Turnover of Nuclear and Cytoplasmic Ribonucleic Acid in Two Types of Animal Cell, with some Further Observations on the Nucleolus

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In a previous paper (Watts & Harris, 1959) some of the principles underlying the measurement of nucleic acid turnover in animal cells were discussed. It was shown that although in the macrophage a rapid turnover of ribonucleic acid occurs, as measured by the incorporation and release of [8-¹⁴C]adenine, the products of turnover are completely re-utilized by the cell in the resynthesis of ribonucleic acid and are thus continuously recycled. Under such conditions the turnover is not detected unless the cells are cultivated in a medium containing some unlabelled compound which has access to the turnover pathway and dilutes or displaces some labelled intermediate in the process of recycling. Of the compounds tested, adenosine was found to be the most effective in this respect.

Measurements of ribonucleic acid turnover in different parts of the cell have been undertaken. These measurements were made possible by the use of tritium-labelled precursors and quantitative radioautographic methods. Two cell types were studied: the connective-tissue cell derived from rat-heart tissue, and the macrophage obtained from the peritoneal cavity of the rabbit. The former multiplies *in vitro*; the latter does not multiply but remains alive for long periods.

In a previous paper (Harris, 1959) some observations were made on the function of the nucleoli, which are prominent structures in the connective-tissue cell. These observations are further elaborated in the present paper.

EXPERIMENTAL

Preparation of cultures

The connective-tissue cells were obtained from the hearts of 5-day-old rats and cultivated as dissociated cells in small coverslip chambers (Harris, 1955). The macrophages were obtained from the peritoneal cavity of the rabbit (Lucké, Strumia, Mudd, McCutcheon & Mudd, 1933) and were maintained as a single-cell layer in similar chambers. The culture medium consisted of 80% (v/v) of Eagle's solution (Eagle, Oyama, Levy, Horton & Fleischman, 1956) with 20% (v/v) of bovine serum for the connective-tissue cells and 20% (v/v) of rabbit serum for the macrophages. The tritium-labelled precursors, adenosine, cytidine and thymidine, were used at a concentration of 15 μ C/ml. of medium. These compounds were obtained from Schwarz Labora-

tories Inc., Mt. Vernon, N.Y., U.S.A., and had specific activities of 1.0c, 0.36c and 1.9c/m-mole respectively; they were all stably labelled in the purine or pyrimidine ring.

Method used for measuring ribonucleic acid turnover

In previous experiments (Watts & Harris, 1959) it was shown that in the macrophage there is no synthesis of deoxyribonucleic acid (DNA). It was also shown that exogenous [¹⁴C]adenine contributes no radioactive carbon to the proteins of the cell. The incorporation of adenosine into compounds insoluble in 0.3N-trichloroacetic acid at 0° can therefore be regarded as incorporation into ribonucleic acid (RNA) in this cell.

In the multiplying connective-tissue cell, however, synthesis of DNA occurs, and adenosine may be incorporated into both DNA and RNA. But DNA is synthesized only during a certain phase of the intermitotic cycle, and it is only in this phase that precursors are incorporated into DNA (Harris, 1959). Cells which are not in the phase of DNA synthesis incorporate adenosine only into RNA. Thus in order to measure turnover of RNA by the incorporation and release of labelled adenosine it is necessary to select cells which are not in the phase of DNA synthesis. This was achieved in the present experiments by adding tritium-labelled thymidine to the medium as well as tritium-labelled adenosine. Cells in the phase of DNA synthesis

EXPLANATION OF PLATE 1

- A. Radioautograph of two connective-tissue cells from the same culture. The culture was exposed to tritium-labelled adenosine and tritium-labelled thymidine for 10 min., washed with three changes of non-radioactive medium containing an excess of unlabelled adenosine and thymidine and then incubated for 3 hr. in non-radioactive medium containing 2 mM-adenosine and 2 mM-guanosine. The cells were fixed at the end of this period. The upper cell was in the phase of DNA synthesis at the time of exposure to the tritium-labelled precursors; the lower cell was not.
- B. Radioautograph of macrophages fixed after 30 min. exposure to tritium-labelled adenosine. The heavy labelling is over the nuclei; the lighter labelling is over the cytoplasm, which is not counterstained.
- C. Radioautograph of macrophages fixed after incubation for 30 min. in medium containing tritium-labelled adenosine and 8 hr. in non-radioactive medium containing 2 mM-adenosine and 2 mM-guanosine. The nuclei are much less heavily labelled and there are also fewer grains over the cytoplasm.

