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

IV. HeLa Tumor Strain Cells

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ABSTRACT

Interferometric and photometric measurements have been made on HeLa cells, a strain of cells originally derived from a human carcinoma. From a study of the relations between successive physical measurements on individual cells, it was confirmed 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 on nuclear metabolism agree with others previously reported in cell strains derived from tumors; they contrast with results from freshly prepared normal cells, where the net syntheses of DNA, nuclear RNA, and protein are closely associated during interphase. Cytoplasmic measurements on HeLa cells showed that much of the net synthesis of cytoplasmic RNA is associated with DNA replication as in normal cells, and they failed to detect transfer from the nucleus of a stable RNA component synthesized independently from DNA replication. In auxiliary experiments, an inhibition of the onset of DNA synthesis was produced by a dose of X-rays; under these conditions it was shown that the major part of the accumulation of nuclear protein was independent of DNA replication and that the accumulation of nuclear RNA was equivalent to or slightly less than that of nuclear protein. About half the accumulation of cytoplasmic RNA was inhibited when DNA synthesis was blocked.

INTRODUCTION

The investigation by physical methods of the relations between DNA, RNA, and protein syntheses during the interphase of replicating animal cells has been described in earlier papers (Seed, 1961, 1962, 1963, 1964, 1966 *a*). The present experiments with HeLa cell cultures had two aims:

1. To investigate the relationship between DNA synthesis and the accumulation of cytoplasmic RNA during the interphase of replicating HeLa cells by making plots of successive physical measurements on individual cells: at the same time to confirm the relations previously published between DNA, RNA, and protein syntheses in the nucleus (Seed, 1963).
2. To prevent the initiation of DNA synthesis by administering a dose of X-irradiation, and then to measure the amounts by which nuclear protein, nuclear RNA, and cytoplasmic RNA had increased after one interphase time. This provided an auxiliary method for the investigation of the relations between the syntheses in replicating cell cultures.

EXPERIMENTAL PROCEDURE

HeLa strain cells were grown in babies' feeding bottles in Eagle's medium (Eagle, 1959) supplemented with

0.25% lactalbumin hydrolysate and 20% human serum. When required for an experiment, the cells were subcultured onto quartz cover slips affixed to small glass slides (Seed, 1962).

The design of the experiments has been described previously (Seed, 1966 *a*); it had been shown in preliminary experiments, where time-lapse photography was used, that a single radiation dose of 1250 R was sufficient to abolish mitosis in HeLa cells for approximately one interphase time and that there was no cell death during this period. At the start of the present experiment, six slide cultures were irradiated with an X-ray dose of 1250 R (3½ min each): the irradiated cells were then reincubated and were later fixed at intervals up to 24 hr after irradiation.

Two unirradiated cultures were fixed at the beginning and two at the end of the experiment (24 hr) for subsequent measurement as controls. During the course of the experiment, an additional control culture was filmed (on a microscope contained in an incubator) by low-power time-lapse cinephotography in order to find the intermitotic time (1250 min) of the cells.

Measurements are given below from one control fixed at the end of the experiment ($\simeq$ 1400 min) and four irradiated cultures fixed at 1107, 1226, 1348 and 1449 min, respectively, after irradiation. Additional control measurements (not shown) were made to confirm that the nuclear dry mass per cell remained constant in the control cultures during the experiment.

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

Physical Measurements

Physical methods of measurement on single cells were used as previously described (Seed, 1966 *a*). Before the measurements on the fixed cultures, low molecular weight compounds were first extracted by immersion in 1% PCA (perchloric acid) at +4°C for 30 min. Four types of measurement were made: (*a*) total UV absorption at 2536 A (total cell nucleic acid); (*b*) nuclear UV absorption at 2536 A (nuclear nucleic acid, consisting of $\simeq$ ⅗ RNA and the remainder DNA (Seed, 1963; Lin and Chargaff, 1964); (*c*) nuclear dry mass ($\simeq$ ⅚ nuclear protein); and (*d*) Feulgen stain (DNA). Values for cytoplasmic RNA of individual cells were obtained by subtraction of the nuclear nucleic acid from the total cell nucleic acid measurements. As before, the excess nucleolar absorption was not included in the nuclear UV and dry mass determinations.

The measurements in Figs. 1 to 6 are expressed in arbitrary units of nucleic acid and separately of dry mass: 1 nucleic acid unit $\simeq 5.7 \times 10^{-13}$ g, and 1 dry mass unit $\simeq 1.4 \times 10^{-12}$ g.

RESULTS

Control (Unirradiated) Cells

NUCLEAR METABOLISM: The interphase growth of nuclear dry mass in HeLa cells is followed in Fig. 1 by plotting the dry mass measurements for individual cell nuclei against the corresponding DNA and nuclear nucleic acid values for the same cells.

It is clear from Fig. 1 that there is an approximate proportionality between the nuclear nucleic acid and nuclear dry mass values within the same cell nuclei: on the other hand, the relation between DNA content and nuclear dry mass is obviously not as good. A least squares analysis of the nuclear measurements gives correlations of $r = 0.900$ between dry mass and nucleic acid, and $r = 0.815$ between dry mass and DNA. Applying Fisher's z-transformation (Fisher, 1936; Fisher and Yates, 1948) confirms a significant difference between the two correlations, giving $t = 2.65$, which exceeds the value to be attained at the $P = 0.01$ level ($t = 2.59$).

From these results we see that, during the interphase of HeLa cells, the growths of nuclear dry mass and nuclear nucleic acid (RNA + DNA) are closely associated, whereas the nuclear dry mass and DNA increase independently to an appreciable extent. These results confirm others already published (Seed, 1962, 1963) where, in addition, it was shown by a time-lapse photographic technique that the time synthesis curves for nuclear RNA and protein were similar and continuous in pattern, and differed significantly from the pattern of DNA synthesis which was not initiated until a delay had elapsed after telophase.

