THE SYNTHESIS OF DNA, RNA, AND NUCLEAR PROTEIN IN NORMAL AND TUMOR STRAIN CELLS

III. Mouse Ascites Tumor Cells

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ABSTRACT

Interferometric and photometric measurements have been made on replicating mouse ascites tumor cell cultures. From a study of the relations between successive physical measurements on individual cells, it was found that whereas the net syntheses of nuclear RNA and nuclear protein are closely associated during interphase, they are dissociated from DNA replication to a significant extent. These results agree with others reported in replicating cell strains derived from tumors. In auxiliary experiments an attempt was made to block the initiation of DNA synthesis by X-irradiation: although large amounts of nuclear protein accumulated in some cells in the absence of DNA synthesis, the inability to hold the DNA block for an interphase time prevented a quantitative analysis of the results.

INTRODUCTION

The investigation of the relations between DNA, RNA, and nuclear protein syntheses during the interphase of replicating animal cells has been described in earlier papers (Seed, 1961, 1962, 1963, 1964, 1966 *a*, 1966 *b*). In the present studies, a strain of cultured cells derived from a mouse ascites tumor was used. The experiments had two aims:

1. To investigate the relations between DNA, nuclear RNA, and nuclear protein syntheses during interphase by making plots of successive physical measurements on individual cells.
2. In line with previous experiments, to prevent the initiation of DNA synthesis for one interphase time (by X-irradiation) and then to measure the amounts by which nuclear dry mass and RNA had increased in that period.

It was not possible to make measurements of cytoplasmic nucleic acid or total cell nucleic acid on this cell type because the cells tended to grow contiguously in colonies on the cover slips.

EXPERIMENTAL PROCEDURE

The ascites tumor cells were grown in babies' feeding bottles in Eagle's medium (Eagle 1959) supplemented with 0.25% lactalbumin hydrolysate and 20% bovine serum. For the experiments the cells were subcultured onto quartz cover slips affixed to small glass slides as previously described (Seed, 1962).

The ascites cells were somewhat rounded 1 day after subculturing; because of this, slide cultures were initially prepared with low cell inocula and were allowed to grow for three days, with appropriate changes of growth medium, before use in the experiments. This allowed ample time for the cells to flatten in readiness for the subsequent physical measurements.

Preliminary experiments were first carried out with low-power time-lapse photography, when it was established that, as with other cell types, a single X-ray dose of 1250 R was sufficient to abolish mitosis for approximately one interphase time and that

there was no cell death during this period. This was in agreement with the work of Klein and Forssberg (1954) and Klein and Revesz (1953) who used a different strain of mouse ascites tumor cells in vivo.

At the start of the experiments described here, five slide cultures were irradiated with 1250 R (3½ min each): the cultures were then replaced in the incubator and were fixed at intervals up to 25 hr after irradiation. Two unirradiated control cultures were fixed at the beginning and two at the end of the experiments. During the course of the experiment an additional control culture was filmed by low-power time-lapse cinephotomicrography, and, from subsequent examination of this film, the intermitotic time (1280 min) of the cells was found. Dry mass measurements were made on four cultures: one control fixed at the beginning and one fixed at the end of the experiment, and two irradiated cultures fixed at about 1200 min after irradiation. The cultures gave consistent results, and measurements from only one control and one irradiated culture are presented here.

All cultures were rinsed briefly in warm saline before fixing in methanol.

Physical Measurements

Before making measurements on the fixed cells, low molecular weight compounds were first extracted in 1% PCA (perchloric acid) at +4°C for 30 min. Three types of measurements were made on the individual cells: nuclear UV absorption at 2536 A (nuclear nucleic acid, constituting $\simeq \frac{2}{5}$ RNA and the remainder DNA), nuclear dry mass ($\simeq \frac{5}{6}$ nuclear protein), and Feulgen stain (DNA) (Killander et al., 1962 *b*, Seed 1966 *a*). The excess nucleolar absorption was not included in the nuclear UV absorption and dry mass measurements.

The measurements in Figs. 1 and 2 are expressed in arbitrary units of nucleic acid and separately of dry mass: 1 nucleic acid unit $\simeq 8.1 \times 10^{-13}$ g, and 1 dry mass unit $\simeq 1.5 \times 10^{-12}$ g.

RESULTS

Control Cells

As was demonstrated for other cell types (Seed, 1963), it is possible to relate the interphase growth of nuclear dry mass to that of DNA by plotting the dry mass measurements for individual cell nuclei against the corresponding DNA and nuclear nucleic acid values for the same cells (Fig. 1).

