Ribonucleic Acid Synthesis and Transport in Animal Cells at 27°C

By MICHEL HORISBERGER* AND HAROLD AMOS

Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass. 02115, U.S.A.

(Received 17 November 1969)

The failure to induce glutamine synthetase in retinal cells with hydrocortisone at 27°C (37°C is the normal temperature for induction) led to a study of some aspects of RNA synthesis at 27°C by using the method of selective labelling with radioactive precursors, sucrose-density-gradient centrifugation and radioautography. The low-temperature treatment not only decreases the rate of RNA synthesis but also interferes with the general pattern of RNA maturation and distribution. It has been found that nuclear-cytoplasmic exchange is severely altered at low temperature and may be an explanation of the failure to induce the enzyme. When the temperature is raised from 27°C to 37°C, the incorporation of exogenous radioactive uridine into the RNA is distorted by a pool-dilution effect that results in an 'overshoot' phenomenon. The kinetics of labelled uridine equilibration into the UMP pool accounts for the difficulty encountered by many investigators in 'chase' efforts with unlabelled nucleosides.

In the hope of further dissociating transcription from translation in the induction of glutamine synthetase in chick retinal cells (Reif-Lehrer & Amos, 1968), low-temperature cultivation was explored (Horisberger & Amos, 1968). Little or no enzyme was synthesized at 27°C under conditions of induction with hydrocortisone that resulted in the rapid appearance of enzyme at 37°C. Indeed, preliminary results strongly suggested (Horisberger & Amos, 1968) that the mRNA specific for glutamine synthetase is not made or, if made, is rapidly destroyed at 27°C. No impairment of the cell's ability to translate preformed glutamine synthetase mRNA was detected in experiments in which glutamine synthetase mRNA accumulated at 37°C was translated at 27°C.

We have examined the species of RNA synthesized at 27°C and compared the pattern with that observed both at 37°C and in the period of transition occasioned by raising the incubation temperature from 27°C to 37°C.

Although all classes of RNA appear to be synthesized at the lower temperature, little processing of the 45S ribosomal precursor was detected. Of considerable interest is the observation that RNA transport from the nucleus to the cytoplasm is markedly restricted at 27°C.

MATERIALS AND METHODS

Whole retinas were removed into flask cultures under conditions specified by Reif-Lehrer & Amos (1968).

* Present address: Biological Department, CIBA Ltd., Basle, Switzerland.

RNA synthesis was studied by labelling cells with [2-¹⁴C]uridine or [5-³H]uridine. Retinal cells do not divide under the conditions of culture; they synthesize RNA at a substantial rate. It is difficult, however, to obtain high specific radioactivities for labelled RNA in relatively short pulses. RNA extraction and sucrose-density-gradient analysis were done by the method of Scherrer, Marcaud, Zajdela, Breckenridge & Gros (1966). The method consists essentially of cell lysis with sodium dodecyl sulphate followed by hot-phenol extraction.

Cell fractionation. Retinal cells were homogenized in Nirenberg buffer (Nirenberg & Matthaei, 1961) including 0.25M-sucrose and 6mM-mercaptoethanol. The homogenate was centrifuged at 15000g for 15 min. The pellet was treated twice in the same way: the buffer for the last homogenization contained a mixture of Tween 40 and sodium deoxycholate as indicated by Penman (1966). The supernatant fractions were combined, treated with sodium deoxycholate (final concn. 0.5%) and centrifuged for 2 h at 130000g. RNA was extracted from the pellet and from the supernatant to give the ribosomal fraction and the cell-sap RNA fraction respectively. The crude pellet containing the nuclei and cellular debris was further fractionated into nucleolar and nuclear-sap fractions by the method of Penman and co-workers (Penman, 1966; Penman, Vesco & Penman, 1968).

