nephrine or norepinephrine and showed alterations in reactivity similar to those observed with hearts from animals infused *in vivo* with norepinephrine. The results suggest that the infused catecholamines interact directly with mammalian hearts to produce persistent metabolic or pathologic alterations in reactivity.

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The Cell Cycle of Leukemia L1210 Cells *in vivo* and *in vitro*.* (32601)

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Leukemia L1210 has been and is being used extensively for evaluation of candidate anticancer agents. Ascitic cells of this neoplasm can be cultured *in vitro*, and it has been demonstrated that when cultured cells are implanted into mice, a disease indistinguishable from Leukemia L1210 results(1). In view of the current interest in the relationship between the phases of the cell cycle and the susceptibility of cells to various drugs and other agents, it seemed desirable to determine the length of the cell cycle and of the different phases of the cycle for these cells. It also seemed worthwhile to compare the cycles of cells proliferating in the mouse, of cells proliferating *in vitro*, and of cells proliferating in the mouse following implantation of cultured cells. If the cycle of cells in culture is similar to that of cells in mice, then the results of experiments with cultured cells would have increased importance.

Methods and materials. For *in vivo* experiments mice (BDF_1) were inoculated intraperitoneally with 10^5 L1210 cells, and on the 6th day thereafter they were given thymidine-(methyl-^{3}H) (specific activity, 6.7 c per millimole) intraperitoneally at a dosage of 1 μc per gram of body weight. Groups of 3 to 5 animals were killed by asphyxiation with carbon dioxide at various times following the injection of the radioactive substrate, and the ascites cells were taken in syringes containing small quantities of heparin and pooled. The cells were separated by centrifugation in a clinical centrifuge and suspended in 4 ml of cold 0.1 M citric acid. The tube containing the suspension was placed in a water bath at 37°C for ½ minute, and the cells were again collected by centrifugation. (In experiments in which the initial number of cells per unit volume was large, only a portion of the suspension of the cells in citric acid was used for the remainder of the procedure.) The cells were suspended in 0.4 ml of ethanol-

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acetic acid (3:1, v/v) and allowed to stand for 4 minutes. Volumes of 0.1 ml of these suspensions were placed on microscope slides and allowed to dry at room conditions. The dried slides were immersed in 1.0 N hydrochloric acid at 60°C for 6½ minutes and then rinsed sequentially in water and ethanol. This procedure for preparing slides is essentially that described by Puck, Sanders, and Petersen(2). The slides were dipped in a 30% aqueous emulsion of Ilford Nuclear Research Emulsion, Type K-2. After an exposure period of 1-4 days, the slides were developed and stained in a 0.3% solution of toluidine blue in a 1.0% borax solution(3). 1000 nuclei per slide were classified and scored as unlabeled interphase, labeled interphase, unlabeled metaphase, or labeled metaphase (for counting purposes no distinction was made between metaphase and anaphase).

Leukemia L1210 cells were taken from BDF_1 mice and used to initiate a line of cultured cells by the procedure of Dixon *et al*(1). In the course of the next 8 days, the cultures were subcultured 6 times with fresh medium being added each time. On the 8th day the cells were inoculated intraperitoneally into mice, each mouse receiving 10^5 cells. The resulting ascites cells were serially transplanted each 6 or 7 days into other mice. Animals of the 3rd serial transplant group and of the 9th serial transplant group were used for experiments similar to those described in the preceding paragraph.

In *in vitro* experiments cultured L1210 cells of the 29th and 58th subcultures were used as inocula for cultures to be used for cell cycle analysis. 400 ml of medium was inoculated with enough cells to give 10^5 cells per ml. 42 hours later thymidine-(methyl-^{3}H) (specific activity, 6.7 c per millimole) was added to give a concentration of 0.015 μc per ml. After an additional 30 minutes, non-radioactive thymidine was added to give a concentration of 3.6 μg per ml. (This is a 6,650-fold dilution of the labeled material.) Thereafter, 3-ml samples of the culture were taken each 30 minutes for the first 2 hours and each hour for the ensuing 36-40 hours. These samples were centrifuged, and the cells were used for the preparation of stained slides and radioautograms as described above, and cells were counted and scored in the same way.

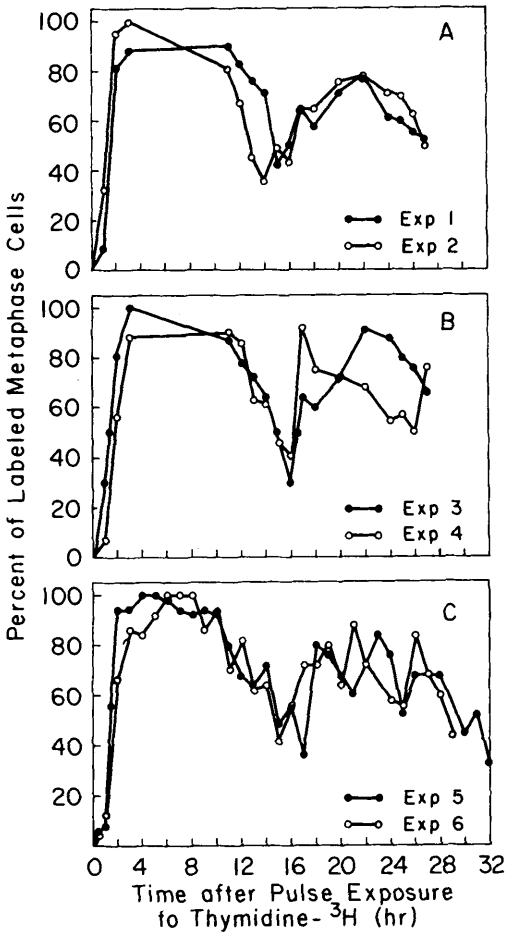

FIG. 1. The percent of labeled metaphase Leukemia L1210 cells at various times following pulse exposure to thymidine-(methyl-^{3}H). A, cell line maintained by serial transplantation in mice; B, cell line obtained by serial transplantation in mice following inoculation of cultured cells; C, cultured cells.

