erned by the anticipated needs of the cultures, according to the appearance of the cells.

The incubation temperature had a decisive effect on the parasite's development. Cultures incubated at 41°C after inoculation supported at least one complete asexual generation of the parasite in bovine kidney cell cultures. These cultures were also grown at 41°C prior to inoculation. Similar cultures held at 37°C both before and after inoculation showed only a few very immature schizonts which failed to develop further. A vacuole-like intracytoplasmic space around the parasite in fixed stained tissue cultures (Fig. 1, *A–E*) was useful in quickly locating the parasite in all its asexual stages. These vacuolar spaces are probably artifacts similar to those seen by Huff (*12*) in his study of malarial parasites in tissue cultures, by Hogan *et al.* (*13*) in their study of *Toxoplasma* in tissue cultures, and by Hogan *et al.* (*14*) in their study of *Toxoplasma* in mouse peritoneal macrophages. Coverslip cultures were fixed for 5 to 10 minutes in 10 percent formalin in Hanks balanced salt solution. Fixed cultures were then taken through a series of increasing concentrations of tertiary butyl alcohol mixed with decreasing concentrations of ethyl alcohol and water. Staining was done with 0.05 percent aqueous toluidine blue O (*p*H 6.2). The developing parasites (that is, beyond the sporozoite stage) always accepted the stain to a greater degree than did their host cells. This also aided in locating the parasite in all its asexual stages. Intracellular structures even remotely resembling the various parasitic stages of *Eimeria tenella* were never found in uninoculated cultures.

Finally, two points deserve emphasis: First, the many manipulations in preparing the inoculum resulted in loss of large numbers of oocysts, sporocysts, and sporozoites. Second, further inefficiency was incurred with the relatively small number of sporozoites which developed after invading the host cells. However, all of the stages in one complete asexual generation of the coccidium, *Eimeria tenella*, were clearly and repeatedly demonstrated in a variety of types of cultured animal cells.

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Lymphoma Growth in vivo: Electronic Discrimination between Tumor and Stroma Cells

Abstract. *Moloney lymphoma cells are larger than normal cells of mouse spleen, lymph-node, and thymus. Use of electronic cell-size anaylsis in conjunction with specific immunocytolysis permits differential counting of host and tumor cells. Increase of lymphoma cells occurs first in spleen and then in lymph nodes; in thymus it occurs only during the terminal stages of tumor growth.*

Lymphoma cells, transplanted to genetically compatible mice, can be characterized by various means (histology, specific immunofluorescence, cytotoxicity, and so on). Such techniques, however, are not convenient for detection of tumor cells during the early stages of growth, or for enumeration of these cells within host organs (for example, for quantitative study of growth characteristics).

Electronic analysis of cell size, combined with specific immunocytolysis for differentiation between host and neoplastic cells, has indicated that the enlarged lymph nodes of mice carrying transplanted lymphomas induced by Moloney virus contain cells of two

