We may now concentrate our attention on the $K_{1,2}$ column in Table 2. The crosses Bahia 2 ♀ × Belém A ♂ and Bahia 6 ♀ × Belém C ♂ produce fertile hybrids, while all other crosses of Bahia females to Belém males give sterile hybrids. The isolation coefficient for the former crosses is significantly lower than for the latter crosses. Belém D is an Andean-Brazilian strain which is sympatric with the Amazonian strains from Belém. The isolation between Belém D males and Belém A, C, and J females is significantly higher than in any of the Bahia ♂ crosses with Belém ♀. The Ceará strains of the Andean-Brazilian race behave in this respect like the Belém D strain. Although no strains of the Amazonian race have been collected in Ceará, this locality is much closer to the known distribution area of the Amazonian race than is the Bahia locality.

Conclusion and Summary.—The Amazonian and the Andean-Brazilian races or incipient species of *Drosophila paulistorum* are sympatric, i.e., occur together, at Belém; in Bahia and Ceará only the Andean-Brazilian race is found. The Amazonian race males are accepted as mates by Andean-Brazilian females more easily than are Andean-Brazilian males by the Amazonian females. The transitional strains of the Andean-Brazilian race from Bahia not only give fertile hybrid progenies when crossed to certain Amazonian race strains from Belém, but also show a reduced ethological (sexual) isolation from the latter. The transitional strains from Ceará do not, however, show any indication of a reduction in ethological isolation. The reproductive isolation between the incipient species is more nearly complete where these incipient species are sympatric or occupy adjacent territories than where they are allopatric and occupy territories farther apart. The transitional strains are not products of an introgressive hybridization between the incipient species; they occur where the likelihood of such hybridization is minimal.

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[1] Dobzhansky, Th., and B. Spassky, these PROCEEDINGS, **45**, 419–428 (1959).

[2] Dobzhansky, Th., L. Ehrman, O. Pavlovsky, and B. Spassky, these PROCEEDINGS, **51**, 3–9 (1964).

[3] Malogolowkin, Ch., *Evolution*, **17**, 187–193 (1963).

[4] Ehrman, L., *Evolution*, **14**, 212–223 (1960).

[5] Ehrman, L., *Genetics*, **46**, 1025–1038 (1960).

[6] Levene, H., *Evolution*, **3**, 315–321 (1949).

UNIDENTIFIED, FILTRABLE AGENTS ISOLATED FROM AFRICAN CHILDREN WITH MALIGNANT LYMPHOMAS

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The malignant lymphomas of children in tropical Africa are noteworthy because of their unusual form and the frequency with which they involve the jaws.[1] They are most common in areas where temperature and rainfall favor mosquitoes and mosquito-borne diseases are prevalent.[2] It has been suggested that the tumors themselves may be caused by an arbor virus,[3] and during the past two years we

have sought filtrable agents in children under observation in the King George VI Hospital in Nairobi, Kenya.

A number of cytopathogenic agents have recently been recovered from six of eight patients. The two most extensively examined have been a four-year-old girl and a seven-year-old boy, both Africans from the western provinces of Kenya where the disease is relatively common. From them isolations have been made from bone marrow, tumor suspension supernate, and feces. The isolations were repeated from the original samples and from fecal specimens and the boy's bone marrow collected after a one-month interval. In two additional patients isolations were made from both tumor samples and stools.

All these patients had tumors that were typical clinically and histologically. Several were judged to be in an unusually early stage of the disease. In all six the tumor had presented in bones of the jaws. Stool specimens from two patients with abdominal tumors did not yield the agent. From one of these an ECHO virus was cultivated. A Group B Coxsackie virus was isolated from the feces of a ward mate whose disease was later identified as osteomyelitis.

The isolations were made by first inoculating primary human amnion cell cultures or cells of the Wistar line (WI-26) of fetal human lung. The human amnion cells used were cultivated from four placentas. The responses of amnion cells have been relatively inconspicuous, consisting chiefly of elongation of the cell bodies that resulted in a fibroblastic appearance. Subsequent passage in primary human amnion cell cultures (or Wistar cells) consistently failed to show recognized morphologic changes, but fluids harvested from such cultures induced severe cytopathogenic changes in either FL or human embryonic kidney cell cultures. The former have been used routinely in the study of the agents which have, so far, been indistinguishable in their behavior and, when tested, seemed to be serologically similar as well. The destructive changes are preceded by striking abnormalities in the nuclei which may best be identified in stained, coverslip preparations (Fig. 1).

