# Growth Characteristics of the Shay Chloroleukemia in Diffusion Chambers[1] (34592)

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Algire (1) demonstrated the feasibility of using diffusion chambers to study *in vivo* cultures of growing cells, and many reports utilizing this technique have appeared (2–6).

More recently, diffusion chambers have been employed to investigate the response of rodent and human leukocytes to phytohemagglutinin (PHA) and to study the kinetics of PHA-stimulated lymphocyte proliferation using tritiated thymidine autoradiography (7–9). The latter studies involved the serial sacrifice of rats bearing intraperitoneal diffusion chambers harvested at specific time intervals after isotope administration. The question has arisen as to whether comparable cell growth occurs in chambers borne by different animal species. Therefore this study was undertaken to investigate the growth characteristics of Shay chloroleukemia cells of rats (10) cultured in diffusion chambers implanted into the peritoneal cavities of rats and mice. Specifically, an attempt was made to determine (1) whether the same initial cell population placed into chambers showed comparable growth in individual rat hosts; (2) if initial cell concentration had any effect on population growth rate; and (3) if rat tumor cells manifested the same growth if chambers bearing them were implanted into mice.

*Methods.* Diffusion chambers were made by cementing two nylon-reinforced Millipore filters of 0.45-$\mu$ porosity to a lucite ring. Chloroleukemia tumors grown subcutaneously for 10 days in suckling rats were passed through a sterile tissue press and the cells dispersed in medium 199 (with 100 units/ml penicillin–streptomycin). One-tenth milliliter of pure tumor cell suspension was injected into chambers previously sterilized under ultraviolet light for 48 hr, and the hole in the ring was sealed with MF cement (Millipore Filter Corp.). The filled chambers were stored for 2 hr in sterile petri dishes containing medium 199 before being implanted into the animals. Feasibility of storage without deleterious effects was verified by the trypan blue dye exclusion technique ( a modification of the Schrek test (11) ). Freshly prepared chambers and those allowed to remain at 4° for as long as 6 hr contained approximately the same proportion (3 %) of dead cells. Cell counts and smears were made from each chamber to determine total cellularity and mitotic indices. Wistar rats and Swiss mice were anesthetized by Metafane inhalation (Pittman-Moore, Indianapolis) and the chambers were introduced into the peritoneal cavity by laparotomy. Two milliliters of medium 199 were injected into the peritoneal cavity after closure of the wound to retard development of connective tissue lesions around the chambers (7, 8).

To determine if rat cells contained within chambers implanted into different hosts grew at comparable rates and if initial cell concentration influenced growth rates, the following experiments were performed in Swiss mice and Wistar rats. Two groups of four animals each of both species, equalized as to sex and weight, received diffusion chambers containing $13 \times 10^6$ cells. Five days later, the chambers were removed and subjected to vigorous shaking in 0.5 % pronase for 30 min to dissolve the clot characteristically found in all

[1] Supported by U.S. Public Health Service Grant 2-RO1-HE03357-12 and a Bio-Medical Science Support Grant to New York University.

TABLE I. Total Counts and Mitotic Indices of Chloroleukemic Cells in Chambers Implanted in Wistar Rats and Swiss Mice.

| Species | Chamber cell inoculum ($\times 10^{-6}$) | 5-Day cell count ($\times 10^{-6}$) | Mitotic index (%) |
|---|---|---|---|
| Rats | 13.0 | 5.26 ± 0.99[a] | 1.6 ± 0.03 |
| Mice | 13.6 | 7.75 ± 0.57 | 1.9 ± 0.12 |

[a] Mean ± standard error of mean.

chambers (after exposure for 30 min to pronase, cells examined microscopically did not appear to have been damaged by this treatment), and cell counts and mitotic indices were determined. One filter from each chamber was removed, and the chamber contents were aspirated into a 1-ml syringe qs to 1 ml with medium 199. Verification of complete cell removal was obtained from additional pronase treatment of some Millipore filters and rings of the chambers. The pronase-containing fluid was centrifuged (2000 rpm) for 10 min, and smears prepared from the sediment revealed few, if any cells.

