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DNA SYNTHESIS IN IRRADIATED ANIMALS

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Through the availability of radioactive tracers, rapid progress has been made in the last fifteen years in our knowledge of the biochemistry of the nucleic acids. The two main types, ribose nucleic acid (RNA) and desoxyribose nucleic acid (DNA), differ considerably in their metabolism. Whereas RNA may have a rapid intracellular renewal, DNA--once formed--appears stable for the life of the cell. Every diploid cell in a species contains the same amount of DNA, and it synthesizes an additional complement of this chromosomal material only in preparation for cell division. The synthesis of new DNA occurs during interphase; the exact time may vary for different cell types. Under normal conditions isotopes are incorporated only into the newly synthesized DNA, and the rate of incorporation is therefore a reliable index of the rate of cell renewal in a tissue.

Since Euler's and Hevesy's original study,[1] in which they described a decrease in the incorporation of P^{32} into DNA of irradiated Jensen sarcoma, many papers have appeared on the influence of radiation on DNA synthesis in mammalian tissues (a complete review cannot be presented here, however).[2] A great many tissues, both normal and neoplastic, have been investigated by use of radiation dosages in the range of 100 to 5000 r. Nearly always the specific activity of DNA has been lowered at some time after radiation, and the inhibition of DNA synthesis has thus come to be considered one of the important biochemical defects produced by radiation. However, more recent experiments on ascites tumors have led us to believe that irradiated cells are still able to synthesize DNA and that the inhibition that is usually observed in tissues with mixed cell populations is largely a secondary effect.

Ascites tumors have been used in many laboratories in recent years for radiobiological investigations because they offer several advantages over most tissues: they may be obtained as nearly pure suspensions of one cell type, and their growth rate can be determined with ease, as also can the cell number and percentage of viable cells in any sample to be studied biochemically. We owe much of our knowledge of these tumors to the work of Klein and associates, and the experiment to be described is an extension of one of their studies.[3]

Mice bearing the Ehrlich ascites tumor 4 to 6 days after transplantation were irradiated with 800 r.[4] After radiation it was found that mitoses were absent for two days, and during this time there was no change in the total cell

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number or in the number of dead cells. In other words, all the cells that had been irradiated were still present, and biochemical changes in them could be studied. The mean cell volume and mean RNA content increased during this time. When the 2-hour incorporation of P^{32} into DNA was measured at various times after radiation, no significant depression was seen until the second day. Determinations of the mean DNA content per cell (Fig. 1) showed that during the first day after irradiation the cells continued to synthesize DNA at a rate closely matching the estimated rate of DNA synthesis and the growth rate of the unirradiated tumor (1.5% per hour). The increased mean DNA content per cell one day after irradiation was confirmed by Feulgen microspectrophotometry, which showed a definite increase in the percentage of cells with the higher DNA content. During the second postirradiation day there was little further increase in the mean DNA content per cell; also there was a depression in the rate of incorporation of P^{32}. Our results therefore would be consistent with the idea that although the cells are unable to go through mitosis after radiation, they continue to synthesize DNA until the majority of cells have reached the premitotic DNA content, at which time DNA synthesis decreases. Howard has recently discussed this question in detail.[5] It is important to point out that, although the irradiated Ehrlich cells synthesize DNA at a normal rate, we have no assurance that this is entirely normal DNA or nucleoprotein. Conger has shown that when mitosis is resumed nearly all cells have anaphase abnormalities after 400 r.[6]

It has frequently been suggested that the well-known radiation inhibition of mitosis is the result of an inhibition of DNA synthesis. However, our results with the Ehrlich tumor and similar observations in regenerating livers,[7] together with the recently discovered time relationships, make it very evident that the mitotic inhibition is due to some radiosensitive process that is independent of any possible effect on DNA synthesis.

