rate as the tube was rotated and tilted back and forth. Attractiveness of samples was based on the percentage of insects trapped in 3 minutes after the sample tube was connected into one of the two lines (the other was the check). All parts were handled with disposable gloves to avoid contamination of the system. Before each test, mosquitoes were exposed to the nitrogen–carbon dioxide mixture; if more than 2.5 percent of the mosquitoes responded, the sleeves and traps were replaced.

With mosquitoes of average avidity, 10 μg of L-lactic acid attracted 29 to 75 percent of the mosquitoes in 3 minutes, and three to four consecutive tests could be made (subjected to air flow for 9 to 12 minutes) before the sample tube lost its attractiveness. The attractiveness of lactic acid did not appear to be enhanced by the addition of any other component isolated from the acid fraction, including the unidentified minor attractant. However, the presence of carbon dioxide is essential (Table 1). Carbon dioxide has long been recognized as a mosquito activator (*4*), but alone in nitrogen or in purified air it does not attract mosquitoes.

Other carboxylic acids containing as many as five carbon atoms were tested for attractiveness, but the response was never more than feeble compared with the responses to L-lactic acid.

The quantity of lactic acid in the acetone rinses of the hands of individuals who differed in their attractiveness to mosquitoes was determined by enzymatic assay. Overall there was good correlation between the amount of lactic acid found in the rinses of these subjects and the percentage of mosquitoes attracted by the rinses from each (Table 2). The rinses showed the same order of attractiveness between subjects that had been shown in many previous tests of the hands of the same individuals.

Lactic acid occurs in sweat and on skin (*5*); as a product of animal muscle metabolism it is invariably in the L(+)-configuration (*6*) (sometimes referred to as "sarcolactic acid"). An old report (*7*) indicated that the compound is slightly attractive to *A. aegypti*. The compound appears to be more volatile than is generally recognized, and conversion to the less volatile ammonium salt was required to obtain satisfactory recoveries in the evaporations of biological material and eluates from chromatograms.

A good attractant in traps is an invaluable aid to the detection of insect infestations. Such an attractant is needed for the worldwide eradication of the yellow-fever mosquito; lactic acid may be useful for this purpose.

Note added in proof: A recent report (*8*) stated that formic, acetic, propionic, and lactic acids attracted *A. aegypti* and that lactic acid (L- or D- not specified) was the most attractive. The acids tested were not isolated from humans, and the concurrent need of carbon dioxide for attraction was not noted.

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Gaucher's Disease: A Genetic Disease Detected in Skin Fibroblast Cultures

Abstract. *Skin fibroblasts from three adult patients with chronic noncerebral Gaucher's disease, three children of one of the patients, three parents, and six normal individuals were grown in cell culture. Giant fibroblasts containing metachromatic material were seen in all cultures derived from affected individuals and heterozygous carriers but not in those derived from normal individuals.*

Gaucher's disease is a rare genetic disorder of cerebroside metabolism which results in the accumulation of glucocerebroside in various tissues of the body (*1*). The metabolic defect appears to be a deficiency of the enzyme which catalyzes the cleavage of glucose from glucocerebroside (*2*). Although there are two known clinical forms, infantile cerebral and adult chronic noncerebral, no biochemical difference between the two has been found (*3*). Family studies suggest an autosomal recessive mode of inheritance (*4*). The clinical manifestations are presumed secondary to organ infiltration of a large storage cell, the Gaucher cell, resulting in hepatosplenomegaly, lymphadenopathy, and bone lesions. The cytoplasm of the Gaucher cell has a unique reticular appearance and permits diagnosis to be made by light microscopy (*5*).

It has become evident as a result of studies in cell culture that diseases associated with the intracellular accumulation of a metabolite and which can be detected either morphologically or chemically should be amenable for cell culture studies.

Skin biopsies were obtained from three patients with the adult form of Gaucher's disease, three clinically unaffected offspring from one patient with the disease, and three parents of affected individuals. Six normal unrelated individuals also were studied. Four different biopsies were taken from one patient on four different occasions over a 12-month period. The establishment of cell lines from biopsy specimens by standard culture methods has been described (*6*). The cell lines were grown as monolayer cultures from 2 to 5 months in culture in modified Eagle's medium (*7*) with 10 percent newborn calf serum. The preparation of slides and subsequent staining of the cells with the metachromatic dye toluidine blue O have been described elsewhere (*6*).

Cultures of skin fibroblasts from patients with Gaucher's disease contained large cells with marked metachromasia (Fig. 1). These cells were observed in the cultures from the first subculture (2 months after establishment of explant culture) through the 20th subculture (9 months in culture). The number of metachromatic fibroblasts was approximately 20 to 40 percent and was relatively constant in replicate cultures de-

rived from different biopsy specimens from the same individual. Four biopsies taken from one affected individual at intervals throughout a 12-month period produced cultures with a similar proportion of metachromatically staining fibroblasts. The metachromatic material was evenly distributed through the cytoplasm. These cells contained cytoplasmic vacuoles which did not stain metachromatically. Although the metachromatic cells appeared much larger than the other fibroblasts in the culture (Fig. 1), wrinkled cytoplasm and eccentric nuclei, the pathognomonic morphological characteristics, were not seen. Gaucher cells from the bone marrow of the affected individuals stained light blue with toluidine blue O and showed no metachromasia. The nonmetachromatic fibroblasts seen in the cultures appeared similar to cells seen in the cultures derived from the six normal, unrelated individuals. The cytoplasm of these normal fibroblasts had a fine granular appearance, stained light blue with toluidine blue O, and occasionally contained a few metachromatic granules (Fig. 1).

