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RADIATION-INDUCED NUCLEIC ACID SYNTHESIS IN L CELLS UNDER ENERGY DEPRIVATION

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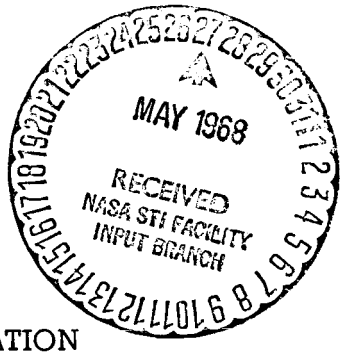

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ABSTRACT

L cells show a brief, accelerated uptake of radioactive precursor into both DNA and RNA following relatively low doses of radiation. This response appeared for cells being starved in a glucose-free salt solution and for cells treated with 2,4-dinitrophenol.

Three distinct features characterize the response. First, a relatively fixed postirradiation time delay seems necessary before the accelerated labeling of the nucleic acids occurs. Second, the labeling of the DNA fraction closely parallels the labeling of the RNA fraction. Finally, the radioactive label appears to enter and then to leave both nucleic acid fractions.

Although the accelerated labeling was most apparent after 100 rads, it also happened at higher doses where several cycles of incorporation and loss of label appeared. Since comparable changes were not found under normal growth conditions, this response presumably results because of the energy-deprived state. Both continuous labeling and pulse methods were used; the nucleic acids were labeled with either inorganic phosphate - P32 or radioactive precursors specific for a given fraction. Survival studies indicated that cells treated with 2,4-dinitrophenol had an increased resistance to X-rays.

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SUMMARY

The results of these studies show that L cells under energy deprivation have an unscheduled nucleic acid synthesis after irradiation. A brief accelerated uptake of radioactive precursor followed by a subsequent loss of label characterizes the response. The labeling of the DNA and RNA fractions closely parallel each other. This labeling appears to be superimposed on the normal replication. While the effect prevails after 100 rads, it also occurs at higher doses. At least 15 minutes must elapse before the accelerated labeling appears. The time delay, as well as the simultaneous DNA and RNA labeling, implies a rigidly controlled cellular mechanism.

Changes in ATP metabolism coincide with the accelerated labeling of the nucleic acids after 100 rads, but not at higher doses. Apparently, ATP is utilized for either energy-requiring reactions or perhaps as a precursor for the increased nucleic acid synthesis.

Cells treated with 2,4-dinitrophenol had a greater survival than cells irradiated in growth medium.

INTRODUCTION

The expanded use of radiation, as well as the radiation hazards of space travel, over the last twenty years has prompted detailed studies on cellular responses to this agent. The cultured mammalian cell has proved a convenient system for these investigations (1). The cell population is homogenous and can be readily controlled. Also, the radiobiologic properties of these cells are similar in many respects to rapidly growing mammalian cells, *in vivo*.

By studying the postirradiation survival of cultured cells, Elkind et al (2,3) demonstrated that mammalian cells can modify radiation damage. Using the single cell technique (4), these investigators performed paired-irradiations. The cells were given an initial dose which was followed sometime later by a second dose. The response to the second dose reflected the damage remaining from the first exposure. It also indicated how radiation damage varies as a function of time. Although a simple exponential term defined the rising survival after six hours, the cells behaved in a rather unusual manner toward the second irradiation during the immediate postirradiation period. Initially the survival increased to a maximum at two hours, and then decreased to a minimum at six hours.

Elkind (5), Whitmore (6) and Sinclair (7) have postulated the initial rise of the paired-dose curve to be a consequence of actual repair of radiation injury. They further speculate that the subsequent decrease in survival is due to the progression of cells through a radiosensitive period of the life cycle. Evidence for repair comes from experiments which showed the initial recovery to be independent of wide temperature variation. The decreased survival, however, was strongly temperature-dependent. Therefore, Elkind (5) contends that while actual repair does occur it is probably passive. Nevertheless, inhibitors of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) syntheses reduce the cell's ability to recover from radiation injury (8,9). This finding suggests that repair is dependent in part on anabolic processes.

A continuous and adequate supply of energy must be available for anabolic processes. A lack of information, however, prevents a correlation between the energic state of the cells and the potential for survival. For this reason, a series of studies were undertaken to establish the effect of energy deprivation on the radiation response of cultured mammalian cells.

The L-929 cell culture line was chosen as a test system because 1) the cell can repair radiation injury (10) and 2) it has been studied with respect to its metabolism (11-13). The approach to this problem has involved measurements of DNA and RNA syntheses, as well as adenosine triphosphate (ATP) levels and formation, under conditions of normal growth, starvation and chemical inhibition. The biochemical analyses performed after irradiation have been compared to results on single cell survival under corresponding conditions.

The investigations have provided evidence that, in energy-deprived cells, relatively low radiation doses induce rapid nucleic acid synthesis which is paralleled by changes in ATP metabolism. The survival studies showed an increased viability for cells under extreme energy deprivation at the time of irradiation.

MATERIALS AND METHODS

Cell Culture. - Stock cultures of the L-929 (14) were maintained as monolayers attached to the surface of glass bottles. Eagle's Minimal Essential Medium (15), supplemented with 10% calf serum, supplied the nutrients required for growth. The cell line, as well as the constituents of the growth medium, was purchased from Microbiological Associates, Bethesda, Md.

Cell survival was quantitated by Puck's single cell technique (4). Usually the percentage of untreated control cells which grew (plating efficiency) was

between 55 - 75%. An 18-hour interval placed between the seeding of the cells and their use in an experiment allowed time for recovery from the transfer process.

Analyses of biochemical changes involved in these studies required relatively large quantities of cells. At confluency a monolayer culture contains approximately 10^7 cells. Since as many as 10^8 cells were needed for some experiments, a means had to be devised for pooling several monolayer cultures. Figure 1 illustrates the essential features of the technique used. Cells from ten to twenty monolayers were detached from the glass surface with a rubber policeman, pooled in a dark bottle, and kept constantly suspended in the desired medium by the action of a magnetic stirrer. A water bath maintained the temperature at 37°C. This method of operation had the distinct advantage of permitting preirradiation and postirradiation samples to be taken from the same homogenous population.

Radioactive Labeling Methods. - As shown in Figure 1, the cells could be pulse labeled with radioactive precursor by transferring a given aliquot of the suspension into a screw cap tube containing the label. A vacuum, created by pulling back on the plunger of a syringe, caused the aliquot to be transferred. In all instances a 2.0 ml volume was sampled. The labeling lasted for 4.0 minutes and was terminated by the addition of cold 0.3 N perchloric acid. The time points in all pulse labeling experiments represent the end of the incorporation period.

Continuous labeling involved adding the radioactive precursor directly to the cell pool and permitting integral labeling over the course of the experiment. At predetermined times, samples were obtained from the pooled cells and labeling was stopped with cold 0.3 N perchloric acid.

The cellular constituents of interest were prelabeled by incubating the cells at 37°C with the appropriate labeled material. The cells were washed with HBSS and then resuspended in a non-radioactive medium. Here also, samples were taken at predetermined times and labeling was stopped with cold 0.3 N perchloric acid.

The radioactive compounds employed in these studies are listed below with the supplier:

Sodium Phosphate-P32, 25-30 mCi/mg-P
Abbott Laboratories, North Chicago, Ill.

Adenosine-8-C14, 32.2 mCi/mM, Schwarz
Bioresearch Inc., Orangeburg, N. Y.

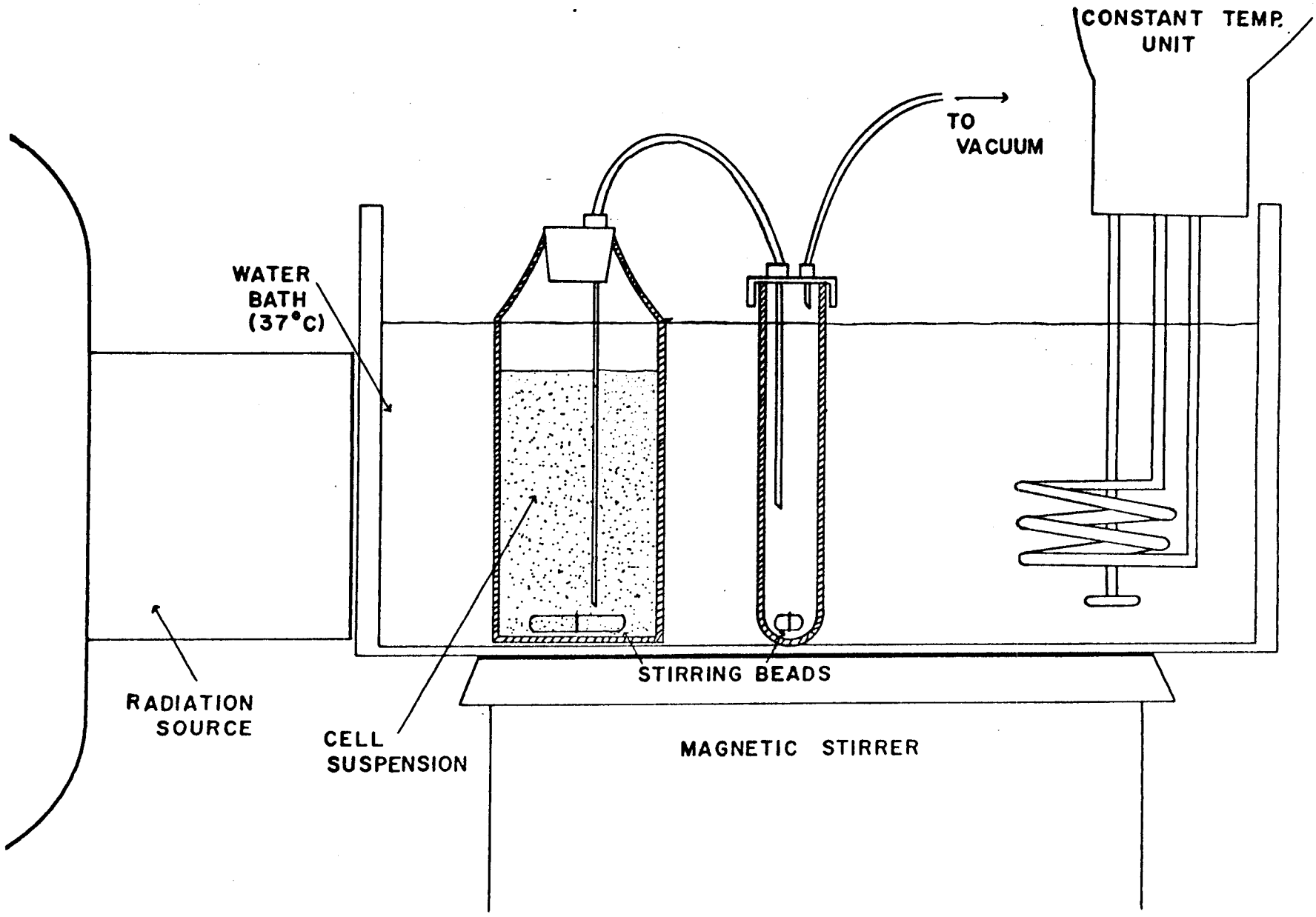

FIGURE 1: SCHEMATIC DIAGRAM OF THE SUSPENSION SYSTEM

Thymidine (methyl-T), 20,600 mCi/mM,
Nuclear Chicago Corp., Des Plaines, Ill.

