and their corresponding precipitins.

Interestingly, although we expected agarose to serve as a suitable medium-gelling agent for our cationic antigen because it is supposed to be a neutral derivative of agar(6), we found it unsatisfactory since it too bound this antigen. Why this happened we do not know.

Summary. A cationic (basic) protein, methylated serum albumin, could not be analyzed by immunodiffusion in agar or agarose gels because it would not diffuse through them. Using gels of gelatin solved this problem. This finding may be applicable to gel-diffusion studies of other basic protein antigens, and it indicates that immunodiffusion tests in gelatin can be employed to complement those performed in agar to detect otherwise overlooked cationic antigens and corresponding precipitins.

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Localization of C-Type Virus Particles in Lymphoid Germinal Centers of C58 Mice.* (32556)

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(Introduced by Walter J. Nungester)
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Various aspects have been investigated of the distribution and ultrastructural properties of C-type virus particles in tissues of mice with spontaneous(1,2) or experimentally virus-induced(3-8) leukemia. Few studies have been reported for C58 mice, a strain with a high incidence of spontaneous lymphoid leukemia(9). Accordingly, a study was undertaken by electron microscopy to trace the frequency and distribution of virus particles in the hematopoietic and lymphoid tissues of C58 mice during the course of spontaneous leukemia. One paramount reason for conducting such a study was to obtain insight into the sequence of events at the preleukemic stage which lead to the evolution of fatal leukemia. Thus, mice were studied immediately after birth and at each succeeding month until they died of lymphocytic leukemia.

This study reports that C-type virus particles are readily detectable by electron microscopy in tissues throughout the preleukemic stage. The most significant finding was the localization of C-type virus particles in the germinal centers of the spleen and lymph nodes.

Materials and methods The lineage of the C58 mice used in this study has been described(10). In accordance with recommended nomenclature(11), they are designated the C58/Wm line. Starting with newborn mice, males and females were killed by cervical dislocation at approximately monthly intervals of age. Bone marrow, mesenteric lymph node, spleen and thymus were removed for histologic and electron microscopic study.

For light microscopy, tissues were fixed in Bouin's fixative and stained with hematoxylin and eosin. Tissues for electron microscopy were fixed in 2.5% phosphate-buffered glutaraldehyde, post-fixed in 1% OsO_4, and embedded in Epon 812. Thick sections (.5 to 1 μ) were cut and stained with toluidine blue for purposes of orientation and identification of germinal centers. Thin sections were

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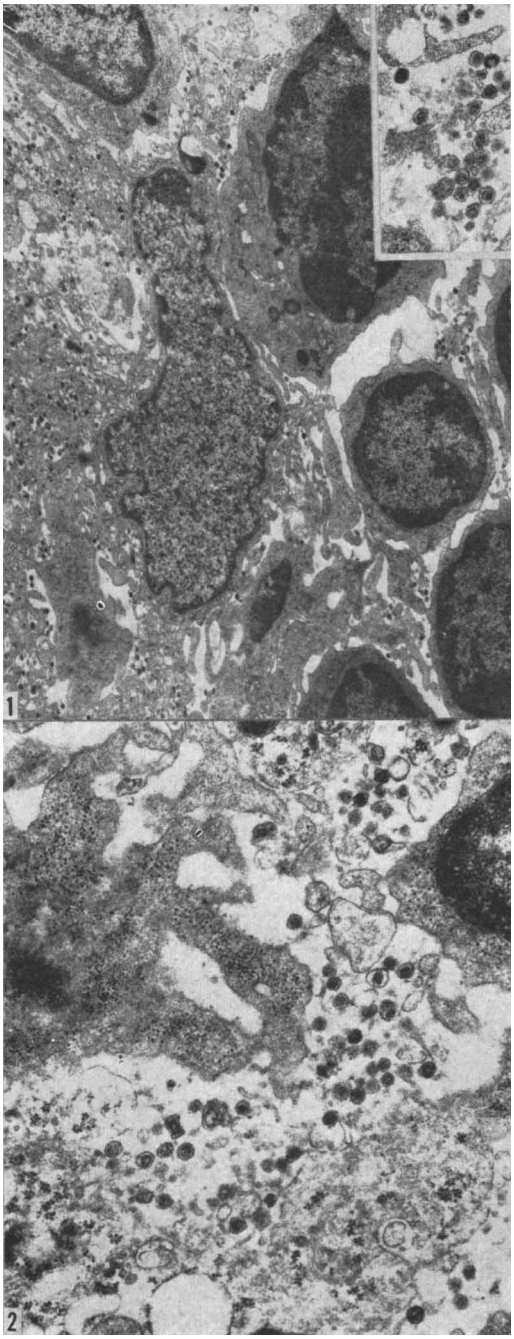