In Fig. 1 it is seen that the dry mass and nucleic acid values in the same cell nuclei are approximately proportional: a least squares analysis gives the high correlation coefficient of $r = 0.879$ between the two. On the other hand, the relation between nuclear dry mass and DNA in the upper part of the figure is obviously not as good, and a least squares analysis gives a lower correlation of $r = 0.739$. Using Fisher's z-transformation (Fisher, 1936; Fisher and Yates, 1948) to test the significance of the difference between the correlations, we obtain $t = 2.98$, which exceeds the value to be attained at the $P = 0.01$ level ($t = 2.60$); the difference is therefore significant.

We conclude, therefore, from the dissimilarity of these correlations, that in ascites tumor cells preparing for a further division the net syntheses of nuclear protein and nuclear nucleic acid (RNA + DNA) are closely associated during interphase, whereas the syntheses of nuclear protein and DNA contents occur independently to an appreciable extent.

The conclusion is similar to that reported previously for HeLa strain cells in culture (Seed, 1962, 1963), where, in addition, it was shown by time-lapse photography that the synthesis curves for nuclear RNA and protein were similar and continuous in pattern, and differed significantly from that for DNA which was discontinuous in time.

Irradiation Experiments Blocking DNA Synthesis

In Fig. 2 the Feulgen-stain distribution for the control culture shows a well defined group at the 2m-DNA (posttelophase) level, with a small accumulation near the 4m-DNA (preprophase) level, together with a number of cells in synthesis. No polyploid cells were found in the control culture.

In the irradiated culture fixed 1223 min after irradiation, there is a large accumulation of DNA values at the 4m (preprophase) level, with relatively few cells in synthesis. This result is again consistent with previous work of other authors (Kelly et al., 1957; Caspersson et al., 1958; Dickson et al., 1958; Painter and Robertson, 1959; Yamada and Puck, 1961; Seed, 1961), who showed that cells already in synthesis went on to completion of the cycle, but that the radiation-induced mitotic block gave a consequent accumulation of cells at the 4m-DNA level. However, in the present case there are a number of cells that have escaped the DNA block: without dividing, they have gone on to synthesize DNA in the next cycle, i.e., from the 4m-DNA to the 8m-DNA level. Now, out of a total of 103 cells in the control