Deoxycytidine-5-T, 14,700 mCi/mM
Nuclear Chicago Corp., Des Plaines, Ill.

Radioisotope Counting - Phosphorous- 32 from all cellular fractions was detected by a gas-flow ionization chamber (Baird-Atomic model 134 scaler, model 318A high voltage power supply, and model 755 automatic sample changer). Samples containing carbon-14 and tritium were counted with a Packard model 3003 liquid scintillation counter. After digestion in hyamine hydroxide (Packard Co., Downer Grove, Ill.), the cellular material was mixed with 15.0 ml of a water-based scintillation fluid. The scintillation fluid consisted, by volume, of 8% Liquifluor (T. M. Pilot Chemicals, Watertown, Mass.), 16% toluene, 2% ethanol and 74% dioxane. Finally, 50 grams of napthalene is added to each liter of the mixture. The count from an external radium source indicated the degree of quenching, which was low and quite consistent for all samples of a given experiment.

Biochemical Analyses - The Schmidt-Thannhauser extraction scheme (16) was followed for separation of the various cell components. This method includes a cold perchloric acid extraction to obtain a low molecular weight fraction containing ATP, a lipid solvent treatment, alkali digestion of RNA, and a hot acid extraction of DNA.

The luciferin-luciferase method described by Addanki, et al (17) represents an extremely sensitive technique for the assay of ATP and was used in these studies. Because the measurements are based on a chemiluminescent reaction requiring ATP, the photomultiplier system of a Packard model 3003 counter served as the detection instrument. The samples containing ATP were loaded into vials with the enzyme system, extracted from firefly lanterns (Sigma Chemical Co., St. Louis, Mo.). The luminescence was measured in the liquid scintillation counter.

Ion exchange chromatography served to separate ATP from other adenine nucleotides (18). The resin employed was Dowex-1-formate (Baker Chemical Co., Phillipsburg, N. J.) with a formic acid-ammonium formate elution system; the recovery of ATP from the columns was $90 \pm 5\%$.

Irradiation Procedures - Irradiations were performed with a Norelco 150 kvp X-ray machine and a Keleket 3000 curie cobalt-60 unit. When operated at 150 kvp and 10 ma, the Norelco machine produced a dose rate of 100 roentgens per minute at 16 cm from the port. The half-value layer of the beam was 6 mm of Al. The cobalt-60 beam delivered a dose rate of 138 roentgens per minute at 30 cm from the source.

All dose measurements were made with a Victoreen R meter. Irradiations were conducted under conditions of full backscatter and electron equilibrium. The roentgen to rad conversion factor is 0.93 for 150 kvp X-rays and 0.975 for cobalt-60 gamma rays (19).

RESULTS

The Effect of Radiation on Cells in Growth Medium - An important point in these studies concerned the stability of cells in the suspension system used for the experiments. Figure 2 indicates the biochemical behavior of cells maintained in growth medium for a three hour period. The ATP level remained constant at about 450 picomoles per 10^6 cells. Phosphate-P32 incorporation into the ATP, DNA and RNA fractions is expressed as counts per minute per 10^6 cells. The measurements started after the cells had been in the suspension system for 40 minutes. Each time point signifies the end of a 4.0 minute labeling period with 12.0 μCi/ml. The mean and standard deviation for the points are shown. During the course of the experiment, the variation in the measurements did not appear random, but instead fluctuated in a cyclic manner which is most apparent in the ATP and RNA fractions.

Although the observations extended up to four hours, an interval sufficient to cover the repair period, radiation doses from 100 rads to 1000 rads failed to produce any change in the precursor uptake for ATP, DNA and RNA when the cells were kept in growth medium.

Figure 3 illustrates the incorporation pattern of phosphate-P32 for cellular fractions being labeled continuously. At 20 minutes prior to irradiation (indicated by time 0), phosphate-P32 was added to a final concentration of 7.0 μCi/ml. The ATP level is expressed as percent of the final measurement. The uptake into DNA is linear; but the labeling of ATP and RNA cannot be described simply. A comparison of the upper and lower panels shows no apparent difference in the labeling for irradiated and control cells. Although the data come from an experiment using 500 rads, they reflect the response which is typical for doses of 100 rads to 1000 rads.

The Effect of Radiation on Cells in Starvation Medium - Pulse Labeling - Since Hank's Balanced Salt Solution (HBSS) is a usual constituent of the growth medium, a glucose-free preparation of this solution served as the starvation medium. The effect of suspending the cells in glucose-free HBSS is shown in the left panel of Figure 4. The measurements are given as percent of growth medium control. Each point stands for the end of a 4.0 minute labeling period with 12.0 μCi/ml of phosphate-P32. Time 0 indicates the point at which the cells were transferred from growth medium to HBSS. Although

