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*See comments on nature of report in Preface, page i.

PREFACE

A semi-annual status report has been prepared instead of a final report because I have submitted a proposal to NASA to extend the current research. The proposed starting date for the extended research is August 1, 1973.

Although we have not been officially awarded a grant to extend the current research, we do have indications from NASA officials that we will be granted funds to undertake another phase of investigation. Hence, a semi-annual report is deemed more appropriate than a final report at this time.

The Effect of Continuous Low Dose-Rate Gamma Irradiation on Cell Population Kinetics of Lymphoid Tissue*

By
Bessie Ruth Foster**

PURPOSE, METHODS, AND DISCUSSION

The following report concerns cellular response and cell population kinetics of lymphoid tissue in mice under the stress of continuous irradiation.

The problem being studied involves the mechanism of cell proliferation in the thymus of mice under continuous irradiation at a dose rate of 10 roentgens (R) per day for 105 days (15 weeks). Our aim is to determine whether or not a steady state of cell population can be established for the indicated period of time, and what compensatory mechanisms of cell population are involved.

Changes in thymus weights, thymic cell counts, distribution of PAS-positive cells, non-PAS-positive reticular cells, distribution of lymphocytes and labeling among these cell types under continuous irradiation have been discussed in previous semi-annual status reports.

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On the basis of thymidine labeling and distribution of various cell types in the thymus under continuous irradiation, it was concluded that at least three compensatory mechanisms serve to maintain a near steady state of cellular proliferation:

1) an increase in the proportion of PAS-positive cells which stimulate mitotic activity

2) "maturation arrest" of precursor cells which tend to replenish the cells damaged or destroyed by radiation, and

3) increase in the "proportion of cells proliferating."

This report deals with findings as they relate to cell cycle times in the thymus under continuous irradiation, and changes in individual classes of thymic lymphocytes by using autoradiographic techniques and specific labeling with tritiated thymidine (TdR-^{3}H).

The Cell Cycle

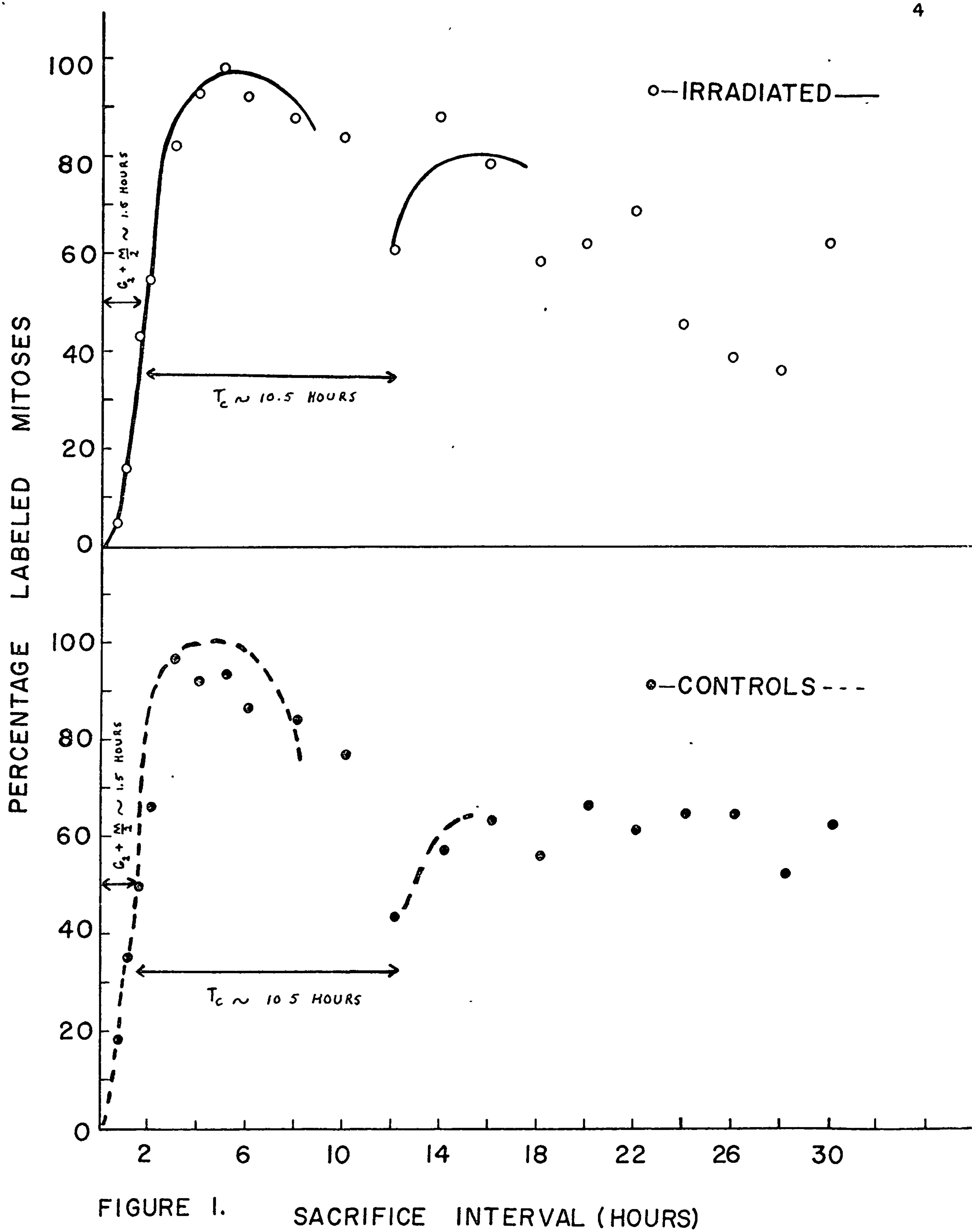
On the basis of thymus weights and thymic cell counts (discussed previously) it was demonstrated that a seemingly steady state of cellular proliferation was achieved by about four weeks of continuous irradiation. In order to determine whether or not there were any changes in the generation time of thymic cells, a group of 40, four-week old BCF_1 male mice were exposed to continuous irradiation at a dose rate of 10 R per day for about 4 weeks, removed from the irradiation unit, injected intraperitoneally with TdR-^{3}H (1.0 μ Ci/gram body weight), and sacrificed at various time intervals from 0.5 to 30.0 hours following TdR-^{3}H. Thymus tissue was dissected, fixed, processed through autoradiography, developed, stained, examined microscopically, and labeled mitoses scored at each sacrifice interval using routine histological and autoradiographic procedures.

Forty unirradiated mice of the same age, sex, and strain were processed similarly, these mice served as controls.

Two hundred mitotic figures were scored at each sacrifice interval, and each data point on a given labeled mitosis curve represents an average taken on two animals.