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incorporated both the adenosine and the thymidine into DNA and the adenosine into nuclear RNA. The nuclei of such cells were therefore much more heavily labelled than the nuclei of cells not in the phase of DNA synthesis, since the latter incorporated the adenosine only into RNA and did not incorporate the thymidine at all. The two groups of cells were easily distinguished, as shown in Plate 1A.

Pre-warmed medium containing tritium-labelled adenosine and, with connective-tissue cells, tritium-labelled thymidine, was introduced into ten chambers in which the cells were being cultivated. The cells were incubated at 37° in the radioactive medium for periods of 5–30 min. The radioactive medium was then withdrawn and the cells adherent to the floor of the chamber were washed with three changes of medium containing an excess of unlabelled adenosine and thymidine. Incubation was then continued for periods up to 12 hr. in non-radioactive medium containing 2 mM-adenosine and 2 mM-guanosine. The guanosine was added because some of the radioactivity from exogenous [¹⁴C]adenine had been found to appear in RNA guanine residues (Watts & Harris, 1959). Similar experiments were carried out with tritium-labelled cytidine as the precursor; in these 2 mM-cytidine and 2 mM-uridine were added to the non-radioactive medium.

At the end of the period of incubation in the radioactive medium and at regular intervals afterwards the coverslip with the adherent cells was removed from one of the chambers. The cells were washed in 0.85% NaCl soln. and then fixed. The connective-tissue cells, which become extremely flat when grown on a glass surface, were fixed by immersion in methanol for 15 min. The macrophages were flattened before fixation. This was done by pressing the cells between the coverslip and a small square of agar and then allowing the methanol to diffuse across the interspace containing the cells (Slider & Downey, 1950).

After fixation the cells were extracted with 0.3N-trichloroacetic acid at 0°, washed under running water and then dried. The coverslips, with the cell-covered surface facing upwards, were mounted on slides. Radioautographs

were prepared by the stripping-film technique with Kodak AR. 10 film (Pelc, 1956). The films were developed after 4–8 days and the cells stained through the emulsion with nuclear-fast red.

Measurement of the amount of radioactive precursor in various parts of the cell

The amount of radioactive precursor incorporated into any part of the cell is reflected by the number of silver grains visible in the emulsion overlying that part of the cell. The high resolution of radioautographs produced by tritium-labelled compounds is well known. With cells cultivated on glass and fixed in the manner described it was possible to resolve not only nuclear and cytoplasmic radiation, but also radiation emanating from the nucleoli. Owing to the extreme thinness of the fixed dried cells the results were not complicated by variation in the internal absorption of the tritium radiation. Below a certain grain density the relation between the number of visible grains in the emulsion overlying a given part of the cell and the amount of tritium-labelled precursor incorporated in that part of the cell was found to be linear. Fig. 1 illustrates the relation between the number of visible grains over the

Fig. 1. Relation between the number of visible grains in the emulsion overlying the nucleus and the nucleoli of connective-tissue cells and the time for which the cells were exposed to a tritium-labelled precursor. Cells were incubated in medium containing tritium-labelled adenosine and tritium-labelled thymidine for the periods shown and then fixed. Cells not in the phase of DNA synthesis were selected for grain counts. The lower line shows the mean number of grains per cell over the nucleoli; the upper line shows the mean number of grains per cell over the rest of the nucleus.