CYTOPLASMIC RNA: Of the 134 cells on which nuclear measurements had been made in Fig. 1, it was possible to make measurements of total cell UV absorption on 75 cells without cytoplasmic overlapping (Fig. 2). The accumulation of cytoplasmic RNA during interphase was followed as described before (Seed, 1966 *a*). Feulgen DNA and nuclear nucleic acid (RNA + DNA) values for individual cells were plotted separately against the corresponding total cell nucleic acid values and the correlations were compared: total nucleic acid was plotted in preference to cytoplasmic RNA because the latter is obtained by subtraction and its use would entail the combination of two errors in the results. (In HeLa cells DNA comprises $\simeq$¼ of total cell nucleic

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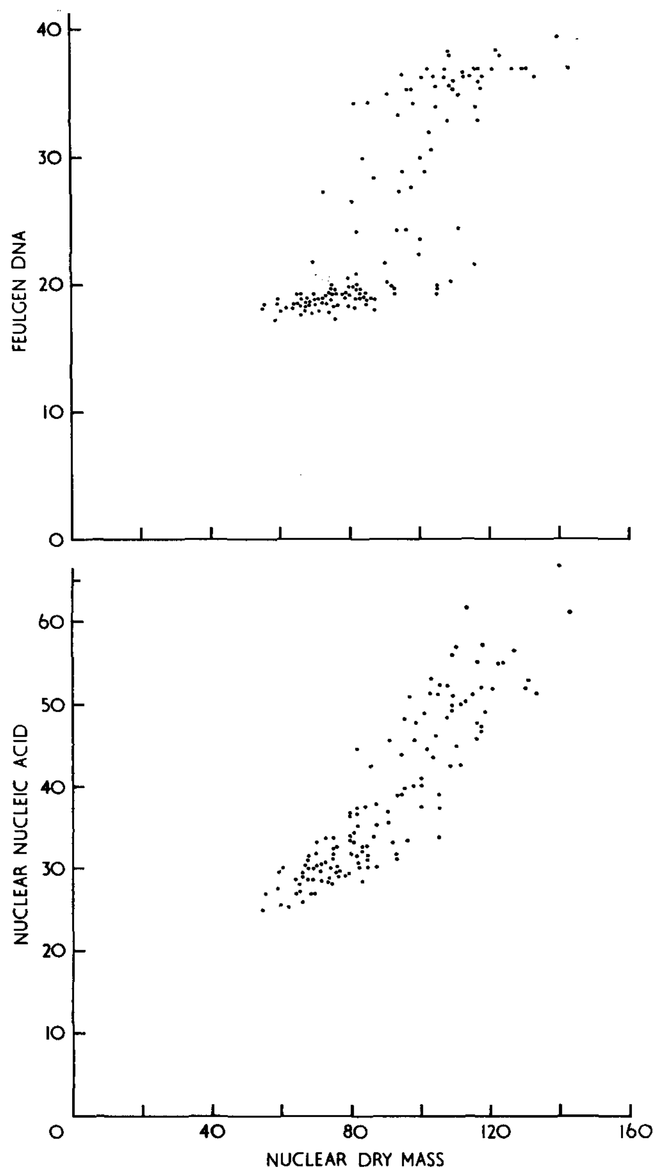

FIGURE 1 Feulgen stain (DNA) and nuclear ultraviolet absorption (at 2536 A, nuclear RNA + DNA) measurements plotted against the corresponding nuclear dry mass values for replicating Hela strain cells. All quantities are expressed in arbitrary units.

acid, and nuclear nucleic acid comprises $\simeq 2/5$ of total cell nucleic acid.)

As in other replicating cells, in HeLa cells the synthesis of cytoplasmic RNA occurs in the nucleus and is dependent on the *presence* of DNA. This conclusion was derived from experiments with labeled precursors (Feinendegen et al., 1960) and from the almost complete inhibition of cytoplasmic RNA accumulation produced by Actinomycin D (Shatkin, 1962; Tamaoki and Mueller, 1962), an antibiotic that binds with DNA, but much less readily with RNA (Rauen et al., 1960; Kawamata and Imanishi, 1961. See also Marshak, 1948; Jeener and Szafarz, 1950; Goldstein and Plaut, 1955; Goldstein and Micou, 1959; Woods and Taylor, 1959; Woods, 1960; Perry, 1960; Zalokar, 1960; Reich et al., 1963). In this section we are interested in whether the accumulation of cytoplasmic RNA is associated with the *replication* of DNA in a cell proceeding to division. Following the arguments used in previous papers (Seed 1966 *a*, *b*), in a cell synthesising RNA it is reason-

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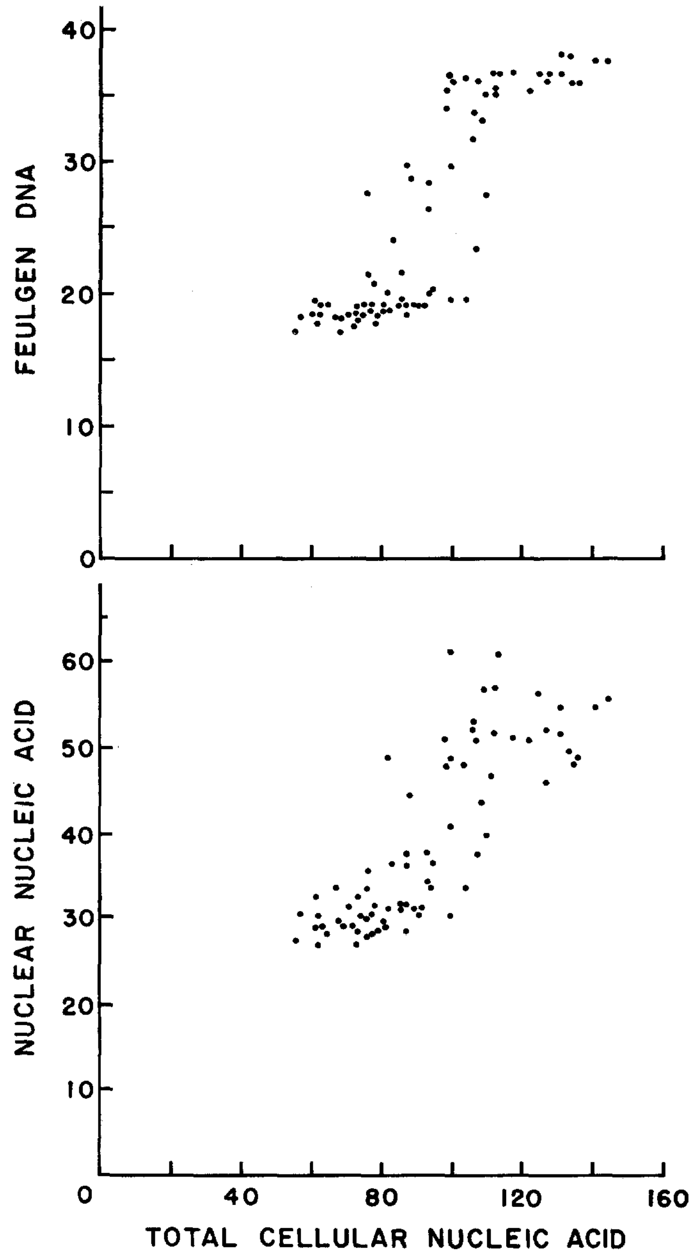