Although radiation in this dose range probably does not cause a biochemical lesion in DNA synthesis, the incorporation of precursors into DNA has been found depressed in nearly every mammalian tissue studied. The observed decrease in DNA specific activity might be the result of a number of processes other than a direct inhibition of DNA synthesis (processes that vary with the tissue, the radiation dose, and the time interval under investigation): (a) The radiation-induced depression of mitosis might prevent cells from entering their next period of DNA synthesis, and the time of appearance of inhibition would depend on the normal time interval between mitosis and DNA synthesis. (b) Since mammalian tissues are composed of mixed cell populations with very different rates of cell division, any loss of the more actively dividing cells would produce a decreased DNA specific activity. (c) In some tissues death of cells shortly after radiation is a prominent phenomenon, and inclusion of their DNA in the isolated material would lower the DNA specific activity, although the remaining viable cells might be synthesizing DNA at a normal rate. The magnitude of this effect depends on the death rate and the rate of removal of dead cells from the tissue. (d) An inhibition of DNA synthesis may be the result of several as yet ill-defined indirect effects, such as the liberation of tissue-breakdown products, reduced food intake, or adrenal hyperactivity.

There are wide variations among tissues with respect to the time course, the ultimate percentage depression, the beginning of recovery, and the dose response. Figures 2 and 3 illustrate these differences. The data were taken from experiments in which mice were irradiated with x-rays and the 2- or 4-hour incorporation of P^{32} into DNA was measured at various times afterwards.[8] In general the results parallel the histologically observed patterns of injury and recovery. It is well known that radiation produces a marked change in the cell population of the spleen, with nearly complete elimination of the myelopoietic and lymphopoietic cells. The low rate of isotope incorporation into DNA is probably due to the absence of those cells which are normally most active in the synthesis of DNA. The marked difference in the intestinal response to 800 and 2500 r (Fig. 3) is in accord with histological observations of early regeneration at 800 r, contrasted with a severe depletion of the intestinal crypts at higher doses.[9] Figure 2 includes data on the liver DNA specific activity. Even though liver cells are considered radioresistant and the liver normally has a very low rate of DNA renewal, renewal is depressed after radiation. Part of the inhibition presumably is due to the lowered food intake after radiation, since fasting alone causes a depression. However, additional factors must be involved. There is no known loss of cells, and the inhibition of synthesis cannot be attributed to mitotic inhibition since the intermitotic time is so long.

A comparison of two transplantable tumors affords a striking example of different reactions to irradiation. These tumors had approximately the same growth rate, mitotic index, and short-term incorporation of P^{32} into DNA before radiation. Figure 4 shows the DNA specific activities at various times after 800 r, and it is apparent that the responses were quite different; they paralleled the histologically observed radiosensitivities. The very low specific activity of the lymphosarcoma one day after radiation was undoubtedly due mainly to the presence of large amounts of cell debris. By three days the tumors had involuted severely, but the remaining cells had a normal DNA specific activity.

In the mammary carcinoma the incorporation into DNA was only very slightly affected at short times after radiation. Mitoses, however, were absent, indicating again that some process other than inhibition of DNA synthesis was responsible for the mitotic inhibition. After the first day, both the DNA specific activity and mitotic activity were approximately 50% of normal. Despite this considerable rate of cell production, there was no increase in tumor weight for 6 days, presumably because of increased cell death. Unfortunately, measurements of total DNA content which would have permitted quantitation of the number of cells synthesizing DNA, were omitted from this experiment.

In radiosensitive tissues the effects of rather low doses can be clearly measured. For example, in rat thymus and spleen the DNA specific activity (2-hour incorporation of P^{32}) measured 24 hours after 100 r was found to be approximately 40% of normal. At the same time the DNA content of these organs, a reflection of the total number of cells, was approximately 60% of normal. It can be estimated by multiplying these percentages that one day after 100 r the number of cells synthesizing DNA was reduced to one-quarter.

SUMMARY

Experiments carried out during the past few years strongly suggest that the generally observed inhibition of DNA synthesis after radiation is a secondary effect principally due to changes in cell populations. Furthermore, the mitotic delay after radiation must be due to some radiosensitive process independent of any possible effect on DNA synthesis.

