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

few human chromosomes, with human chromosome 7 being present in all the cells. These hybrid clones expressed SV40-induced T antigen and behaved as transformed cells in culture (*3*).

To investigate whether hybrid cells which contain a diploid complement of mouse chromosomes are tumorigenic, we injected nude mice with two different BALB/c MPM × LN-SV hybrid clones. One (series A) contained a near diploid complement of mouse chromosomes (less than 10 percent of the cells were near tetraploid) and only human chromosomes 7 (53-87-3 clone 10); and the other (series B) contained a near diploid complement of mouse chromosomes (less than 20 percent of the cells were near tetraploid) and human chromosomes 7, 5, and 11 (53-87-3 clone 43).

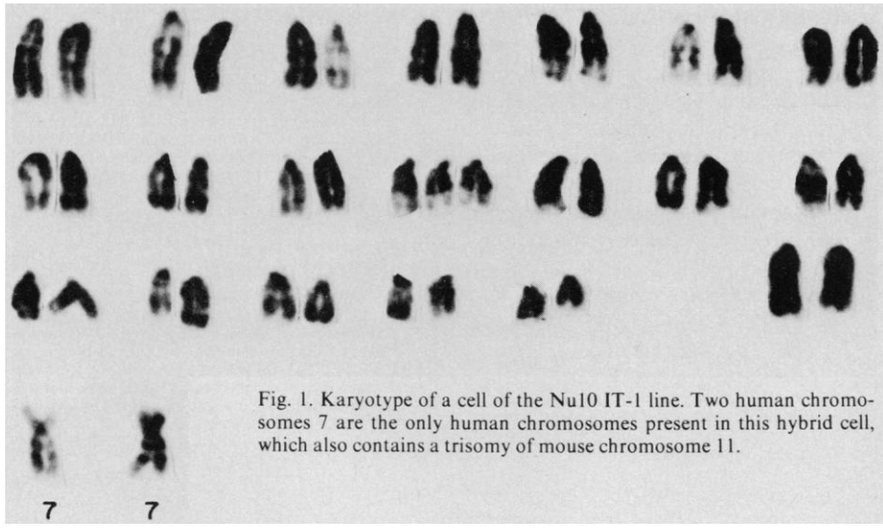

Fig. 1. Karyotype of a cell of the Nu10 IT-1 line. Two human chromosomes 7 are the only human chromosomes present in this hybrid cell, which also contains a trisomy of mouse chromosome 11.

Hybrid cells (10^7 per mouse) were injected under the abdominal skin of nude (BALB/c *nu/nu*) mice, 6 to 8 weeks of age. Ten mice were injected with cells from the hybrid clone containing only human chromosome 7, and 12 mice were injected with cells from the hybrid clones containing human chromosomes 7, 5, and 11. Twenty inoculated mice developed tumors and two died before the experiment was completed at 8 weeks after the injection. Two tumors of series A and five tumors of series B were transferred to tissue culture, and all the resulting cell lines were analyzed for expression of SV40 T antigen (*1*) and for the presence of human chromosomes by Giemsa banding staining (*6*).

All the tumor cells were positive for SV40 T antigen (Table 1). Karyological examination indicated that tumor cells derived from the inoculation of 53-87-3 clone 10 contained one to three human chromosomes 7 per cell and a near diploid complement of mouse chromosomes. The tumor cells retained all the mouse chromosomes (Fig. 1). Tumor cells originating from five tumors induced by inoculation of 53-87-3 clone 43 cells retained one to four human chromosomes 7 per cell. Human chromosome 5 was retained in four of the tumor cell lines; human chromosome 11 was present in two of the five tumor cell lines examined. The Nu43 IT-2 cells contained only one human chromosome, the 7. All tumor cells maintained a near diploid complement of mouse chromosomes in at least 80 percent of the cells examined (Table 1). Again, no loss of any mouse chromosome was observed.

In the case of the tumor lines Nu10 IT-1, Nu10 IT-2, and Nu43 IT-1, a single human chromosome 7 per cell was observed in the great majority of the cells analyzed. In three of 325 metaphases analyzed it was impossible to recognize an intact human chromosome 7; but since 100 percent of the cells were positive for SV40 T antigen [the gene for SV40 T antigen has been assigned to chromosome 7 in LN-SV cells (*1*)] it is likely that failure to detect human chromosome 7 was due to rearrangements, involving this chromosome.