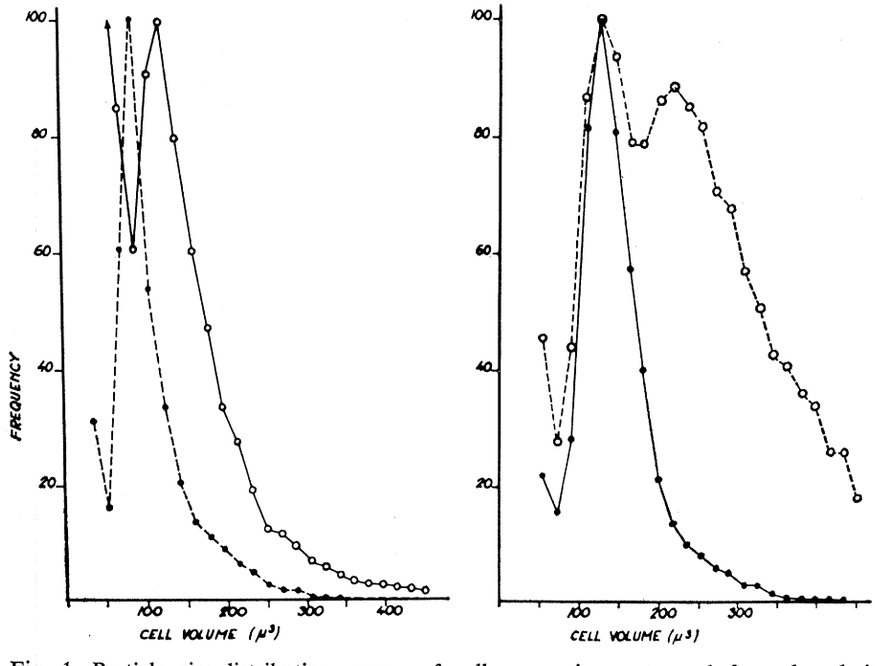

Fig. 1. Particle size-distribution curves of cell suspensions prepared from lymphoid organs of adult mice. Suspensions in BSS containing about 10^5 cells per milliliter were examined with model B Coulter counter. (Left) ●---● thymus, and ○—○ spleen, of normal mice. (Right) Lymph nodes from a normal mouse (●—●) and from a mouse inoculated with YHA lymphoma (○---○). Frequency is on an arbitrary scale.

size groups; the smaller cells are of host origin, and the larger cells are of tumor origin (*1*). We now substantiate this claim, give details of the method used, and show that the method may be used to discriminate between host and tumor cells in other lymphoid tissues of the mouse. We have also used the method to study the growth characteristics of intravenously transplanted Moloney lymphomas within the lymph nodes, spleen, and thymus.

The lymphomas we used had been induced by neonatal infection of mice with Moloney virus; Y7A originated in C57Bl/K1 and YHA in C3H/K1. Hosts were adult F_1 hybrids between the strain of origin and a strain of different H-2 genotype. Lymphoma Y7A was preferred for its higher sensitivity to Moloney tumor-specific cytotoxic antibody (*1*), YHA for the more ready availability of suitable hosts. Cells of the two lymphomas were of similar size (170 to 250 μ^3).

Tumor cells (10^6 per mouse) were inoculated into the tail veins. Organs were cut into small pieces with scissors, and cells were liberated in balanced salt solution (BSS) by sucking up and down with a syringe. From the cell suspensions so produced, dead cells and debris were removed by incubation with 0.5 percent trypsin in BSS for 30 minutes at 37°C. By this method, only dead cells are broken down, but some living cells may be trapped in the viscous DNA liberated from the digested dead cells. Suspensions were adjusted to between 10^4 and 10^5 cells per milliliter with BSS. Curves showing the size distribution were plotted with a "particle size distribution plotter" attached to a model B counter (Coulter Electronics, Hialeah, Fla.) with a 100-μ aperture. Mouse erythrocytes were used for volume calibration.

Table 1. Appearance of transplanted YHA lymphoma cells within mouse lymphoid tissues as a function of time after inoculation of 10^6 cells per mouse. Results are presented as the ratio of the number of mice with detectable tumor cells to the number of mice examined.

Days after inoculation	Spleen	Lymph nodes	Thymus
2	0/6	0/6	0/6
4	4/6	0/6	0/6
6	5/7	4/7	1/7
8	6/6	6/6	2/6
10	6/6	6/6	3/5
13	7/7	7/7	7/7

The large numbers of small particles (less than 70 μ^3) in some samples, notably spleen, were mostly contaminating erythrocytes. Apart from these small particles, each normal tissue contained cells of a single size-group. There was no obvious difference in cell size between corresponding normal tissues taken from seven different inbred strains and various F_1 hybrids between them. Figure 1 shows typical curves obtained with cell suspensions prepared from spleen, thymus, and lymph nodes of normal adult mice and from lymph nodes of a mouse carrying YHA lymphoma. The tumorous lymph-node suspension contained cells of two different sizes, the smaller cells corresponding in size to the cells of normal lymph nodes. Similar double peaks were seen in curves obtained with cell suspensions prepared from the lymph nodes of mice bearing other Moloney lymphomas.

The cytotoxin-sensitive lymphoma Y7A (*H-2^b*) was chosen for studying the nature of the cells comprising the two peaks. The tumor was grown in an (A × C57B1) F_1 (*H-2^{a}H-2^b*) mouse, and a cell suspension was prepared from the enlarged lymph nodes. Host cells carry histocompatibility antigens determined by the alleles *H-2^a* and *H-2^b*; lymphoma cells lack H-2^a antigen, but carry H-2^b and an antigen specific for tumors induced by the Moloney virus (*2*). Specific antiserums are available which, in the presence of guinea pig complement, will kill cells carrying H-2^a antigen, H-2^b antigen, or antigens characteristic of Moloney tumors. Portions of cell suspension (0.2 ml, containing about 10^7 cells) were incubated with equal volumes of antiserums—*H-2^b* antiserum to H-2^a, *H-2^a* antiserum to H-2^b, or antiserum to Moloney tumor antigen (*1*). Control cells were incubated with normal mouse serum. After 15 minutes at 37°C the centrifuged supernatant was removed, and the cells were resuspended in 0.2 ml of 50 percent guinea pig serum in BSS as source of complement. After further incubation (45 minutes at 37°C) the cells were once again collected by centrifugation, and dead cells were digested by incubation in 1 ml of 0.5 percent trypsin at 37°C for 30 minutes. The remaining viable cells were washed once and resuspended in 70 ml of BSS; then a size-distribution curve was plot-