Figure 1 illustrates the curve of labeled mitoses. Although there was no well defined descending limb of the first wave of mitosis nor was there a well defined second wave in irradiated nor in control thymuses, it was possible to roughly approximate a cell cycle time of about 10.5 hours in both groups as indicated in the figure.

Theoretically, all cells in DNA synthesis at the time of TdR-^{3}H injection should have been labeled and should have passed through mitosis to give 100% labeling (Johnson, 1961). However, in the present study labeling reached only about 98% on the ascending limb of the initial wave in irradiated thymuses and about 96% in control thymuses at 5 hours and 3 hours after TdR-^{3}H, respectively. Less than 100% labeling during the first wave of the cell cycle has been reported in spontaneous breast cancer (Mendelsohn, Dohan and Moore, 1960), transplanted tumors (Steel, Adams and Barrett, 1966), and the regenerating liver (Fabrikant, 1964; 1967a); this may be due, in part, to the spread in the duration of the G_2 + M/2 complex (Fabrikant, 1967a) and the failure of a proportion of labeled cells (false negatives) lying deep in a section to give a radiographic image because of the very short range of



PERCENTAGE LABELED MITOSES
100
80
60
40
20
0
o—IRRADIATED——
$G_2 + \frac{M}{2} \sim 1.5$ HOURS
$T_c \sim 10.5$ HOURS
●—CONTROLS - - -
$G_2 + \frac{M}{2} \sim 1.5$ HOURS
$T_c \sim 10.5$ HOURS
2
6
10
14
18
22
26
30
SACRIFICE INTERVAL (HOURS)

FIGURE I.

tritium beta particles (Johnson, 1961; Fabrikant, 1967a; Fry, personal communication).

The mean duration of the S phase in both the irradiated and control groups was approximated to be about 6.5 hours; since the seemingly descending limb of the initial wave of both curves is less steep than the rise, the S phase in individual cells (reticular cells, large, medium, and small lymphocytes) is of unequal lengths. The low labeling among mitotic figures following the peak is possibly due to cells which were in the G_1 phase at the time of labeling. The variability in the duration of the G_1 phase must be extremely great, which partly accounts for the absence of a well-defined peak for the S phase of the daughter cells.

Curves of this type are characteristic of several tissues such as the small intestine (Quastler and Sherman, 1959), bone marrow (Cronkite, Bond, Fliedner, and Rubini, 1959), tumors (Mendelsohn, et al., 1960; Mendelsohn, 1962; 1963), hair follicles (Cattaneo, Quastler, and Sherman, 1961; McCarter and Quastler, 1962; Griem, 1966), skin (Gelfant, 1963), and regenerating liver (Fabrikant, 1967a; 1967b, 1967c; 1968a; 1968b).

Furthermore, the radiation response of a cell system may very well be more marked in autoradiographic studies due to intranuclear radiation effects from incorporated TdR-^{3}H in cells preparing for division (Painter, Drew and Hughes, 1958; Drew and Painter, 1959; Johnson and Cronkite, 1959; Wimber, 1959; 1964; Post and Hoffman, 1961).

Another factor which should be taken into account is that the thymus is a multicompartmental cell system with at least 4 major cell types, each with a different generation time. Therefore, the findings reported here are, at best, a rough approximation of the cell cycle time. Nonetheless, there were no apparent changes in the generation time of cells in irradiated thymuses compared to controls. It was concluded, therefore, that changes in the cell cycle time in thymuses irradiated at 10 R per day were not contributing compensatory mechanisms.

Since there was no apparent change in the generation time of thymus cells in irradiated mice compared to controls, the next line of investigation was to determine the distribution of cell types in the thymus population and the incorporation of TdR-^{3}H at various intervals under continuous irradiation.

Distribution and Labeling Among Thymus Cell Types

One hundred, 29-day old BCF_1 male mice were irradiated at a dose rate of 10 R per day for 105 days. Four mice were removed from the irradiation unit and sacrificed one hour following an intraperitoneal injection of TdR-^{3}H at various time intervals until 105 days had elapsed. Standard techniques were used to process the tissue through autoradiography with subsequent staining and microscopic examination.

One hundred unirradiated mice of the same strain, sex, and age as the irradiated group were processed in a similar manner. These mice served as controls.

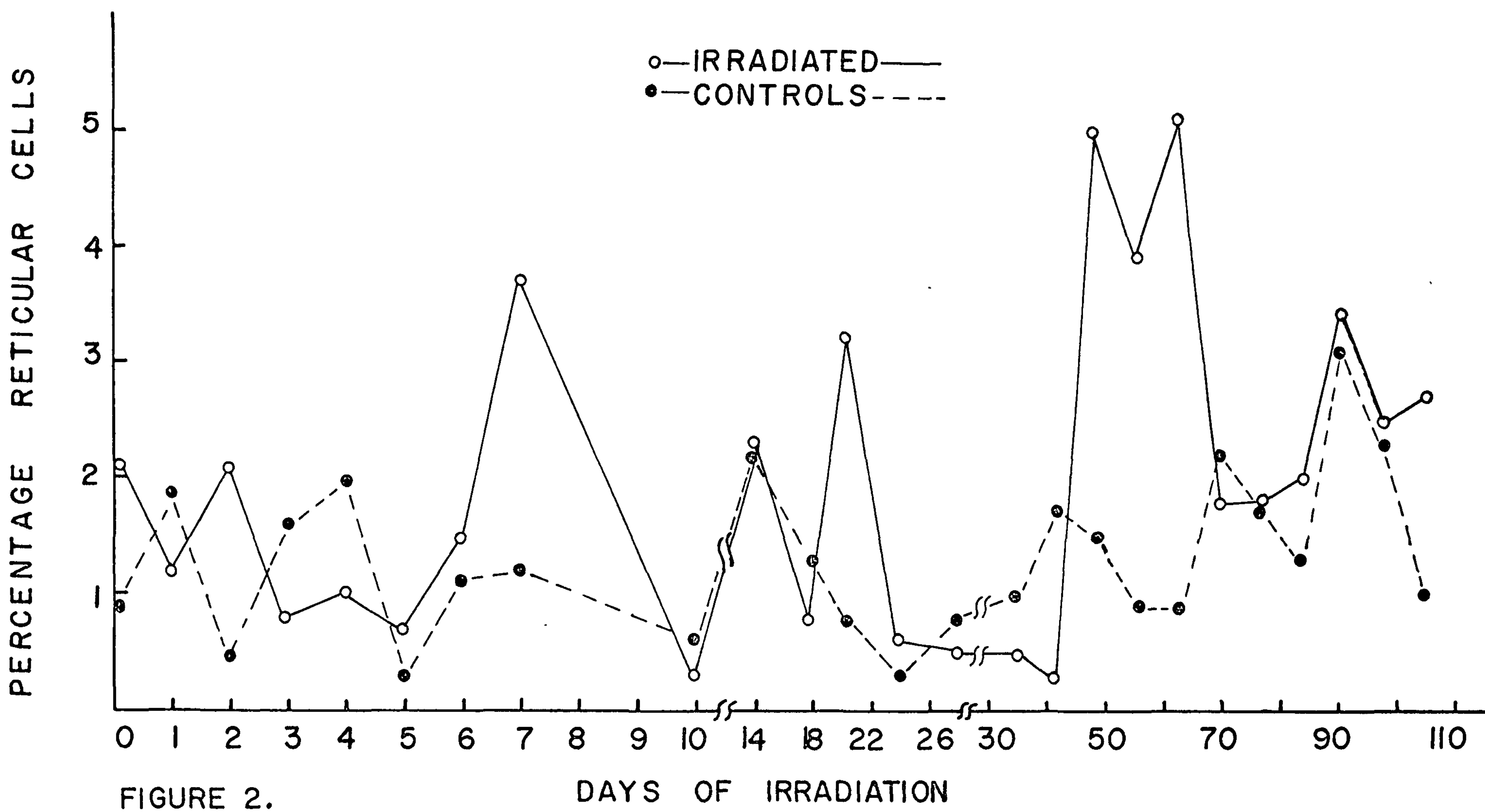
Each data point on any given graph represents an average on 4 animals, and at least 1000 thymus cells were counted and categorized per microscopic examination per animal. Cells were classified on the basis of morphology and size as reticular cells, large ($>11.0\mu$ in smears, $>6.0\mu$ in sections), medium (7.0 to 11.0μ in smears, 4.5 to 6.0μ in sections), and small ($<7.0\mu$ in smears, $<4.5\mu$ in sections) lymphocytes after descriptions of Metcalf and Wiadrowski (1966) for tissue smears, and of Sainte-Marie and Leblond (1958) for sections.

