mentally by immunologic or chemical means (1,2,4). Usually homologous rather than human neoplasms have been employed. Lymphocyte-borne immunity probably represents the first recognized host reaction induced by foreign tissues, including neoplasms(1).

Cortisone preconditioning and irradiation have made heterologous tumor transplantation into hamsters successful allegedly partly because they have inhibitory effects upon host lymphocytes. But host resistance appears incompletely inhibited by cortisone, since the growth of transplanted human melanoma was enhanced after antilymphocyte sera. The injection of antiserum was given at the critical time when tumor was beginning the logarithmic growth phase. Lymphocytes were decreased for a period of only 6 days, but this was sufficient for increased tumor growth.

Tumor transplant growth was often prevented by prior footpad injections of frozen melanoma and adjuvant. Transplant rejection occurred more frequently than restricted tumor growth. Some successful transplants under these conditions suggested that the enhanced host resistance might later be overcome by cortisone conditioning.

Independent of immune reactions, melanoma growth could be intrinsically altered by Methotrexate in subsequent untreated transplantations. Chemotherapy clearly affected the melanoma cells rather than the hamster lymphocytes. The alteration was functionally but not morphologically demonstrable. These experiments suggest that a more effective control of some neoplasms might be achieved by therapy directed simultaneously at enhancing host resistance and reducing tumor growth activity.

Summary. Human malignant melanoma grew significantly larger than usual in hamsters treated with antilymphocyte sera. Footpad injections of frozen tumor with adjuvant resulted in increased melanoma transplant rejections. Methotrexate chemotherapy of donor melanoma reduced the subsequent growth in untreated hosts.

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Distribution of Virus-Like Particles in the Lymphatic Tissues of "Nonleukemic" CFW$_W$ Conventional Mice.* (31592)

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Virus-like particles have been described not only in leukemic mice(1-4) but also in "nonleukemic" conventional and germfree mice(5-7). Most of the particles indicated in these reports were located in the thymus. The purpose of this study is to compare the particle distribution in the thymus with the spleen and lymph nodes of "nonleukemic" conventional CFW$_W$ mice.

Materials and methods. The mice used in this study were randomly inbred CFW mice raised locally for 8 years and designated as CFW$_W$ mice. An examination was made of 18 uninoculated conventional CFW$_W$ mice ranging in age from 1 to 6 months. In our laboratory these conventional mice show a 19% spontaneous incidence of lymphocytic leukemia by 12 months and 29% by 24 months of age. All the uninoculated animals

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were "nonleukemic" by gross observation and the majority were checked by light microscopy.

The tissues were in a 2 or 4% phosphate buffered glutaraldehyde solution, pH 7.2(8), and post fixed in osmium tetroxide. The tissues were embedded in epon 812(9), or an epon-araldite mixture, sections were cut with the Porter-Blum (MT-1) or Cambridge microtomes and stained with uranyl acetate (10) and lead citrate(11). The sections were examined and photographed with an RCA EMU-3G electron microscope.

The particle distribution was determined by counting the number of nuclear sections in an area of tissue(12) and a minimum of 2400 nuclear sections were observed in the lymph node, thymus, and spleen of each animal. The virus-like particles will be designated as either A^2 or C type according to a classification by de Harven(13).

The thymus tissue removed for electron microscopy was routinely taken from the right lobe, unless otherwise indicated, and the left lobe was used for light microscopic examination.

Results. The results have been summarized in Fig. 1. Thin sectioned areas were observed throughout the spleen, thymus, and lymph node of each "nonleukemic" animal. Examination of the thymus showed 61% with C type and 28% with A^2 type particles (Fig. 1), while one animal contained both A^2 and C particles. The C type virus particle in the thymus were found exclusively in close association with or within vacuoles of epithelial cells (Fig. 2), while the A^2 particles were located within the cytoplasm of lymphocytes or lymphoblasts (Fig. 4).