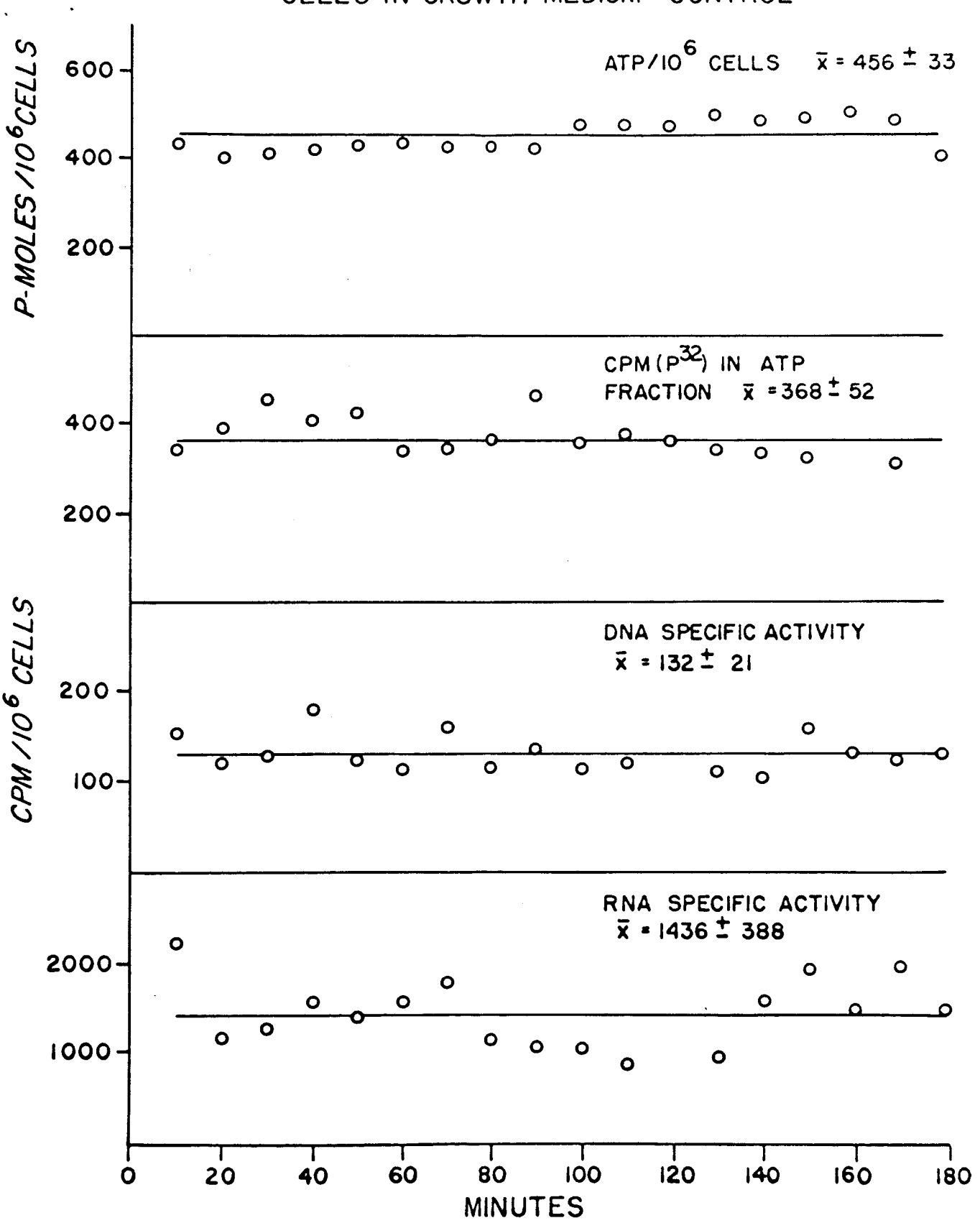

FIGURE 2: CELLS IN GROWTH MEDIUM-CONTROL

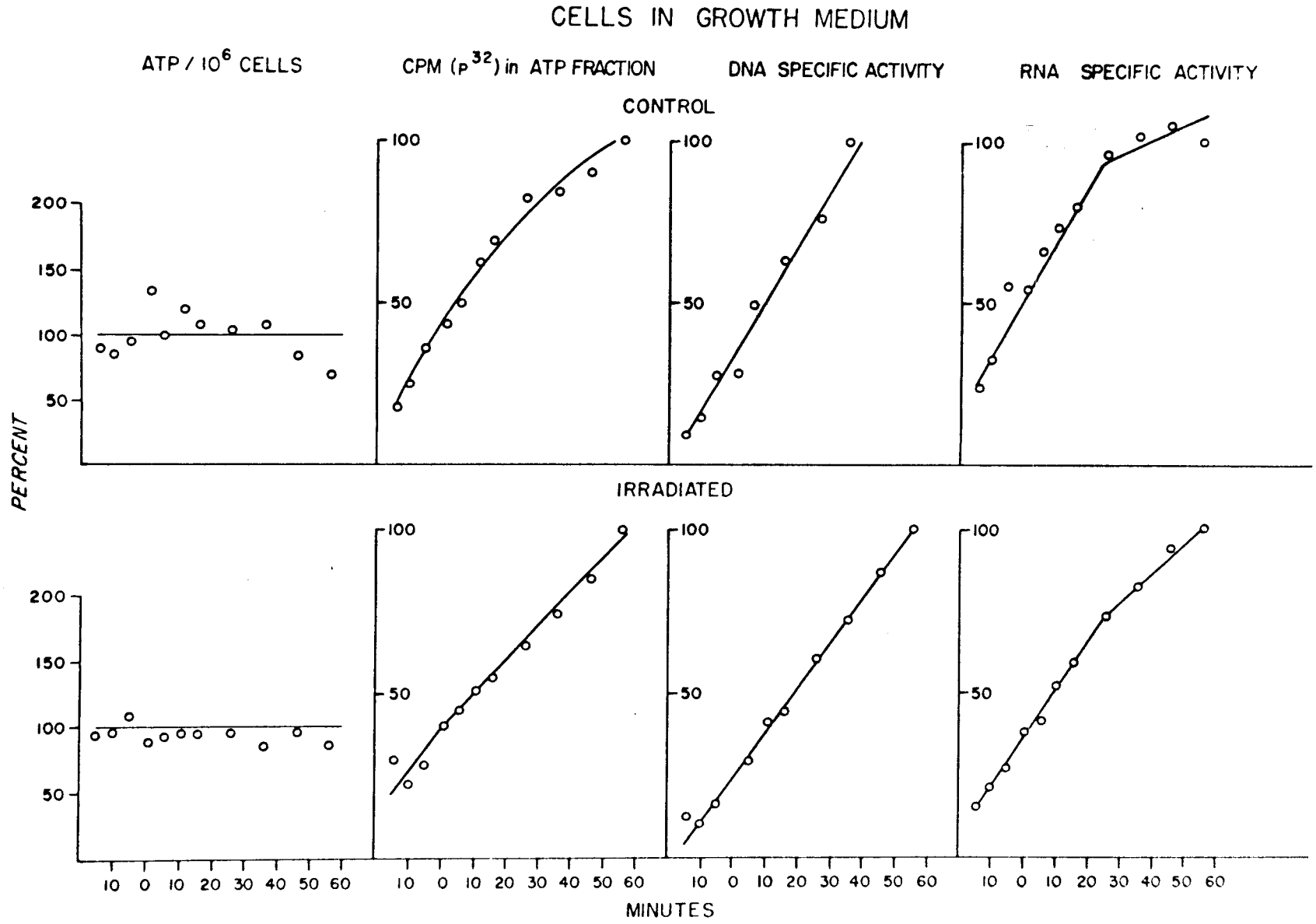

FIGURE 3: CELLS IN GROWTH MEDIUM - CONTINUOUS LABELING

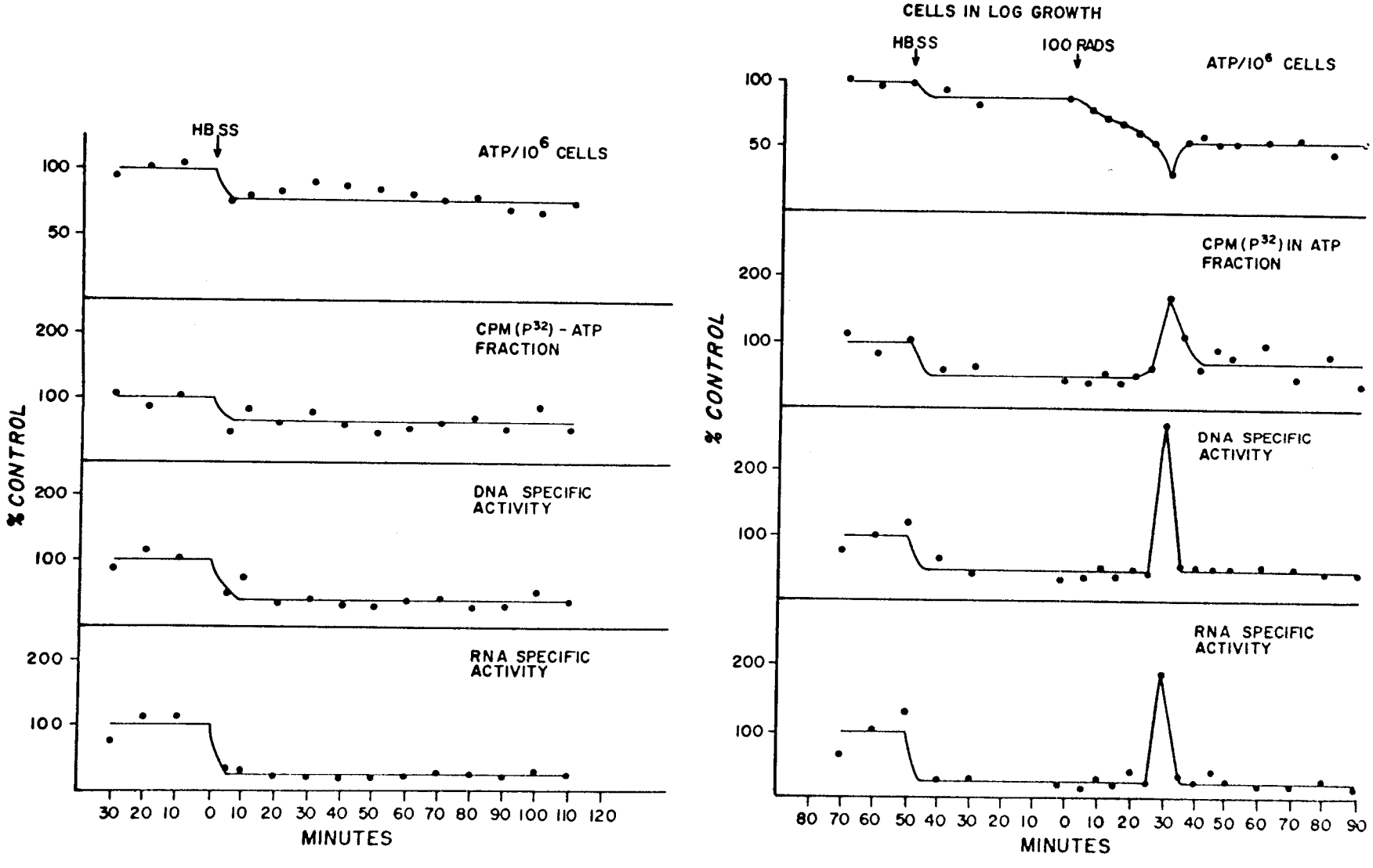

FIGURE 4: CELLS IN STARVATION MEDIUM

starvation produced an immediate depression of precursor incorporation into the macromolecular fractions, the ATP level as well as phosphate-P32 uptake into ATP remained high.

The right panel of Figure 4 indicates the effect of 100 rads on starved cells. After 50 minutes in HBSS, the cells were irradiated and samples were collected for another 90 minutes. Again the measurements are standardized to a growth medium control. A period of rapid phosphate-P32 uptake into the ATP, DNA and RNA fractions with a corresponding decrease in the ATP level is the most prominent feature. This response, characteristic for cells in logarithmic growth at the time they are harvested for the experiment, appeared around 30 minutes postirradiation, but only for low doses. Doses of 250 rads or more did not produce similar changes.

Cells which are permitted to remain in a confluent state for one or two days enter a lag phase since the nutrients in the growth medium are rapidly depleted under these circumstances. These "prestarved" cells were studied with respect to their nucleic acid synthesis and ATP metabolism after irradiation. Figure 5 compares representative experiments in which the cells received 100 rads. The essential aspects do not differ from those observed for cells in log growth except the period of accelerated uptake occurs somewhat earlier. The nucleic acid specific activities in the right panel correspond to the data represented by triangles in the left panel.

The Effect of Radiation on Cells in Starvation Medium - Continuous Labeling - In a effort to understand better the postirradiation activity of starved cells, a continuous labeling technique was used for studying precursor uptake into the different cellular fractions. The left panel of Figure 6 contains the control curves for ATP, DNA and RNA. After the cells had been starved for 40 minutes in HBSS, phosphate-P32 was added to a final concentration of 10.0 μCi/ml and aliquots of the suspension were sampled at varying times later. The ATP/10^6 cells is expressed as percent of the average of all the values. The observed patterns of incorporation closely resemble those found for cells in growth medium, although the specific activities are lower.

As the right panel of Figure 6 shows, 100 rads produces a brief period of nucleic acid synthetic activity at about 30 minutes postirradiation. The concomitant loss of label from the ATP fraction at this time probably results from the reduced ATP content. Because inorganic phosphate immediately enters ATP, continuous labeling effectively prelabels the ATP fraction; consequently, the decrease is not surprising. The nucleic acids are most interesting because the phosphate-P32 seems to enter and then immediately to leave both fractions. After 30 minutes, however, the labeling returns to a normal pattern.