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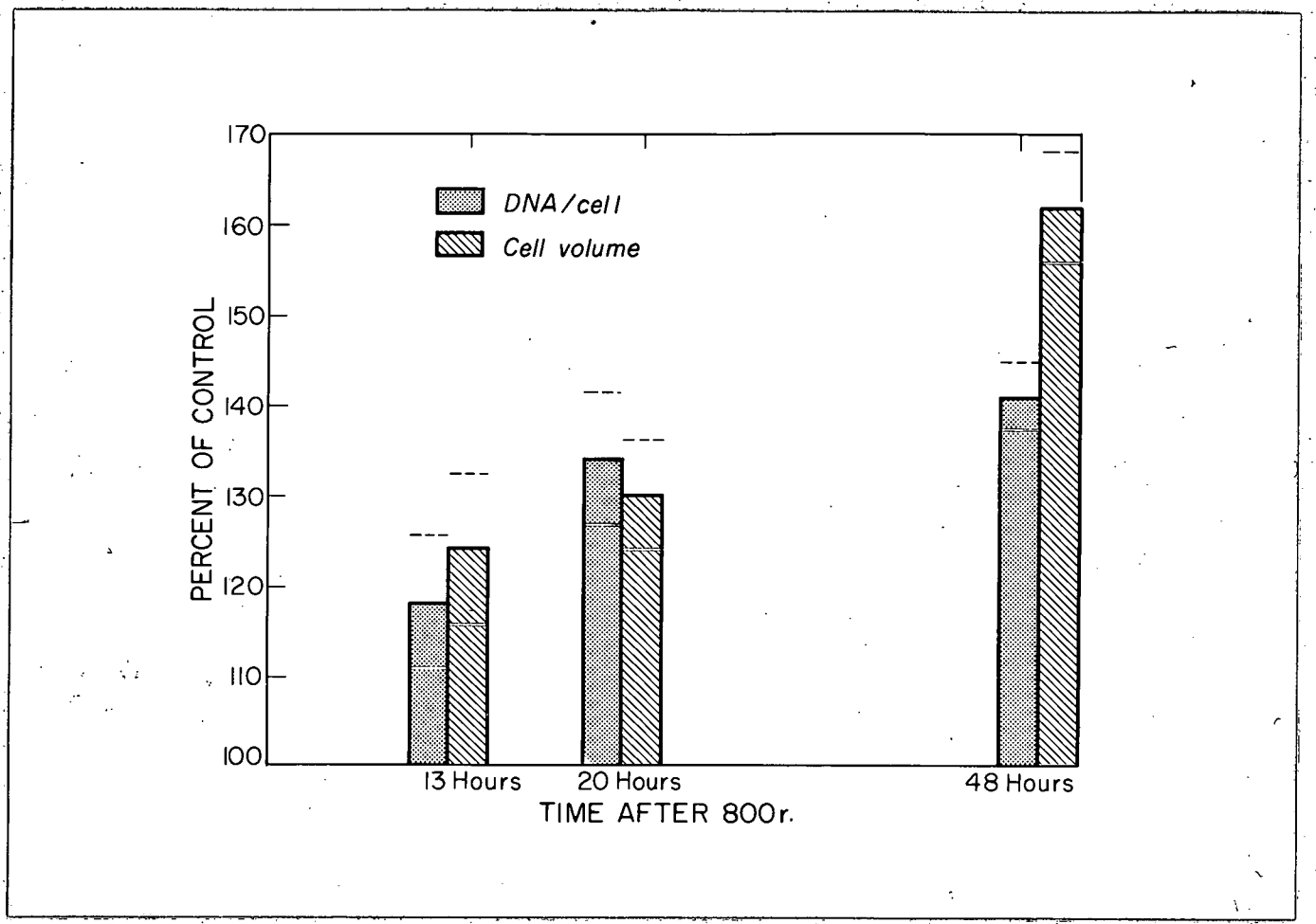

Fig. 1. Increase in mean cell volume and DNA content per cell of irradiated Ehrlich ascites tumor.