Cell degeneration, granularity and elongation, and cell death occur within a day or two of exposure of FL cells to the agents. The end point in titrations has frequently been indefinite and has occurred as late as five or seven days. This has made titrations and neutralization tests difficult to quantitate precisely. Fluids harvested from infected cultures characteristically have titers of 10-3 or greater. The agents are filtrable through Selas 03 candles and Gradacol membranes of 100-mμ pore diameter. They have so far failed to induce disease in newborn mice or hamsters or in immature monkeys of several species. Vervet monkeys inoculated intramuscularly had appreciable neutralizing antibodies in serum samples collected after 7 and 14 days, and one pair of such sera have been tested in complement fixation tests with adenovirus, influenza A, B, and C, Psitticosis-lymphogranuloma venereum, measles, herpes simplex, mumps, EEE, WEE, St. Louis encephalitis, LCM, Parainfluenzas 1, 2, and 3, and respiratory syncytial virus antigens without evidence of activity. Hemagglutination inhibition tests were also made using antigens of EEE, WEE, St. Louis, and Powassan antigens without evidence of activity.

The sera of patients have exhibited neutralizing capacity of modest degree. This has also been true of patients' sera from Uganda. Neutralization has been noted in

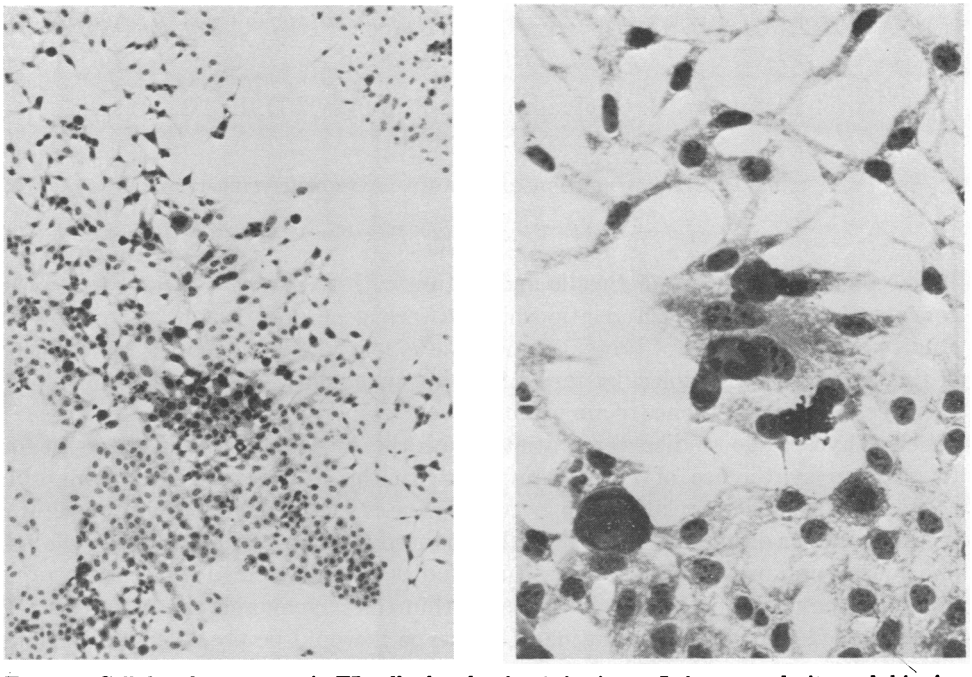

FIG. 1.—Cellular changes seen in FL cells shortly after infection. *Left:* granularity and thinning of the cell sheet and nuclear changes (× 200). *Right:* the latter in greater detail (× 500).

sera from patients with leukemia. These specimens were collected in New York. Sera from healthy Kenya children have frequently been without activity, and it is hoped that serum surveys will help in determining whether these agents are significantly related to the disease.

The circumstances suggest that they deserve consideration by workers interested in malignant lymphomas, and our early experience is being reported in the hope that others will test for their presence.

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[1] Burkitt, D., *Postgrad. Med. J.*, **38**, 7179 (1962).

[2] Haddow, A. J., *E. African Med. J.*, **40**, 429 (1963).

[3] Davies, J. N. P., quoted by Burkitt.