Nucleotide determination. Ten retinas were pooled for each determination. Cells were washed in cold 0.9% NaCl, then precipitated with 5% trichloroacetic acid; 300 µg of unlabelled uridine and 300 µg of unlabelled UMP were added to each sample as carrier and as marker to measure from the extinction the loss of uridine and UMP during the purification process. After precipitation overnight at 4°C the samples were centrifuged: the protein content of the pellet was determined (by the method of Lowry, Rosebrough, Farr & Randall, 1951). The supernatants were treated with ether to eliminate the trichloroacetic acid, then the ether was evaporated off. The samples

were treated with $M-HCl$ for 10 min at boiling temperature to give a mixture of monophosphates only. The hydrolysates were neutralized with aq. NH_3 (sp.gr. 0.88), then adsorbed on charcoal (5mg/ml). The charcoal was washed with cold water and eluted with ethanol-aq. NH_3 (sp.gr. 0.88)-water (5:3:12, by vol.). After evaporation of the eluent the samples were dissolved in mild acid and applied to Whatman no. 3 paper. Two-dimensional chromatography was carried out with: first solvent (descending), propan-2-ol- HCl , as described by Wyatt (1951); second solvent (ascending), butan-1-ol-aq. NH_3 (MacNutt, 1952). Spots were eluted, the extinctions measured and the radioactivities determined. The recovery was 10–20% for UMP and 30–60% for uridine.

Radioautography. Leighton tubes containing cover-slips supporting cell monolayers received $[5-^3H]$ uridine ($10\mu Ci/ml$) in Eagle's (1955) basal medium supplemented with calf serum (3%, v/v). Incorporation of the radioactive uridine was allowed to proceed for 3–6 h, at which time the cover-slip was removed, washed in 0.9% $NaCl$, fixed with ethanol and dried before being dipped in photographic emulsion (Messier & Leblond, 1957). The film was exposed to the cells in sealed black boxes at $4^\circ C$ for 7–10 days, at which time the radioautographs were developed. The cells were stained after developing by the Giemsa staining procedure.

Materials. Sucrose (ribonuclease-free) was from Mann Research Laboratories Inc., New York, N.Y., U.S.A. $[2-^{14}C]$ Uridine (specific radioactivity 53.5 mCi/mmol) and $[5-^3H]$ uridine (specific radioactivity 20 Ci/mmol) were from Schwarz BioResearch, Orangeburg, N.Y., U.S.A. $H_3^{32}PO_4$ (carrier-free) was from New England Nuclear Corp., Boston, Mass., U.S.A. The photographic emulsion used was Kodak NTB-2.

RESULTS

Failure of induction of glutamine synthetase at $27^\circ C$. Induction of glutamine synthetase in cultivated retinal cells from the 11-day chick embryo is dependent on hydrocortisone or an analogue of hydrocortisone (Moscona & Piddington, 1966; Reif-Lehrer & Amos, 1968). Specific mRNA for the enzyme accumulates when protein synthesis is prevented by cycloheximide under conditions otherwise favouring induction of the enzyme (Reif-Lehrer & Amos, 1968; Moscona, Moscona & Saenz, 1968). Evidence obtained indicates that the mRNA for glutamine synthetase formed in the absence of protein synthesis was less stable than that accumulated while protein synthesis continued at the normal rate.

In an effort to avoid the complications introduced by chemical inhibitors of protein synthesis, we investigated the effect of lowering the incubation temperature during induction. Should translation of glutamine synthetase mRNA be selectively inhibited at the low temperature, the phases of and requirements for translation of accumulated mRNA could be studied in detail at $37^\circ C$. We selected $27^\circ C$ as the highest temperature of several

tried (Horisberger & Amos, 1968) in which no significant induction was detected after 48 h incubation under conditions favourable to induction at $37^\circ C$.

Failure to induce the enzyme at $27^\circ C$ was ascribed to inability to form detectable amounts of glutamine synthetase mRNA (Horisberger & Amos, 1968). This conclusion was drawn from experiments in which glutamine synthetase mRNA accumulated at $37^\circ C$ was translated almost as well at $27^\circ C$ as at $37^\circ C$. [Interpretation of the comparative efficiency of translation at $37^\circ C$ and $27^\circ C$ is complicated by the results obtained when actinomycin D was introduced to prevent further accumulation of mRNA during translation. At $27^\circ C$ the 'paradoxical effect' (Garren, Howell, Tomkins & Crocco, 1964) of actinomycin was more pronounced than at $37^\circ C$.] However, no evidence of significant mRNA accumulation after any interval at $27^\circ C$ was produced by attempts to translate such mRNA at $27^\circ C$ or at $37^\circ C$. Moreover, results obtained on lowering the temperature from $37^\circ C$ to $27^\circ C$ indicated that induced cells stopped making glutamine synthetase mRNA immediately they were transferred to the lower temperature. We interpreted the failure to demonstrate accumulation of translatable glutamine synthetase mRNA at $27^\circ C$ to represent either a failure to transcribe or very rapid destruction of the newly synthesized mRNA. As shown below the interpretation of the result is