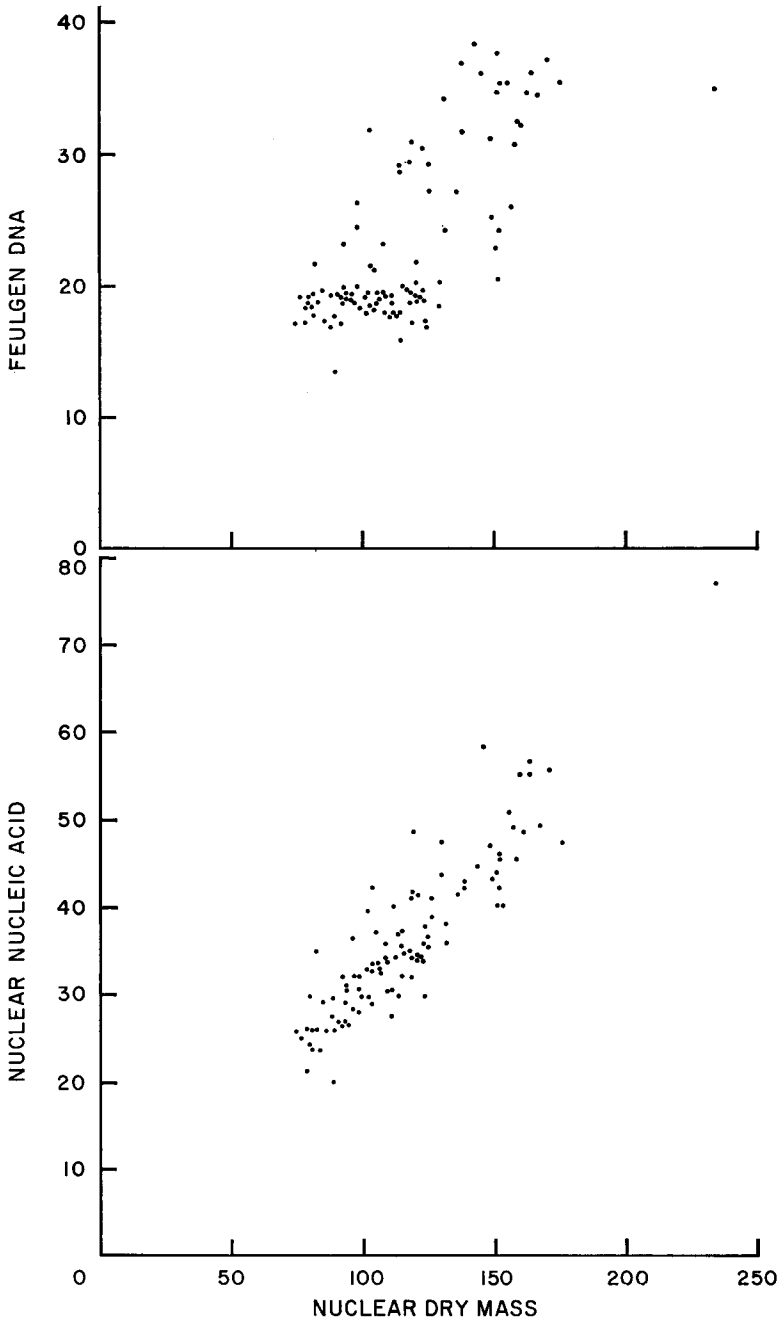

FIGURE 1 Feulgen stain (DNA) and nuclear ultraviolet absorption (at 2536 A, nuclear RNA + DNA) measurements plotted against the corresponding nuclear dry mass values for replicating ascites tumor cells. All quantities are expressed in arbitrary units.

culture, there are 19 cells at around the 4m-DNA value: in Fig. 2 these cells are shaded vertically in the Feulgen-stain and dry mass distributions for the control culture. In the irradiated culture, since mitosis has been abolished, there should be out of a total of 122 cells a proportion $19/103 \times 122 \simeq 22$ cells that have been blocked at the 4m-DNA level since the time of irradiation: these cor-

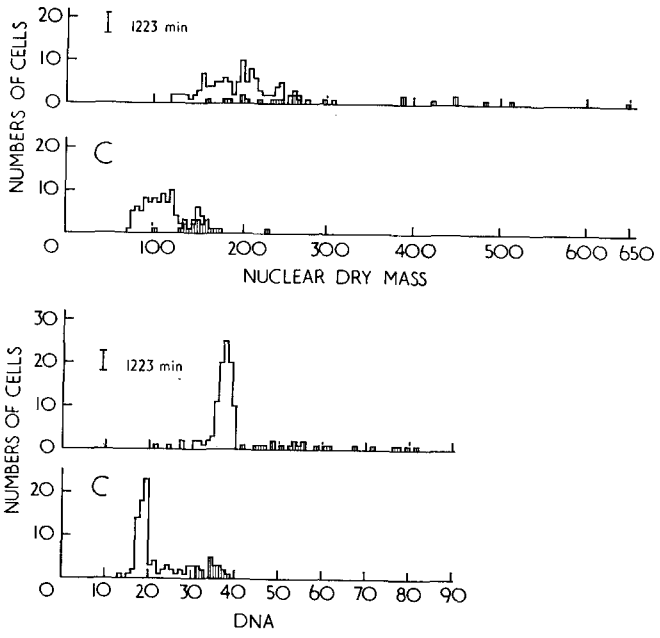

FIGURE 2 Distributions of Feulgen stain (DNA) and nuclear dry mass measurements on individual ascites tumor cells, intermitotic time 1280 min: C control cells (from Fig. 1); I cells fixed 1223 min after X-irradiation with 1250 R. No polyploid cells were found in the control culture, but measurements are shown for those found in the irradiated culture. ||| cells with 4m to 8m DNA values.

respond to the 19 cells at the 4m-DNA level in the control culture. However, we see from the DNA distribution for the irradiated culture that 22 cells have in fact escaped the block and are synthesizing on the 4m-DNA to 8m-DNA cycle: in Fig. 2 these cells are shaded vertically in the Feulgen-stain and dry mass distributions.

Because of the large-scale failure in the block of DNA synthesis, with consequent polyploidy, it is not possible to make a quantitative estimate of the fraction of nuclear protein synthesis dissociated from DNA synthesis in this particular experiment. However, in spite of the leak in the block, it is seen in Fig. 2 that there are still an additional 12 cells at the 4m-DNA level which have increased in nuclear dry mass by about ⅔ in the absence of DNA synthesis: this is a considerable increase, similar to that observed in HeLa strain cells and reported in the following paper (Seed, 1966 *c*).

DISCUSSION

The evidence here presented from measurements on the replicating control cells shows that, during the interphase of ascites tumor cells proceeding to a further division, the syntheses of nuclear protein and RNA are closely associated; whereas they are disconnected, to a significant extent, from the replication of DNA. As far as the nuclear protein and DNA syntheses are concerned, the result confirms the conclusions of Richards and Davies (1958) on ascites tumor cells in vivo: it is also consistent with the results reported with HeLa strain cells (Seed, 1961, 1962, 1963).

