mals. 2. No significant removal of either free or complexed Hp from the perfusate occurred during 4 hours of liver perfusion. 3. In intact animals, the T½ of I^{125} Hp-Hb was considerably shorter (2 hours) than that of free I^{125} Hp (19 hours); in both groups, hepatic uptake per gram of tissue was less than splenic uptake, and was minimal. 4. It is concluded that the liver, the major site of Hp synthesis, is of limited importance in the degradation of this protein and of its hemoglobin complex.

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Electron Microscopic Quantitation of Viral Particles in Tissues of Leukemic Mice.* (31315)

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There have been numerous reports in the literature concerning the presence of virus-like particles in the lymphatic tissue of leukemic mice(1). Investigators have made use of techniques to determine the frequency of particles in thin sections(2), which have proved to be of use when relatively large numbers of particles were present. However, when small numbers of cells and/or particles are inoculated, the number of viruses in the initial stages of leukemia may be quite small. While evaluating certain preliminary observations in mice, it became necessary to determine the presence and frequency of virus-like particles when few were present in the non-inoculated controls and inoculated mice. There was, therefore, a need to indicate an appropriate number of sections that should be observed before concluding the tissue "negative" of particles.

Description of inoculation. In the preliminary study one- to five-week-old axenic CFW_w mice, to date free of spontaneous leukemia, were inoculated intraperitoneally with a suspension of bone marrow from 4 human leukemic patients. Diagnosis of the leukemic patients was as follows: chronic myelocytic leukemia, acute reticulum cell leukemia, acute lymphoblastic leukemia, and acute leukemia. Serial passages of resultant leukemias were maintained by the intraperitoneal inoculation of 0.1 ml of a 20% homogenate of leukemic cells in 0.85% saline.

Electron microscopic techniques. Some of the tissues were fixed in 1% osmium tetroxide, pH 7.3, and buffered with a Palade's(3), or a phosphate buffer(4). Other tissues were fixed in a 4 or 6% phosphate buffered glutaraldehyde solution, pH 7.2(5), and post-fixed in osmium tetroxide. The tissues were

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TABLE I. Particle Frequency—Determination.

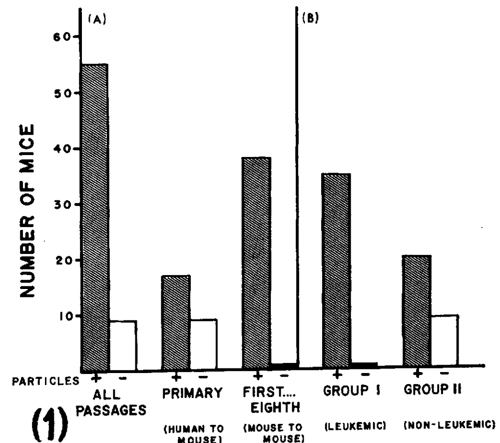
Particle frequency (P.F.)	Nuclear sections Minimum	Maximum
+4	1	120
+3	121	241
+2	242	361
+1	362	2,400
0	More than 2,400	

embedded in epon 812(6), or an epon-araldite mixture and sections cut with the Porter-Blum (MT-1) or Cambridge microtomes and stained with uranyl acetate(7) or lead citrate (8). The sections were examined and photographed with an RCA-EMU-3G electron microscope.

Determination of particle frequency. The particle frequency was defined as the presence or absence of particles within a specified sectioned area, which was dependent upon the number of nuclear sections (only a small portion of any nucleus or cell is viewed) within the tissue area examined with the electron microscope. Based on the finding of particles in the sections of leukemic mouse tissue, the following arbitrary method for establishing particle frequency was devised. An area of tissue containing 2,400 nuclear sections was examined from the tissue of any one mouse before that animal was declared "negative" of particles. These nuclear sections included all cells that might be encountered in any lymphatic tissue. The term "negative," in our opinion, indicated that further study of these tissues with the electron microscope was not a practical means of determining the presence or absence of particles. The frequency of particles was rated according to Table I.

Using the above system, a +4 indicated that at least one particle was found before tissue areas with 120 nuclear sections had been examined.