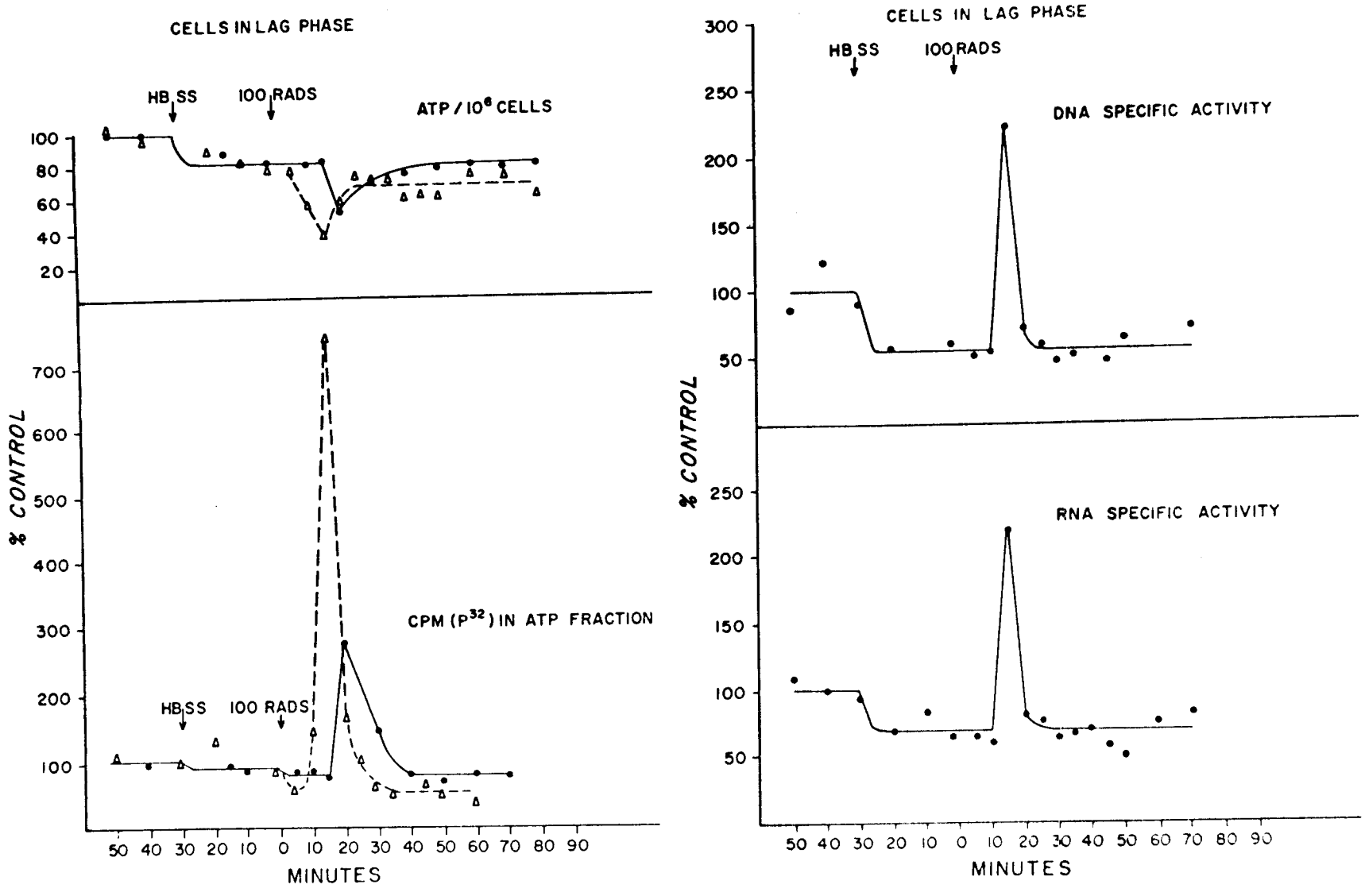

FIGURE 5: CELLS IN STARVATION MEDIUM - LAG PHASE

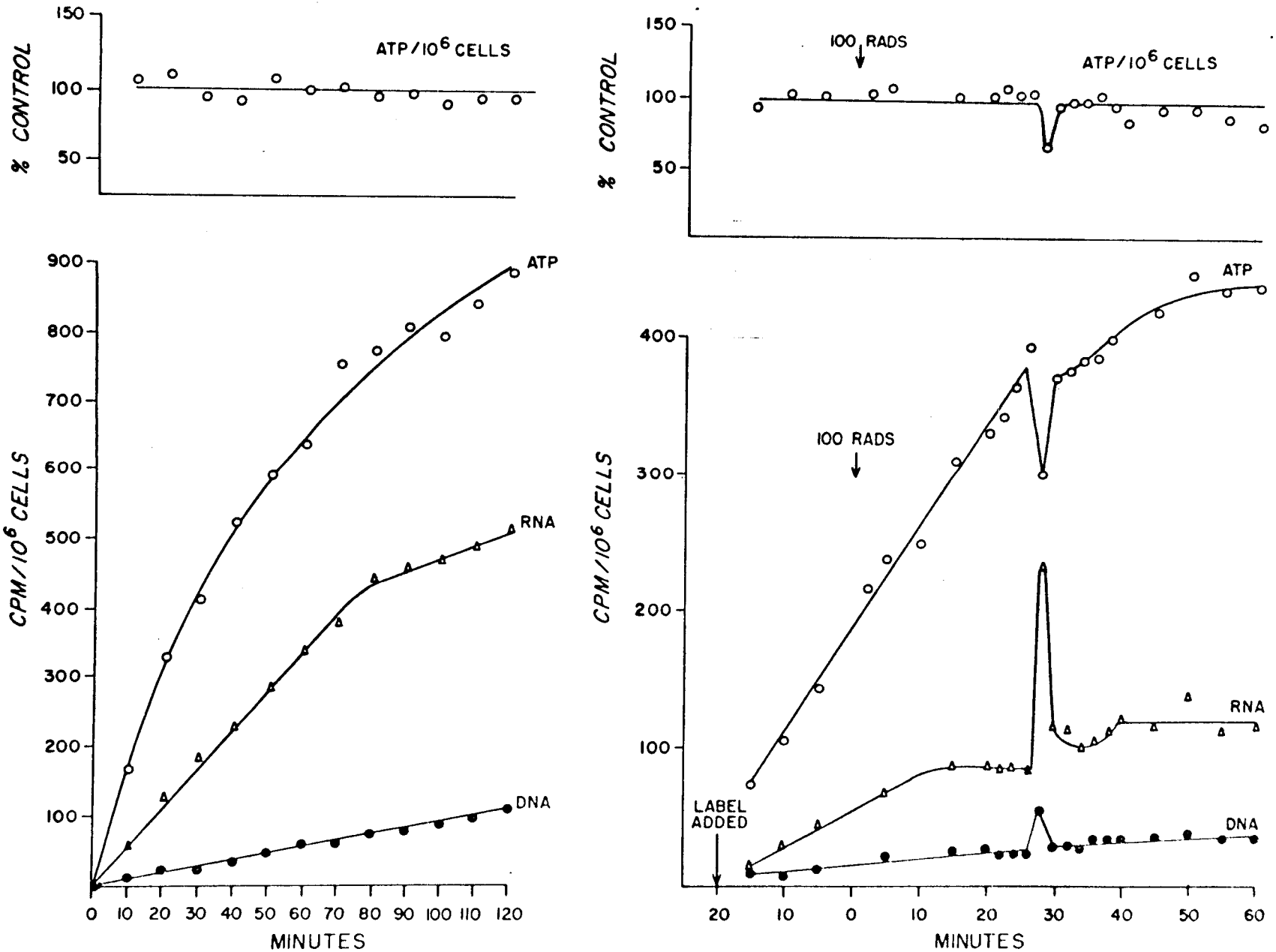

FIGURE 6: CELLS IN STARVATION MEDIUM - CONTINUOUS LABELING

As in the case of pulse labeling, continuous labeling did not show ATP metabolism to be altered by higher doses. Figure 7 illustrates the lack of change from control seen for ATP metabolism after 250 rads. The ATP content does not differ significantly from preirradiation levels; the incorporation of phosphate-P32 into ATP follows a pattern similar to that for the control uptake shown in Figure 6.

Unlike the results with pulse labeling, however, higher dose markedly affected the precursor incorporation into nucleic acids when continuous labeling was used. Figure 8 compares the DNA and RNA specific activities for different doses. Labeling started 20 to 25 minutes before irradiation with the addition of inorganic phosphate-P32 to a final concentration of 10 μCi/ml. The results are presented as percent of the final measurement. After 250 and 500 rads, the specific activities followed a cyclic pattern of incorporation and loss of label. The loss, however, never fell below the level of the unirradiated control.

While higher doses produced essentially the same results as those found after 100 rads, the period of activity and the extent of uptake is greater in the latter case. The marked similarity in the behavior of DNA and RNA represents another unusual feature of these data.

Labeling with Specific Precursors - Although phosphate is an immediate precursor for ATP formation, its entry into the de novo synthesis of nucleic acids begins with glucose-6-phosphate. In order to establish more fully the nature of the radiation-induced biochemical changes, labeled precursors specific for given fractions were used for measuring the synthesis of ATP, DNA and RNA in starved cells. Because 100 rads produced a noticeable change in ATP metabolism and a period of accelerated DNA and RNA labeling centered around 30 minutes postirradiation, this was the dose employed for these experiments.

Figure 9 contains the results for adenosine-C14 uptake into the ATP fraction. Continuous labeling started at - 20 minutes with a concentration of 0.010 μCi/ml. As indicated, the labeled ATP decreased precipitously around 30 minutes after irradiation, remained low for several minutes, and then recovered completely. The values for the uptake are adjusted to the final measurement.

Figure 10 is a compilation of data from experiments designed to specifically label either DNA or RNA. As shown, continuous labeling with tritiated deoxycytidine indicated a change in DNA metabolism at 25 minutes postirradiation. The deoxycytidine uptake correlates well with similar observations using inorganic phosphate. Again, label appears to enter and