As already stated, the failure to maintain a block in DNA synthesis for an interphase time renders impracticable an analysis of the present radiation results along the lines of those of the preceding papers. However, it was of some interest that, when this series of experiments (Seed, 1961) was in its early stages, the work of Killander et al., (1962 *a*, *b*) appeared. These workers used in vivo a strain of ascites tumor cells different from that used here, and in their system it proved possible to produce a block in DNA synthesis for 48 hr.

In their experiments, Killander et al., (1962 *a*, *b*) showed that at times up to 48 hr after irradiation, DNA synthesis was blocked at the 4m-DNA (premitotic) level whilst the accumulation of nuclear protein and RNA continued. Because of the lack of a quantitative analysis, in their paper, along the lines of that published here, one cannot make an immediate comparison with the present work. However, considering their first experiment (1962 *a*) on ascites cells measured 24 and 48 hr after irradiation, there is, in each case, a number of cells corresponding to the 4m-DNA group in the controls ($\simeq$ 26/61 of the total population) that has increased in mean nuclear dry mass by $\simeq$ 0.35

and $\simeq$ 1.5 times, respectively, the original dry mass value of cells at the 4m-DNA level in the control culture, without accompanying DNA synthesis. In their second experiment (Killander et al., 1962 *b*), there are only $\simeq$ 8/44 cells at the 4m-DNA level in the control culture, which is consistent with a dry mass increase of $\simeq$ 0.7 at 48 hr.

Now, from the earlier work of Klein and Revesz (1953) with the same tumor cells, it appears that, with an initial inoculum of 4×10^6, the cells in the experiments of Killander et al. performed 6 to 8 days after innoculation would have an intermitotic time of 38 to 50 hr: this indicates an increase of $\simeq$ 0.6 to 1.0 in nuclear protein in the absence of DNA synthesis. Although it seems, from the variation in these two experiments of Killander et al. (1962 *a*, *b*), that the cells in vivo are not growing as consistently as those in culture, their results appear compatible with the earlier work of Richards and Davies (1958) on replicating ascites tumor cells in vivo, which demonstrated the independence of a major part of nuclear protein synthesis from DNA replication.

The results for ascites tumor cells are discussed along with others (Seed, 1966 *a*, *b*) in the following paper (1966 *c*).

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REFERENCES

CASPERSSON, T., KLEIN, E., and RINGERTZ, N. R., 1958, *Cancer Research*, **18,** 857.

DICKSON, M., PAUL, J., and DAVIDSON, J. N., 1958, *Biochem. J.*, **70,** 18P.

EAGLE, H., 1959, *Science*, **130,** 433.

FISHER, R. A., 1936, Statistical Methods for Research Workers, London, Oliver & Boyd, Ltd, 1936.

FISHER, R. A., and YATES, F., 1948, Statistical Tables, London, Oliver & Boyd Ltd., 1948.

KELLY, L. S., HIRSCH, J. D., BEACH, G., and PETRAKIS, N. L., 1957, *Proc. Soc. Exp. Biol. and Med.*, **94,** 83.

KILLANDER, D., RIBBING, C., RINGERTZ, N. R., and RICHARDS, B. M., 1962 *a*, Exp. Cell Research, **27,** 63.

KILLANDER, D., RICHARDS, B. M., and RINGERTZ, N. R., 1962 *b*, *Exp. Cell Research*, **27,** 321.

KLEIN, G., and FORSSBERG, A., 1954, *Exp. Cell Research*, **6,** 211.

KLEIN, G., and REVESZ, L., 1953, *J. Nat. Cancer Inst.*, **14,** 229.

PAINTER, R. B., and ROBERTSON, J. S., 1959, *Radiation Research*, **11,** 206.

RICHARDS, B. M., and DAVIES, H. G., 1958, *in* General Cytochemical Methods. I. (J. F. Danielli, editor), New York, Academic Press Inc., 1958, 130.

SEED, J., 1961, *Nature*, **192,** 944.

SEED, J., 1962, *Proc. Roy. Soc., London, Series B*, **156,** 41.

SEED, J., 1963, *Nature*, **198,** 147.

SEED, J., 1964, *J. Cell Biol.*, **20,** 17.

SEED, J., 1966 *a*, *J. Cell Biol.*, **28,** 233.

SEED, J., 1966 *b*, *J. Cell Biol.*, **28,** 249.

SEED, J., 1966 *c*, *J. Cell Biol.*, **28,** 263.

YAMADA, M., and PUCK, T. T., 1961, *Proc. Nat. Acad. Sc.*, **47,** 1181.