Fig. 1. RNA synthesis after the shift from $27^\circ C$ to $37^\circ C$. Cultures were preincubated for 24 h at $27^\circ C$, then the temperature was raised to $37^\circ C$ (indicated by the arrow). At the time-intervals indicated cultures were labelled for 2 h with $[2-^{14}C]$ uridine. Trichloroacetic acid-insoluble radioactivity is reported per mg of cell protein. O, Retinas; □, L cells (monolayer); ●, Chick-embryo fibroblasts (monolayer).

Fig. 2. UMP pool equilibration. The endogenous UMP pool was equilibrated with $^{32}\text{PO}_4^{3-}$ for 24 h at either 37°C or 27°C. $[2\text{-}^{14}\text{C}]\text{uridine}$ was then added (a) without change of medium at 27°C (●) and at 37°C (○) (□, 6 h after transfer to 37°C), and (b) at 30 min (early) (○), 6 h ('overshoot') (●) and 10 h (late) (□) after the temperature rise. Cells were harvested 1 and 2 h after the addition of $[2\text{-}^{14}\text{C}]\text{uridine}$.

complicated by the finding that little or no RNA having the properties attributed to mRNA is transported into the cytoplasm at 27°C. Indeed little RNA of any species except possibly cell-sap RNA moves out of the nucleus or nucleolus at 27°C.

RNA synthesis at 27°C and (after) transfer to 37°C. At 27°C the rate of RNA synthesis, determined by the incorporation of $[^{14}\text{C}]\text{uridine}$ into acid-insoluble nucleic acid, is about 25–30% of that at 37°C. As reported earlier (Horisberger & Amos, 1968), when retinal cells maintained for some time at 27°C are transferred to 37°C the apparent rate of RNA synthesis increases with time until it reaches a rate considerably higher than that normally observed at 37°C. In the earlier paper we referred to this phenomenon as an 'overshoot'. The same pattern of recovery was observed with chick-embryo fibroblasts and with strain L mouse fibroblasts (Fig. 1). The conditions of cultivation before the test varied with the cell type, as noted in the Fig. 1 legend. A constant feature of the 'overshoot' phenomenon is the timing. In all cells studied the peak occurs at about 6 h after the transfer to the higher temperature. In retinal cells the peak is sharper and the return to normal more rapid than with the other two.

Explanation of the 'overshoot' nucleotide pools. The 'overshoot' phenomenon could reflect either a UMP-pool dilution effect or a true increase in the rate of RNA synthesis by the cells after transfer to 37°C. Direct measurement of RNA accumulation was not useful since the rate of its synthesis is very low compared with the large reservoir of RNA

in the cells. It was therefore necessary to accept $[^{14}\text{C}]\text{uridine}$ incorporation into RNA as a measure of RNA synthesis. Estimation of the $[^{14}\text{C}]\text{uridine}$ nucleotide pools and of the kinetics of their equilibration was essential to an interpretation of the apparent uridine incorporation. Inasmuch as nucleotide recovery is erratic and the cell populations are relatively small, we decided to equilibrate ^{32}P in the endogenous nucleotides for 24 h at either 27°C or 37°C before determining $[^{14}\text{C}]\text{uridine}$ entry as the $^{14}\text{C}/^{32}\text{P}$ ratio in the recovered UMP.