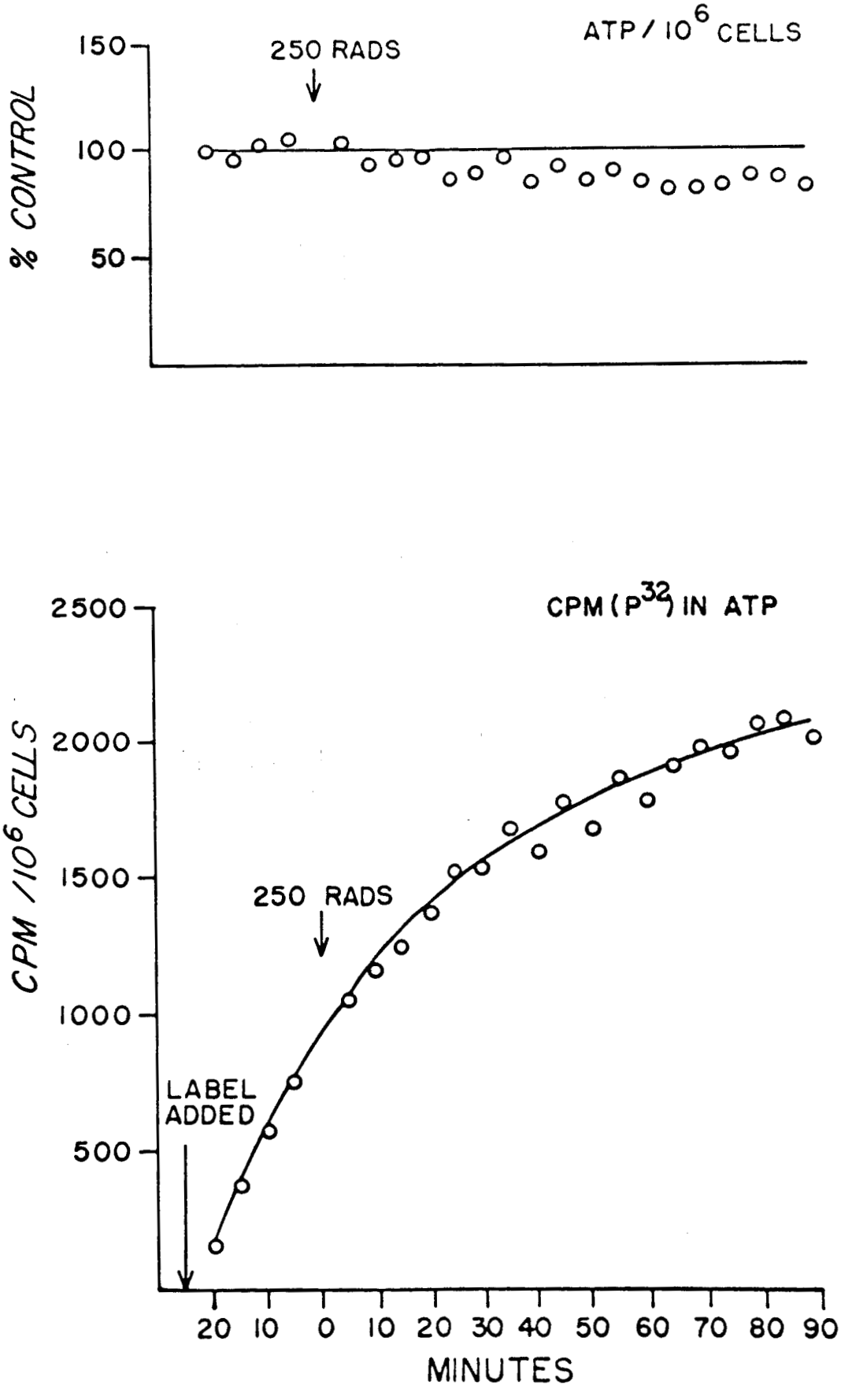

FIGURE 7: ATP METABOLISM AFTER 250 RADS - CONTINUOUS LABELING

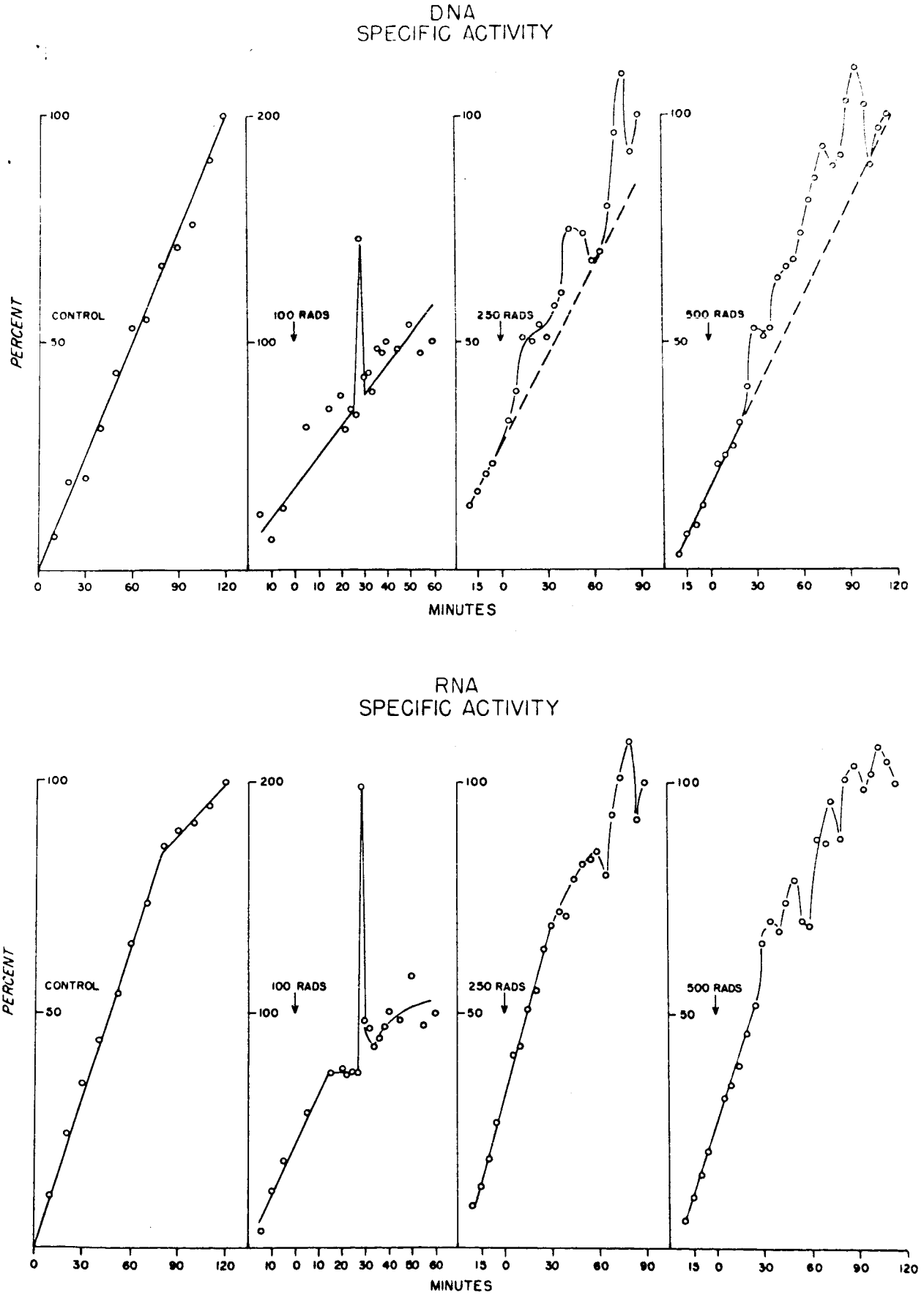

FIGURE 8: COMPARISON OF DNA AND RNA SPECIFIC ACTIVITIES - CONTINUOUS LABELING

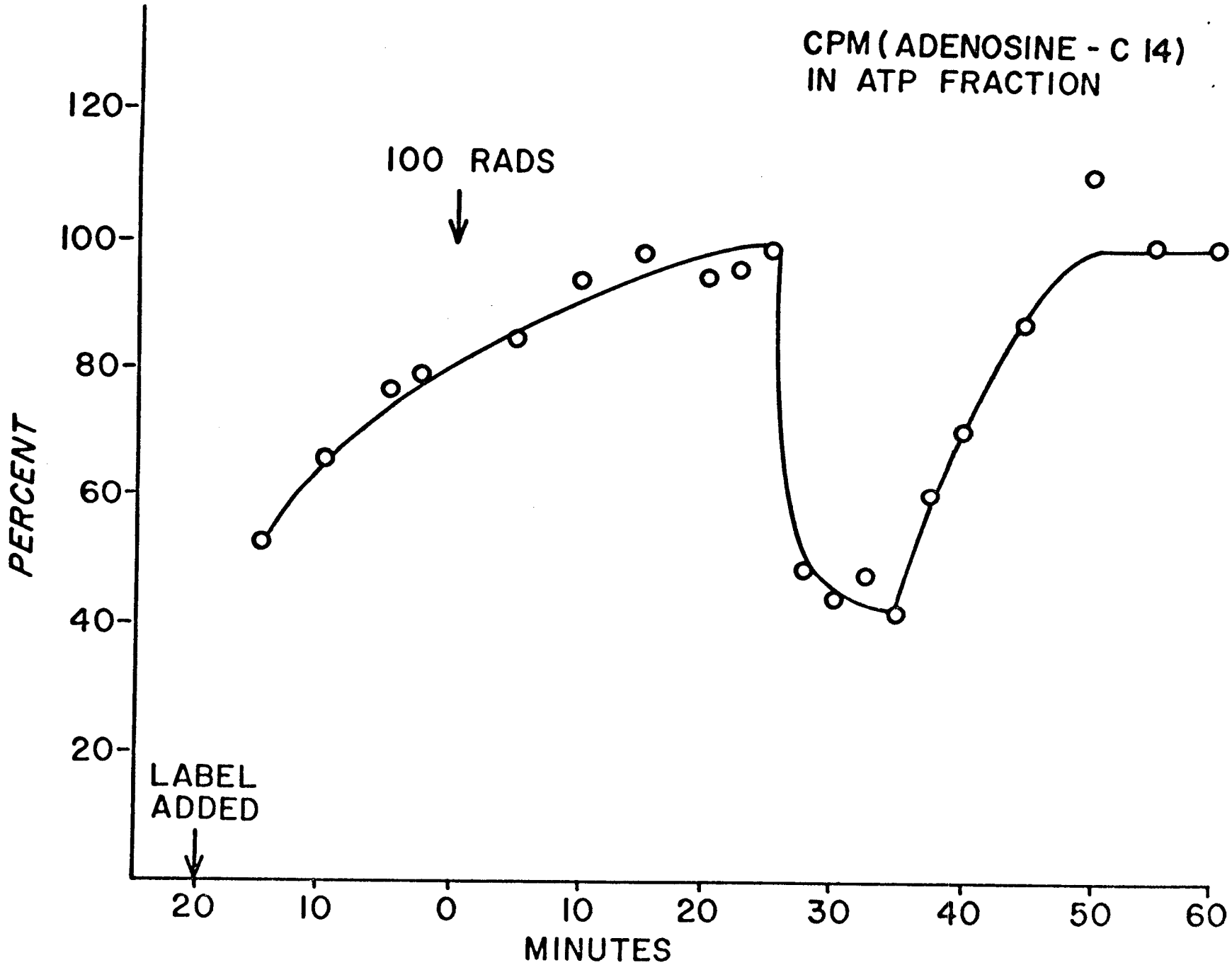

FIGURE 9: UPTAKE OF ADENOSINE-C14 INTO ATP AFTER 100 RADS

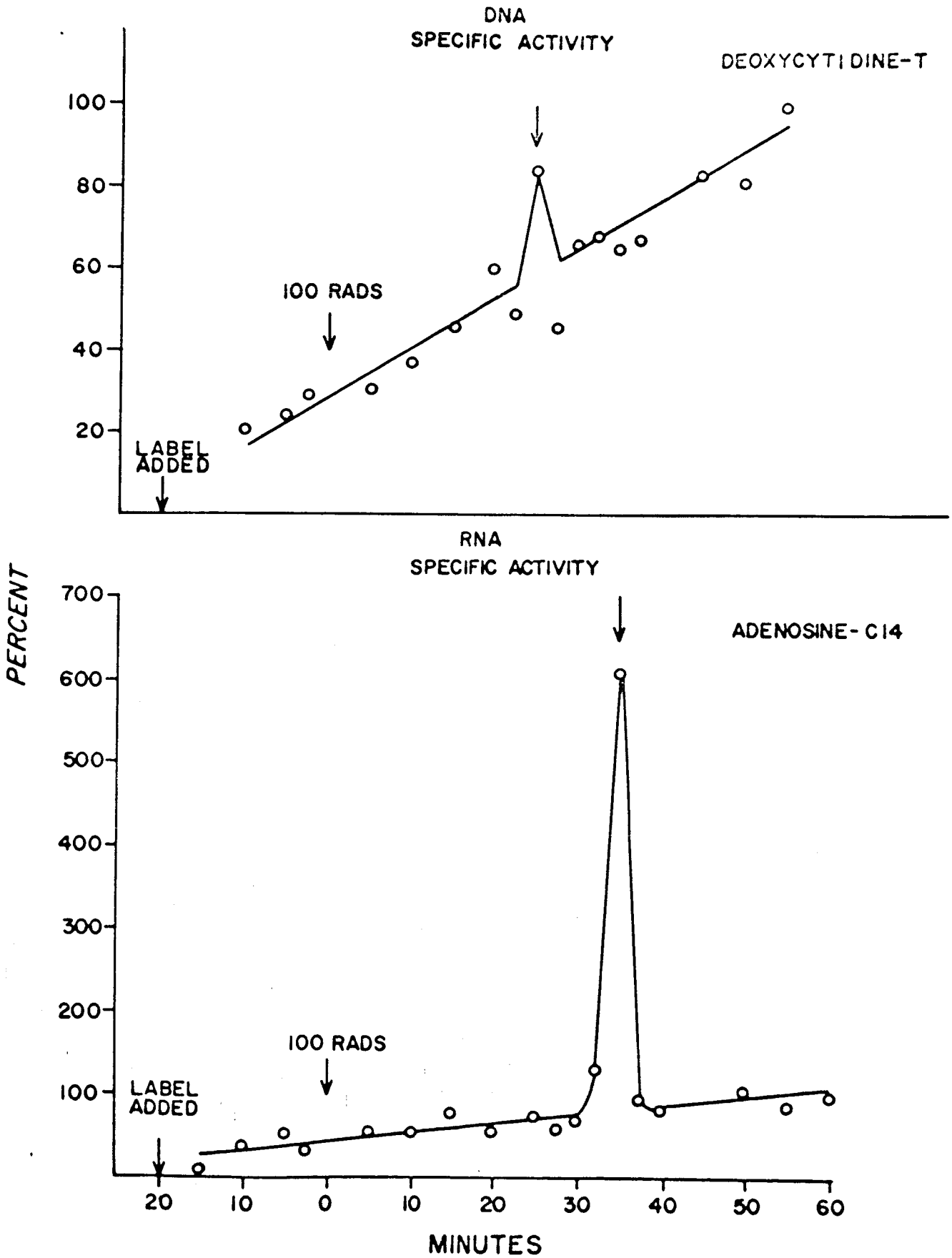

FIGURE 10: LABELING WITH SPECIFIC PRECURSORS OF THE NUCLEIC ACIDS

and then to leave the DNA fraction. The arrows point out the periods of reduced ATP content. The concentration of label was 0.2 μCi/ml; the uptake is expressed as percent of the terminal value.

As seen in Figure 10, the continuous uptake of adenosine-C14 into the RNA fraction agrees well with the results obtained using inorganic phosphate. Label enters and then leaves. The concentration of the labeling medium in this case was 0.010 μCi/ml.

Prelabeling of Nucleic Acids - The possibility of nucleic acid degradation, suggested from the continuous labeling experiments, led to studies on the behavior of prelabeled DNA and RNA. Figure 11 shows results for a 30 minute and a 17 hour prelabeling with inorganic phosphate-P32. Concentrations of 1.0 μCi/ml and 0.10 μCi/ml were used. After labeling in growth medium, the cells were transferred to HBSS and were allowed to starve for 40 minutes before sampling began. Following 100 rads, no obvious change from the preirradiation level appeared in either nucleic acid fraction.