A recent report shows a lack of particles in spleens and lymph nodes of normal C3Hf mice(7), however, we found C type particles in 22% of the spleens and 17% of the lymph nodes of CFW_w mice (Fig. 1). The above C type particles were found only in those animals containing virus particles in the thymus. The C type particles in the spleen and lymph node were associated primarily with lymphocytes (Fig. 3).

Discussion. Our results showed particles, not only in the thymus of "nonleukemic"

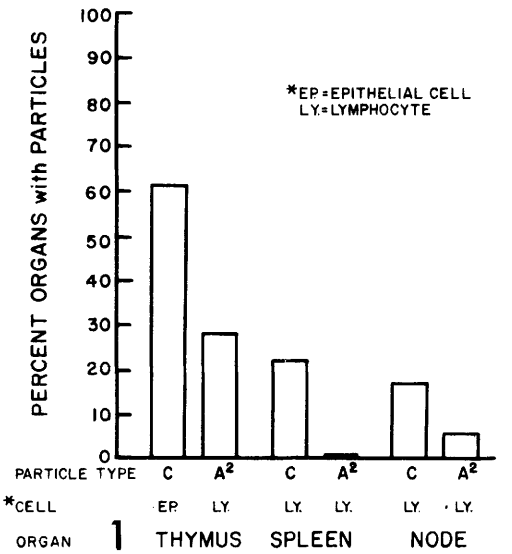

FIG. 1. Estimate of percent organs and cells with virus-like particles from 18 nonleukemic conventional CFW_w mice. Results were based on electron microscopic examination of thin sections.

CFW_w mice, but also in the spleen and lymph node. The higher incidence of particles in the spleen and node, when compared with the C3Hf(7), may be partially related to the higher incidence of leukemia in the CFW_w mice. Gross(14) indicated the C type particle to be most frequently involved in spontaneous leukemia. Our results did show that the percent of mice with C type particles in the spleen closely approximated the spontaneous incidence of leukemia in the CFW_w mice. However, the incidence of A^2 particles was low as exemplified by A^2 particles being present in the lymph node of only 1 animal, although 28% of the thymus glands were positive. The relationship of particles in "nonleukemic" mice to particles in leukemic mice is not known, although various alternatives have been proposed. Feldman and Gross(7) indicated that the presence of similar particles in both healthy and leukemic mice may indicate the particles are unrelated to mouse leukemia. However, particles might be present before there is any gross evidence of leukemia, accounting for the presence of particles in our "nonleukemic" animals. It is also possible that both types of particles (A^2 and C) are needed before "spontaneous" leukemia can be initiated.