EXPLANATION OF PLATE 2

- A. Radioautograph of a connective-tissue cell fixed after incubation for 20 min. in medium containing tritium-labelled adenosine. The cell was not in the phase of DNA synthesis. The nucleus is more heavily labelled than the cytoplasm and the heaviest labelling is over the nucleolus.
- B. Radioautograph of a connective-tissue cell fixed after incubation for 20 min. in medium containing tritium-labelled adenosine and 3 hr. in non-radioactive medium containing 2 mM-adenosine and 2 mM-guanosine. The cell was not in the phase of DNA synthesis at the time of exposure to the tritium-labelled precursor. The nucleus is less heavily labelled than the nucleus in A, but the cytoplasm is much more heavily labelled. The nucleolus is still the most heavily labelled part of the cell.
- C. Radioautograph of a connective-tissue cell fixed after incubation for 20 min. in medium containing tritium-labelled adenosine and 12 hr. in non-radioactive medium containing 2 mM-adenosine and 2 mM-guanosine. The nucleus is now much less heavily labelled than in A and B, but the cytoplasm shows about the same degree of labelling as in B.

nucleus and the nucleoli of connective-tissue cells and the time for which the cells were incubated in the presence of tritium-labelled adenosine. The incorporation of the precursor into RNA had previously been shown to be virtually linear over periods of this duration. The length of exposure of the radioautographs was regulated to give grain densities within this linear range.

In each preparation forty macrophages or forty connective-tissue cells not in the phase of DNA synthesis were chosen at random for grain counts. This was found to be a large enough sample: the standard error of the mean number of grains over the nucleus, nucleolus or cytoplasm was always less than 10% of the mean.

RESULTS

Macrophages

Since it has been shown that the amount of RNA per cell remains constant (Watts & Harris, 1959), the release of labelled adenosine from the RNA is a measure of RNA turnover. The turnover of nuclear and cytoplasmic RNA in the presence of exogenous unlabelled adenosine and guanosine is shown in Fig. 2. The observed half-life of the loss of radioactivity from the nucleus is less than 2 hr.; the observed half-life of the loss from the cytoplasm is between 5 and 7 hr. Plate 1B shows a radioautograph of cells fixed after incubation for

30 min. in the radioactive medium. Plate 1C shows a radioautograph of cells from the same experiment which were incubated for 30 min. in the radioactive medium and then for 8 hr. in the non-radioactive medium before fixation. It will be seen that there has been a much greater fall in the number of grains over the nucleus than in the number over the cytoplasm. For purposes of illustration these radioautographs were exposed for a longer period than was optimum for grain counting.

The curves shown in Fig. 2 appear to set very low limits to the amount of nuclear RNA which could have passed into the cytoplasm, unless the nuclear RNA were rapidly destroyed on entering the cytoplasm. If as little as 10% of the labelled RNA in the nucleus had passed unchanged into the cytoplasm in an hour, it should have been detected by the techniques used.

Connective-tissue cells

Fig. 3 shows the turnover of the nuclear and cytoplasmic RNA of the connective-tissue cell in the presence of exogenous unlabelled adenosine and guanosine. In this cell the number of grains over the cytoplasm continued to rise for about 2 hr. after the introduction of the non-radioactive medium into the chambers. The number of grains over the nucleus did not fall during this period, or fell only slightly; in some experiments there was actually a rise during the first hour. In this respect the connective-tissue cell differed from the macrophage, in which the number of grains over both nucleus and cytoplasm began to fall as soon as the non-radioactive medium was introduced into the chambers. This difference appears to indicate that in the connective-tissue cell there is a pool of acid-soluble RNA precursors which does not rapidly come into equilibrium with exogenous adenosine; if there is such a pool in the macrophage it is presumably very much smaller.