The Effect of Radiation on Cells in Dinitrophenol - While starvation in glucose-free HBSS produced a sharp decrease in macromolecular synthesis, it did not lower the ATP content or ATP labeling with inorganic phosphate-P32 much below that observed in growth medium. In order to study the cells under complete energy deprivation, they were irradiated while exposed to DNP prepared to a final concentration of 5×10^{-5} molar in glucose-free HBSS.

In Figure 12 the results for the control and irradiated cells, studied by pulse labeling with 12.0 μCi/ml of inorganic phosphate-P32, are expressed as percent of the growth medium control. As the left panel shows, DNP produced a precipitous drop in the ATP content and the precursor uptake for all fractions. The center panel shows that the only change from the unirradiated control after 100 rads is an increased ATP labeling at 15 minutes. This finding differs from the results obtained with HBSS where an increased incorporation was also found for the DNA and RNA fractions. Because inorganic phosphate enters the nucleic acid synthetic pathway at a point quite distant from the final step, the possibility existed that the lack of incorporation resulted from a deficiency of label in the immediate precursor pool. This hypothesis was tested by pulse labeling with 4.0 μCi/ml of tritiated thymidine, a precursor specific for DNA synthesis. As seen in the center panel, 100 rads produced an increased uptake of tritiated thymidine into DNA at 15 and 25 minutes postirradiation. This supports the contention that a lack of labeled precursor, caused by the DNP treatment, is responsible for the failure to see accelerated uptake into the nucleic acids when inorganic phosphate is used.