FIGURE 2 Feulgen stain (DNA) and nuclear ultraviolet absorption (at 2536 A, nuclear RNA + DNA) measurements plotted against the corresponding total cell ultraviolet absorption (at 2536 A, total cell nucleic acid) for replicating Hela strain cells. There are 75 cells on the plot, measurable out of the 134 cells in the population in Fig. 1.

able to expect a correlation between the amounts of nuclear RNA and total cell RNA, because the synthesis of cellular RNA occurs in the nucleus. On the other hand, one would not expect much correlation between DNA and total cell RNA if these two syntheses are largely independent: in this case, one would expect a higher correlation between nuclear nucleic acid and total cell nucleic acid than between DNA and total cell nucleic acid.

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Referring to the measurements in Fig. 2, a least squares analysis of the nucleic acid plots shows correlations of $r = 0.812$ between total cell nucleic acid and nuclear nucleic acid, and of $r = 0.858$ between total cell nucleic acid and DNA: applying Fisher's z-transformation (Fisher, 1936; Fisher and Yates, 1948), the difference between the correlations is found not to be significant, giving $t = 0.919$ only, whereas $t = 1.98$ must be attained at the $P = 0.05$ level, and $t = 1.66$ at the $P = 0.1$ level.

This similarity of the correlations is at first sight unexpected in view of the evidence, from the nuclear measurements in the preceding section, demonstrating that a large component of nuclear RNA synthesis was dissociated from the synthesis of DNA (see also Seed, 1963). It seems likely therefore that the part of nuclear RNA synthesis dissociated from DNA replication is not destined for the cytoplasm in stable form. On reflection, this conclusion is less surprising because the function of this RNA is associated with synthesis of the *nuclear* protein disconnected from DNA replication (see Fig. 1).

Now, previous radioautographic studies (Seed, 1963) of the uptake of tritiated cytidine during interphase had indicated the presence of two components of nuclear RNA synthesis in HeLa cells: one associated with and the other independent of DNA *replication*. From the correlations in Fig. 2, therefore, it appears that in the HeLa strain cell much of the accumulation of cytoplasmic RNA is derived from the nuclear RNA component associated with DNA synthesis. The difference in the cytoplasmic RNA/DNA ratios between the 4m-DNA and 2m-DNA levels (1.82 and 2.47, respectively, from Fig. 6) is consistent with a considerable time shift between the two (stepwise) accumulation curves of cytoplasmic RNA and of DNA. Thus after a short incubation period in tritiated cytidine, during which the nuclear RNA became labeled, Feinendegen et al., (1960) found that the labeled RNA in the cytoplasm increased continuously up to and after 13 hr of subsequent incubation in unlabeled cytidine. Moreover 7 to 8 hr elapsed before the cytoplasmic RNA label had reached 80% of the 13 hour value. A delay of this order would be comparable with the period of DNA synthesis ($\lesssim 10$ hr) in these cells (Seed, 1962, 1963). Because of this time lag the experiment cannot be as conclusive as the cytoplasmic results for other types of cells (Seed, 1966 *a*, *b*), where near equality of the cytoplasmic RNA/DNA ratios was observed at the 2m-DNA and 4m-DNA levels. In these cases, this ratio result afforded confirmation of the conclusions from the correlations.

By subtracting the nuclear nucleic acid values from total cell nucleic acid values for HeLa cells, it is found that the cytoplasmic RNA shows correlations of $r = 0.520$ and $r = 0.639$, respectively, with nuclear nucleic acid and DNA. There is no significant difference between the correlations ($t = 1.09$, whereas $t = 1.98$ at the $P = 0.05$ level). These correlations are lower than those for total cell nucleic acid, for the reasons outlined above: it is more informative to compare the total nucleic acid correlations.

It should be noted that the above measurements refer to RNA accumulation; they do not exclude transfer from nucleus to cytoplasm of an unstable RNA. The neglect of the excess nucleolar UV absorption ($\simeq 1/10$ of the total nuclear absorption) in the nuclear measurements is not expected to influence the results, because the uptake of cytidine into nucleolar RNA followed a time course, during interphase, similar to that of the remainder of the nucleus (Seed, 1963). The nucleolar syntheses will form the subject of a separate communication (Seed, in preparation).

Irradiation Experiments Blocking DNA Synthesis

In this section, by comparing control with irradiated cultures, we shall study the relations between the syntheses when the initiation of DNA replication is blocked by a dose of X-radiation. The relations between the nuclear syntheses in an irradiated culture (I_3) are plotted in Fig. 3. The distributions of values of DNA, nuclear dry mass, and nuclear nucleic acid for control and irradiated cultures are plotted in Figs. 4 and 5.