For convenience and due to their relative proportions, data on medium and small lymphocytes were sometimes pooled.

Figures 2-4 illustrate the distribution of the various cell types in the thymus under continuous irradiation. There was generally an increase in the proportion of reticular cells among irradiated thymuses compared to controls. Since lymphopoiesis in the thymus appears to occur as a result of asymmetric division (Osgood, 1957; 1961) of reticular cells into lymphocytes of progressively decreasing sizes (Leblond and Sainte-Marie, 1960; Yoffey, 1960; Yoffey, et al., 1961), an additional compensatory mechanism under continuous irradiation seems to be an increase in the proportion of precursor cells.

There was generally a smaller proportion of large lymphocytes among irradiated thymus tissue compared to controls with a few exceptions as indicated in Figure 3.

Data on medium and small lymphocytes were pooled. Figure 4 illustrates that there was a very slight increase in the proportion of medium and small lymphocytes among irradiated thymuses compared to controls throughout most of the irradiation period. Due to the relative numbers of medium and small lymphocytes, and since the small lymphocyte is generally non-dividing in vivo, these findings suggest that the medium lymphocyte is contributing an appreciable amount to the proliferative activity of the cell



PERCENTAGE RETICULAR CELLS
5
4
3
2
1
o—IRRADIATED——
•—CONTROLS- - - -
0 1 2 3 4 5 6 7 8 9 10 14 18 22 26 30 50 70 90 110
DAYS OF IRRADIATION

FIGURE 2.