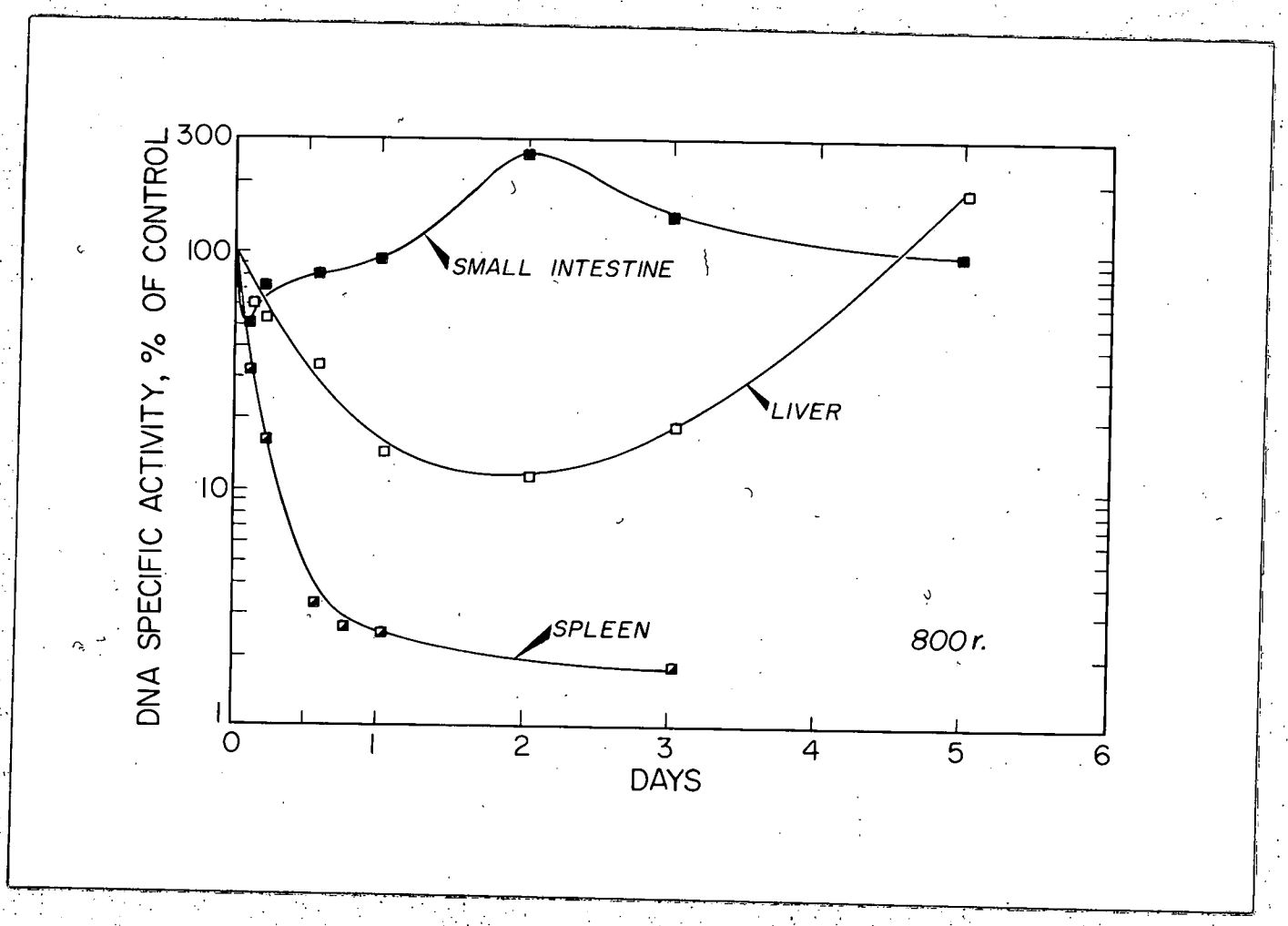

Fig. 2. Short-term incorporation of P^{32} into DNA of mouse spleen, liver, and small intestine as a function of time from radiation to injection of P^{32}.