In a representative experiment $[^{14}\text{C}]\text{uridine}$ ($0.03 \mu\text{Ci}/\text{ml}$) was added without change of medium to cells previously exposed to $^{32}\text{PO}_4^{3-}$ for 24 h. Then 1–2 h later retinas were harvested and the 5% trichloroacetic acid-soluble fraction was treated as described in the Materials and Methods section. The result of such an experiment is shown in Fig. 2. There the 'specific radioactivities' of $[^{14}\text{C}]\text{uridine}$ are compared for cells cultivated at 27°C, at 37°C and after transfer from 27°C to 37°C. There is essentially no difference between the $[^{14}\text{C}]\text{uridine}$ entry into the nucleotide pool at 27°C and 37°C. It should be noted that equilibration of the nucleotide pool with exogenous uridine is very slow and not completed in 2 h except for 'late' in the transition from 27°C to 37°C. Cells transferred to 37°C after 24 h at 27°C do increase the entry of $[^{14}\text{C}]\text{uridine}$ into the nucleotide pool sufficiently to account for the apparent increase in the rate of RNA synthesis that we called an 'overshoot' (Horisberger & Amos, 1968). In the experiment (Fig. 1) in which the rate of incorporation of $[^{14}\text{C}]\text{uridine}$ into the RNA of retinal cells is twice as high at the peak of the

Fig. 3. Uridine uptake. $[2\text{-}^{14}\text{C}]\text{Uridine}$ was added to each culture without change of medium at 27°C (●), at 37°C (○) and at 7 h after the temperature rises from 27°C to 37°C (□). Retinas were harvested after 5, 15 and 30 min. Trichloroacetic acid-soluble radioactivity in uridine is reported per mg of cell protein (see the Materials and Methods section).

'overshoot' as that normally observed at 37°C , the $[^{14}\text{C}]\text{uridine}/^{32}\text{P}$ ratio is also twice as high (Fig. 2). We have not attempted to account for the increased rate of entry after the transfer to a higher temperature, which could result either from increased uridine kinase activity or more effective negative feedback on UMP biosynthesis by the intact cell system. $[^{14}\text{C}]\text{Uridine}$ equilibration with the uridine pool is rapid in all cases and is established within 5 min (Fig. 3). The effect is clearly beyond the level of exogenous uridine.

If one compares the $[^{14}\text{C}]\text{uridine}$ entry into the nucleotide pool as a function of time after raising the temperature to 37°C , it appears that the increase is immediate (Fig. 2) and remains so during the peak of the 'overshoot' period. These results are fundamental to the interpretation of studies dealing with the quantitative comparison of the species of RNA made early and during the 'overshoot' period. It is shown below that different species of RNA are synthesized at various rates as a function of time after the shift from 27°C to 37°C .

The evidence that the rate of $[^{14}\text{C}]\text{uridine}$ incorporation into RNA (Fig. 1) is low from 0–4 h after the change of temperature from 27°C to 37°C reflects an even slower recovery of RNA synthesis in the light of the rapid nucleotide equilibration observed. Otherwise stated, the complete recovery of RNA synthesis requires about 6 h. The timing compares rather well with the recovery of protein synthesis reported earlier (Horisberger & Amos, 1968).

RNA synthesis at 27°C . Our interest is in the rate of synthesis of the various classes of RNA at 27°C

compared with so-called normal synthesis at 37°C . In other words we are concerned with distortion of the pattern of RNA synthesis at the lower temperature, given the decrease of some 70–75% in the overall rate of RNA synthesis. To effect the comparison $[^{14}\text{C}]\text{uridine}$ -labelled RNA from cells incubated at 37°C was used as reference RNA for $[^3\text{H}]\text{uridine}$ -labelled RNA from cells incubated at 27°C . The cells at both temperatures were labelled by exposure to radioactive uridine for 1 h.

The two uridine-labelled cell populations were mixed immediately after harvesting, then separated into cytoplasmic, nuclear and nucleolar fractions before phenol extraction of the RNA from each fraction (see the Materials and Methods section). Each phenol extract was applied to a sucrose density gradient and centrifuged with precautions taken to minimize the ribonuclease activity during centrifugation. By their sedimentation properties and the cell fraction from which they were derived, the RNA species were classified as cell-sap RNA, nucleolar RNA, nuclear-sap RNA and polyribosomal RNA. The profile of each of these is illustrated in Fig. 4. After 1 h of incorporation of radioactive uridine little or no labelled RNA was detected in the cell-sap fraction even at 37°C . A similar delay in the appearance of RNA was described in HeLa cells (Penman, 1966). The profiles at 27°C for such a pulse are quite similar to those at 37°C . Also at 27°C the rate of uridine incorporation into the nucleolar and the nuclear-sap fractions is about the same. No conclusion can be proposed about the relative rates of synthesis of the cell-sap and polyribosomal RNA fractions since these are defined by sucrose-density-gradient distribution of material extracted from the cytoplasm. From evidence presented below there is reason to believe that their migration into the cytoplasm is significantly decreased at 27°C . Thus their appearance in the cytoplasm may not reflect the rate of synthesis in the nucleus.