Our results agree with the previous evidence that somatic cell hybrids between normal diploid mouse cells and human cells transformed by SV40 are tumorigenic in nude mice and that the tumor cells derived from the mouse tumors retained the human chromosome 7 carrying the SV40 genome. In addition, we have now shown that hybrid cells containing a near diploid complement of mouse chromosomes are tumorigenic in nude mice and that the tumor cells retain the near diploid complement of mouse chromosomes. Our results also show that one copy of human chromosome 7 [Nu10 IT-1, Nu10 IT-2, and Nu43 IT-1 (Table 1)] is sufficient for the expression of the tumorigenic phenotype in the hybrid cells. Since no loss of any specific mouse chromosome was detected in the tumorigenic hybrid cell clones

Table 1. Correlation of the expression of SV40 T antigen and the presence of human chromosome 7 in tumorigenic hybrid clones and tumor cells.

Cells	T antigen-positive cells (%)	Human chromosomes (average No. per cell)			Average No. of rearranged chromosome 7 per cell	No. of mouse chromosomes per cell		No. of metaphases analyzed*
		5	7	11		Average	Range	
Series A								
53-87-3 clone 10	100	0	1.0	0	0	41.9	39–44	25
Nu10 IT-1	100	0	1.3	0	0.6	41.8	38–44	49
Nu10 IT-2	100	0	1.0	0	0.1	42.0	40–43	27
Series B								
53-87-3 clone 43	100	0.9	1.2	0.5	0	40.3	39–42	30
Nu43 IT-1	100	0.6	1.1	0.3	0.1	41.1	39–43	39
Nu43 IT-2	100	0	2.0	0	0	41.4	38–44	24
Nu43 IT-3	100	0.1	1.9	0	0.1	41.6	40–43	37
Nu43 IT-4	100	0.2	2.6	0	0.0	40.3	39–42	39
Nu43 IT-5	100	0.4	1.9	0.2	0.1	41.8	39–47	55

*Less than 10 percent of the cells of 53-87-3 clone 10, Nu10 IT-1, and Nu10 IT-2 were near tetraploid, and they were not included in the computation of the averages. Less than 20 percent of the cells of 53-87-3 clone 43 and its tumor derivatives were near tetraploid, and they were not included in the computation of the averages.

and in the tumor cells derived from all tumors induced in nude mice, we conclude that no suppression of the malignant phenotype takes place in somatic cell hybrids between normal diploid mouse cells and SV40-transformed human cells.

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Retention of Nonhelical Procollagen Containing *cis*-Hydroxyproline in Rough Endoplasmic Reticulum

Abstract. *Fibroblasts freshly isolated from embryonic tendons were incubated with a proline analog,* cis-*4-hydroxy-*L*-proline, which is incorporated into protein and which leads to the intracellular accumulation of nonhelical procollagen. Evidence is presented here that the nonhelical procollagen containing the analog is retained within the rough endoplasmic reticulum and does not pass to the smooth endoplasmic reticulum or Golgi vacuoles at a normal rate.*

Collagen is first synthesized as a precursor molecule called procollagen, and assembly of procollagen involves several steps: (i) incorporation of amino acids into the three pro-α chains of procollagen; (ii) hydroxylation of some of the prolyl and lysyl residues in the newly synthesized polypeptides to hydroxyprolyl and hydroxylysyl residues and the subsequent glycosylation of some of the hydroxylysyl residues; (iii) association of the three pro-α chains largely through interactions among the globular, peptide extensions on the chains; (iv) folding of the collagen portions of the pro-α chains into the triple-helical conformation characteristic of collagen (*1, 2*). The triple-helical conformation is required for procollagen to be secreted at a normal rate (*1, 2*). The role of the triple-helical conformation in secretion has largely been established by experiments in which cells or tissues synthesizing procollagen are incubated without oxygen or with the iron chelator α,α'-dipyridyl under conditions that allow polypeptide synthesis to continue at about the control rate for several hours but which completely prevent the hydroxylation of proline and lysine (*3*). Under such conditions, cells accumulate the unhydroxylated form of procollagen, which has been called protocollagen and which cannot fold into a stable triple-helical conformation at physiological temperatures because it lacks hydroxyproline (*4*). In cells incubated with α,α'-dipyridyl, protocollagen accumulates within the cisternae of the rough endoplasmic reticulum (*5, 6*); this observation suggested that the triple-helical conformation is required for the protein to pass at a normal rate from the endoplasmic reticulum to the smooth endoplasmic reticulum and Golgi vacuoles. We now provide further evidence for this conclusion by incubating cells with a proline analog, *cis*-4-hydroxy-L-proline, which is incorporated into protein (*7*). Our experiments provide a chemically independent test for the effect of conformation on