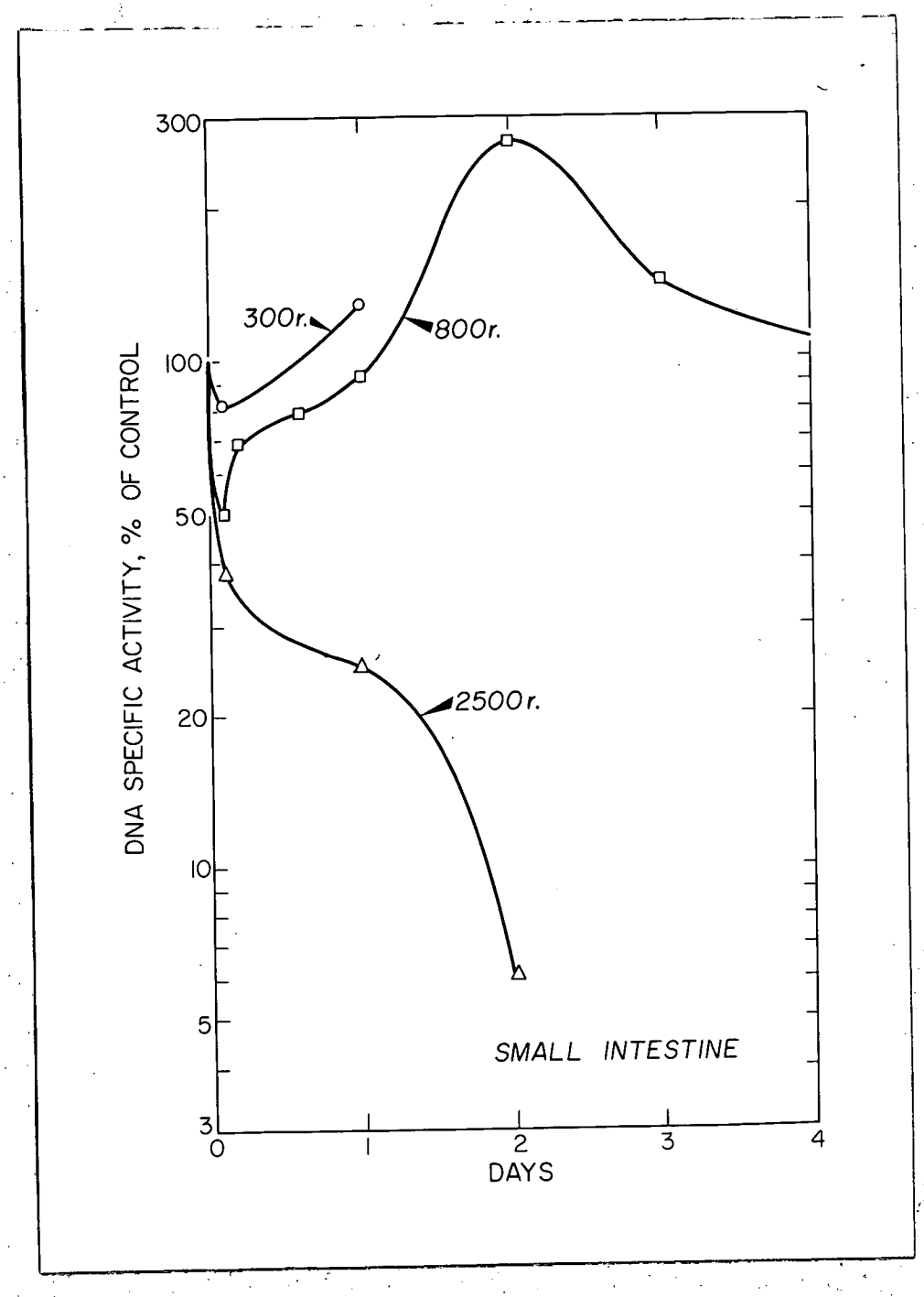

Fig. 3. Short-term incorporation of P^{32} into DNA of mouse small intestine as a function of time from radiation to injection of P^{32}.