In conclusion, we can say that at 27°C overall RNA synthesis is 25–30% of the rate at 37°C . Nucleolar RNA, i.e. 45S RNA, is made at the diminished rate, proportional to its rate of synthesis at 37°C . As shown previously (Horisberger & Amos, 1968), labelled 28S and 18S RNA are not demonstrable even after 8 h incorporation at 27°C , which means that little of the 45S RNA is converted into 28S or 18S rRNA or that the latter are destroyed as rapidly as they are formed. Polyribosomal RNA may be made at a rate equivalent on a comparative basis to that in other species. If it is, it is not transported from the nucleus to the cytoplasm as effectively as at 37°C . The latter proposal is confirmed by radioautographic studies.

Transport of RNA from nucleus to cytoplasm at 27°C and 37°C . The finding from the cell-fractiona-

EXPLANATION OF PLATE I

Migration of labelled RNA. Chick-embryo fibroblasts grown on cover slips were labelled with [5-³H]-uridine (10 μ Ci/ml) and processed for radioautography as described in the Materials and Methods section. (a) 37°C for 3 h; (b) 37°C for 6 h; (c) 27°C for 3 h; (d) 27°C for 6 h. Cells were unstained.

Fig. 4. RNA synthesis at 27°C. Retinas cultivated for 24 h at 27°C were labelled for 1 h with [5-³H]uridine, then mixed before fractionation with retinas cultivated at 37°C and labelled for 1 h with [2-¹⁴C]uridine. RNA extraction and analysis were as indicated in the Materials and Methods section. (a) Polyribosomal RNA; (b) nucleolar RNA; (c) cell-sap RNA; (d) nuclear-sap RNA. (a) and (b), 37°C samples; (c) and (d), 27°C samples. About 25–30 fractions were separated by the drop-collection method. —, ³H radioactivity (27°C sample); ----, ¹⁴C radioactivity (37°C sample). The relation between the scales for ³H and ¹⁴C is the same in each graph. The top of the gradient is on the right.

tion studies that relatively little labelled rRNA or mRNA appeared to reach the cytoplasm led us to examine the distribution of [³H]uridine-labelled RNA in retinal cells attached to cover-slips. Cells were exposed to [³H]uridine for 3 or 6 h at 37°C or at 27°C. At the end of the exposure period the cover-slips were treated for radioautography as described in the Materials and Methods section. The evidence presented in Plate 1 shows that very little labelled RNA is found in the cytoplasm even after 6 h of synthesis in the presence of [³H]uridine at 27°C. It is also evident that the nucleoli are heavily labelled at 27°C, presumably with rRNA precursor or a degradation product.

RNA synthesis after the transfer from 27°C to 37°C. The effect of raising the temperature to 37°C on the synthesis of the various classes of RNA was examined early (at 1–2 h) and at the peak (6–8 h) of the 'overshoot'. Comparison in each case was made by labelling cells at 27°C with [³H]uridine, whereas those at 37°C received [¹⁴C]uridine for the same period. From the results presented (Fig. 5) it appears that synthesis of polyribosomal RNA, cell-sap RNA and rRNA recovers very soon. Either the synthesis of rRNA precursor or the rate of processing of rRNA is higher at the peak of the 'overshoot' than a control kept at 37°C (Fig. 5).

At the peak both 28S and 18S RNA appear in the

cytoplasm after a 1 h labelling period. Normally at 37°C no 28S and very little 18S RNA are detectable under these conditions.

The rapid recovery of polyribosomal RNA synthesis fits well with the kinetics of enzyme induction under the same conditions (Horisberger & Amos, 1968), i.e. enzyme induction proceeds without delay when the temperature is raised to 37°C. It should be noted that the values as obtained for uridine label cannot be compared directly at 27°C and during the 'overshoot' because of pool differences. We decided not to make an arbitrary normalization and we were unable to obtain comparisons of pools in each experiment.