DNA SYNTHESIS: The Feulgen-DNA value distribution (Fig. 4) for the control culture shows well defined groups at the 2m-DNA (posttelophase) and 4m-DNA (preprophase) levels, together with a number of intermediate values corresponding to synthesizing cells. These 2m-DNA and 4m-DNA values belong to cells in the presynthetic and postsynthetic DNA periods: in HeLa cells DNA synthesis takes place during only a part of interphase, and it is most probable to find DNA values corresponding to one of the delay periods.

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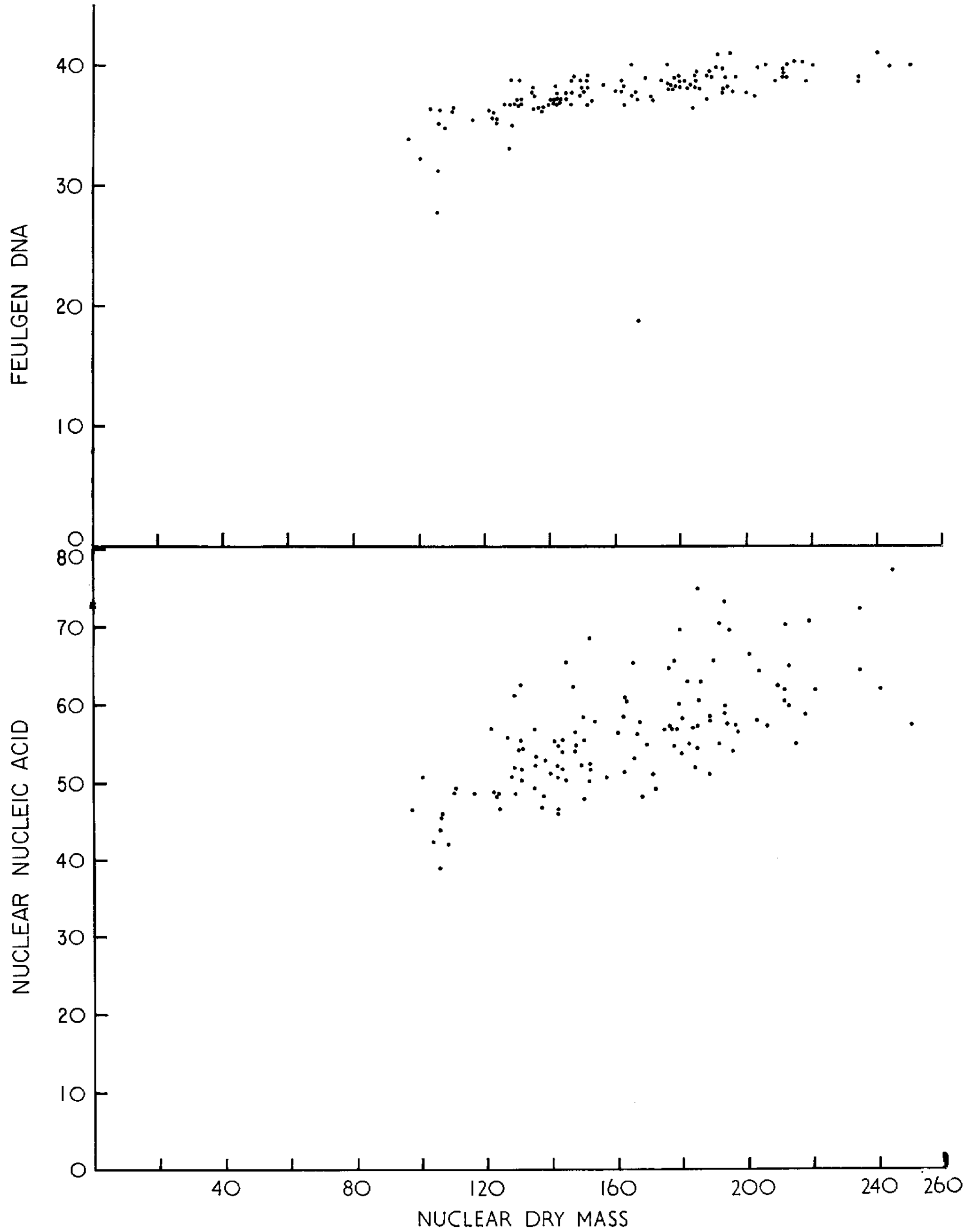

FIGURE 3 Feulgen stain (DNA) and nuclear ultraviolet absorption (at 2536 A, nuclear RNA + DNA) measurements plotted against the corresponding nuclear dry mass values for Hela strain cells fixed 1348 min after X-irradiation with 1250 R (I_3).

In all the irradiated cultures there is a large accumulation of Feulgen-DNA values at the 4m-DNA (preprophase) level, with relatively few cells elsewhere in synthesis. This block of DNA synthesis at the preprophase value is consequent on the abolition of mitosis (Painter and Robertson, 1959; Seed, 1961). In addition, there are no cells blocked at the 2m-DNA level, in agreement with

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the work of Painter and Robertson on HeLa strain cells.

NUCLEAR DRY MASS: It is clear from the distributions that in the irradiated cells there has been a general increase in nuclear dry mass. We are interested in the cells where the initiation of DNA synthesis has been blocked at the 4m-DNA level since the time of irradiation. Now, in the control culture (Fig. 4) there are 39 cells in the group at the 4m-DNA level out of a total number of 134 cells. Thus, in the irradiated culture I_1, out of a total of 167 cells there will be a number $39/134 \times 167 \simeq 49$ cells in which the initiation of DNA synthesis has been prevented at the 4m-DNA level since the time of irradiation (1107 min), mitosis having been abolished. Similar considerations apply to the $39/134 \times 147 \simeq 43$; $39/134 \times 126 \simeq 37$; $39/134 \times 126 \simeq 37$ cells out of totals of 147, 126, and 126 cells in the I_2 (1226 min), I_3 (1348 min), and I_4 (1449 min) cultures, respectively.