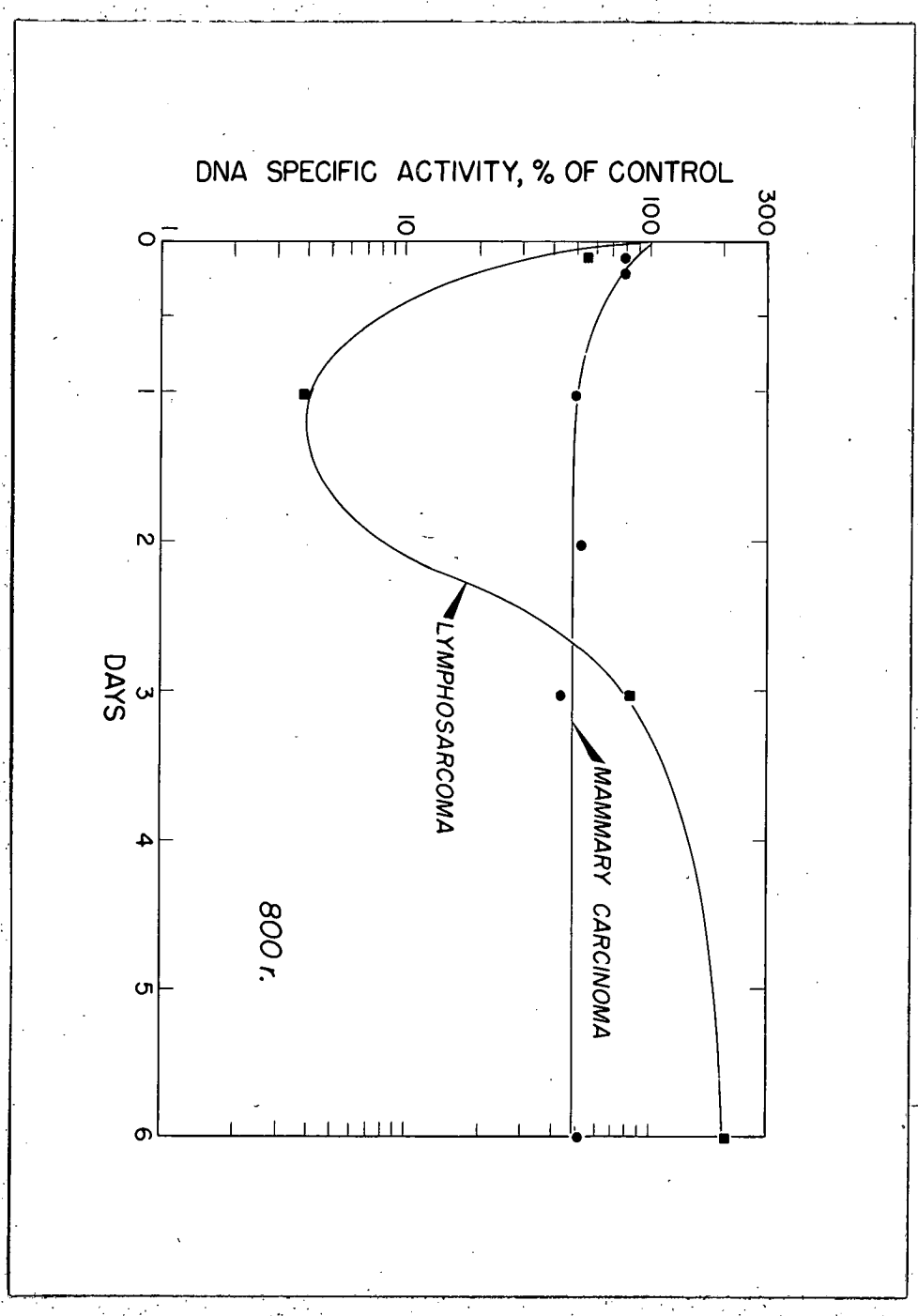

Fig. 4. Short-term incorporation of P^{32} into DNA of two transplanted tumors as a function of time from radiation to injection of P^{32}.