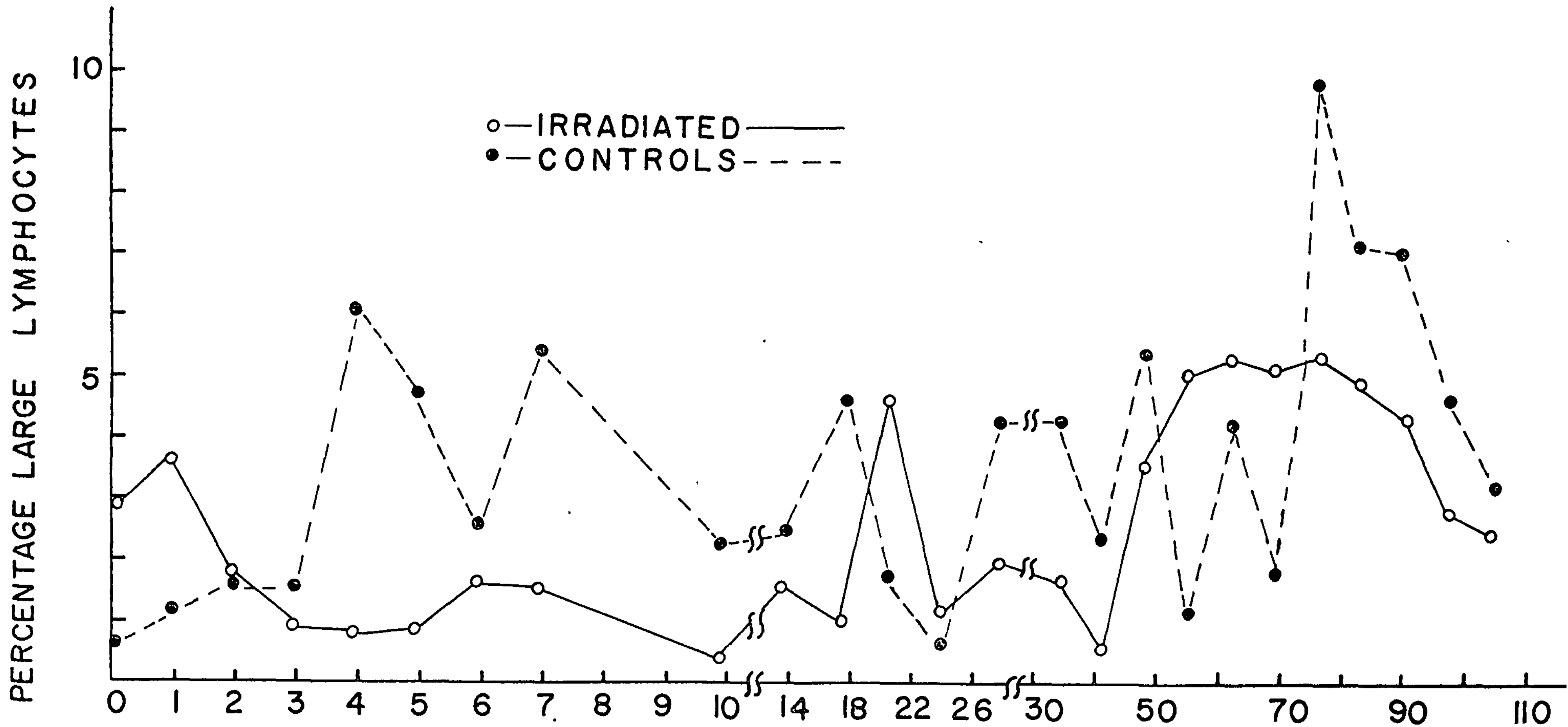


FIGURE 3. DAYS OF IRRADIATION

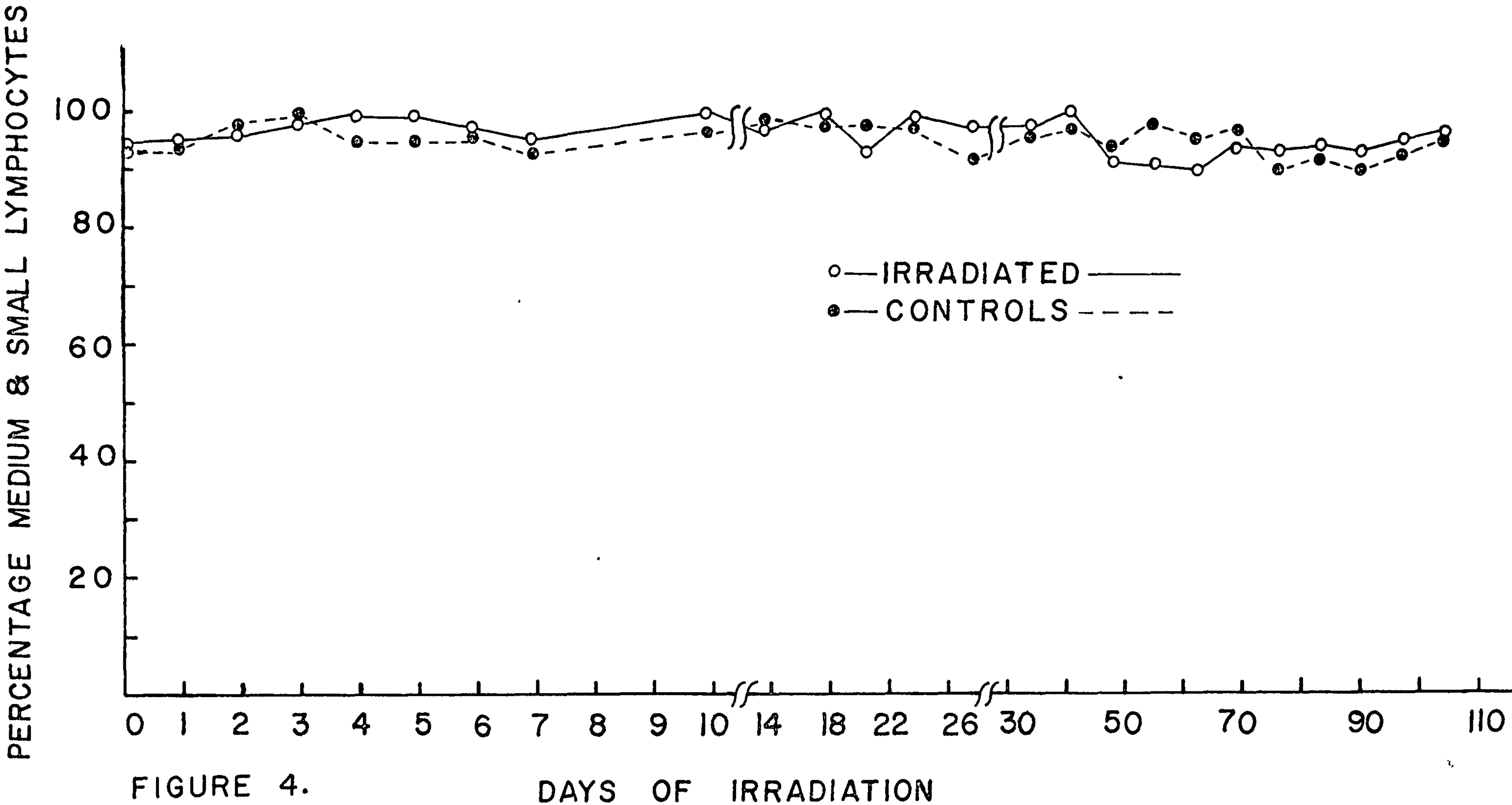

PERCENTAGE MEDIUM & SMALL LYMPHOCYTES
100
80
60
40
20
○— IRRADIATED ———
●— CONTROLS - - - -
0 1 2 3 4 5 6 7 8 9 10 14 18 22 26 30 50 70 90 110
DAYS OF IRRADIATION

FIGURE 4.

system. The relative proliferative capacity of each cell type will be discussed later in this report.

Because of the volume of data accumulated on the distribution of each cell type at each sacrifice interval, an average was taken over the entire 105-day irradiation period and tabulated in Table I below.

TABLE I

PERCENTAGES OF VARIOUS THYMIC CELL TYPES
UNDER CONTINUOUS IRRADIATION

	Reticular Cells	Large Lymphocytes	Medium and Small Lymphocytes
Irradiated	2.0	2.7	95.3
Controls	1.3	3.8	94.9

Table I shows that there was an overall increase in the percentage of reticular cells in irradiated thymuses to almost twice the value observed in controls. There was a decrease in the overall percentage of large lymphocytes among irradiated thymus, and a slight increase in the proportion of medium and small lymphocytes.

Thymidine labeling among various thymic cell types is illustrated in Figures 5-7. There was generally less labeling in irradiated reticular cells and among large lymphocytes compared to controls. However, among the medium and small lymphocyte category there was an increase in labeling in irradiated groups during the initial and final phase of the irradiation period.

