temperature. The enhancement of CPE with respect to the methods of incubation varied with the virus strains employed. At an incubation temperature of 24°C only reovirus type 2 produced CPE and HA to a limited extent in WI-38 cells. 4. Reovirus types 1 and 2 HA antigens prepared in WI-38 cells showed no significant differences in titers when HA was carried out at temperatures of 4, 24 and 37°C. Antigens prepared with 3 strains of type 3 failed to agglutinate erythrocytes at 4°C but demonstrated HA at 24 and 37°C.

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Received October 30, 1964. P.S.E.B.M., 1965, v118.

Virus-Like Particles Associated with Chloroleukemia in the Rat.* (29874)

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Many investigators have now succeeded in inducing leukemia in mouse and rat with mouse leukemia virus which was initially isolated from tumors, from mice with spontaneous leukemia or from mice with leukemia induced with carcinogenic agents(1,2). Attempts to isolate leukemogenic viruses from rats with spontaneous or chemically induced leukemias have, however, been unsuccessful. These failures can be due either to an absence of such viruses in the rat or to the use of unsatisfactory techniques. This report deals with the results of an electron microscopic study in which virus-like particles were observed in leukemic rats.

Materials and methods. Chloroma cells, harvested originally in 1958 from a non-inbred Wistar rat with Shay chloroma, have been subtransferred subcutaneously in Wistar rats for the last 7 years(3). Three such rats, two from generation 52 and one from generation 55, were used in this study. Peripheral blood smears, organ imprints and histologic study of the tissues of these animals revealed that each animal had acute myelogenous leukemia with involvement of spleen, thymus and liver at time of sacrifice. Several rats from later generations, also bearing myelogenous leukemia, were examined using negative staining techniques but were not studied with thin sections.

Small pieces of chloromas were fixed in 2% osmic acid buffered with veronal-acetate at pH 7.2. Tissues were then dehydrated with ethanol, passed through graded dimethyl sulfoxide (DMSO) and propylene oxide and

* This investigation was supported by USPHS Research Grant CA-05691 from Nat. Cancer Inst., and by USPHS Research Grant CA-07368-01 from Nat. Inst. Health.

embedded in Epon(4). Thin sections were cut on glass or diamond knives. Sections were stained with lead hydroxide(5) and examined with a Siemens Elmiskop I electron microscope.

Negative staining was carried out using the spread cell method of Parsons(6). The staining solution used was a 3% phosphotungstate solution containing 0.05% bovine serum albumin to facilitate spreading. The final pH of the staining solution was adjusted to 4.9 with normal potassium hydroxide.

Results and discussion. Thin sections of all 3 chloromas examined revealed electron dense virus-like particles in the intercellular spaces of the chloromas (Fig. 1). This location is similar to that found in rats infected with mouse leukemia virus(7). The quantity of particles present varied considerably among