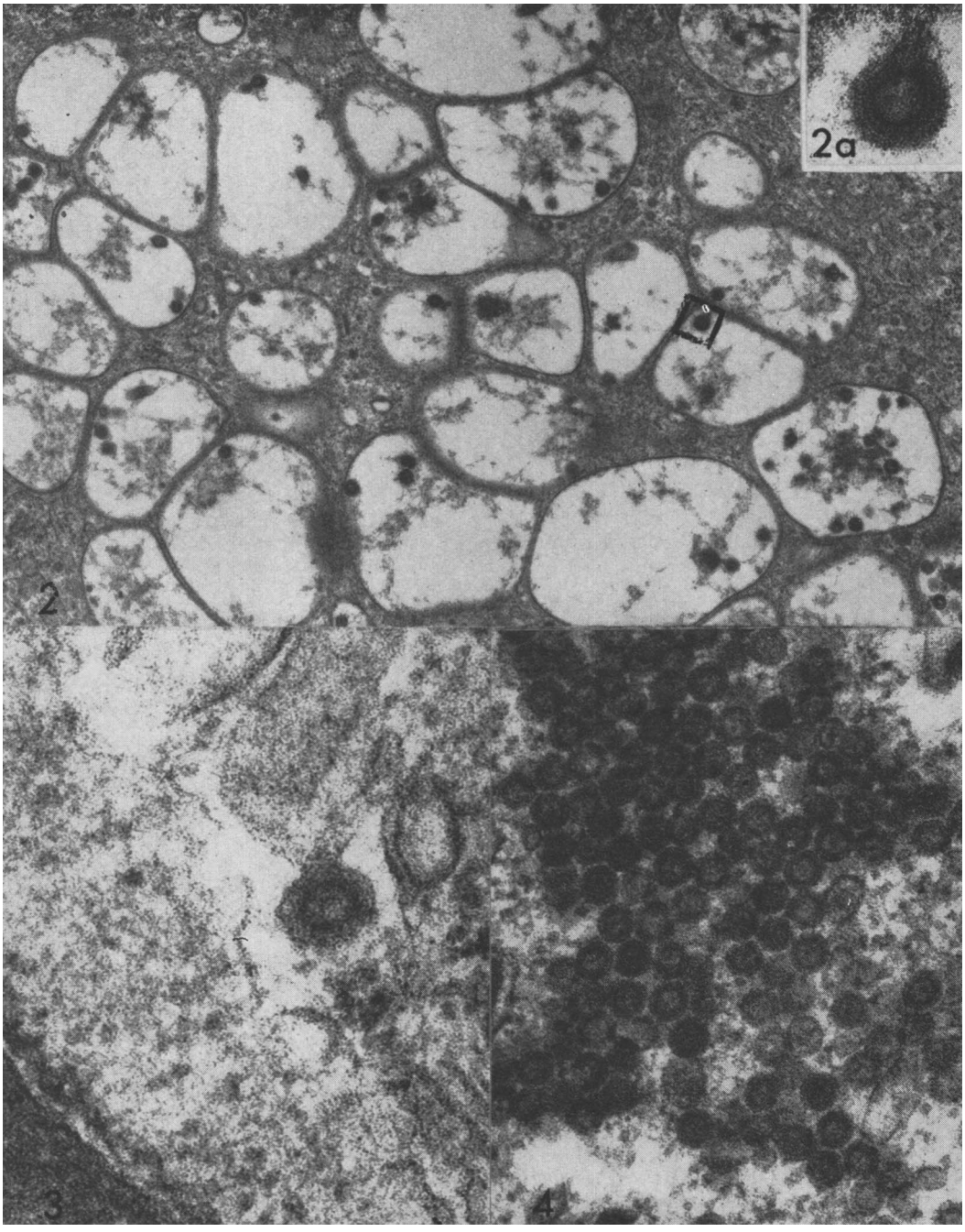

FIG. 2. Section from the thymus of nonleukemic conventional CFW_w mouse. Shows budding immature C type particles within vacuoles of epithelial cell. × 20,880. 2a. Detail showing budding of immature C type particles (approximately 100 mμ in diameter). × 140,000.

FIG. 3. Section from spleen of nonleukemic conventional CFW_w mouse. Shows immature C type particle budding within a lymphocyte. × 140,000.

FIG. 4. Section from thymus of nonleukemic conventional CFW_w mouse. Shows smaller A^2 type particle within the cytoplasm of a lymphocyte. (Approximately 70 mμ in diameter). × 73,000.

The seemingly high incidence of virus particles in the thymus gland, when compared with the spleen and lymph node, is suggestive of some means for maintaining a concentration of particles in the thymus. There are reports that the thymus may be involved in the development of leukemia in mice(5,6,17), possibly serving as a source for the virus. Because of this postulated ability of the thymus to act as a reservoir for virus, a discussion of possible reasons for finding a higher number of mice with particles in the thymus gland would be appropriate. It is recognized that there is a morphologically identifiable thymic blood barrier(15,16). This barrier may retard the movement or "seeding" of the particles from the thymus in much the same manner that it resists the entrance of particulate substances, such as thorium dioxide into the thymus(15). In addition to the barrier, the reduced mobility of the particle-producing epithelial cells, possibly due to desmosomes, may also tend to reduce the transfer of particles from the thymus. In addition to the above mechanisms, however, the possibility of sampling error cannot be overlooked when results are based entirely on electro microscopy of thin sectioned material. For example, the localization of virus-like particles in the epithelial cells of the thymus may increase the likelihood of finding them, whereas the lack of a cell that would concentrate particles in the spleen and lymph node may make the finding of these structures more difficult, thereby giving us an apparent, but unreal, difference in the particle content between organs.