After the second hour in non-radioactive medium the number of grains over the nucleus fell rapidly, the half-life of the process being less than 2 hr. But no turnover of cytoplasmic RNA was observed over the period studied. Although a very slow turnover of cytoplasmic RNA may not have been detected within 10 hr., a turnover of the magnitude observed in the cytoplasm of the macrophage would have been easily measurable. Plate 2A shows the radioautograph of a connective-tissue cell fixed after incubation for 20 min. in the radioactive medium. Plate 2B shows a cell which was fixed after incubation for 20 min. in the radioactive medium and then 3 hr. in the non-radioactive medium. Plate 2C shows a cell which was fixed after incubation for 20 min. in the radioactive medium and then 12 hr. in the non-radioactive medium. For purposes of illustration these radio-

Fig. 2. Turnover of nuclear and cytoplasmic RNA in the macrophage. ○, Nuclear RNA; ●, cytoplasmic RNA. Cells were exposed to tritium-labelled adenosine for 30 min., washed with three changes of non-radioactive medium containing an excess of unlabelled adenosine and then incubated for 8 hr. in non-radioactive medium containing 2 mM-adenosine and 2 mM-guanosine. The incubation in non-radioactive medium was begun at zero time.

autographs were also exposed for a longer period than was optimum for grain counting.

The curves in Fig. 3 again appear to indicate that most of the nuclear RNA does not pass intact into the cytoplasm. The large rise in the number of grains over the cytoplasm during the first 2 hr. is due to incorporation of labelled material from acid-soluble pools. This rise cannot be due to movement of RNA from nucleus to cytoplasm because during this period the number of grains over the nucleus remains more or less constant; and even if all the labelled RNA in the nucleus had been transferred to the cytoplasm in the first 2 hr. it would still not have accounted for the increase in the number of grains over the cytoplasm. Owing to the complications introduced by the continued labelling of RNA from acid-soluble pools it is not possible to measure the movement of labelled RNA from nucleus to

cytoplasm during this initial period. In the following 10 hr., however, there was an average loss of about 35 grains/cell from over the nucleus, but no detectable increase in the number of grains over the cytoplasm. Although the magnitude of the counting errors might have masked an increase of about 10 grains/cell over the cytoplasm, an increase of much more than this would have been detected. It therefore appears that in the connective-tissue cell also at least two-thirds of the nuclear RNA does not pass into the cytoplasm in a stable form.

Nucleolar ribonucleic acid

Experiments on the turnover of nucleolar RNA could only be carried out on the connective-tissue cell, since the macrophage has no visible nucleoli. When the connective-tissue cells were fixed after a period of incubation in medium containing tritium-labelled adenosine radioautographs always showed the heaviest labelling over the nucleoli. This is illustrated in Plate 2A and B. From radioautographic results of this sort obtained with other cells it has been concluded that the turnover of nucleolar RNA is more rapid than the turnover of RNA elsewhere in the cell (Ficq, 1953, 1955; Vincent, 1955). But the number of grains over any

Fig. 3. Turnover of nuclear and cytoplasmic RNA in the connective-tissue cell. ○, Nuclear RNA; ●, cytoplasmic RNA. Cells were exposed to tritium-labelled adenosine and tritium-labelled thymidine for 20 min., washed with three changes of non-radioactive medium containing an excess of unlabelled adenosine and thymidine and then incubated for 12 hr. in non-radioactive medium containing 2 mM-adenosine and 2 mM-guanosine. The incubation in non-radioactive medium was begun at zero time. Cells not in the phase of DNA synthesis were selected for grain counts.

Fig. 4. Rate of turnover of nucleolar RNA in the connective-tissue cell compared with the rate of turnover of RNA in the rest of the nucleus. Cells were exposed to tritium-labelled adenosine and tritium-labelled thymidine for 20 min., washed with three changes of non-radioactive medium containing an excess of unlabelled adenosine and thymidine and then incubated for 8 hr. in non-radioactive medium containing 2 mM-adenosine and 2 mM-guanosine. The incubation in non-radioactive medium was begun at zero time. Cells not in the phase of DNA synthesis were selected for grain counts. ●, Ratio of the mean number of grains over the nucleoli to the mean number of grains over the nucleus; ○, expected change in this ratio if the rate of turnover of nucleolar RNA exceeded the rate of turnover of RNA in the rest of the nucleus by 20%.

part of a cell is a reflexion of the total amount of labelled precursor incorporated at that site. Heavier labelling over the nucleoli may indicate either that the nucleolar RNA is incorporating the precursor more rapidly or merely that there is a greater concentration of RNA at the nucleolus. It is possible to distinguish between these possibilities by measuring the rate at which the labelled precursor is released from the nucleolar RNA.