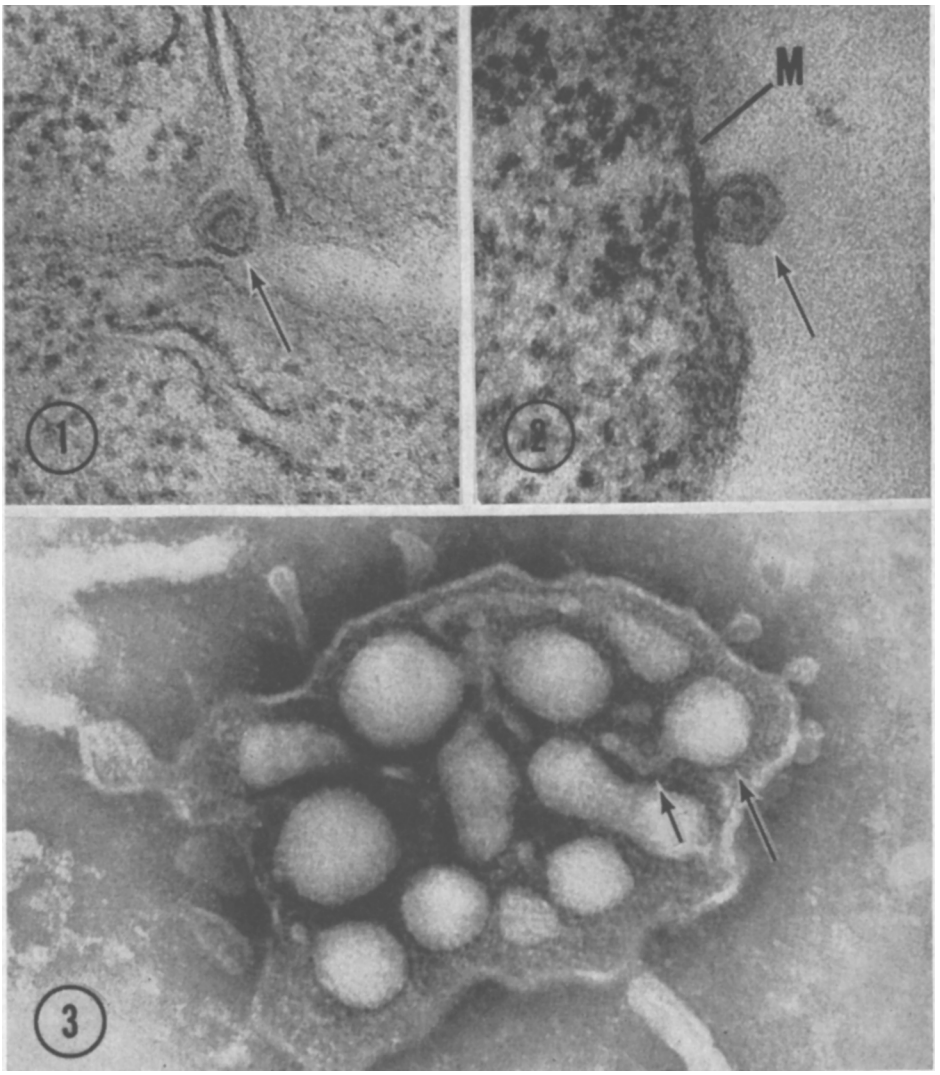

FIG. 1. Intercellular particle (arrow) in a chloroma. The particle has both an outer and an inner limiting membrane. × 100,000.

FIG. 2. A particle (arrow) which appears to be at an end stage of budding out through the plasma membrane (M) of a cell. × 120,000.

FIG. 3. Negative stain preparation from the spleen of a rat with Shay chloroleukemia. What is interpreted as a fragment of cytoplasm contains many particles. A typical particle (long arrow) has a tail-like structure (short arrow). × 180,000.

the 3 tumors. The tumor in which particles were most plentiful had 5 to 10 particles per thin section while the tumor with the least had an average of one particle for every 10 to 20 thin sections examined. Also observed were particles which appeared to be budding out from the plasma membranes of the chloroma cells (Fig. 2).

The electron dense particles, as seen in thin sections, are approximately 70 mμ in diameter. Incomplete particles budding from plasma membranes are slightly larger. All of the particles have an outer limiting membrane and some have an inner limiting membrane while others do not. This may be a function of the degree of maturation of the particles(8). The particles have a core of variable density. Some of the particles appear hexagonal in shape.

Negative stain preparations of the chloromas have not been convincing as a variety of particles of widely differing dimensions are seen. Similar preparations of spleen from animals with Shay chloroleukemia are more convincing. What are interpreted to be fragments of cytoplasm contain many particles (Fig. 3). Some of these particles have tail-like processes. The length of the tails is variable but the diameter is quite constant. The particles seen in these negative stain preparations are of comparable shape and dimension to those seen in thin sections.

Summary. An electron microscopic study of non-inbred Wistar rats with acute myelogenous leukemia has revealed the presence of intercellular particles with the morphologic features usually ascribed to type C virus particles(9). These particles are approximately 70 mμ in diameter, hexagonal in shape, and may have tail-like structures. Their distribution is similar to that reported for the murine leukemia viruses. Although attempts to isolate leukemogenic viruses from rats with spontaneous leukemia or chemically induced leukemia have been unsuccessful, this study provides morphologic evidence for the existence of such particles in leukemic rats.

The authors gratefully acknowledge the useful suggestions and continual encouragement given them by Dr. Stanley Bullivant, and thank Mr. Richard R. Hebert and Mr. Vincent King for their valuable technical assistance.

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Received November 2, 1964. P.S.E.B.M., 1965, v118.

Further Studies on the Hemolytic Properties of Arboviruses.* (29875)

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In a previous report on the hemolytic properties of eastern and western equine encephalomyelitis viruses propagated in tissue culture, it was noted that other serogroup A arboviruses grown in a similar manner also possessed this property(1). The present report is concerned with the study of the hemolysins of these other serogroup A viruses as well as representatives of B, C, Bunyamwera and California serogroups(2,3,4,5,6,7). It appears that hemolytic activity is a com-

* This study was conducted under the auspices of Commission on Viral Infections of Armed Forces Epidemiological Board and was supported in part by Office of Surgeon General, Dept. of the Army, and in part by a grant (AI-04566) from U.S.P.H.S.