DISCUSSION

The principal interest in this series of experiments derives from the failure of corticosteroids to induce glutamine synthetase at 27°C under conditions favourable to induction at 37°C. On closer examination the failure was assigned to specific polyribosomal RNA synthesis, since translation of preformed polyribosomal RNA was shown to be effective at the lower temperature (Horisberger & Amos, 1968).

We decided to examine RNA synthesis at both temperatures as well as when the temperature was

Fig. 5. RNA synthesis during the transfer from 27°C to 37°C. Retinas were preincubated at 27°C for 24 h, then the temperature was raised to 37°C. Retinas were labelled for 1 h with [5-³H]uridine at 27°C (a and d), and at 1–2 h (b and e) or 6–7 h (c and f; ‘overshoot’) after the transfer. (a)–(c) Polyribosomal RNA; (d)–(f) cell-sap RNA. Before fractionation the samples were mixed with retinas cultivated at 37°C and labelled for 1 h with [2-¹⁴C]uridine. Fractions were separated by the drop-collection method. E_{260} values (not shown in the graph) and radioactivity were measured. —, ³H radioactivity; ----, ¹⁴C radioactivity. The top of the gradient is on the right.

raised from 27°C to 37°C. Temperature elevation produced a pattern of RNA synthesis (measured by incorporation of labelled uridine) that suggested a release of RNA synthesis and a restoration of control some 6–8 h later (Horisberger & Amos, 1968). From estimation of uridine nucleotide pools it is clear that the apparent ‘overshoot’ is an artifact of pool differences. What accounts for the relatively more efficient use of exogenous uridine after the transfer from 27°C to 37°C we have not tried to examine. Many factors could play a role, including kinase activity, feedback inhibition on endogenous synthesis of UMP, the rate of turnover of RNA etc. Scholtissek (1967) has shown that a feedback mechanism active at the level of UTP at 37°C is not active at lower temperatures in chick fibroblasts *in vitro*. The inability to effect ‘chase-pulse’ experiments with nucleosides in vertebrate cells has been a common problem (Darnell, 1968). Labelled precursor continues to be incorporated despite the addition of a large excess of unlabelled nucleoside. The slow equilibration of exogenous uridine with the UMP pool observed at both 27°C and 37°C in these experiments could account for the persistence of labelling commonly encountered.

Except for some of the nuclear-sap RNA, under normal circumstances most of the RNA synthesized in the nucleus eventually appears in the cytoplasm (Darnell, 1968). The processing of RNA even in the nucleus and certainly in the nucleolus depends on the availability of specific proteins. It has been proposed (Spirin, 1969; Girard, Latham, Penman &

Darnell, 1965) that transport of mRNA and rRNA from the nucleus to the cytoplasm requires specific classes of protein. The processing may also be affected by a sequence of reactions as diverse as methylation or acetylation of RNA and of protein and specific transport through the nuclear membrane.

At 27°C the various classes of RNA appear to be made, albeit at a lower rate than that at 37°C. Cell-sap RNA is transported reasonably well, although we have no evidence as to its functional integrity; polyribosomal RNA and rRNA appear in the cytoplasm at much lower concentrations than would be predicted from the overall rates of RNA synthesis at 27°C. Nuclear-sap RNA predominates at 27°C; a similar observation has been reported for the RNA synthesized in hibernating animals (Satake, Matsukawa & Miyazawa, 1968). In the case of rRNA the 45S precursor is made, but very little of it undergoes maturation. This situation is reminiscent of the effect of puromycin on rRNA synthesis in HeLa cells (Soeiro, Vaughan & Darnell, 1968). We have no information as to the factors involved in the failure to process rRNA precursor. Depletion of the ribosomal protein pool of the nucleolus may be a contributing factor.

The failure of polyribosomal RNA to migrate into the cytoplasm may be due to a lack of formation of the transport complex or to a conformational barrier at the level of the nuclear membrane. Indeed the evidence from radioautography that relatively little RNA crosses from the nucleus or

nucleolus into the cytoplasm at 27°C is a strong new factor in the interpretation of the failure to induce glutamine synthetase.

