[16] Peachey, L. D., and H. Rasmussen, *J. Biophys. Biochem. Cytol.*, **10**, 529 (1961).

[17] Ito, S., and R. J. Winchester, *J. Cell Biol.*, **16**, 541 (1963).

[18] Novikoff, A. B., E. Essner, S. Goldfisher, and M. Heus, in *Interpretation of Ultrastructure* (New York and London: Academic Press, 1962).

[19] MacRobbie, E. A. C., and H. H. Ussing, *Acta Physiol. Scand.*, **53**, 348 (1961).

[20] Engbaek, L., and T. Hoshiko, *Acta Physiol. Scand.*, **39**, 348 (1957).

[21] Farquhar, M. G., S. L. Wissig, and G. E. Palade, *J. Exptl. Med.*, **113**, 47 (1961).

[22] Palade, G. E., and R. Bruns, in *Small Blood Vessel Involvement in Diabetes*, ed. M. Siperstein, in press (1963).

[23] Koefoed-Johnsen, V., and H. H. Ussing, *Acta Physiol. Scand.*, **42**, 298 (1958).

[24] Curran, P. F., F. C. Herrera, and W. J. Flanigan, *J. Gen. Physiol.*, **46**, 1011 (1963).

[25] *Note added in proof:* The exact relationship between the cell membrane nucleoside triphosphatase activity that we have demonstrated histochemically in frog skin and the $Na^+ + K^+$-dependent, oubain-sensitive ATPase activity demonstrated in homogenates of frog skin by Bonting *et al.* [*Arch. Biochem. Biophys.* **98**, 413 (1962)] remains to be established by further experiments.

[26] This functional interpretation of the frog epidermis agrees in many respects with the modifications recently introduced by Ussing (Harvey Lectures, 1963, in press), in his earlier model.

[27] Farquhar, M. G., and G. E. Palade, *J. Cell Biol.*, **19**: No. 2, 22A (1963).

---

*MESSENGER RNA IN CELLS INFECTED WITH VACCINIA VIRUS**

BY YECHIEL BECKER† AND WOLFGANG K. JOKLIK

DEPARTMENT OF CELL BIOLOGY, ALBERT EINSTEIN COLLEGE OF MEDICINE, NEW YORK

Communicated by Harry Eagle, February 18, 1964

In a cell infected with a vaccinia virion two genomes code for and control the synthesis of DNA, RNA, and protein: that of the host cell and that of the virus. The host genome, in the nucleus, serves as template for ribosomal precursor RNA,[1] mRNA,[2] and tRNA, which are all rapidly labeled, but only the last of which is transferred to the cytoplasm within 30 min. Transcription of mRNA from the viral genome, on the other hand, proceeds in the cytoplasm, since it is there that the DNA genome of vaccinia virus becomes established.[3] Pulse-labeling of infected cells with uridine-2-C^{14} for periods of less than 30 min therefore provides a technique for the study of the patterns of RNA transcription from the host cell and viral genomes: for, excepting tRNA (which appears in both the nuclear and cytoplasmic cell fractions, but the properties of which are so distinctive as to cause no ambiguities) RNA transcribed from the host cell genome will be found in the nuclear fraction, whereas RNA transcribed from the viral genome will be located in the cytoplasmic cell fraction. We present evidence in this paper that the rapidly labeled RNA found in the cytoplasm of HeLa cells infected with vaccinia virus is indeed mRNA. It associates rapidly with ribosomes to form polyribosomes; its base composition is analogous to that of the DNA of vaccinia virus; it hybridizes with denatured viral DNA but not with denatured DNA of HeLa cells; and the RNA species transcribed from the viral genome at various stages of the infection cycle differ in size. Further, transcription of RNA from the host cell genome is not detectably depressed for the first 3 hr following infection, but is pro-

gressively inhibited thereafter; and polyribosomes disappear from cells at about the time when viral maturation begins.

Materials and Methods.—Cells and virus: HeLa S3 cells were propagated in spinner culture with a generation time of 18–24 hr in Eagle's medium[4] supplemented with 5% fetal calf serum. The WR strain of vaccinia virus (kindly supplied by Dr. N. P. Salzman) was grown in HeLa cells and purified in sucrose density gradients.[5]

Mode of infection: Cells, grown to a population density of 5–6 $\times$ 10^5 cells/ml, were harvested and concentrated to 10^7 cells/ml in Puck's saline A[6] containing 20 mM Mg^{++} and 1% fetal calf serum (adsorption medium) and incubated with an inoculum of 400 virus particles per cell for 15 min. Under such conditions 50% of the inoculum is adsorbed.[7] After this adsorption period, the cells were diluted tenfold with growth medium and further incubated (Procedure A). To infect cells under growth conditions the virus/cell ratio was increased to 1,000 which permitted adsorption of 10% of the inoculum within 15 min (Procedure B).

Pulse-labeling with uridine-2-C^{14}: Uridine-2-C^{14} (New England Nuclear Corp., 30 mC/mM) was added to cells at successive intervals after infection, at the rate of 5 μC per 5 $\times$ 10^7 cells. Incorporation was stopped by pouring the labeled cells onto crushed frozen saline.