In order to identify these cells in the nuclear dry mass distributions of the irradiated cultures, the reasonable assumption has been made that they will have increased most in dry mass: thus, distributions of values containing 49, 43, 37, and 37 cells, respectively, have been drawn in the right ends of the dry mass distributions for the I_1, I_2, I_3, and I_4 irradiated cultures shown in Fig. 4. In drawing these distributions, the attempt has been made to include as many high values as possible, consistent with maintaining a general similarity in shape to the dry mass distribution for the 4m-DNA cells in the control culture. These

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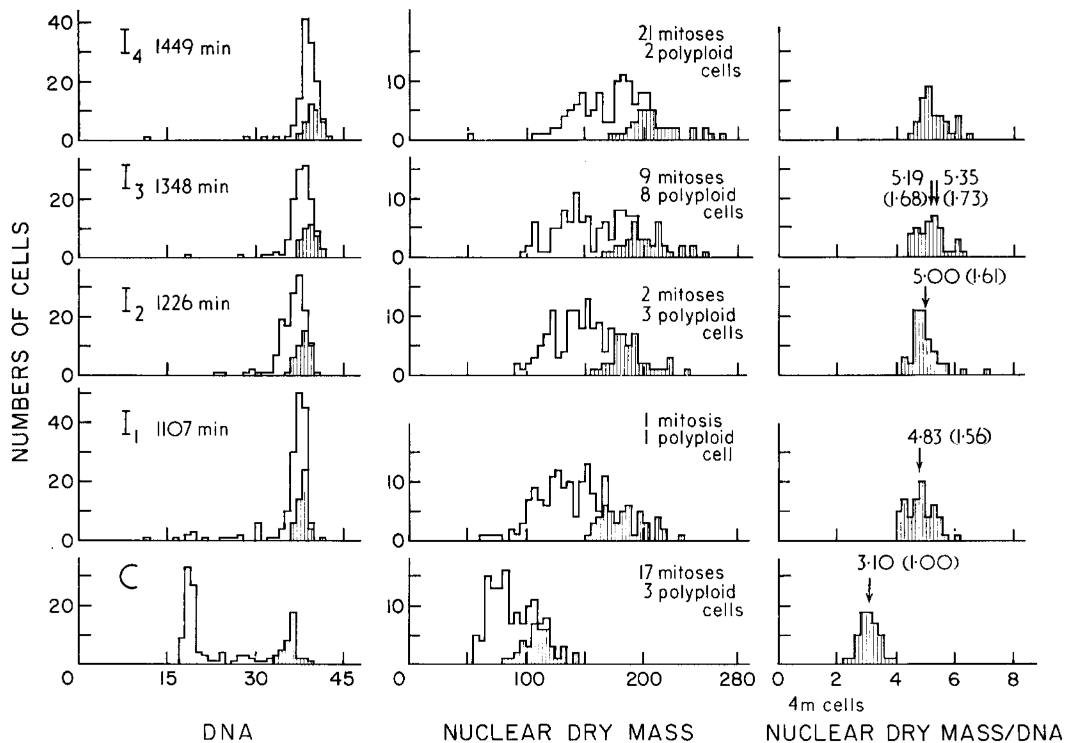

FIGURE 4 Distributions of Feulgen stain (DNA) and nuclear dry mass measurements on individual Hela strain cells, intermitotic time 1250 min. At the right of the figure are plotted the nuclear dry mass/DNA ratios for controls and for cells having DNA synthesis blocked since the time of irradiation: |||| (vertical shading), cells with 4m-DNA values. Lower section, C control cells (from Fig. 2); section above, I_1 cells fixed 1107 min after X-irradiation with 1250 R; upper sections, I_2 cells fixed 1226 min after irradiation; I_3 cells fixed 1348 min after irradiation; I_4 cells fixed 1449 min after irradiation. All quantities are expressed in arbitrary units. Cells in mitosis and polyploid cells are not included in the measurements, but the numbers of such cells found among those measured are given above the dry mass distribution for each culture.

cell distributions are identified by vertical shading in Fig. 4, along with the corresponding Feulgen-DNA values: the ratios of nuclear dry mass/DNA for these cells are also plotted at the right of the figure, together with the corresponding ratios for the 4m-DNA group in the control cells. The mean ratio values, in arbitrary units, and the amounts of increase (in parentheses) relative to the controls as 1.00, are indicated by arrows.

Now, as already mentioned, in preliminary experiments it was found by time-lapse photography that mitosis began again at around one interphase time after irradiation. No correction has been made for the dry mass increases from the 1 and 2 mitoses found among the I_1 and I_2 cells, respectively, as the correction involved is small. However, a correction is necessary for the 9 mitoses escaping the block in the I_3 irradiated culture fixed 1348 min after irradiation: if these 9 cells have come from the group of 37 blocked at the 4m-DNA level since the time of irradiation, then we must take a dry mass distribution of only 28 cells to determine the mean increase of dry mass (1.73): on the other hand, if the 9 cells have not come from this group, no correction is necessary (1.68). Because we do not know which is the case, there is an ambiguity in the result between 1.68 and 1.73, and in fact we take the mean of the two values shown in the figure.

No correction has been made for the presence of the 5 extra polyploid cells in I_3: their presence was not consistently observed in other cultures. It is clear that at 1449 min after irradiation (I_4) the block has largely broken down. No attempt has been made to derive a mean dry mass increase for this culture (I_4), because the inaccuracy in the result caused by the 21 mitoses escaping the block is too great. However, the dry mass/DNA ratios for a group of 37 cells are shown in the figure.

When the irradiated cultures are compared with the control cultures, it is seen that the cells under consideration have increased in nuclear dry mass by 0.56, 0.61, and 0.71, in 1107 (I_1), 1226 (I_2), and 1348 (I_3) min, after irradiation in the absence of DNA synthesis at the 4m-DNA level. With a constant rate of increase assumed, these values, weighted for the number of cells representing each, give a mean increase of 0.64 in nuclear dry mass in an interphase time of 1250 min: this corresponds to an increase of 0.72 in nuclear protein, allowing for the presence of nucleic acid in the nuclear dry mass.