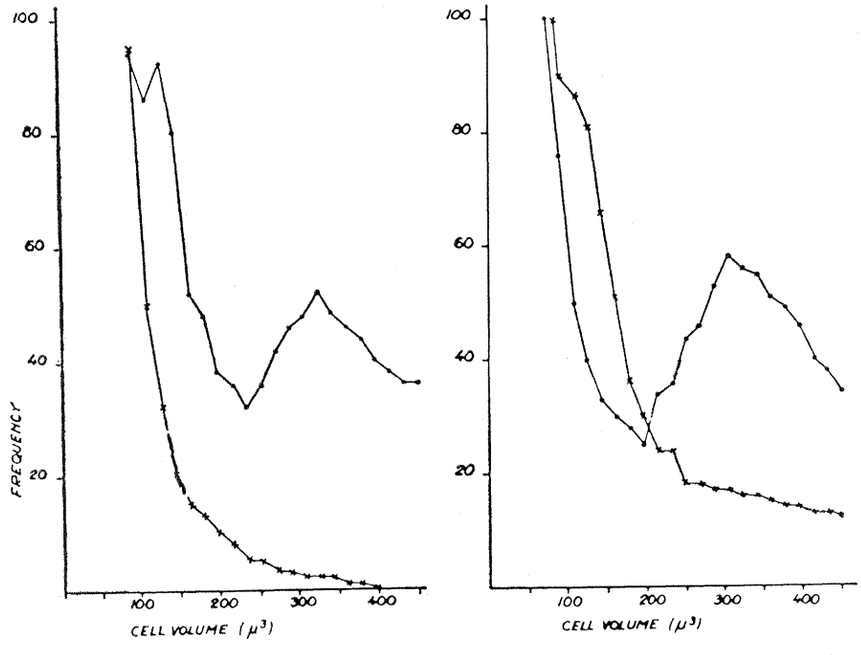

Fig. 2 Size-distribution curves of cells from the enlarged lymph nodes of an A × C57B1 (*H-2^{a}H-2^b*)F_1 mouse, inoculated intravenously with Y7A (*H-2^b*, lymphoma induced by Moloney virus). (Left) Cell suspension first treated with normal mouse serum (●—●) and then with *H-2^a* antiserum to H-2^b (reactive with both host and lymphoma cells) (×—×). (Right) *H-2^b* antiserum to H-2^a (reactive only with host cells) ●—● and antiserum to Moloney tumor-specific antigen (reactive only with lymphoma cells) ×—×. Cells which had reacted with antibody were subsequently killed with guinea pig complement and removed by digestion with trypsin. Frequently is on an arbitrary scale.

ted for each sample (Fig. 2). The control, treated with normal mouse serum, showed the characteristic double peak. Treatment with *H-2*^{a} antiserum to H-2^b (reactive with both host and tumor cells) abolished both peaks, a demonstration that both classes of cells were sensitive to cytotoxic antibody. However, the antiserum to H-2^a (reactive only with host cells), killed only the smaller cells, and the antiserum to Moloney antigen (reactive only with tumor cells) killed only the larger ones. Thus, tumor cells are confined to the second peak, and the first peak represents cells derived from host tissues.

Similar second peaks also appear in the curves from spleen and thymus cells of mice inoculated with lymphoma. We have used this fact to study the growth of YHA in *H-2*^{a}*H-2*^{b} (F_1) hybrids (C3H × C57B1 or C3H × A.BY). The results, similar with both genotypes, were pooled. Groups of mice, inoculated from the same suspension with 10^6 tumor cells, were killed at intervals. Size-distribution curves plotted for cells from spleen, pooled peripheral lymph nodes, and thymus of each mouse, were used together with the curves from corresponding tissues from untreated animals, to calculate the percentage of neoplastic cells within each organ. We have used this value, rather than the absolute number of lymphoma cells in the preparations, in order to reduce errors due to variation in the proportion of cells recovered from the organs, the number of cells lost upon removal of dead cells by trypsin, and other nonspecific factors. We have expressed only the percentage of tumor cells within the organs and have taken no account of the probable changes in the number of host cells within these organs during growth of the lymphoma.