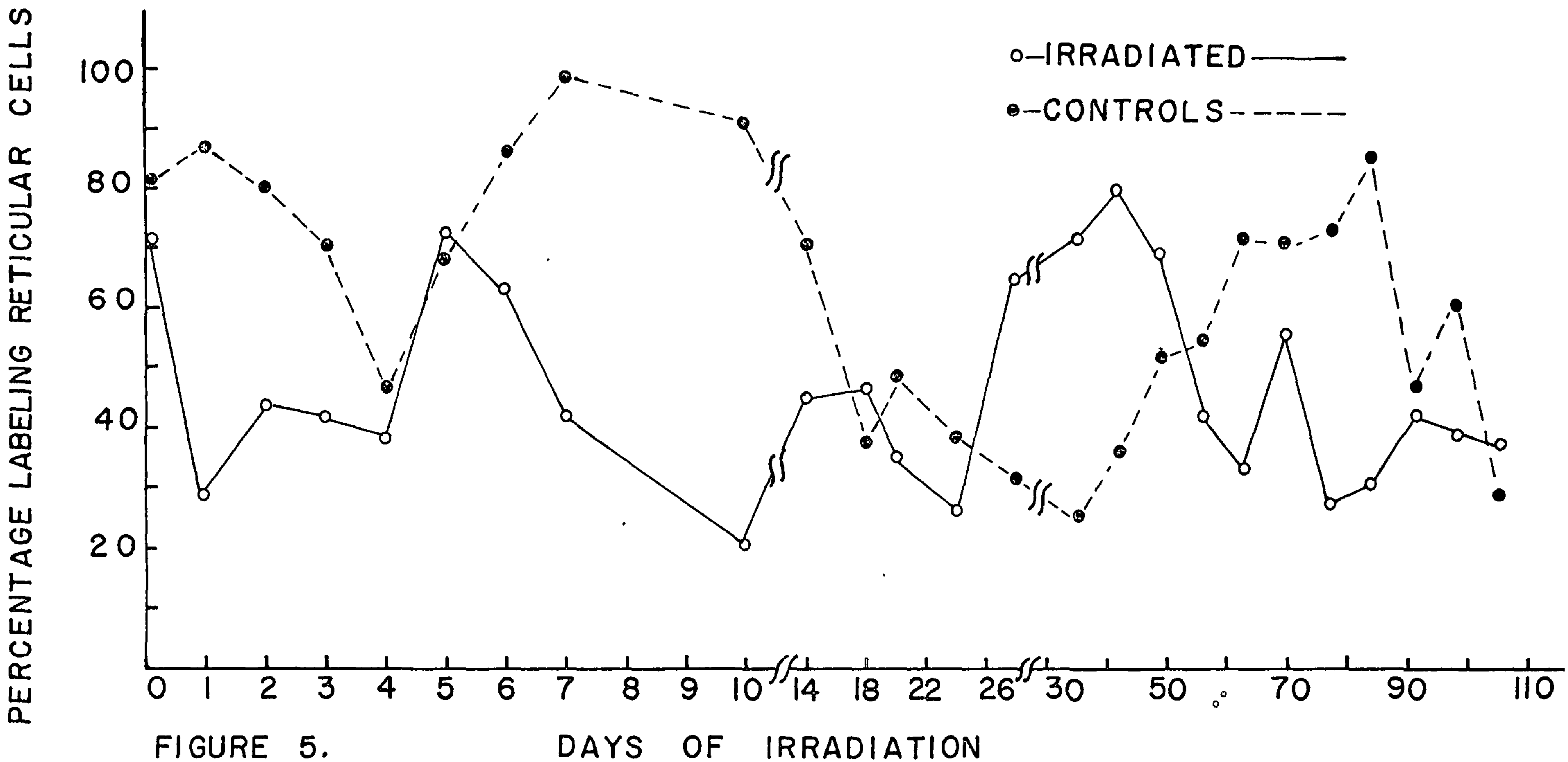
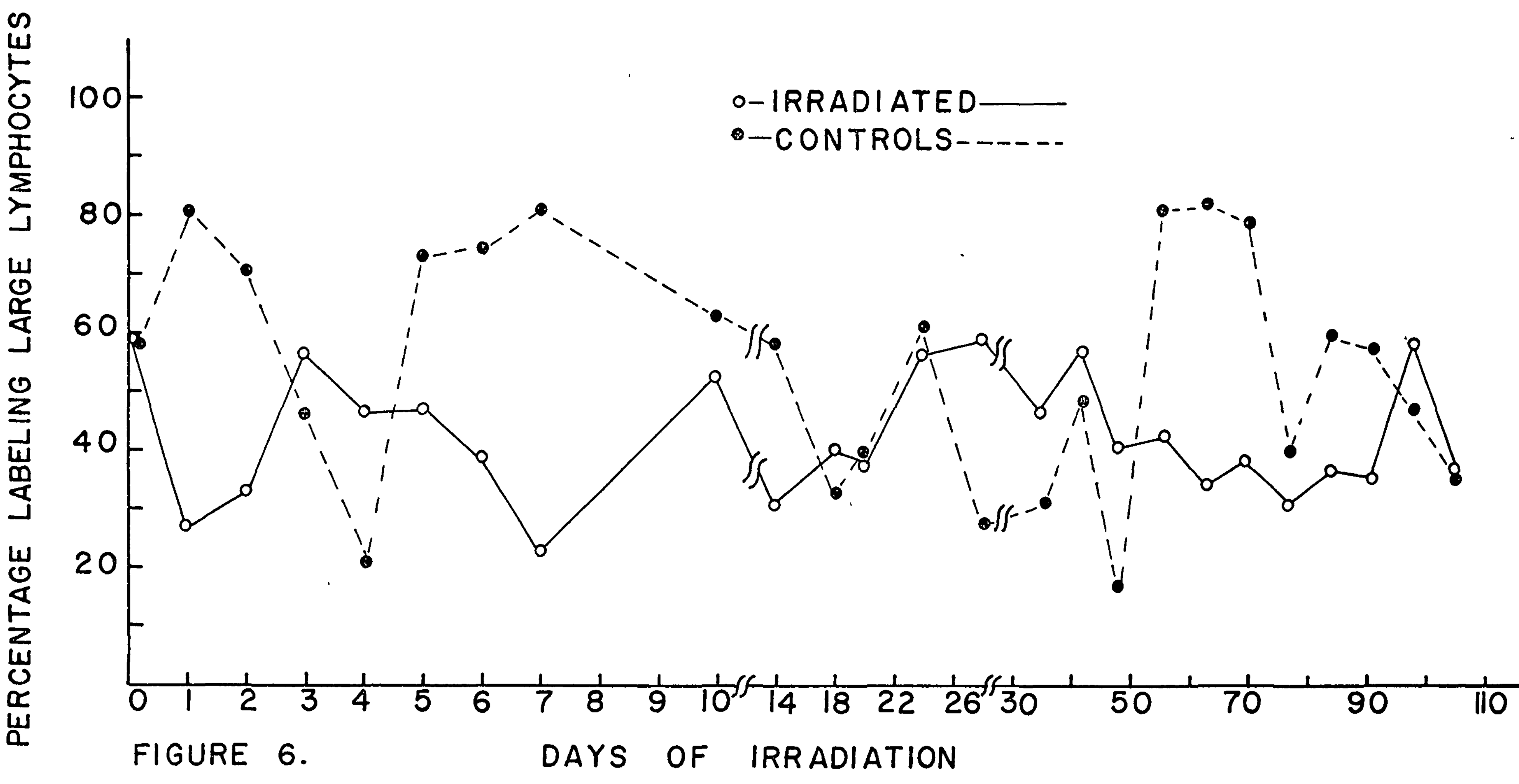
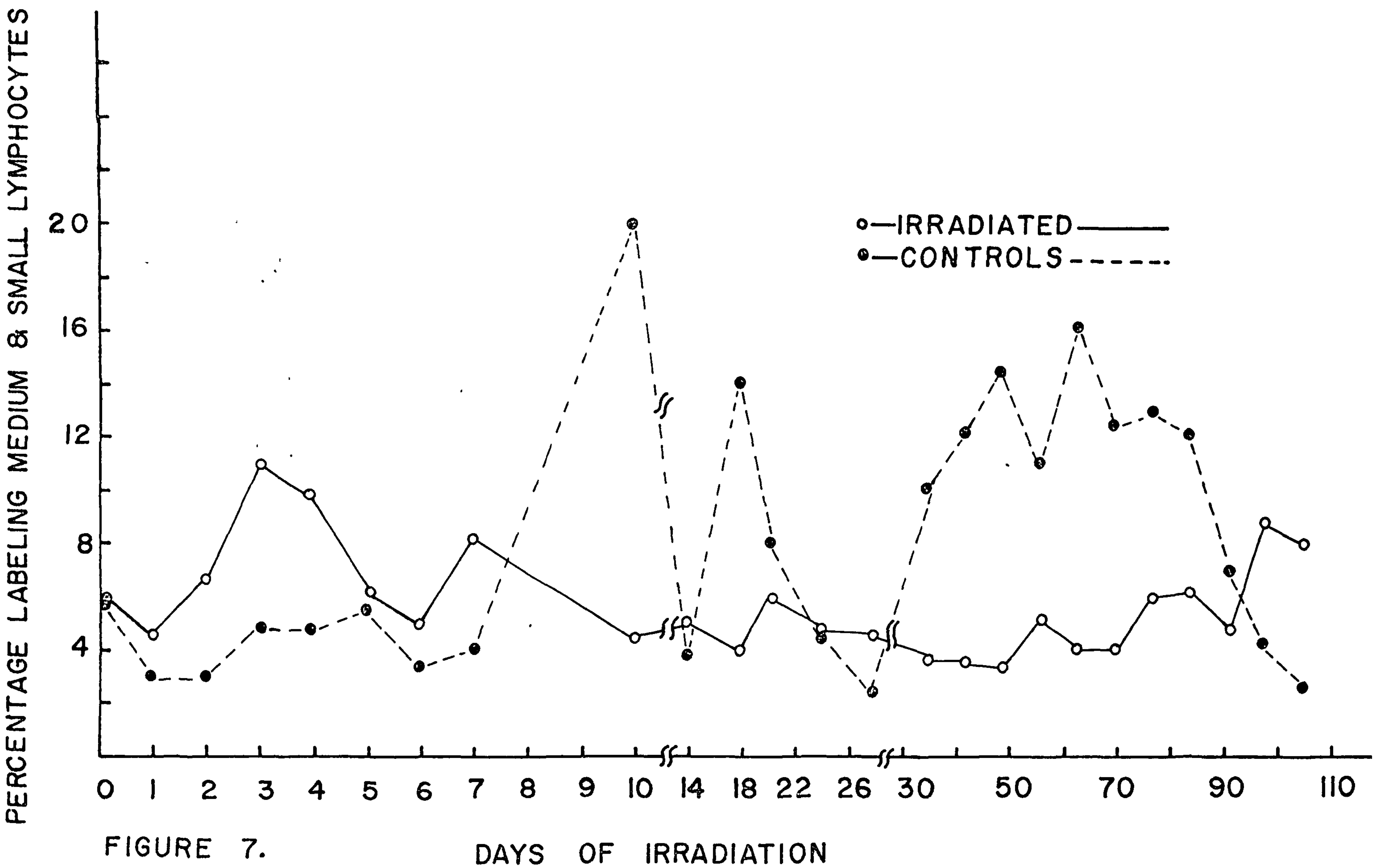