FIG. 1. Electron micrograph of germinal center from mesenteric lymph node of 5-month-old C58 mouse. Note accumulations of C-type particles in the network of cellular processes, elongated reticular cell nucleus and adjacent lymphocytes. × 6,000. Inset shows budding C-type particle and mature particles at higher magnification. × 20,000.

FIG. 2. Numerous C-type particles from splenic germinal center of 3.5-month-old C58 mouse illustrating apparent cytopathic changes on the reticular network. × 24,000.

mounted on copper grids and stained with aqueous uranyl acetate and lead citrate prior to examination in a Philips EM200 electron microscope.

Results. Tissues from more than 30 C58 mice of various ages have been examined. The representative results presented here were obtained from studies done on 5 mice that were 3, 3.5, 5, 6, and 6.5 months of age, respectively. Histologic examination of the spleens and lymph nodes of C58 mice in these age groups disclosed the presence of well-defined germinal centers in the splenic white pulp and lymph node cortex. Such centers were noticeably larger in mice that were 6.5 months old compared with 3 month old mice. None of the mice examined displayed overt signs of leukemia, *i.e.*, grossly enlarged spleen, thymus or lymph nodes.

Electron microscopic study of the germinal centers in the spleens and lymph nodes of the same mice revealed the presence of many C-type virus particles associated with the branching processes of the reticular network interspersed with free cells in such germinal centers (Fig. 1). C-type particles were extracellular or within vacuoles of the cytoplasm of the reticular network. The particles have the structural features(12) classical for C-type virus particles: a dense nucleoid surrounded by an outer envelope approximately 100 mμ in diameter, and typical budding. Budding particles were associated with elongated cellular processes of the reticular network and also with the cell membranes of free cells in the germinal center. C-type particles were more numerous in the germinal centers of the older (6.5 months of age) mice than in the 3 to 3.5 month old animals.

Electron microscopic study of splenic white pulp, other than germinal centers, disclosed very few C-type particles. Thus, in the spleens of C58 mice, virus-like particles were selectively localized in the germinal centers. In the lymph nodes on the other hand, C-type particles were not strictly localized in germinal centers, but also were associated with macrophages and littoral cells lining sinuses and with endothelial cells. Budding C-type par-

ticles were relatively scarce in the splenic red pulp, bone marrow and thymus. No large aggregates of particles similar to those seen in germinal centers were found.

Extensive vacuolization was noted in the cytoplasm of some reticular cells containing C-type particles. Alterations were observed in the cytoarchitecture of the network of cell processes in some cases (Fig. 2) indicating cytopathic changes. The severity of the alterations seemed to correlate with the number of C-type particles per unit area and was observed in germinal centers of all the mice examined.

Discussion. From the studies summarized here, it is clear that C-type virus particles are present in the hematopoietic and lymphoid tissues of C58 mice throughout the preleukemic stage of disease. The most interesting observation was the localization of C-type particles in splenic and lymph node germinal centers. The fine structure of the reticular cell network in germinal centers, in which C-type particles were concentrated, has been previously described(13-15) and conforms to criteria recognized for specialized reticular cells with dendritic processes. The elaborate, lacy network of fine cell processes is effective as an antigen-retaining or trapping mechanism (16,17) and is a conspicuous ultrastructural feature of the germinal center.