Cells were incubated for 20 min. in the presence of tritium-labelled adenosine and thymidine, washed with three changes of non-radioactive medium containing an excess of unlabelled adenosine and thymidine and then incubated for 8 hr. in non-radioactive medium containing 2 mM-adenosine and 2 mM-guanosine. At the end of the period of incubation in radioactive medium and at hourly intervals afterwards the cells from one of the chambers were fixed and radioautographs were prepared in the usual way. Cells not in the phase of DNA synthesis were selected and grain counts made over the nucleoli and over the rest of the nucleus. The results are shown in Fig. 4. Over 8 hr. there appeared to be no significant change in the ratio of the number of grains over the nucleoli to the number over the rest of the nucleus. If the turnover of nucleolar RNA had been either faster or slower than the turnover in the rest of the nucleus, a change in this ratio would have been observed. The open circles in Fig. 4 show the expected change in this ratio if the nucleolar RNA had turned over 20% more quickly than the RNA in the rest of the nucleus. This slope was calculated from the observation that the fall in the number of grains over both nucleus and nucleoli was, to a first approximation, an exponential process and that this process in the nucleus had a half-life of 2 hr. or less. It appears therefore that the rate of turnover of nucleolar RNA does not differ greatly, if at all, from the rate of turnover of RNA in the rest of the nucleus. The heavy labelling over nucleoli observed in radioautographs is thus to be explained by a greater concentration of RNA at these sites.

Turnover of cytidine

Similar experiments were carried out with tritium-labelled cytidine as the precursor. The rates of turnover of nuclear, nucleolar and cytoplasmic RNA as measured by the release of labelled cytidine were essentially similar to those obtained with labelled adenosine. In one respect, however, the results obtained with cytidine differed from those obtained with adenosine. In radioautographs of connective-tissue cells which had been incubated in the presence of tritium-labelled adenosine the ratio of the number of grains over the nucleoli to the number over the rest of the nucleus was about 1:3. In radioautographs of cells which had been incu-

bated in the presence of tritium-labelled cytidine, this ratio was about 1:1.4. Since the rates of turnover of adenosine and cytidine in both nuclear and nucleolar RNA were essentially the same, this difference appears to indicate that the ratio of adenine to cytosine in the nucleolar RNA is substantially lower than the ratio of adenine to cytosine in the RNA of the rest of the nucleus.

Further observations on the role of the nucleolus in the initiation of deoxyribonucleic acid synthesis

In a previous paper (Harris, 1959) radioautographic evidence was produced in support of the view that the synthesis of DNA was initiated at the nucleolus. When connective-tissue cells in the phase of DNA synthesis were exposed for a short period to tritium-labelled thymidine their nuclei showed three patterns of labelling: (i) labelling over the nucleoli only; (ii) labelling over the nucleoli together with labelling over the rest of the nucleus; (iii) labelling over the rest of the nucleus without labelling over the nucleoli (Plate 1, Harris, 1959). It was found that the phases showing nucleolar labelling preceded the phase showing no nucleolar labelling. During mitosis, however, when the nucleoli are no longer present as discrete structures, all the labelled thymidine which has been incorporated is found to be associated with the chromosomes. It may therefore be concluded that when a cell shows nucleolar labelling after exposure to tritium-labelled thymidine the thymidine has actually been incorporated into chromatin which is intimately associated with the nucleolus. The experiments now to be described were designed to determine whether the chromatin associated with the nucleoli is a specific and constant part of the chromosomal material, as appears to be the case in certain plants (McClintock, 1934), or whether the association between the nucleoli and the chromatin is random.