PERCENTAGE LABELING RETICULAR CELLS
100
80
60
40
20
o–IRRADIATED
●–CONTROLS
0
1
2
3
4
5
6
7
8
9
10
14
18
22
26
30
50
70
90
110
DAYS OF IRRADIATION

FIGURE 5.



○—IRRADIATED——
⊗—CONTROLS- - - -
PERCENTAGE LABELING LARGE LYMPHOCYTES
100
80
60
40
20
0 1 2 3 4 5 6 7 8 9 10 14 18 22 26 30 50 70 90 110
DAYS OF IRRADIATION

FIGURE 6.



PERCENTAGE LABELING MEDIUM & SMALL LYMPHOCYTES
20
16
12
8
4
0
1
2
3
4
5
6
7
8
9
10
14
18
22
26
30
50
70
90
110
DAYS OF IRRADIATION
o—IRRADIATED
●—CONTROLS

FIGURE 7.

The relative increase in labeling among the medium and small lymphocyte category further suggests that the medium lymphocyte, in particular, is contributing appreciably to the proliferative activity of the thymus cell population.

The next line of investigation was to determine the "proliferative fraction" among the various thymus cell types to gain some idea of the proportion of a given cell category that is proliferating. That is to say, a cell in a population may or may not be proliferating. Therefore, in order to determine which cell type is contributing most to the proliferative activity, one needs to know the proliferative fraction of that category, and also some indication of its relative proliferative capacity.

The Proliferative Fraction of Thymic Cell Types

The proliferative fraction of various thymic cell types under continuous irradiation was determined using a modification of the method of Lala and Patt (1966). Figure 8 illustrates the findings. Only in the medium and small lymphocyte categories was there an appreciable increase in the proliferative fraction during the initial and final phase of the irradiation period. These findings further suggest that it is the medium lymphocyte category that is contributing most to the proliferative activity of the thymus.