Before a study of the inoculated mice was made it became necessary to determine a base-line for comparison in the uninoculated axenic mice, as there have been reports of viral particles in untreated axenic Swiss and C3H mice(9). Tissue areas with approximately 25,000 nuclear sections were examined from the spleen, thymus, and lymph nodes from a total of 13 axenic mice varying in age from 6 to 10 months. The examined cells included epithelial cells from the thymus, megakaryocytes from the spleen, lymphocytes and reticular cells from thymus, spleen, and lymph nodes. There were no particles of any known type found in the above tissue sections from these uninoculated mice. Though this technique does not prove the absence of definitive viral particles in these



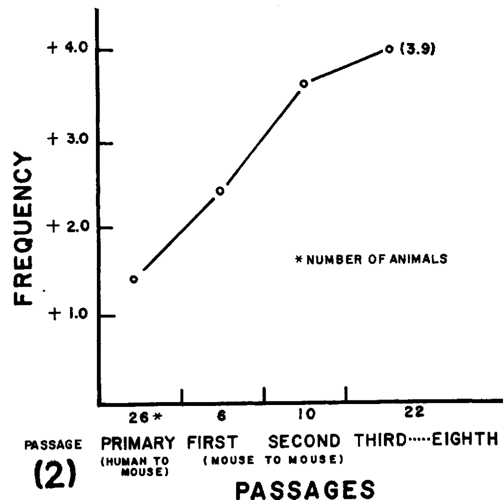


FIG. 1. Presence or absence of particles within various groups of axenic mice inoculated with leukemic cells. (A) Comparison of the primary with the subsequent passages showing that most of the mice in the former and all in the latter group contained particles. (B) A comparison between inoculated leukemic and non-leukemic mice showing that all leukemic mice demonstrated particles.

FIG. 2. Estimate of the particle frequency (P.F.) value for each of the indicated cell passages in inoculated axenic mice.

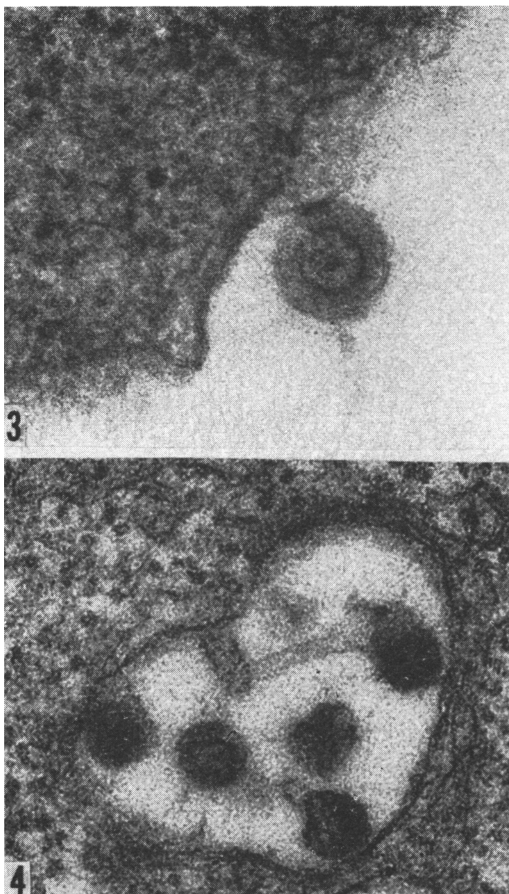


FIG. 3. Section through spleen from one of the leukemic mice inoculated with bone marrow cells from the reticulum cell leukemia. Virus particle adjacent to the plasma membrane of a lymphoid cell. Mag. 123,000 ×.

FIG. 4. Section through lymph node from a leukemic mouse of one of the second serial passage inoculated with the above cell line. Exemplifies the same type particle and an increase in the number of particles as the P.F. value increased. Mag. 88,150 ×.

uninoculated mice it did indicate the lack of particles as compared with mice inoculated with leukemic cells.

Results of inoculations. Most of the results utilizing this technique are summarized in Fig. 1 and 2. With this method it was possible to find particles in all leukemic mice and 68% of non-leukemic mice inoculated with human leukemic cells (Fig. 1). In contrast to this, no particles were found in the uninoculated axenic mice. It was possible to correlate the particle frequency with serial mouse passage of reticulum cell leukemia derived from the patient with acute reticulum cell leukemia. There was a definite increase in the number of particles as the cells were serially passed in the mice (Fig. 3, 4). This increase was correlated with a concomitant decrease in the time for development of leukemia from 5 months in the primary passage to approximately 3 weeks by the second passage. It was possible that additional observations of more than 2,400 nuclear sections per animal would have increased the percentage with particles; however, in our opinion these additional studies would not have appreciably changed the results.

Summary. A simple method for estimating the relative number of viral particles in thin sections of tissue is described. By this method particle frequency was defined as the presence or absence of particles within a specified sectioned area, which was dependent upon the number of nuclear sections examined with the electron microscope. This technique was applied to CFW_w axenic mice inoculated with human leukemic cells. It was observed that the number of viral particles appeared to increase as the cells were serially transferred in mice.

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