A similar study was made on the germ-free counterpart of these CFW_w mice(12), with no spontaneous leukemia to date, and no virus-like particles were found in the thymus, spleen, and lymph node from these mice. This is an apparent disagreement with results obtained by other workers(6,18) in which it was indicated that all germ-free mice contain virus. It is recognized that the absence of particles by using this technique does not prove the complete lack of particles but it does demonstrate a markedly reduced incidence between the germ-free CFW_w and the conventional counterpart.

Investigations are in progress to determine what factors are needed to vary the number and type of particles as well as the incidence of leukemia in the germ-free and conventional CFW_w mice.

Summary. A study was made on the "non-leukemic" conventional CFW_w mice which show a 29% incidence of spontaneous leukemia by 24 months of age. An estimate on the presence of virus particles in the thymus showed 61% with C type particles and 28% with A^2 particles. In addition, 22% of the spleens and 17% of the lymph nodes examined contained C type particles. The C type particles in the thymus were associated with epthelial cells while the A^2 particles were within lymphocytes. This particle concentration within certain cell types, as it may relate to sampling error, was discussed. The implication of the thymus as a source of virus was discussed, including the possible role of the thymic blood barrier and epithelial cells in the "release" of particles to other lymphatic tissues.

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Serum Electrophoretic Changes Following Heart Allotransplantation.* (31593)

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Serum electrophoretic changes in animals receiving allotransplants have been observed by a number of investigators; however, the results are not uniform and depend upon the animal species and the type of graft used. In earlier studies using cornea and skin allografts(1,2), no consistent effect on serum electrophoretic patterns of the recipient animals was found. On the other hand, West and his co-workers(3) demonstrated a definite change in the level of alpha-2 globulin during the course of kidney allograft rejection. They explained this phenomenon as a response to the inflammatory reaction present in the rejected organ. Peacock and Biggers(4), using skin allograft and xenograft, found a progressive rise in alpha-2 globulin levels after repeated grafting and thought that this represent a cause-and-effect relationship between the development of foreign tissue immunity and a rise in this serum protein fraction. Paronetto and his associates(5), using liver allograft, reported an early increase in alpha-2 and a rise of gamma globulin fraction following rejection of the graft. They stated that this indicates the possibility of production of graft specific antibodies. Pressman(6) has suggested that even though such antibodies may be present, their immediate fixation to the grafted tissue may prevent any alteration in the protein fraction in which those antibodies reside. If this is true, the removal of the graft should trigger a rise in the level of that fraction.

In the present study, experiments were performed to determine changes in serum electrophoretic patterns following allotransplantation of the heart, and their possible modification by antimetabolite treatment; the effect of the removal of the graft on serum protein levels was also followed.

TABLE I. Experimental Design.

Group	Description	No. of animals
I	First set grafting	9
II	Second set grafting	8
III	First set grafting + azathioprine injection	4
IV	Azathioprine injection only	2
V	Sham operation	5

Material and methods. Twenty-eight adult mongrel dogs weighing from 10 to 20 kg were used. Animals were first observed for at least 3 weeks to confirm their general health. During the observation period, total serum protein, serum electrophoresis, and body weight were recorded once a week, and those showing an abnormality were eliminated. The animals were divided into 5 groups (Table I). Group I, 9 dogs, received first set allografts of the heart; Group II, 8 animals, received second set grafts; Group III, 4 animals, was subjected to first set grafting and received daily treatment with azathioprine (Imuran®) starting on the day of transplantation; Group IV, 2 dogs, was subjected to daily azathioprine injections alone; and Group V, 5 dogs, sham operations were performed.

Transplantation of the heart was performed by a modification of Mann's technique as described previously(7). Puppies weighing from 3 to 5 kg served as heart donors. The transplanted hearts were observed daily and rejection was considered complete when the

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