Again, because of the volume of data accumulated an average was taken over the entire period of irradiation for each cell type, and these data are reported here. On an average there was about 85% of the reticular cell population proliferating, 75% of the large lymphocytes, 20% of the medium lymphocytes and about

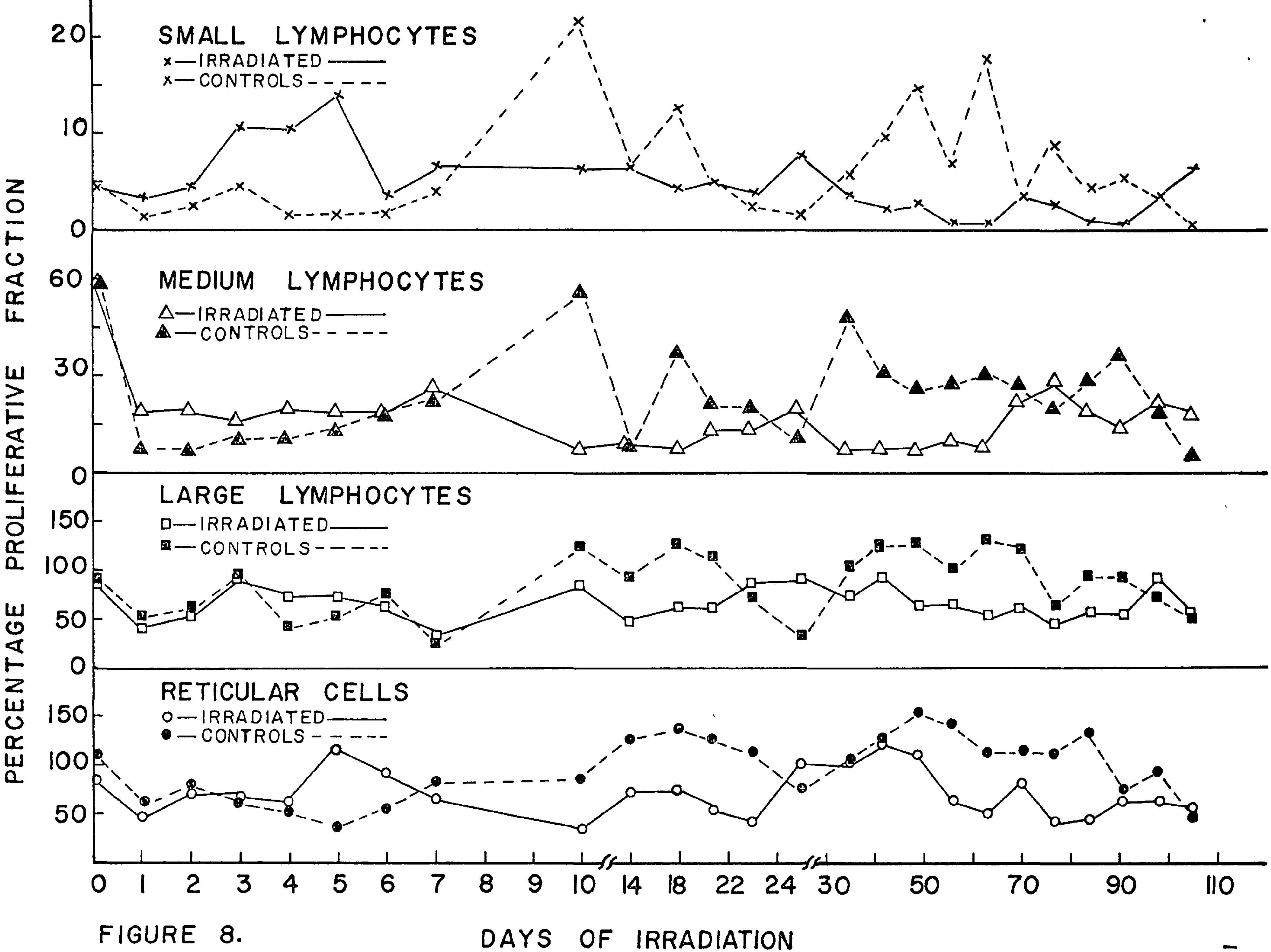

SMALL LYMPHOCYTES
×—IRRADIATED ——
×—CONTROLS - - - -
MEDIUM LYMPHOCYTES
△—IRRADIATED ——
▲—CONTROLS - - - -
LARGE LYMPHOCYTES
□—IRRADIATED ——
■—CONTROLS - - - -
RETICULAR CELLS
○—IRRADIATED ——
●—CONTROLS - - - -
PERCENTAGE PROLIFERATIVE FRACTION
DAYS OF IRRADIATION

FIGURE 8.

5% of the small lymphocytes.

Although only a small percentage of the medium and small lymphocyte population was proliferating, their relative numbers play an important role in the proliferative activity of the total population as will be seen in the following discussion.

Relative Proliferative Capacity of Thymic Cell Types

The relative proliferative capacity of each thymic cell type was determined using the method of Berman, Winter and Newby (1966). These data are shown in Figure 9. This figure illustrates that there was generally an increase in the relative proliferative capacity among irradiated reticular cells compared to controls throughout most of the irradiation period. Also, there was an increase in the relative proliferative capacity among medium and small lymphocytes during the initial and final phases of the irradiation period. Most importantly, the figure shows that the medium lymphocyte has a relative proliferative capacity of about 4 times that of reticular cells, and about 2 times that of large and small lymphocytes.

Whether or not any of these findings are statistically significant will be known when the computer analysis of the data is completed.

PROGRESS REPORT

All phases of this study are nearing completion with the exception of a part of the computer analysis which is currently being processed, and the publication of several papers in scientific journals. The research papers are in preparation.