To estimate the cell growth rates, 52 rats divided into two groups received chambers containing $2.7 \times 10^6$ cells and $5.5 \times 10^6$ cells, respectively. On Days 1, 2, 3, 4, and 7, three to five rats were sacrificed, the cells harvested, and total cell counts made. On Days 2 and 4, one rat from each group was given 0.25 ml of a 0.1 % Colcimide solution by injection 4 hr prior to sacrifice to estimate the mitotic time using a stathmokinetic method (12).

*Results.* After an initial decrease in cell count, chamber cell growth in both rats and mice was approximately the same for populations of similar initial cell concentrations ($13 \times 10^6$) [Table I]. Thus Day 5 cell counts for these two groups were about 5–7 million, and mitotic indices were closely similar. Cell growth, however, appeared to be related to the original number of cells introduced into the chamber, smaller inocula (after initial decreases) growing more efficiently. This is indicated in Table II, which suggests a delayed doubling time before plateau (48 hr as against 24 hr) for the larger inoculum population.

The average mitotic time computed from the stathmokinetic mitotic index of 2- and 4-day cultures was approximately 40 min. Since these measurements were arbitrarily made before and after population numbers had stabilized, these data suggest a similar mitotic time/generation time ratio for both culture systems at these times.

*Discussion.* Once the chamber cell numbers underwent their initial decline period, the rate of growth in all groups was consistent and related primarily to the size of the initial inoculum. Studies of other types of cell populations (2) also indicated a large initial drop in cell number during the first 48 hr of culture.

Growing chloroleukemic cells in diffusion chambers has many advantages over studying tumors grown directly in the host. In the latter case, abnormal changes in nutrition appear as the tumor outgrows its blood supply. The accompanying necrosis and nonuniform growth make the quantitative studies of cell proliferation kinetics difficult. For example, tritiated thymidine was found to be unevenly distributed in solid growing tumors (13), unvascularized areas of the tumor having lower concentrations of the isotope. On the other hand, using the chamber, metastasis is eliminated, uniformity of growth assured, and quantitation of growth facilitated. Moreover,

TABLE II. Numbers of Chloroleukemic Cells Grown in Diffusion Chambers in Rats over 7-Day Period ($\times 10^{-6}$).

| Initial count | Day number 1 | 2 | 3 | 4 | 7 |
|---|---|---|---|---|---|
| 2.7 | 1.41 ± 0.56 | 0.89 ± 0.10 | 1.93 ± 0.84 | 2.20 ± 0.60 | 2.46 ± 0.46 |
| 5.5 | 5.02 ± 1.32[a] | 4.31 ± 0.49 | 4.46 ± 0.11 | 7.75 ± 0.77 | 7.89 ± 0.23 |

[a] Mean ± standard error of mean.

since the initial cell concentration can be controlled, any changes in chamber cell numbers may be interpreted without the complications arising from intrusion of nontumorous host cells.

In the present study, the growth characteristics of the Shay chloroleukemia, which originated in Wistar rats, was unaffected when studied in mice. This is important because obviously the study of rat tumors in mice is both technically easier and more economical.

Mitotic times (calculated for 2- and 4-day cultures) were similar in both the small and large initial inoculum populations. Therefore, it is of interest that the doubling time was significantly longer for the larger inoculum cultures. This suggests that mitotic indices would probably have been lower in these cultures had they been measured during the period of population expansion (*i.e.*, between 2 and 4 days) unless, of course, mitotic times were greater at this time. Since average mitotic durations are fairly constant, this latter possibility appears unlikely.

The need for absolute sterility when working with diffusion chambers must be emphasized. In chambers possessing the same number of cells but bearing microbial infection, the variation in total cell count from chamber to chamber was great, with many of the cells abnormal in shape, size, and nuclear morphology. When scrupulously sterile techniques were employed, the chamber cell growths were uniform, and the morphological characteristics of the cells were identical to those of the young solid tumor cells used to initiate the cultures.

*Summary.* Chloroleukemic cells placed within diffusion chambers grew at a fairly uniform rate in both rats and mice. Both mitotic indices and mitotic times (about 40 min) were the same in all chambers regardless of the number of cells in the initial population. The numbers of cells declined moderately during the first 48 hr and increased slowly until harvest on Day 7.

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Received Sept. 10, 1969. P.S.E.B.M., 1970, Vol. 133.