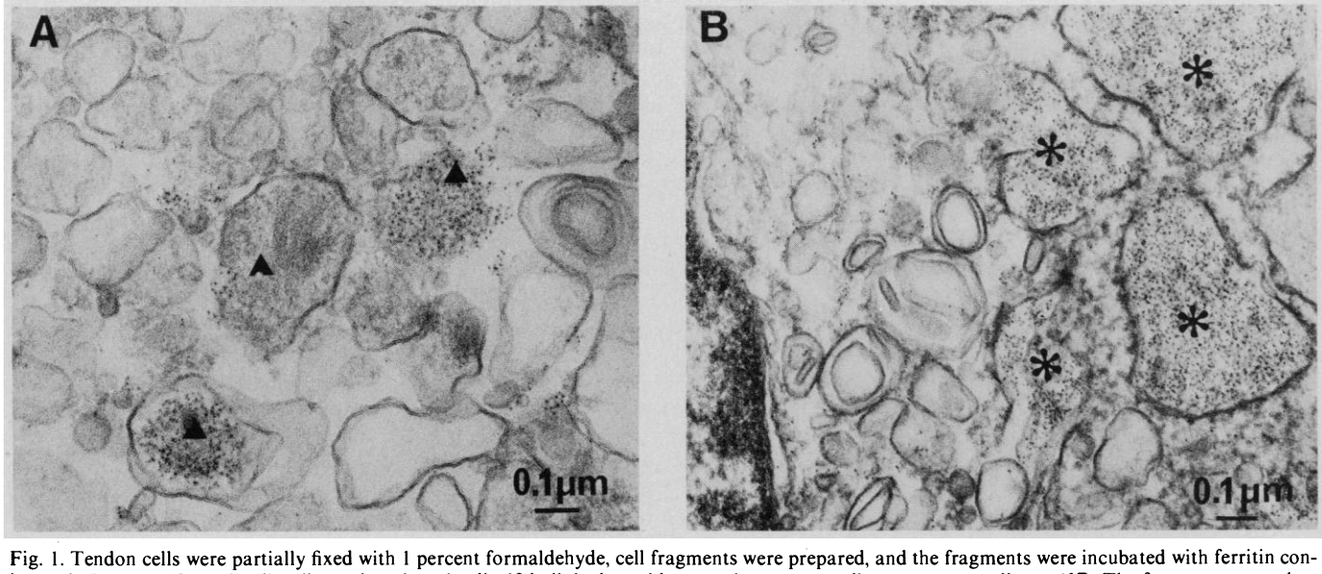

Fig. 1. Tendon cells were partially fixed with 1 percent formaldehyde, cell fragments were prepared, and the fragments were incubated with ferritin conjugated (*5*) to specific antibodies directed against the disulfide-linked peptide extensions on procollagen or protocollagen (*17*). The fragments were then washed, further fixed with 3 percent glutaraldehyde, and postfixed with 1 percent osmium tetroxide. The samples were stained with 1 percent uranyl acetate (Polysciences) for 180 minutes at room temperature, dehydrated with ethanol and acetone, and embedded in Epon 812 (Electron Microscopy Sciences) (*5*). Ultrathin sections were prepared and examined with a JEM-100B electron microscope (JEOL). (A) Fragments from cells incubated with 1 μM colchicine for 2 hours. Golgi vacuoles are large and many contain fibrillar material. Triangles indicate Golgi vacuoles labeled with ferritin-antibody conjugates. Cisternae of the rough endoplasmic reticulum were also labeled (not shown). (B) Fragment from cells incubated with 1 μM colchicine and 1.53 mM *cis*-hydroxyproline for 2 hours. Golgi vacuoles are small and not specifically labeled with the ferritin-antibody conjugates. Asterisks indicate cisternae of the rough endoplasmic reticulum which are extensively labeled with the conjugates.