. In contrast, none of the guinea pigs with flap lesions that did not produce metastases and none of the guinea pigs infected on intact skin showed significant levels of antibody as measured by either technique, and none had increased levels of γ-globulins.

It has been suggested that *L. enriettii* parasitizes the cooler parts of body skin (*9*). It is possible, therefore, that reduced skin temperature of the skin flap might permit excessive multiplication of organisms in the flap. Skin temperatures were determined at sites of skin flap lesions and at lesions in intact skin at comparable locations in the flank by using a Thermister thermometer with a YS1 series 700 probe (United System Corp.). The mean flap temperature was 35.47°C, whereas that for intact skin was 35.66°C. The difference between the temperatures of the skin flap and the intact skin was not significant.

The results can be interpreted as indicating that the interruption of lymphatic drainage to local lymph nodes prevents the host from mounting an effective cell-mediated response to the lesion on the skin flap. This permits the organisms to divide rapidly and infiltrate the alymphatic skin massively. The factors responsible for the metastasis of such lesions to other cutaneous sites are unknown. Blocking antibody is suggested by Garnham and Humphrey (*10*), and damage or compromise to the local lymphatic drainage is suggested by Bryceson (*1*).

In man, disseminated cutaneous leishmaniasis and oriental sore are associated with varying levels of antibody and thus resemble the situation seen in guinea pigs with comparable manifestations of cutaneous leishmaniasis. Thus, antibody is present in small amounts or is absent in guinea pigs infected in the intact skin with *L. enriettii*, and such antibodies are detectable by an indirect fluorescent antibody technique and not by indirect hemagglutination. In contrast, guinea pigs with metastatic lesions show increased levels of antibodies detectable by indirect fluorescence (*4*), hemagglutination, and direct agglutination of promastigotes. Whether the increased level of antibody acts in a blocking manner requires further study. However, this study does provide direct experimental evidence for Bryceson's hypothesis (*1*), that compromise of the lymphatic drainage may lead to metastatic lesions.

Of particular interest is the fact that organisms which are generally regarded as dermatropic may be found in lymph nodes and the liver in animals with extensive metastatic lesions. Therefore, this model may shed light on the mechanism for dissemination of cutaneous and possibly visceral leishmaniasis in man and the role of antibody in this infection. Furthermore, our results would tend to exclude the possibility that a different (*11*) or less antigenic strain (*1*) of *Leishmania* is the causative agent of disseminated cutaneous leishmaniasis.

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Tumorigenicity of Mouse-Human Diploid Hybrids in Nude Mice

Abstract. *Somatic cell hybrids between normal mouse cells and simian virus 40 (SV40)–transformed human cells, which contained a diploid complement of mouse chromosomes and the human chromosome 7 carrying the genome of SV40, were tumorigenic in nude mice. One single copy of human chromosome 7 per hybrid cell appeared to be sufficient for the tumorigenicity of the hybrids.*

Consequent to the discovery of the integration of the simian virus 40 (SV40) genome into chromosome 7 of SV40-transformed human cell lines (*1*), we have shown that somatic cell hybrids between normal diploid mouse cells and LN-SV cells, an SV40-transformed human cell line, behave as transformed cells in culture (*2*) and retain human chromosome 7.

Of clones derived from the hybridization between mouse peritoneal macrophages (MPM) and LN-SV cells, 75 percent contained a near tetraploid complement of mouse chromosomes and human chromosome 7, often in multiple copies (*2, 3*). Cells of tumors resulting from the inoculation of these hybrid cells into nude mice (*4*) contained a near tetraploid complement of mouse chromosomes and multiple copies of human chromosome 7 carrying the SV40 genome (*5*). All tumor cells also expressed SV40-induced T antigen (*5*).

In contrast to the near tetraploid complement of mouse chromosomes observed in the majority of the MPM × LN-SV hybrid clones, 25 percent of the clones isolated from hybrid cultures between MPM and LN-SV cells contained a near diploid complement of mouse chromosomes and a