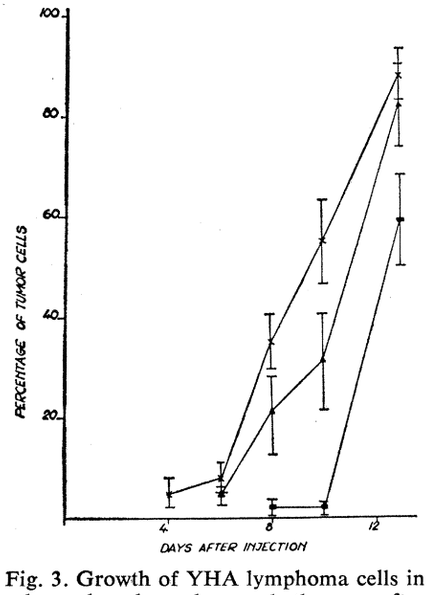

Fig. 3. Growth of YHA lymphoma cells in spleen, lymph nodes, and thymus after intravenous injection (10^6 cells per mouse) into (C3H × C57B1) or (C3H × A.BY)F_1 mice. Proportions of normal and tumor cells were calculated from the heights of the corresponding peaks in size distribution curves (Fig. 1). A correction was applied for overlapping of the peaks by reference to size distribution curves of cell suspensions prepared from corresponding organs of uninoculated mice. Arithmetical mean and standard error of the mean are given for each point; groups of five to seven mice were used. Spleen (×), lymph nodes (▲), and thymus (■) of each mouse were examined.

The three curves are displaced in time (Fig. 3) and indicate that lymphoma cells increase in the spleen about 1 day sooner than in the lymph nodes. Tumor cells appeared in the thymus 3 to 4 days later and were only found in large numbers 13 days after inoculation. At this late stage of tumor growth, other tissues of the host were also invaded and neoplastic cells could be detected, for example, in cell suspension prepared from lung. The data suggest that multiplication of the lymphoma cells takes place within the spleen and lymph nodes, but the late increase in the thymus may be accounted for by massive invasion from without.

Samples of the cell suspensions prepared from the organs of F_1-hybrid hosts inoculated with YHA were also treated with cytotoxic *H-2*^{k} antiserum to H-2^b (reactive only with host cells) in an attempt to detect the first appearance of lymphoma cells. Details of the technique were as described for treatment of Y7A. Less than 5 percent of lymphoma cells admixed with stroma cells can be detected in this way, but more precise quantitation is unreliable. Table 1 shows the ratio of the number of mice with detectable tumor cells to the number of mice examined. These figures also show earliest appearance of tumor cells in the spleen, followed by lymph nodes, followed by thymus (Fig. 3).

Attempts to apply the same technique for the detection of lymphoma cells to bone marrow have failed, because there are two sets of cells in normal bone marrow; the larger of these cells are similar in size to the tumor cells and are also resistant to the cytotoxic action of antibody to H-2 in the presence of complement. Lymphoma cells are thus masked.

This technique for detection and counting of tumor cells within organs offers (i) objectivity; (ii) sensitivity; (iii) speed; (iv) a method for measuring rate of tumor increase within the tissues of the host; and (v) wide applicability for study of tumor growth. The cells of sarcomas, carcinomas, and similar tumors are even easier to detect than are leukemic cells because of their even larger volume.

In order to avoid possible misinterpretations of size-distribution curves, it is, however, important that neoplastic cells be positively identified as described for each new tumor examined.

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Absence or Singular Specificity of Carotenoids in Some Lower Fishes

Abstract. *No colored carotenoids were recovered from the scales or liver of the coelacanth,* Latimeria chalumnae, *or from skin, immature eggs, or liver of the Pacific hagfish,* Eptatretus stoutii. *Zeaxanthin, present as esters in the skin and largely unesterified in the liver, was the only carotenoid identified in the thornback ray,* Platyrhinoidis triseriata, *and in the horned shark,* Heterodontus francisci, *whereas the skin of the Pacific mako shark,* Isurus glaucus, *yielded no carotenoids.*

An earlier review (*1*), surveying the wide occurrence of carotenoids in fishes, indicated the preponderance of the oxygenated or xanthophyllic class, while the hydrocarbon or carotene type was represented uncommonly and in minor proportions. Two xanthophylls resembling taraxanthin and lutein, which are common in plants, were encountered most frequently; astaxanthin