The accumulation of C-type particles in the dendritic reticular network and the occasional cytopathic changes observed, suggest that virus may have an effect on the capacity of the animal to respond to oncogenic virus infection and thus contribute in an immunologic sense to the pathogenesis of leukemia. One could hypothesize that destructive effects of C-type particles on a family of cells in germinal centers correlates with the depression of immune responses observed in rodents inoculated with mouse leukemia viruses(18-20). There is evidence also from *in vitro* studies that C-type viruses induce cytopathic changes on phagocytic cells(21).

It is considered of importance to identify virus-containing cells in leukemic tissues. The effect of thymectomy on lowering the incidence of spontaneous leukemia(22) has directed attention to the thymus. Reports describing virus-like particles in other strains of nonleukemic conventional and germfree mice(23-25) have emphasized their occurrence in reticular-epithelial cells of the thymus. In contrast, the C58 mouse appears unique in that accumulations of C-type particles were found in lymphoid germinal centers and infrequently, by comparison, in the thymus during the preleukemic stage of disease. However, C58 mice have a high incidence of spontaneous leukemia in contrast to the mouse strains examined in the above mentioned studies. It is known that neonatal thymectomy exerts a powerful influence on the development of lymphoid tissues and contributes significantly to a reduction of germinal centers (26). Our findings that C-type particles were present in the hematopoietic tissues of C58 mice at birth and later localized in lymphoid germinal centers suggests that the role of the thymus in leukemogenesis should not be restricted simply to it as a source of virus containing cells.

In a recent study, virus-like particles were also observed in splenic germinal centers of nonleukemic $BC3F_1$ mice in the same sites as extraneous antigen(27). The possibility exists that functionally adapted cells in key sites, such as specialized reticular cells in germinal centers, performing a specialized role in the retention of antigens, concentrate large numbers of virus particles. This concentration of antigens, beneficial to the animal in evoking an immune response, is a normal biologic function; but in the case of viruses which have biologic activity, may lead to infection of these cells and thus contribute directly to the pathogenesis of disease.

Summary. A systematic electron microscopic study was done on bone marrow, spleen, lymph node, and thymus tissues of C58 mice which have a high incidence of spontaneous lymphocytic leukemia. The primary objective was to determine the frequency and distribution of virus particles over the lifespan of these mice. The unique finding was made that C-type virus particles were localized in germinal centers of the spleen and lymph node of young (3 to 6.5 months old) preleukemic mice. C-type particles were associated with the lacy network of reticular cell dendritic processes in germinal centers. Virus particles were found infrequently in other areas of the spleen or

lymph node, thymus and bone marrow of preleukemic mice. Alterations were observed in the cytoarchitecture of the reticular cell networks that contained many particles. The possible importance of the occurrence of C-type particles in lymphoid germinal centers was discussed in terms of the pathogenesis of spontaneous leukemia.

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Prenatal Glucocorticoid Administration and the Development of the Epinephrine-Forming Enzyme.* (32557)

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The conversion of norepinephrine to epinephrine is catalyzed by the enzyme phenylethanolamine-N-methyl transferase (PNMT). The enzyme is highly localized in the adrenal medulla but lesser activity is detectable in heart and brain(1). Animals with a larger adrenal cortex and greater proximity of the cortex and medulla have a higher ratio of epinephrine to norepinephrine(2). Recent studies have shown that PNMT activity is controlled by the pituitary *via* the adrenal cortex. PNMT activity and epinephrine content are decreased following hypophysectomy and are restored by treatment with ACTH or glucocorticoids(3). The present report describes the effects of prenatal injection of physiological(4,5) and higher doses of glucocorticoids on PNMT activity and epi-

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