Cultures of connective-tissue cells were incubated for 15 min. in medium containing tritium-labelled thymidine. The cells were then washed with three changes of non-radioactive medium containing an excess of unlabelled thymidine and incubated for a further 10 hr. in non-radioactive medium containing no added nucleic acid precursors. The cultures were then fixed and radioautographs made in the usual way. Many of the cells which were in the phase of DNA synthesis at the time of exposure to the labelled thymidine had completed mitosis during the 10 hr. period (Harris, 1959). For some time after mitosis it is possible to recognize the two daughter cells by their relatively small size, their proximity or contiguity and their similar morphology. The preparations were scanned for recognizable pairs of daughter cells and the pattern of nuclear and nucleolar labelling in these cells was observed.

Of fifty pairs of daughter cells which were examined, forty-two showed a similar pattern of labelling in both members of the pair, that is, both members showed either nucleolar labelling only, or nucleolar labelling together with labelling over the rest of the nucleus or labelling over the rest of the nucleus without labelling over the nucleoli. In eight pairs of daughter cells the pattern of labelling was not clear enough to establish similarity or dissimilarity, but not one pair was found in which one member clearly showed a different pattern of labelling from the other. These results appear to rule out the possibility that the association between the nucleoli and the chromatin is a random one. But they do not necessarily show that the pattern of labelling in the two daughter cells was similar to the pattern of labelling in the cell from which they were derived. This question was resolved in the following way.

It has previously been shown that when growing connective-tissue cells are dissociated by treatment with trypsin and then introduced into chambers most of them are not in the phase of DNA synthesis immediately after treatment with trypsin but enter this phase during the next 24 hr. (Harris, 1959). As increasing numbers of cells pass into the

phase of DNA synthesis the percentage of labelled cells showing nucleolar labelling after a short exposure to tritium-labelled thymidine rises. Five pairs of partially synchronized cultures were prepared by treatment with trypsin in this way and, 12 hr. after introduction of the dissociated cells into the chambers, one pair of cultures was exposed for 15 min. to medium containing tritium-labelled thymidine. One culture from this pair was fixed at the end of the 15 min. period. The other was washed with three changes of non-radioactive medium containing an excess of unlabelled thymidine, incubated for 24 hr. in non-radioactive medium containing no nucleic acid precursors and then fixed. At 15, 18, 21 and 24 hr. after introduction of the cells into the chambers one pair of cultures was treated in the same way. Almost all the cells which were in the phase of DNA synthesis at the time of exposure to the labelled thymidine would have completed mitosis within 24 hr. under these conditions (Harris, 1959), so that the labelled cells in the cultures fixed after incubation for 24 hr. in the non-radioactive medium corresponded to the daughter cells of the labelled cells in the cultures fixed immediately after incubation in the radioactive medium. The percentage of labelled cells showing nucleolar labelling in the two groups is shown in Fig. 5. It will be seen that the rise in this percentage in the group of cultures fixed immediately after incubation in the radioactive medium is mirrored in the group fixed 24 hr. later. This indicates that the pattern of labelling in the parent cells is reproduced in the daughter cells. This finding, together with the observation that the two members of a pair of daughter cells have the same pattern of labelling, appears to establish the fact that the DNA associated with the nucleoli is a specific and constant part of the chromosomal material. It is apparently in this part of the chromosomal material that the replication of DNA begins.

Fig. 5. Pattern of nuclear and nucleolar labelling in partially synchronized cultures of connective-tissue cells exposed for short periods to tritium-labelled thymidine. The curve on the left illustrates the rise in the percentage of labelled cells showing nucleolar labelling which takes place as increasing numbers of cells pass into the phase of DNA synthesis. These cultures were fixed immediately after exposure to the labelled thymidine. The curve on the right shows a corresponding rise in cultures exposed to the labelled thymidine at the same time as the first group, but fixed after further incubation for 24 hr. in non-radioactive medium.