NUCLEAR NUCLEIC ACID: From Fig. 3, where the measurements of nuclear nucleic acid and DNA are plotted against the corresponding dry mass values, there is a relation between the increases in nuclear dry mass and in nuclear nucleic acid in cells blocked at the 4m-DNA level, although the increase in nucleic acid is relatively small. In order to find the mean increase in nuclear nucleic acid, it is therefore valid to use the same distributions of 43 and 37 cells as were used in the

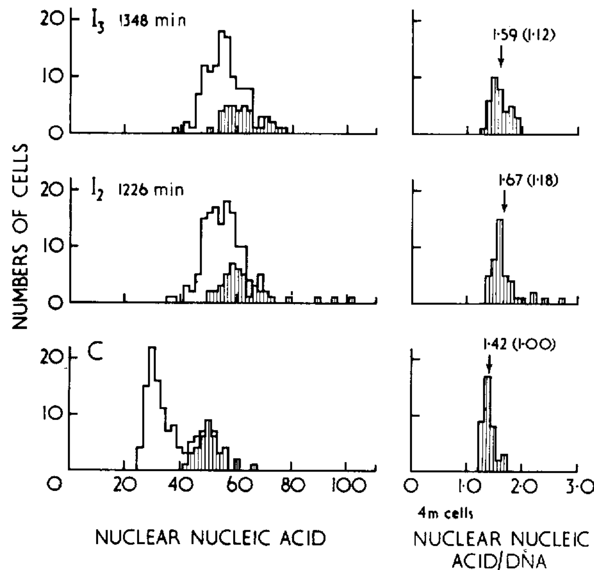

Figure 5 Distributions of nuclear ultraviolet absorption (at 2536 A, nuclear RNA + DNA) measurements on Hela strain cells. At the right of the figure are plotted the nuclear nucleic acid/DNA ratios for controls and for cells having DNA synthesis blocked since the time of irradiation: |||, cells with 4m-DNA values. Lower section, C control cells; center section, I_2 cells fixed 1226 min after X-irradiation with 1250 R; upper section, I_3 cells fixed 1348 min after irradiation.

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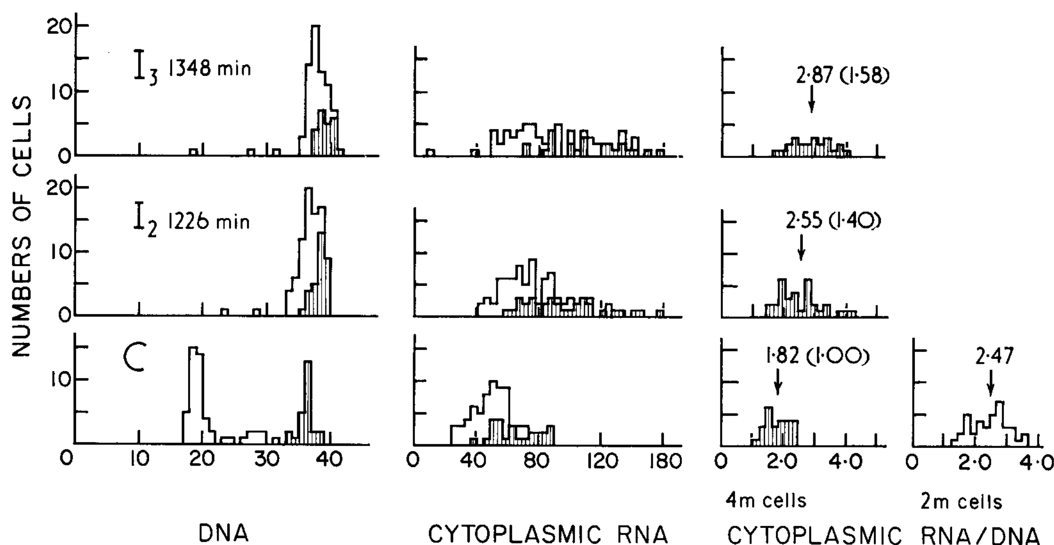

FIGURE 6 Distributions of Feulgen stain (DNA) and cytoplasmic ultraviolet absorption (cytoplasmic RNA) on individual Hela strain cells. At the right of the figure are plotted the cytoplasmic RNA/DNA ratios for controls and for cells having DNA synthesis blocked since the time of irradiation: ||||, cells with 4m-DNA values. Lower section, C control cells; center section, I_2 cells fixed 1226 min after X-irradiation with 1250 R; upper section, I_3 cells fixed 1348 min after irradiation.

dry mass determinations in the I_2 and I_3 cultures, respectively: in these cells, DNA synthesis has been blocked at around the 4m-DNA level since the time of irradiation. The distributions of their nuclear UV absorptions are shaded vertically in Fig. 5, as are also the nuclear nucleic acid/DNA ratios plotted in the figure for the same cells. Again, the mean ratios and amounts of increase (in parentheses), relative to the control as 1.00, are indicated by arrows. No correction for the 9 mitoses has been applied to the 1348 min culture because the correction is small compared with the error in measurement. From the figure, increases of 0.18 and 0.12, respectively, are observed in nuclear nucleic acid (nuclear UV absorption) 1226 min and 1348 min after irradiation in the absence of DNA synthesis at the 4m-level: this corresponds to a mean increase of 0.15 in one interphase time (1250 min).

If the nuclear RNA had increased in proportion to the nuclear protein in the irradiated cultures I_2 and I_3, one would expect the increases of 0.69 and 0.76 in nuclear protein to be accompanied by corresponding increases of $\simeq 0.69 \times 2/5 = 0.28$ and $\simeq 0.76 \times 2/5 = 0.30$ in nuclear nucleic acid (Fig. 5). The observed increase in I_2 (0.18) is not significantly different from that predicted, but the increase in I_3 (0.12) appears to be smaller ($\pm$ 0.07).