The role of temperature decrease in the complex phenomena under study finds support from many examples of alterations in protein tertiary or quaternary structure or biological activity and in self-assembly of biological structures (Kushner, 1969). These changes are in general reversible when the temperatures include: (1) dissociation of the spindle protein of mammalian cells (Inoué & Sato, 1967); (2) disaggregation of the glycogen phosphorylase of rabbit skeletal muscle (Kastenschmidt, Kastenschmidt & Helmreich, 1968); (3) increased sensitivity of a histidine biosynthetic enzyme to feedback inhibition by histidine (O'Donovan & Ingraham, 1965); (4) changes in lipoprotein structure (Scanu, Pollard, Hirz & Kothary, 1969); in other cases a decrease in temperature represses a gene (Hartman-Goldstein, 1967), or interferes with the maturation of a virus (Rott & Scholtissek, 1968).

Temperature variation may prove a very useful method in the study of the interdependence of the nucleus, nucleolus and the cytoplasm for the regulation of cellular events in the cells of higher organisms.

The work was supported by the American Cancer Society (Grant E-348) and U.S. Public Health Service Grant AI-00957.

REFERENCES

- Darnell, J. E. (1968). *Bact. Rev.* **32**, 262.
 Eagle, H. (1955). *J. biol. Chem.* **214**, 839.
 Garren, L. D., Howell, R. R., Tomkins, G. M. & Crocco, R. M. (1964). *Proc. natn. Acad. Sci. U.S.A.* **52**, 1121.
 Girard, M., Latham, H., Penman, S. & Darnell, J. E. (1965). *J. molec. Biol.* **11**, 187.
 Hartman-Goldstein, I. J. (1967). *Genet. Res.* **10**, 143.
 Horisberger, M. & Amos, H. (1968). *Biochem. biophys. Res. Commun.* **33**, 61.
 Inoué, S. & Sato, H. (1967). *J. gen. Physiol.* **50**, 259.
 Kastenschmidt, L. L., Kastenschmidt, J. & Helmreich, E. (1968). *Biochemistry, Easton*, **7**, 4543.
 Kushner, D. J. (1969). *Bact. Rev.* **33**, 302.
 Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
 MacNutt, W. S. (1952). *Biochem. J.* **50**, 384.
 Messier, B. & Leblond, C. P. (1957). *Proc. Soc. exp. Biol. Med.* **96**, 7.
 Moscona, A. A., Moscona, M. H. & Saenz, N. (1968). *Proc. natn. Acad. Sci. U.S.A.* **61**, 160.
 Moscona, A. A. & Piddington, R. (1966). *Biochim. biophys. Acta*, **121**, 409.
 Nirenberg, M. W. & Matthaei, J. H. (1961). *Proc. natn. Acad. Sci. U.S.A.* **47**, 1588.
 O'Donovan, G. A. & Ingraham, J. L. (1965). *Proc. natn. Acad. Sci. U.S.A.* **54**, 451.
 Penman, S. (1966). *J. molec. Biol.* **17**, 117.
 Penman, S., Vesco, C. & Penman, M. (1968). *J. molec. Biol.* **34**, 49.
 Reif-Lehrer, L. & Amos, H. (1968). *Biochem. J.* **106**, 425.
 Rott, R. & Scholtissek, C. (1968). *J. gen. Virol.* **3**, 239.
 Satake, M., Matsukawa, S. & Miyazawa, N. (1968). *Nature, Lond.*, **218**, 768.
 Scanu, A., Pollard, H., Hirz, R. & Kothary, K. (1969). *Proc. natn. Acad. Sci. U.S.A.* **62**, 171.
 Scherrer, K., Marcaud, L., Zajdela, F., Breckenridge, B. & Gros, F. (1966). *Bull. Soc. Chim. biol.* **48**, 1037.
 Scholtissek, C. (1967). *Biochim. biophys. Acta*, **145**, 238.
 Soeiro, R., Vaughan, M. H. & Darnell, J. E. (1968). *J. Cell Biol.* **36**, 91.
 Spirin, A. S. (1969). *Eur. J. Biochem.* **10**, 20.
 Wyatt, G. R. (1951). *Biochem. J.* **48**, 584.