DISCUSSION

The results of the present investigation are difficult to reconcile with some current views about the functions of nuclear and nucleolar RNA. Several investigators have shown in animal experiments that certain precursors are incorporated more rapidly into nuclear than into cytoplasmic RNA (for review of literature see Smellie, 1955). But in animal experiments it is seldom possible to measure to what extent turnover is the result of concomitant cell multiplication and cell death, and the observed rate of turnover is also greatly influenced by the fact that the products of turnover are re-utilized. Interpretation of the data under such conditions is difficult.

Barnum, Huseby & Vermund (1953) and Smellie, McIndoe, Logan, Davidson & Dawson (1953) obtained results in animal experiments which were difficult to reconcile with the view that nuclear RNA was a precursor of cytoplasmic RNA, but the possibility could not be directly excluded. The curves obtained in the present experiments appear to be incompatible with the view that nuclear RNA can be a precursor of cytoplasmic RNA. It appears that in the intact cell only a small fraction of the nuclear RNA can pass into the cytoplasm in a stable form. If nuclear RNA is thought to function as a macromolecular vehicle carrying 'information' from the genes to the cytoplasm, the present findings indicate that during the greater part of the life of the cell most of the nuclear RNA is not engaged in this function. The significance of the rapid turnover of nuclear RNA remains obscure; it does not, however, appear to be directly related to cell multiplication since it occurs in both multiplying and non-multiplying cells.

It is tempting to suppose that the difference between the connective-tissue cell and the macrophage in the observed turnover rate of cytoplasmic RNA represents an essential difference between a multiplying and a non-multiplying cell. But the apparent absence of cytoplasmic turnover in the connective-tissue cell must be regarded with caution. The fact that this cell contains a large pool of nucleic acid precursors may mean that labelled intermediates of cytoplasmic RNA turnover are less effectively diluted by unlabelled nucleosides in this cell than in the macrophage, where such a pool, if it exists at all, is much smaller. And even if the difference in the observed rates of cytoplasmic turnover does reflect a difference in the true rates of turnover, this difference may have little connexion with the fact that one of the cells multiplies and the other does not.

The present experiments show that in the connective-tissue cell nucleolar RNA has much the same turnover rate as the RNA in the rest of the nucleus. Any theories of nucleolar function which involve the notion of a more rapid turnover of RNA at the nucleolus than elsewhere can therefore hardly apply to this cell. While there is some indication that the base composition of the nucleolar RNA is different from the base composition of the RNA in the rest of the nucleus, this difference does not necessarily imply a difference in function. It is possible that different parts of the RNA in the rest of the nucleus also have different base compositions. In assessing the significance of the higher concentration of RNA at the nucleolus, it should be remembered that there is also a higher concentration of DNA intimately associated with it, and that the structure itself consists of an extremely high concentration of protein (Dounce, 1955). The

function of the nucleoli remains obscure; but it is difficult to suppose that this function is unrelated to the observation that it is in the chromatin associated with the nucleoli that the reduplication of DNA is initiated.

SUMMARY

1. Quantitative radioautographic techniques were used to measure the turnover of ribonucleic acid in the nucleus and cytoplasm of two animal cells: the connective-tissue cell, which multiplies *in vitro*, and the macrophage, which does not multiply but remains alive for many hours.

2. Tritium-labelled adenosine and cytidine were used as the precursors and turnover was measured by the rate of release of these precursors from the ribonucleic acid.

3. In both cells there was a rapid turnover of nuclear ribonucleic acid. The observed half-life of the nuclear ribonucleic acid involved in turnover was less than 2 hr. In the macrophage there was also a turnover of cytoplasmic ribonucleic acid; the observed half-life of this ribonucleic acid was 5-7 hr. No turnover of cytoplasmic ribonucleic acid could be detected in the connective-tissue cell over the period studied.