CYTOPLASMIC RNA: Out of the 134, 147, and 126 cells on which nuclear measurements had been made in the control and irradiated cultures (C, I_2, and I_3, respectively), it was possible to make total UV absorption measurements on 75, 86, and 72 cells not suffering from cytoplasmic overlapping. The distributions of cytoplasmic UV absorptions along with the corresponding DNA values for these cells are plotted in Fig. 5.

Now, the increase in cytoplasmic UV absorption in the irradiated cells at the 4m-DNA level is roughly correlated with increases in nuclear nucleic acid and in nuclear dry mass (Seed, unpublished). In order to find the mean increase in cytoplasmic nucleic acid it is therefore valid to use the same cells as were used in the distributions in Fig. 4 to find the mean dry mass increase. In these cells, the initiation of DNA synthesis has been blocked at the 4m-DNA level for roughly one interphase time, and of the 43 and 37 cells which were used in the nuclear dry mass distributions in I_2 and I_3, respectively, it was possible to make cytoplasmic measurements on 32 and 23 nonoverlapped cells. These cells are shaded vertically in Fig. 6, along with the corresponding

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DNA values and cytoplasmic RNA/DNA ratios. The corresponding quantities for the control cells are also shown, together with the mean ratios and amounts of increase (in parentheses) relative to the controls as 1.00.

It is seen that, since the time of irradiation (1226 and 1348 min), the cytoplasmic RNA has increased by 0.40 and 0.58, respectively, over the controls in the absence of DNA synthesis at the 4m-level, corresponding to a mean increase of 0.46 in one interphase time (1250 min).

DISCUSSION

At this stage it is useful to summarize the results from the present series of experiments. In the replicating control cultures freshly prepared from normal tissues, embryo human (Seed, 1966 *a*), monkey kidney (Seed, 1963), and embryo mouse (Seed, 1966 *b*), similar high correlations were found between DNA and nuclear dry mass and between nuclear nucleic acid and nuclear dry mass. From these results, taken in conjunction with others with time-lapse photography (Seed, 1962, 1963), it was concluded that in these replicating cells the net syntheses of DNA, nuclear protein, and nuclear RNA (in the chromatin) are closely associated during interphase. On the other hand, the correlation experiments for the replicating ascites tumor (Seed, 1966 *c*) and HeLa strain cultures (originally derived from a human carcinoma) showed in each case a high correlation between nuclear dry mass and nuclear nucleic acid but a significantly lower correlation between DNA and nuclear dry mass. From these results, taken in conjunction with others with time-lapse photography (Seed, 1962, 1963), it was concluded that whereas the syntheses of nuclear RNA and protein occur closely together during interphase, they are dissociated to a significant extent from the synthesis of DNA. Similar conclusions regarding DNA and nuclear protein syntheses in ascites tumor cells in vivo had been expressed previously by Richards and Davies (1958).

It should be noted that these differences in cell metabolism cannot be caused by protein secretion in the Hela tumor strain cells. Eagle et al. (1959) found that with cultured Hela cells the amino acid uptake corresponding to protein secretion was not more than $\frac{1}{20}$ to $\frac{1}{40}$ of the uptake corresponding to net synthesis of protein. Moreover, it is clear that in Hela cells the protein component dissociated from DNA replication is of considerable magnitude (being at least comparable with that associated with DNA synthesis; e.g. the time synthesis curves in Seed, 1962, 1963) and it would therefore readily have been detected by Eagle et al.

The problem of the tumor is, to a large extent, a problem of growth. Once it has been established that, in normal replicating cells, the majority of the net syntheses of nuclear protein and RNA are coupled with the replication of genetic material DNA (and in particular that they are initiated by the same event, Seed, 1962, 1966 *a*, *b*), then it is clear that the dissociation of much of the processes of nuclear protein and RNA syntheses from initiation of DNA replication in tumor strain cells is of extreme significance for cells that are commonly supposed to be growing out of control. I have previously suggested (Seed, 1961, 1962, 1963) that this dissociation in tumor strain cells reflects a fundamental loss of control in these cells: the probable nature of this loss and the consequences at the chromosomal level have been discussed elsewhere (Seed, 1963, 1965). Similar differences in metabolism were also found to exist in vivo (Seed, 1964).

The large spread or range in nuclear sizes in tumors is of course well known and was one of the features first to be noticed in microscope studies, after tumors had been recognised to be a disease of the cell. Since protein is the main constituent of the nuclear dry mass in the fixed cell, this scatter in nuclear sizes is hardly surprising if a large part of protein accumulation is dissociated from DNA replication (Seed, 1965).

Concerning the radiation experiments of the present series (Seed 1966 *a*, *b*, *c*): it should be reiterated that, because of the possible diversion of metabolites after the blocking of DNA synthesis, it is not possible to deduce, from the values found, the fractions of protein and RNA synthesis normally dissociated from DNA synthesis in an unirradiated cell. However, it is reasonable to suppose that these values provide a basis for comparison of the different cell types.

A radiation-induced block of the initiation of DNA synthesis showed nuclear protein fractions dissociated from DNA synthesis of 0.13 and 0.27 in embryo human cells and 0.13 and 0.40 in embryo mouse cells at the 2m-DNA and 4m-DNA levels, respectively. Considering the 2m-DNA and 4m-DNA levels separately, the differences in the protein fractions between the two types of freshly

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prepared normal cells are not significantly outside the errors ($< \pm 0.09$) involved (Seed 1966 *a*): however, the increases at the 4m level in both cell types are significantly less than that observed (0.72) in HeLa strain cells. Thus, taken as a whole, the entirely different approach afforded by blocking the *initiation* of DNA synthesis gives confirmation of the conclusions previously derived from a study of nuclear protein and DNA synthesis in replicating cultures (Seed, 1961, 1962, 1963). In addition, although in the current experiments it was not possible to obtain a quantitative result for the cultured ascites tumor cells because of the failure of the DNA block (Seed, 1966 *c*), the work of Killander et al. (1962 *a*, *b*) already quoted appears compatible with accumulation of the major part of nuclear protein when initiation of DNA synthesis is blocked in ascites tumor cells in vivo.

