VIRUS-LIKE PARTICLES IN HUMAN LEUKEMIC CELLS

Sir:

Following our previously published results on human leukemia transmitted by embryonal chicken,1 in 1969 we took our experiments over again. The chicken eggs we had previously used came from common fowls reared in the annex of the Medical Clinic and of the Cancer Institute in Rome; this time we used fertile chicken eggs, virus- and mycoplasm-free, from two different breedings, one in England and the other in Germany.2

Our technique has not much changed: always believing that the presumed virus can only be raised in very undifferentiated cells, we continued to inoculate the eggs before incubation. As inoculum, we usually employed leukocyte-rich human plasma from patients with chronic myeloid or chronic lymphoid leukemia, either untreated or in relapse. Successive egg-to-egg transmissions were obtained with a mash of whole decapitated embryo with its membranes.

Using the inoculum after the fourth egg-to-egg transmission, acidified to pH 1 and readjusted to pH 7 after air exposure under a germicidal lamp for 24 hours, we tried an intradermal reaction in leukemic patients: positive specific results were obtained. Simultaneous intradermal injection, to the same patients, of a control material obtained from eggs inoculated with inactivated material, gave negative results.

We have now examined inoculated embryo membranes at the electron microscope: virus-like particles could be observed, even if the first inoculum and successive transmissions had been done with ultrafiltered matter. No viral particles could be observed in controls or in eggs inoculated with inactivated material.

**Electron microscopy.** The specimens were fixed in a glutaraldehyde solution at 2% and postfixed with OsO4, rapidly dehydrated and embedded in Epon resin 812. Sections cut with diamond knives were stained with Uranyl acetate and PbOH and observed with Philips 300 electron microscope. Virus-like particles were observed in perivascular connective tissue cells, only in the infected group. The particles were rather scarce, sometimes lying in the cell sap, more often in the RER cisternae. Their shape was round, their average diameter ranging from 800 to 900 A units; they appeared constituted by a dense core surrounded by a lighter zone delimited by a double membrane. Structures of comparable size, composed by minute membrane whorls, were observed in the same samples, both in the cytoplasm and the nucleus. Such observations deserve further ultrastructural as well as biochemical and immunological investigations.

We are now endeavoring to assess whether these images can be ascribed to any avian or other known virus somehow raised by our processing mechanism, and we plan to soon publish a complete report. Meanwhile, the present photographic documentation is available.

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