Results and discussion. Fig. 1 shows the percent of labeled metaphase cells at various times following injection of thymidine-(methyl-^{3}H) into the animals bearing serially transplanted L1210 cells (upper portion of the figure) and into animals bearing L1210 cells derived from cultured cells (middle portion of figure). The bottom portion of the figure shows the % of labeled metaphase cells following the introduction of thymidine-^{3}H into the growth medium of the cultured cells. The similarities of the 3 sets of curves are striking.

TABLE I. Analysis of Waves of Labeled Mitoses.

Experiment	A Midpoint of 1st ascent %	A Time (hr)	B Midpoint of 1st descent %	B Time (hr)	C Midpoint of 2nd ascent %	C Time (hr)	T_C* (C—A)	T_{G_2} (A)	T_M†	T_S (B—A)	T_{G_1} [T_C—(T_{G_2} + T_M + T_S)]	Mitotic index (%)	Labeling index (%)‡ Observed	Labeling index (%)‡ Calculated
Cell line maintained by serial transplantation in mice														
1	45	1.4	66	14.2	60	17.6	16.2	1.4	0.60	12.8	1.4	2.6	52.6	
2	50	1.3	68	12.1	57	16.6	15.3	1.3	0.46	10.8	2.7	2.1	57.2	
Average							*15.8*	*1.4*	*0.58*	*11.8*	*2.1*	*2.4*	*54.9*	68.3
Cell line obtained by serial transplantation in mice following inoculation of cultured cells														
3 (3rd transplant generation)	50	1.4	65	14.0	60	18.0	16.6	1.4	0.28	12.6	2.3	1.2	63.3	
4 (9th transplant generation)	45	1.7	65	12.9	58	16.6	14.9	1.7	0.36	11.2	1.6	1.7	51.6	
Average							*15.7*	*1.6*	*0.32*	*11.9*	*2.0*	*1.5*	*57.5*	70.0
Cultured cells														
5 (29th subculture)	50	1.4	68	13.1	60	17.6	16.2	1.4	0.6	11.7	2.5	2.4	67.4	
6 (58th subculture)	50	1.7	71	12.6	65	16.6	14.9	1.7	0.5	10.9	1.8	2.5	62.7	
Average							*15.6*	*1.5*	*0.6*	*11.3*	*2.2*	*2.5*	*65.7*	66.1

* T_C = Total cycle time in hr; T_{G_2} = G_2-phase time in hr plus prophase time in hr; T_M = metaphase plus anaphase time in hr; T_S = S-phase time in hr; T_{G_1} = G_1-phase time in hr.

† $T_M = \dfrac{T_C[\log(1 + \text{mitotic index})]}{0.301}$.

‡ Per cent of all nuclei that were labeled.

Analysis of these curves for the estimation of the duration of the cell cycles and the various phases of the cycles was accomplished by a method that is essentially the same as that which has been used by numerous investigators(4), but the midpoints of the respective ascents and descents of the curves were used rather than the 50% points. The lengths of the total cycles were measured by the times between the midpoints of the 1st and 2nd ascents of the curves rather than the times between the midpoints of the plateaus, since less error resulting from differences in the rates of progress of individual cells through the phases of the cycle would be involved. The duration of metaphase plus anaphase was calculated from the total cycle time and the observed mitotic index. Table I contains data obtained in the analysis of the curves and the values obtained for the lengths of the phases.

These data indicate that the cells transplanted serially in animals, the cultured cells, and the cells transplanted serially in animals following several generations in culture have essentially the same cell cycles. These results are consistent with the previous observation that the number of cells from culture required to cause death of animals in a given length of time is essentially the same as that observed for cells grown *in vivo*(1,5). It is of interest that the cells synthesize DNA during about 76% of the cycle, and thus a large portion of the population of cells should be affected by agents that inhibit only cells in this phase of the cycle. This information has been used advantageously in devising a dosage schedule for the administration of 1-β-D-arabinofuranosylcytosine to animals bearing Leukemia L1210(6).

The generation time observed in these experiments (15.7 hours or 0.65 day) is comparable to the observed time required for the number of L1210 cells to double in the mouse (0.55 day)(5,6,7). This doubling time is the average based upon a number of doublings in mice following inoculation of various numbers of cells. There was some evidence that the doubling time increased when the number of cells in the animal became large (8). Since the determinations of generation times in the present study were made on the 6th day following the inoculation of 10^5 cells, there would be 10^7-10^8 cells in the animal at the time of the experiments, and the generation time might be longer than that which would be observed at a lower cell density.

The data for the cell cycle and its phases obtained in these experiments were used to calculate the expected labeling index for thymidine-^{3}H by the method of Cleaver(9). The values in the last 2 columns of Table I indicate that in the *in vivo* experiments the observed labeling index was considerably below the calculated index, but for the cultured cells the observed and calculated indices were approximately the same. The reason for the low labeling index *in vivo* is not presently known.

Summary. The cell cycles of Leukemia L1210 cells proliferating in mice after serial transplantation, proliferating in mice following inoculation of cultured cells, and proliferating *in vitro* in spinner cultures have been examined by the method of waves of labeled mitoses following pulse exposure to thymidine-(methyl-^{3}H). The cycles for the 3 groups of cells were essentially the same. The following mean values were obtained: T_C, 15.7 hr; T_{G_2}, 1.5 hr; T_M, 0.5 hr; T_S, 11.7 hr; T_{G_1}, 2.0 hr.

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