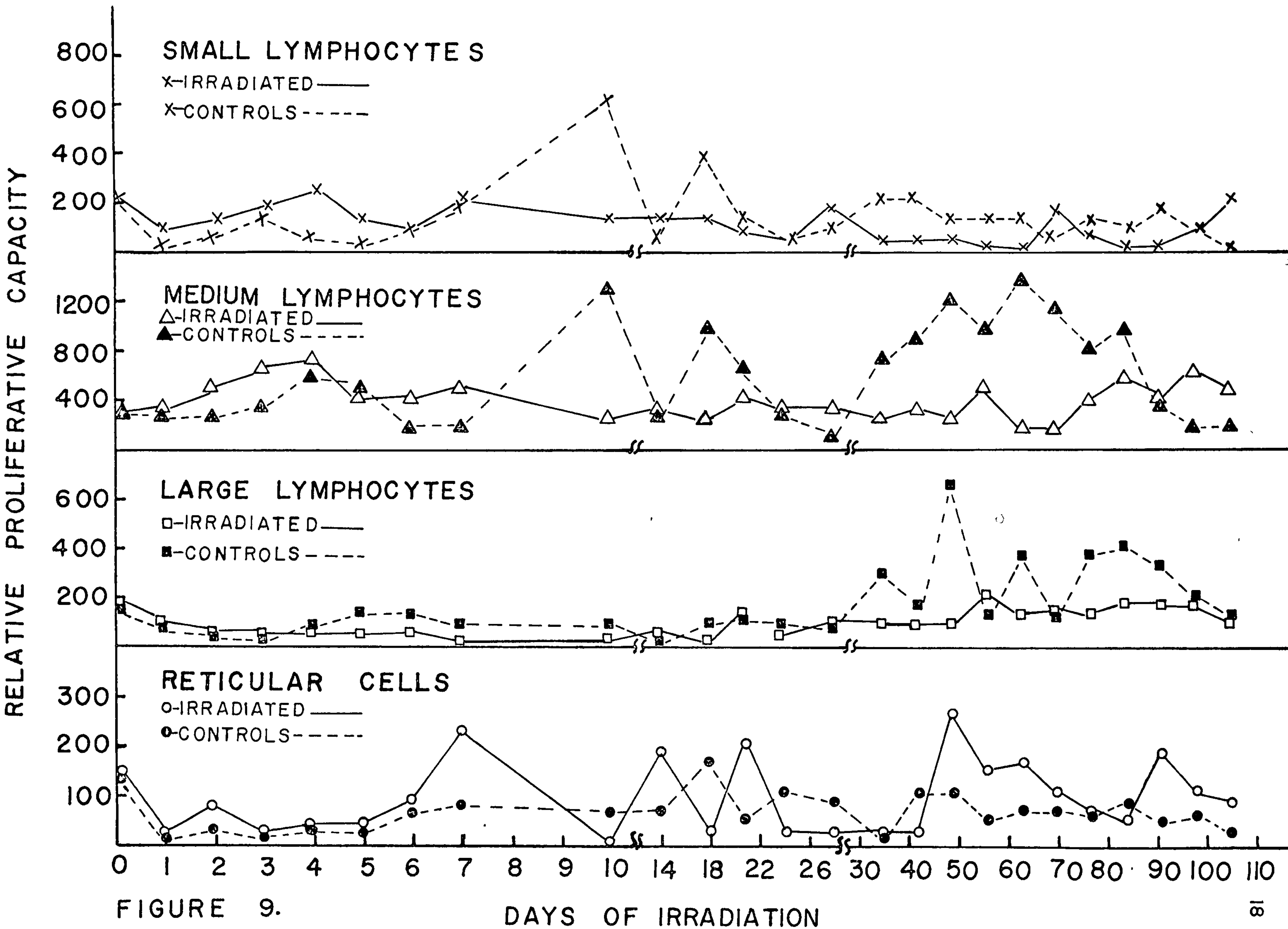

SMALL LYMPHOCYTES
X-IRRADIATED
X-CONTROLS
MEDIUM LYMPHOCYTES
△-IRRADIATED
▲-CONTROLS
LARGE LYMPHOCYTES
□-IRRADIATED
■-CONTROLS
RETICULAR CELLS
○-IRRADIATED
●-CONTROLS
RELATIVE PROLIFERATIVE CAPACITY
DAYS OF IRRADIATION
800
600
400
200
1200
800
400
600
400
200
300
200
100
0 1 2 3 4 5 6 7 8 9 10 14 18 22 26 30 40 50 60 70 80 90 100 110

FIGURE 9.

SUMMARY AND CONCLUSIONS

On the basis of labeled mitoses, distribution of various cell types, and relative proliferative capacities of thymus cells under continuous irradiation, it is concluded that an additional compensatory mechanism which serves to maintain a near-steady state of cellular proliferation is an increase in the proportion of precursor cells.

Also, it is the medium lymphocyte which contributes most to the proliferative activity of the thymus.

COMMENTS

When we are granted support from NASA to extend our current research, a similar study will be carried out using the lymphoid component of the spleen (white pulp) of mice to make a comparison study with findings we have observed in the thymus.

REFERENCES

Berman, I., Winter, S. R., and Newby, E. J., (1966). Anat Rec, 154, 635.

Cattaneo, W. M., Quastler, H., and Sherman, F. G., (1961). Nature (London), 190, 923.

Cronkite, E. P., Bond, V. P., Fliedner, T. M., and Rubini, J. R., (1959). Lab Investigation, 8, 263.

Drew, R. M., and Painter, R. B., (1959). Radiat Res, 11, 535.

Fabrikant, J. I., (1964). Ph.D. Thesis, University of London.

Fabrikant, J. I., (1967a). Radiat Res, 31, 304.

Fabrikant, J. I., (1967b). Brit J Radiol, 40, 487.

Fabrikant, J. I., (1967c). Radiat Res, 32, 804.

Fabrikant, J. I., (1968a). J Cell Biol, 36, 551.

Fabrikant, J. I., (1968b). American J Roentgenal Radium Therapy Nuc Med 102, 811.

Fry, R. J. M., personal communication.

Gelfant, S., (1963). Exp Cell Res 32, 521.

Griem, M. L. (1966). Nature (London), 210, 213.

Johnson, H. A., (1961). Int J Cytol, 26, 32.

Johnson, H. A., and Cronkite, E. P., (1959). Radiat Res, 11, 825.

Lala, P. K., and Patt, H. M., (1966). Proc Nat Acad Sci, 56, 1935.

Leblond, C. P., and Sainte-Marie, G., (1960). In Haemopoiesis, 152, edited by Wolstenholme, G. E. W., and O'Connor, M., Boston: Little Brown.

McCarter, J. A., and Quastler, H., (1962). Nature (London), 194, 873.

Mendelsohn, M. L., (1962), J Nat Cancer Inst 28, 1015.

Mendelsohn, M. L., (1963). In Cell Proliferation, 190 edited by Lamerton, L. F., and Fry, R. J. M. Oxford: Blackwell.

Mendelsohn, M. L., Dohan, F. C., Jr., and Moore, H. A., Jr., (1960). J Nat Cancer Inst, 25, 477.

Metcalf, D., and Wiadrowski, M. (1966). Cancer Res 26, 483.

Osgood, E. E., (1957). J Nat Cancer Inst, 13, 155.

Osgood, E. E., (1961). Ann N Y Acad Sci, 95, 828.

Painter, R. B., Drew, R. M., and Hughes, W. L., (1958). Science, 127, 1244.

Post, J., and Hoffman, J., (1961). Radiat Res, 14, 713.

Quastler, H., and Sherman, F. C. (1959). Exp Cell Res, 17, 420.

Sainte-Marie, G., and Leblond, C. P., (1958). Proc Soc Exp Biol Med, 98, 909.

Steel, C. G., Adams, K., and Barrett, J. C. (1966). Brit J Cancer, 20, 784.

Wimber, D. E., (1959). Proc Nat Acad Sci, 45, 839.

Wimber, D. E., (1964). In Advances in Radiation Biology, 85, edited by Augenstein, L. G., Mason, R., and Quastler, H., New York: Academic Press.

Yoffey, J. M., (1960). Quantitative Cellular Haematology, edited by Bethell, F. H., Springfield, Ill: Charles C. Thomas.

Yoffey, J. M., Reinhardt, N. O., and Everett, N. B., (1961). J Anat 95, 293.