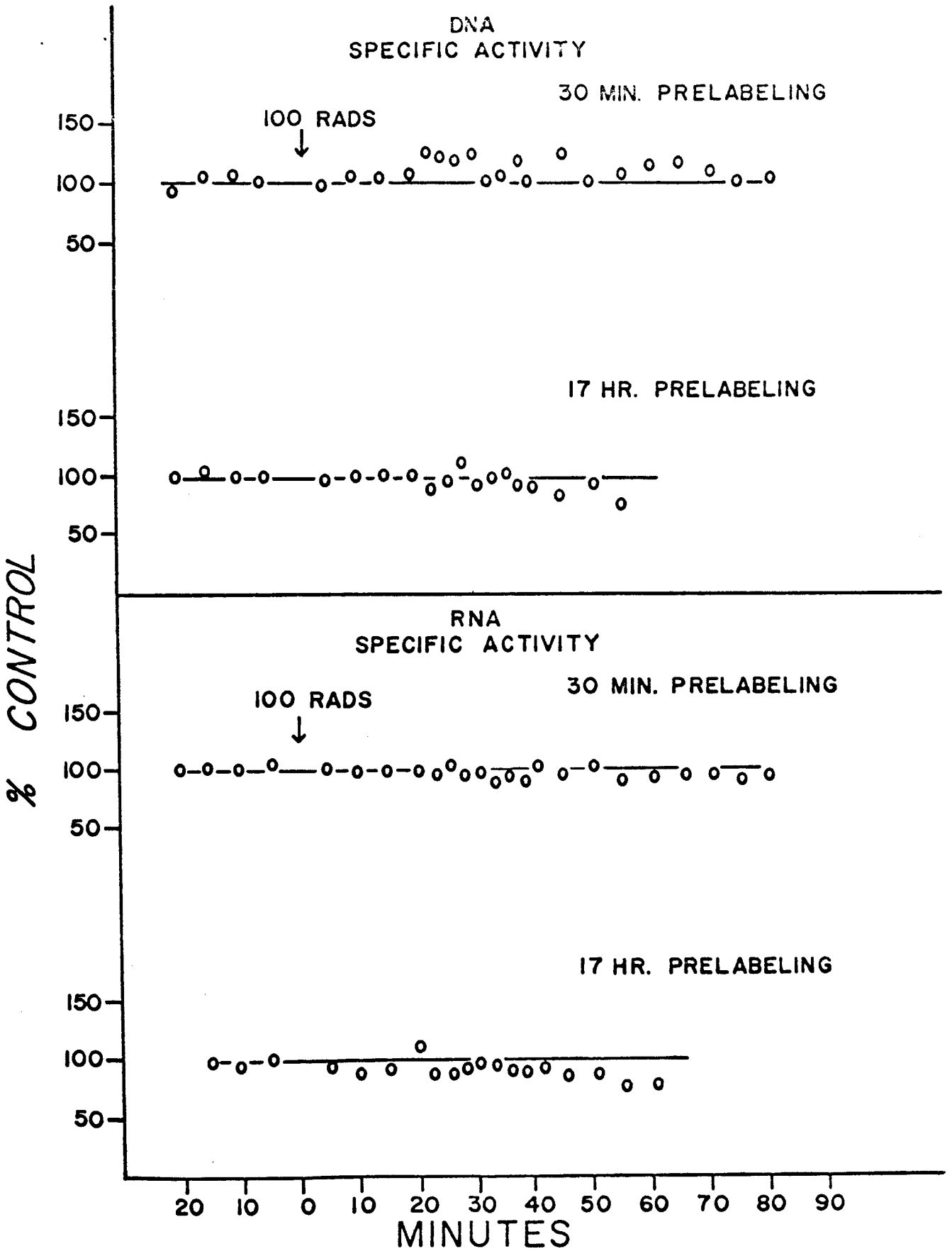

FIGURE 11: PRELABELING OF NUCLEIC ACIDS

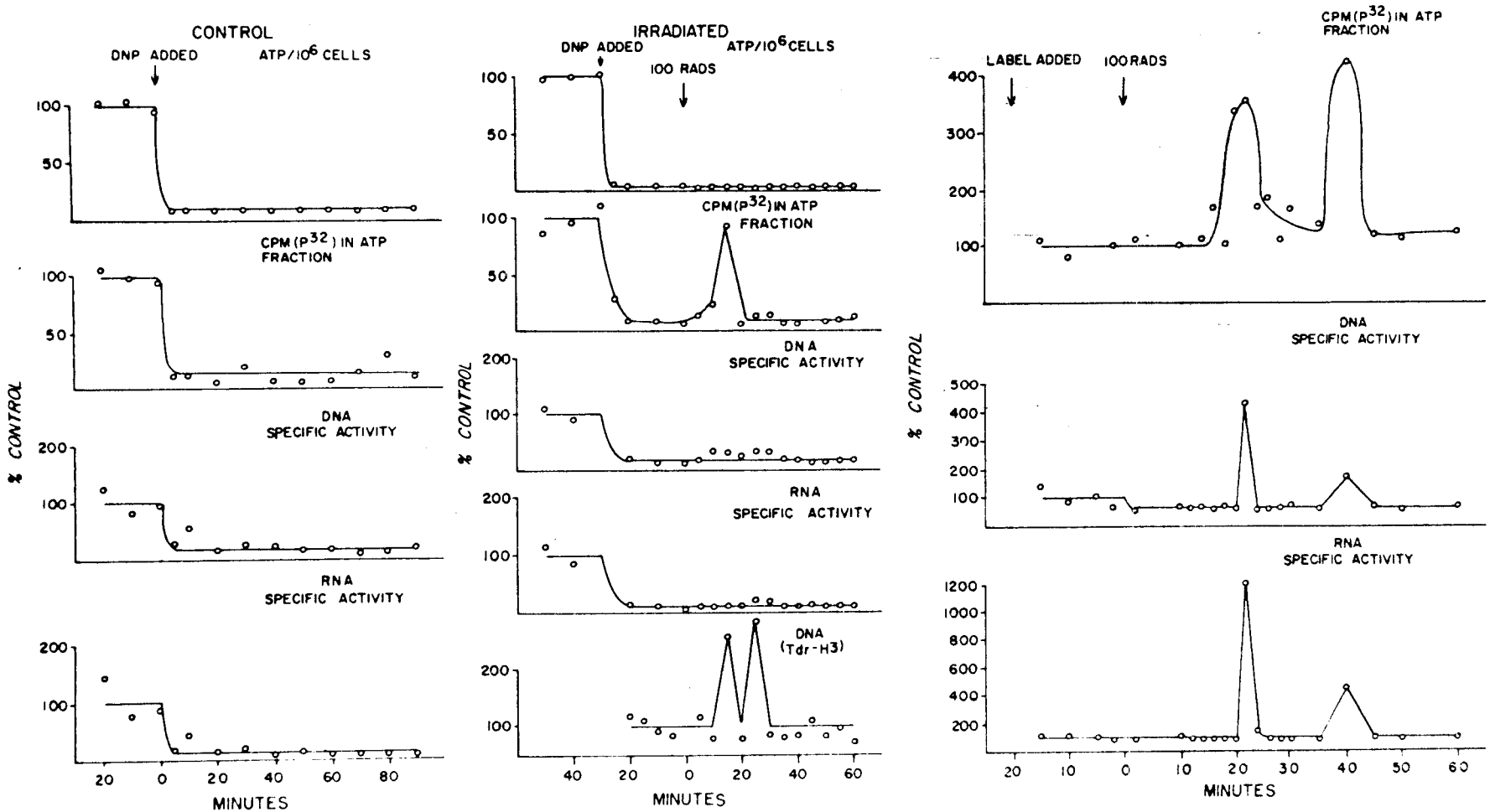

FIGURE 12: CELLS IN DINITROPHENOL

Results from continuous labeling with inorganic phosphate-P32 in DNP also suggest that a lack of label in the immediate precursor pool could be the reason for failure to see stimulated DNA and RNA synthesis after 100 rads. The right panel of Figure 12 indicates increased uptake around 20 and 40 minutes postirradiation. The concentration of the inorganic phosphate-P32 was 5.0 μCi/ml. Since the cells had been exposed to the label for over 40 minutes, there would have been sufficient time for the label to enter the precursor pool. As in the case with HBSS, the label enters and then leaves the nucleic acid fractions. The appearance of two peaks in the continuous labeling experiment agrees with the observation of two peaks of uptake when tritiated thymidine was used for pulse labeling.

Survival Properties under Energy Deprivation - While energy deprivation changes the biochemistry of irradiated cells, its effect on cell survival remains to be established. The dose-response relationships for cells under energy deprivation are shown in Figure 13. The ordinate is surviving fraction, and the upper abcissa is the dose in rads. After one hour in HBSS, or 30 minutes in DNP, the cells received doses ranging from 200 rads to 750 rads. At two hours postirradiation, the HBSS and DNP were replaced with growth medium. Neither treatment reduced the viability of the cells. Although cells starved in HBSS did not vary from the control in their survival properties, cells treated with DNP did show a significant increase in survival which became most pronounced at the higher doses.

The paired-dose responses for cells in growth medium and DNP are also indicated in this figure. The lower abscissa shows the time between two doses of 300 rads each. By 4 hours, the survival for cells in growth medium had become three times greater than for the single exposure. Although cells irradiated in DNP had a higher survival, no time-dependent rise in the surviving fraction appeared. Upon being transferred to growth medium, the DNP-treated cells acquired a radiosensitivity characteristic of cells in growth medium.

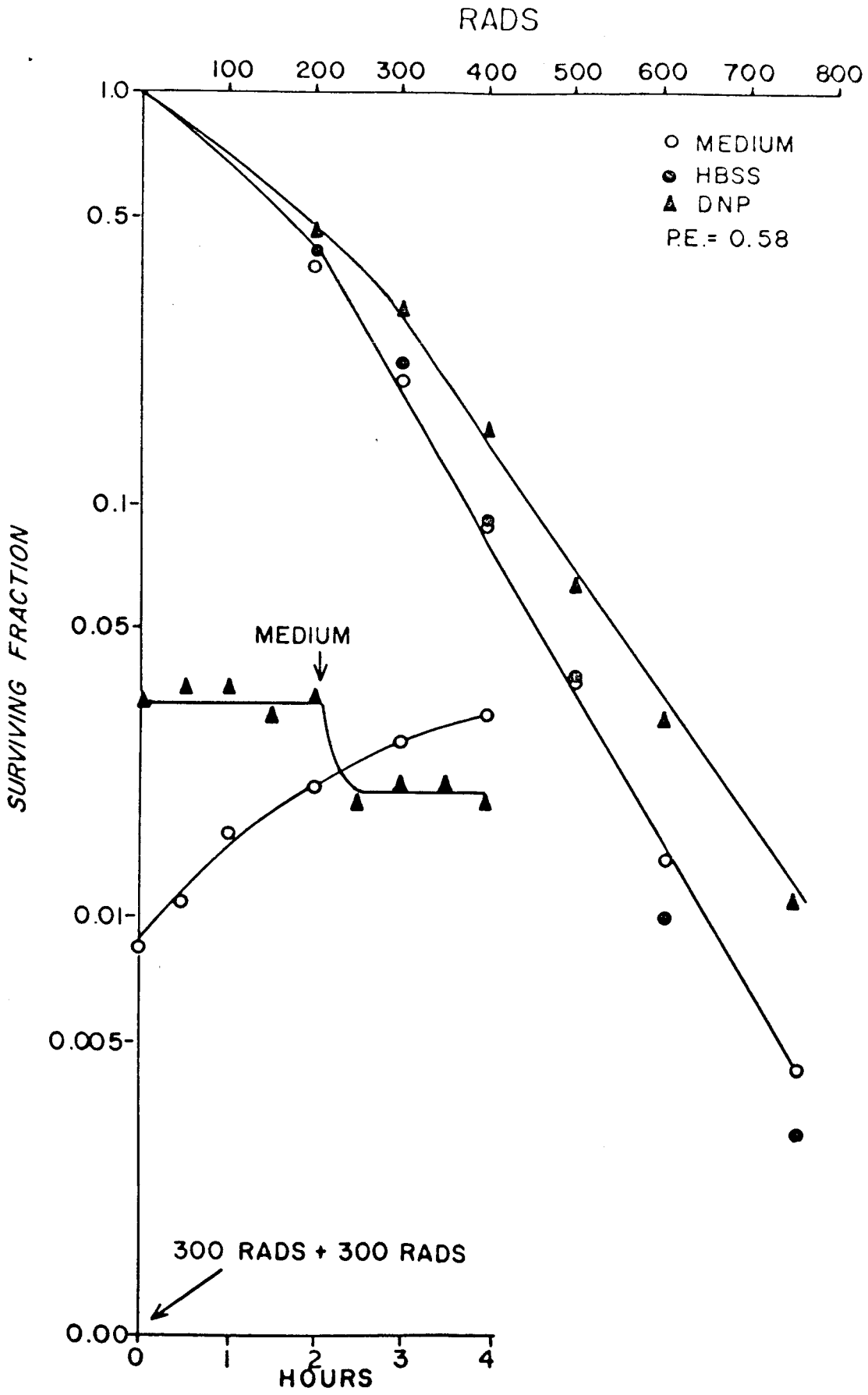

FIGURE 13: SURVIVAL PROPERTIES UNDER ENERGY DEPRIVATION

DISCUSSION

Although events leading to increased survival of the cells must occur during the immediate postirradiation period, a close examination of nucleic acid synthesis and ATP metabolism under growth conditions did not reveal an altered biochemistry in these fractions. The data shown in Figure 3 represent an effort to find a biochemical phenomenon related to recovery from radiation injury. The failure to find such a phenomenon, however, does not eliminate the possibility that it exists. Perhaps the changes associated with repair constitute such a small part of the total metabolism that they cannot be detected with the methods used.

The intent of the present study has been to determine the effect of energy deprivation on the radiation response of L cells. The results of these investigations indicate that irradiated cells being deprived of energy have unusual patterns of radioactive precursor uptake into the nucleic acid fractions. A brief, accelerated labeling of DNA and RNA characterizes the response. Both pulse labeling (Figures 4 and 12) and continuous labeling (Figures 8 and 12) show the effect. A delayed action seems inherent since the response was never detected before 15 minutes postirradiation (Figures 5 and 12). There are two unusual features in the data. First, as indicated from the continuous labeling experiments, the radioactive precursor enters and then leaves the nucleic acids. Second, the labeling of the DNA fraction closely parallels that of the RNA fraction. Although this behavior prevails at 100 rads, it also happens at higher doses (Figure 8), where several cycles of incorporation and loss of label occur.