4. The results in both cells appeared to be incompatible with the view that the nuclear ribonucleic acid was a precursor of the cytoplasmic ribonucleic acid.

5. In the macrophage the findings indicated that only a small proportion, if any, of the nuclear ribonucleic acid could have passed into the cytoplasm during the course of the experiments, unless the nuclear ribonucleic acid were rapidly destroyed on entering the cytoplasm. In the connective-tissue cell the magnitude of the counting errors might have permitted more of the nuclear ribonucleic acid to pass into the cytoplasm without being detected; but, even so, it could be shown that in this cell also most of the nuclear ribonucleic acid did not pass into the cytoplasm in a stable form.

6. The observed rate of turnover of nucleolar ribonucleic acid was not significantly different from the observed rate of turnover of the ribonucleic acid in the rest of the nucleus. There was, however, a higher concentration of ribonucleic acid in the region of the nucleoli and there was indirect evidence suggesting that the base composition of this ribonucleic acid was different from that of the ribonucleic acid in the rest of the nucleus.

7. The chromatin associated with the nucleoli represents a specific part of the chromosomal material which maintains its association with the nucleoli from one cell generation to the next. It is apparently in this chromatin that the reduplication of chromosomal deoxyribonucleic acid is initiated.

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Intermediate Reactions in Protein Synthesis by the Isolated Cytoplasmic-Membrane Fraction of *Bacillus megaterium*

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Studies of the uptake of labelled amino acids into the various cellular fractions of *Bacillus megaterium* have shown that the primary site of protein synthesis in this organism is located in the fraction which sediments when broken protoplasts are centrifuged at 10 000 g. The activity probably resides in the principal component of this fraction, which is the cytoplasmic membrane (Hunter, Crathorn & Butler, 1957; Butler, Crathorn & Hunter, 1958). This may hold true for bacteria in general, as similar findings have now been reported for *Bacillus subtilis* (Nomura, Hosoda & Nishimura, 1958) and *Escherichia coli* (Spiegelman, 1959).

It was further shown (Butler *et al.* 1958) that the isolated cytoplasmic-membrane fraction was capable of incorporating labelled amino acids into proteinaceous material at rates comparable with those of the intact protoplast. Two aspects of the role of the isolated membrane system are considered here: (1) the relationship between normal protein synthesis in the whole cell and the incorporation of labelled amino acids into the cell-free system; (2) the incorporation process itself, with a view to identifying intermediate reactions. As previous studies of protein synthesis in other systems (Hoagland, Keller & Zamecnik, 1956; Hoagland, Stephenson, Hecht & Zamecnik, 1958; Nismann, Bergmann & Berg, 1957; De Moss & Novelli, 1955)

have demonstrated the probable importance of amino acid-activating enzymes and of ribonucleic acid as factors controlling the course of amino acid incorporation into protein (so that peptides containing the amino acids can subsequently be obtained), particular emphasis has been laid on the investigation of their roles in this system.

A preliminary account of some of this work has already been given (Brookes, Crathorn & Hunter, 1959).

METHODS

Organism. The strain of *B. megaterium* (originally KM) was cultured as previously described (Butler *et al.* 1958). For all the experiments described here the organism was grown at 30° in a glucose-salts medium (C medium; McQuillen, 1955).

Preparation of subcellular fractions. It was found that conversion of whole cells into protoplasts was best effected in a medium containing 0.02% of lysozyme and 0.5M-KH₂PO₄, brought to pH 7.0 with NaOH. The lysozyme treatment was continued until a microscopic examination of a sample failed to reveal the presence of more than an occasional whole cell. The upper limit for whole-cell content was about 0.01%, although in many experiments not a single whole cell was observed in the protoplast preparations. Before carrying out any biochemical experiments, the protoplasts were usually aerated at 30° for 1 hr. in C medium containing, in addition, 0.5M-KH₂PO₄ and 1% of glucose, the whole brought to pH 7.0 with NaOH. Lysis of the protoplasts was effected by diluting this medium with