For the nuclear nucleic acid (omitting the nucleoli), the corresponding fractions dissociated from DNA synthesis were 0 and 0.02 in embryo human cells, and 0 and 0.06 in embryo mouse cells at the 2m-DNA and 4m-DNA levels, respectively. An increase in 0.15 (mean of two cultures) in nuclear nucleic acid was observed at the 4m-DNA level in HeLa cells. Although the nuclear nucleic acid increases for embryo human and embryo mouse cells appear smaller than those for HeLa cells, these differences are not significant with the present measurements: in each cell type the nuclear nucleic acid fractions correspond to RNA increases similar to those observed for protein within the same cells, with the possible exception of HeLa cells where the RNA increase may be slightly smaller (± 0.07) after irradiation.

Returning to the experiments with the replicating HeLa cells, it is of some interest that the synthesis of the nuclear protein proceeding independently of DNA replication is associated with a parallel synthesis of RNA, accumulation of which in stable form in the cytoplasm was not detected. The experiments of Feinendegen et al. (1961) and Harris et al. (1962, 1963) had previously indicated synthesis of a component of nuclear RNA which was not transferred to the cytoplasm and in fact was ultimately broken down in the HeLa cell nucleus. Roberts (1965) has recently reported considerable breakdown of nuclear RNA in ascites tumor cells. Whether or not this RNA turnover in HeLa cell nuclei is associated with the nuclear RNA and protein accumulation referred to above (Figs. 1 and 2) is at present a matter for conjecture. The current experiments give no information on this because they measure net amounts of substance only, although it is possible that the nuclear RNA accumulation is slightly less than that of the protein in HeLa cells when DNA synthesis is blocked for an interphase time (see above). Synthesis of protein associated with RNA turnover has been reported in more primitive organisms (Volkin and Astrachan, 1956; Volkin et al., 1958; Ycas and Vincent, 1960; Gros et al., 1961; Hayashi and Spiegelman, 1961; Hall and Spiegelman, 1961; Brenner et al., 1961).

It is clear that further experiments are necessary in order to characterize the base compositions of the RNA components associated with and dissociated from the synthesis of DNA. Although, in the past, various suggestions (Zubay, 1958; Leslie, 1961) have been made concerning the mode of transfer of information in DNA-primed RNA synthesis in animal cells, in order to explain the inverse correlation of the RNA base composition with the base composition of DNA, the possibility that this inversion arises because the RNA synthesis is associated with simultaneous DNA replication appears to have been neglected. Investigations in vitro of DNA-primed RNA synthesis *alone* have yielded RNA of a similar or complementary base composition (Chamberlin and Berg, 1962), in agreement with that found for part of the DNA-primed RNA synthesis in microorganisms (Volkin and Astrachan, 1956; Volkin et al., 1958; Ycas and Vincent, 1960; Hall and Spiegelman, 1961).

Referring to the cytoplasmic measurements in the present series of experiments, in the embryo human, monkey kidney, and embryo mouse cells (Seed 1966 *a*, *b*, *c*), the correlation plots of the nucleic acid components showed, in each case, similar high correlations between DNA and total nucleic acid and between nuclear nucleic acid and total nucleic acid: in addition, the ratios of cytoplasmic RNA/DNA were similar at the 2m-DNA and 4m-DNA levels. From these results it was concluded that the net syntheses of DNA and of cytoplasmic RNA were closely associated in the replicating cells freshly derived from normal tissues. In the HeLa strain cells also, similar correlations were observed between DNA and total nucleic acid and between nuclear nucleic acid and total nucleic acid, from which it was concluded that a large part of the net synthesis of cytoplasmic

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RNA is associated with DNA synthesis. Transfer from nucleus to cytoplasm of a stable RNA synthesized independently from DNA replication was not detected. Where measurements of cytoplasmic RNA were carried out on the irradiated cell cultures (in embryo human and HeLa strain cells), similar results were observed in each case: roughly half of the accumulation of cytoplasmic RNA was inhibited when the initiation of DNA synthesis was blocked.

Thus, in all the experiments, no differences between cell types were observed in the cytoplasm within the accuracy of the measurements. An increase in cytoplasmic RNA after irradiation is of course in agreement with the early work of Mitchell (1942) on the disturbance of nucleic acid metabolism by radiation: Mitchell reported an increase in UV absorption of the cytoplasm after irradiation in both proliferating cultured cells and tumors in vivo. Later experiments of Caspersson et al. (1958) also confirmed an increase in cellular UV absorption in irradiated ascites tumor cells.

There is little work by other authors with which the present results on nuclear metabolism (Seed 1966 *a*, *b*, *c*) may be compared. The success of the methods described lies in their ability to detect changes within a randomly growing population: it would be difficult to demonstrate similar results by normal bulk biochemical methods except by using a population of cells with synchronised mitosis (e.g. the method of Terasima and Tolmach, 1963). Reference has already been made to the work of McLeish (1959, 1960), who made photometric measurements of Feulgen stain (DNA) and of the Sakaguchi reaction (bound arginine) successively on nuclei of replicating plant cells: from his results he concluded that the two syntheses occurred closely together in interphase.

In the present series of experiments, it is apparent that the bulk of the syntheses of DNA, RNA, and protein already in progress are relatively insensitive to radiation, in contrast with the sensitivity of the process initiating synthesis. The present work gives no indication of the nature of this radiosensitive step. However, the existence of a block in DNA synthesis at the 2m-DNA level in embryo human, monkey kidney, and embryo mouse cells, and its absence in the HeLa and ascites tumor cells are in agreement with the present author's earlier suggestion regarding the different nature of the processes initiating DNA synthesis in early interphase, in normal and tumor strain cells (Seed, 1961, 1966 *a*, *b*, *c*, and in preparation).

The nature of these processes is currently under investigation.

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