While these studies give no indication of why a delay is necessary before the accelerated labeling of the nucleic acids, they do suggest that it depends on the metabolic state of the cells prior to their use in the experiment. Cells which had been prestarved (Figure 5) or treated with DNP (Figure 12) gave an earlier response than cells in logarithmic growth (Figures 4 and 6). Since there were no apparent changes in the labeling of DNA, RNA and ATP during this period, the reason for the delay remains an unsolved problem.

The labeling of RNA in the present study parallels to a great extent the labeling characteristics which have been described for messenger RNA (20). Both have a rapid precursor uptake and both show a fast turnover. A similar response occurring in the DNA fraction, however, is most unusual. The loss of labeled DNA following the accelerated uptake could indicate 1) a breakdown of the rapidly synthesized DNA or 2) a loss of preincorporated label from other sites in the molecule. The evidence strongly favors the first possibility. As indicated

by the experiments in Figures 8 and 12, the loss of label frequently exceeds the total amount incorporated prior to the accelerated uptake. In fact, the loss usually equals the amount incorporated. Furthermore, the results of the prelabeling experiment (Figure 11) do not indicate a breakdown of nucleic acids existing before the accelerated labeling. All this points to a rapid formation and breakdown of the same DNA, which is superimposed on the normal replicative process (Figure 8).

While there are reports of unscheduled DNA synthesis in X-irradiated cells (21), this effect has been described only for doses of 10^4 rads or more. Certain strains of bacteria also display an unscheduled DNA synthesis after UV-irradiation (22), which constitutes part of the "cut-patch" repair system. This mechanism, however, differs from the present biochemical events in that it involves degradation of DNA followed by resynthesis. Furthermore, RNA synthesis is not implicated in the "cut-patch" process.

The close parallelism in the labeling of DNA and RNA suggests that the two processes are related. Although DNA serves as a template for RNA formation, new DNA synthesis presumably is not required. Possibly, one strand of the double helical DNA is disrupted either directly by radiation or through the action of an endonuclease (22). This break then leads to the rapid nucleic acid synthesis with the intact strand serving as a template. The newly synthesized nucleic acid, be it DNA or RNA, would code for the synthesis of the other nucleic acid. Finally the last nucleic acid formed acts to repair the damaged site of the DNA molecule through a mechanism which, unfortunately, remains unclear. The results of these studies do not establish a hierarchy of interaction for the rapidly formed DNA and RNA.

The brief interval of accelerated labeling after 100 rads could represent nucleic acid synthesis at a localized point in the genome. Certain regions of the DNA molecule might be more susceptible to radiation damage than others. Since 100 rads produces relatively little lethal damage, as evidenced by a high surviving fraction for the cells (0.8), most of the injury at this dose would center in the sensitive region; the increased nucleic acid synthesis then indicates the cell's effort to reverse the damage. Higher doses (250 and 500 rads) affect even more regions of the genome, resulting in simultaneous nucleic acid synthesis at multiple foci. In this case, the uptake curve at higher doses reflects a composite of several uptake curves such as the type seen after 100 rads. If the synthetic activities at the different foci are somewhat out of phase, the loss of label at one point would tend to cancel the uptake of label at another point. This effect then leads to the diminished response seen at the higher doses.

The reduced ATP content for starved cells irradiated with 100 rads could represent 1) utilization of ATP to supply the energy needed for nucleic acid

synthesis and/or 2) incorporation of the adenine moiety into the nucleic acids. The increased uptake of inorganic phosphate-P32 at this time probably indicates new ATP synthesis since the level recovers rapidly (Figures 4 and 5). The continuous labeling with inorganic phosphate-P32 (Figure 6) and adenosine-C14 (Figure 9) suggest that this method effectively prelabels the ATP fraction. Again, in this case, an increased utilization of ATP is indicated.

While pronounced changes in ATP metabolism occurred after 100 rads for cells in HBSS, higher doses did not produce differences. This could result from the time needed for accelerating labeling. If ATP is necessary for the DNA and RNA labeling, then the uptake curve in Figure 8 shows that the requirement must be met rapidly when 100 rads is used. In contrast, the labeling at higher doses seems to extend over a much longer period. Consequently, the labeling would not be so extensive in a given interval. Possibly the cell has time to adjust its ATP level when the longer periods of labeling are involved, but it cannot meet the demand after 100 rads. Regardless of the situation at higher doses, measurable changes in ATP metabolism do occur after 100 rads, even under extreme ATP deficiency (Figure 12).

The question arises as to why radiation-induced changes appear in energy-deprived cells but not in cells irradiated in growth medium. The answer could lie in the relative rates of anabolic processes under these conditions. Cells starved in glucose-free HBSS or treated with DNP show a reduced macromolecular synthesis compared to growing cells. Perhaps the radiation-induced changes compete with normal metabolic processes. If this is the case, energy-deprivation might reduce the competition in favor of the former, resulting in responses not apparent under normal growth conditions.

As Figure 13 shows, DNP treatment markedly enhanced the survival of cells. The dose-response curves indicate that the effect is present at all dose levels. This increased survival presumably results from physicochemical or metabolic events occurring after irradiation. Figure 12 contains evidence for radiation-induced changes in nucleic acid metabolism under conditions which lead to an increased survival.

CONCLUSIONS

The radiation induced changes in nucleic acid metabolism reported here are unprecedented. Since comparable responses did not appear for cells in growth medium, these changes presumably occur because of the energy-deprived state.

The accelerated nucleic acid labeling appears to be under close cellular control as shown by the relatively fixed delay (25 - 40 minutes for cells in log growth)

and the brief interval of labeling (less than 4.0 minutes) following 100 rads. Although higher doses gave similar responses, they were not so clearly seen.

While no hierarchy of interaction can be established from the present study, the close parallelism of labeling for DNA and RNA indicates a cause-effect relationship. The accelerated labeling of RNA has the characteristics of messenger RNA; the labeling of DNA is novel. The evidence points to a DNA which is rapidly synthesized and then rapidly degraded. The decreased ATP content and increased labeling for cells irradiated with 100 rads further indicates that energy-requiring reactions (nucleic acid synthesis) are occurring.

Survival studies showed that cells treated with DNP have a higher post-irradiation survival than cells in growth medium. This increased survival may result from the metabolic changes observed after irradiation.

REFERENCES

1. M. M. Elkind and G. F. Whitmore. The radiobiology of cultured mammalian cells. Gordon and Breach, New York, 1967.

2. M. M. Elkind and H. Sutton. X-ray damage and recovery in mammalian cells in culture. Nature, 184: 1293-1295, 1959.

3. M. M. Elkind and H. Sutton. Radiation response of mammalian cells grown in culture. I. Repair of X-ray damage in surviving Chinese hamster cells. Radiation Res., 13: 556-593, 1960.

4. T. T. Puck and P. I. Marcus. Action of X-rays on mammalian cells. J. Exptl. Med. 103: 653-666, 1956.

5. M. M. Elkind, H. Sutton-Gilbert, W. B. Moses, T. Alescio and R. W. Swain. Radiation response of mammalian cells grown in culture. V. Temperature dependence of the repair of X-ray damage in surviving cells (aerobic and hypoxic). Radiation Res. 25: 359-376, 1965.

6. G. F. Whitmore, S. Gulyas and J. Botond. Radiation sensitivity throughout the cell cycle and its relationship to recovery. In Cellular Radiation Biology (published for the University of Texas, M. D. Anderson Hospital), pp. 423-441, Williams and Wilkins Co., Baltimore, 1965.

7. W. K. Sinclair and R. A. Morton. Survival and recovery in X-irradiated synchronized Chinese hamster cells. In Cellular Radiation Biology (published for the University of Texas, M. D. Anderson Hospital) pp. 418-422, Williams and Wilkins Co., Baltimore, 1965.

8. R. A. Phillips and L. J. Tolmach. Repair of potentially lethal damage in X-irradiated HeLa cells. Radiation Res. 29: 413-432, 1966.

9. M. M. Elkind, W. B. Moses and H. Sutton-Gilbert. Radiation response of mammalian cells grown in culture. VI. Protein, DNA and RNA inhibition during the repair of X-ray damage. Radiation Res. 31: 156-173, 1967.

10. A. Han, B. Miletic, D. Petrovic and D. Jovic. Survival properties and repair of radiation damage in L cells after X-irradiation. Intern. J. Radiation Biol. 8: 201-211, 1964.

11. M. K. Johnson and E. J. Johnson. Carbohydrate metabolism in strain L-929 mouse fibroblast cells. Proc. Soc. Exptl. Biol. Med. 111: 149-152, 1962.

12. C. de Luca. Effects of mode of culture and nutrient medium on cyclic variations in enzyme activities of mammalian cells cultured in vitro. Exptl. Cell Res. 43: 39-50, 1966.

13. A. Zetterberg. Nuclear and cytoplasmic nucleic acid content and cytoplasmic protein synthesis during interphase in mouse fibroblasts in vitro. Exptl. Cell Res. 43: 517-525, 1966.

14. K. K. Sanford, W. R. Earle and G. D. Likely. The growth in vitro of single isolated tissue cells. J. Natl. Cancer Inst. 9: 229-231, 1948.

15. H. Eagle. Amino acid metabolism in mammalian cell cultures. Science 130: 432-437, 1959.

16. G. Schmidt and S. J. Thannhauser. A method for the determination of deoxyribonucleic acid, ribonucleic acid, and phosphoproteins in animal tissues. J. Biol. Chem. 161: 83-89, 1945.

17. S. Addanki, J. F. Sotos and P. D. Rearick. Rapid determination of picomole quantities of ATP with a liquid scintillation counter. Anal. Biochem. 14: 261-264, 1966.

18. R. B. Hurlbert, H. Schmitz, A. F. Brumm and V. R. Potter. Nucleotide metabolism. II. Chromatographic separation of acid-soluble nucleotides. J. Biol. Chem. 209: 23-39, 1954.

19. H. E. Johns. The Physics of Radiology, pp. 703, Charles C. Thomas, Springfield, Ill., 1961.

20. E. Volkin and L. Astrachan. Phosphorous incorporation in Escherichia coli ribonucleic acid after infection with bacteriophage T-2. Virology 2: 149-161, 1956.

21. R. B. Painter. Unscheduled DNA synthesis following X-irradiation of HeLa cells (Abstract). Radiation Res. 31: 608, 1967.

22. R. B. Haynes. The interpretation of microbial inactivation and recovery phenomena. Radiation Res. Suppl. 6: 1-29, 1966.