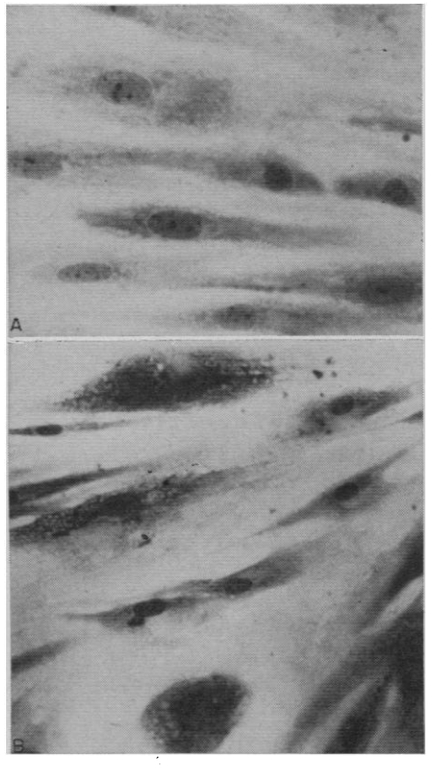

Fig. 1. Monolayers of skin fibroblasts grown in cell culture. Preparations fixed in methanol and stained with toluidine blue O. (A) Normal individual. (B) Patient with Gaucher's disease. Note large cells with metachromatic cytoplasm (× 2000).

Cultures of skin fibroblasts from the the three unaffected carrier parents contained the same large metachromatic cells. These cultures could not be distinguished from those derived from affected individuals either by morphological appearance or metachromatic staining. Cultures derived from all three of the normal children of an affected individual contained cells indistinguishable from those seen in the patient and were thus considered to be carriers of the genetic trait (Fig. 2).

Several genetic disorders (*8, 9*) can be investigated in cultured fibroblasts. The addition of Gaucher's disease to this list demonstrates that the basic defect is not limited to the Gaucher cells but probably involves somatic cells with the ability to synthesize and metabolize cerebrosides. This is in accord with observations indicating that the enzyme defect can be detected in peripheral leukocytes (*10*).

Although a peculiar yellow pigmentation of the skin, presumed secondary to an increase in iron-containing pigment and melanin, occurs in Gaucher's disease, Gaucher cells have not been found in histological sections of skin (*11*). The large metachromatic cells seen in cell cultures were morphologically distinct from other fibroblasts. This metachromasia was markedly different from that seen in cultured cells derived from patients with the genetic mucopolysaccharidoses (*6*) or cystic fibrosis (*12*). Except for the large size, the cells did not resemble typical Gaucher cells. The stringy, fibrillar cytoplasm and small pyknotic eccentric nuclei, characteristic of Gaucher cells, were conspicuously absent (*5*). The marked metachromasia of the giant fibroblast cells may indicate increased sulphation of the intracellular cerebrosides during storage (*13*).

Since Collier first described Gaucher's disease in sibs (*14*), pedigree studies have suggested that the mode of inheritance was an autosomal recessive (*15*). In our study, the known carriers showed cellular metachromasia. In one family (Fig. 2) the abnormal gene could be detected histochemically in three generations. Although these data are limited to two families with the adult chronic type of Gaucher's disease, it is suggested that cellular metachromasia of skin fibroblast cultures can be used to detect both affected individuals and carriers. However, it was not possible to distinguish the affected homozygous individual from the heterozygous carrier by means of cell cultures. Additional studies should be undertaken to determine whether similar abnormalities can be found in the fibroblasts cultured from other inherited storage lipidoses.

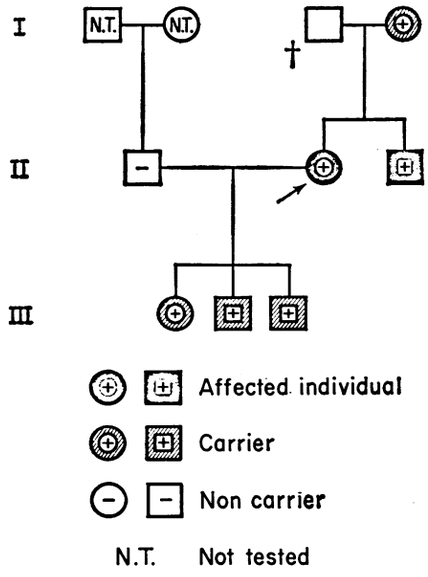

Fig. 2. Pedigree of two patients with Gaucher's disease.

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