Preparation of nuclear and cytoplasmic fractions: The harvested cells were washed twice with cold Earle's salt solution and treated for 10 min with hypotonic buffer[8] (RSB: 10^{-2} *M* Tris pH 7.4, 10^{-2} *M* KCl and 1.5 $\times$ 10^{-3} *M* $MgCl_2$). The cells were homogenized in a Dounce glass homogenizer and were examined microscopically to determine the presence of unbroken cells. Under suitable conditions, all cells are broken. Nuclei were separated from the cytoplasm by centrifuging for 2 min at 600 *g*. The cytoplasm was pipetted off the nuclei, and the latter were washed twice with RSB buffer. If cells labeled with thymidine-2-C^{14} are treated in this manner, not more than 1–2% of the label is found in the cytoplasm, indicating that breakage of nuclei is negligible.

Analysis of cytoplasmic RNA: Polyribosomes were obtained by centrifuging the cytoplasmic fraction into 15–30% w/w sucrose density gradients.[9] The size of the rapidly labeled cytoplasmic RNA species was determined by rendering the cytoplasm 1% with respect to SDS and spinning into 15–30% w/w sucrose density gradients which contained 0.5% SDS, 5 mM Tris pH 7.3 and 0.1 *M* NaCl[10] (rotor SW 25, 23,000 rpm, 18 hr). The gradients were collected from the bottom, the effluent passed through a Gilford flow cell, and the absorbancy at 260 mμ recorded automatically. Fractions of 0.5–1 ml were collected. After addition of carrier RNA and protein, radioactive material insoluble in 15% TCA was collected on Millipore membranes[9] and counted in a Nuclear-Chicago low background gas-flow counter, using a thin end window. S values were approximated according to the method of Martin and Ames,[11] using single HeLa cell ribosomes (74 S), and ribosomal RNA (16 S and 28 S) as markers.

Analysis of nuclear RNA: Nuclei, labeled with uridine-2-C^{14} and washed twice with RSB containing NaCl instead of KCl, were treated with DOC (final concentration, 0.5%), the DNA digested with 100 μg DNase (Worthington) for 10 min at 0°, and then treated with SDS (final concentration, 2%). Cytoplasmic fraction from unlabeled cells was added to this nuclear preparation to provide O.D. markers of 28 S and 16 S ribosomal RNA. The material was layered on 15–30% w/w sucrose density gradients containing 0.5% SDS, 5 mM Tris pH 7.3, and 0.1 *M* NaCl, and centrifuged for 16 hr at 19,000 rpm in rotor SW 25 in a Spinco Model L centrifuge. The gradients were analyzed as above.

Hybridization of cytoplasmic RNA with viral and cellular DNA: Viral DNA was prepared from purified WR vaccinia virions as previously described.[12] HeLa cell DNA was prepared according to the method of Philipson.[13] Both DNA samples were dissolved in 2 $\times$ SSC (0.3 *M* sodium chloride, 0.03 *M* sodium citrate, pH 7.0). Cytoplasmic RNA labeled with uridine-2-C^{14} was obtained from sucrose gradients after a sample from each fraction had been plated and its radioactivity determined. Fractions containing radioactivity were pooled according to their position in the gradients. The pooled fractions were precipitated with ethanol, centrifuged, and dissolved in 2 $\times$ SSC. Each of the different cytoplasmic RNA species prepared from cells at 1, 3, and 5 hr after infection was annealed with 25 μg of denatured (100° for 10 min followed by quick cooling) viral or cellular DNA, in a total volume of 1 ml. The mixtures of DNA and labeled RNA were heated at 70° for 5 min, then incubated at 57° $\pm$ 1° for 15 hr, and then slowly cooled during the

next 9–10 hr.[14] "Nonhybridized" controls were samples which were cooled rapidly after being heated at 70° for 5 min. The RNase-resistant TCA-precipitable radioactivity was determined after incubation for 15 and 30 min at 37° with 10 μg RNase. Digestion was always complete within 15 min.

Base analysis of P^{32}-labeled cytoplasmic RNA: Two and a half hr after infection, cells suspended in medium lacking inorganic phosphate were labeled for 1 hr by the addition of carrier-free P^{32} (5 mC/10^8 cells). Uninfected control cells were similarly labeled at the same time. The cytoplasmic fractions were prepared, and the polyribosomes of each preparation isolated after centrifugation in sucrose density gradients. The polyribosome fractions were treated with SDS (final concentration, 1%), carrier RNA was added, and RNA precipitated with 2 volumes of ethanol. The precipitates were dissolved, precipitated with TCA (final concentration, 5%), and washed by repeated centrifugation until the supernatants were free of radioactive label. The residues were then digested with 0.3 *M* KOH for 18 hr at 37°. After precipitation with PCA and centrifugation, the pH was adjusted to 3.5 with KOH, and samples were subjected to electrophoresis (3,500 volts for 50 min, in pyridine-acetate buffer, pH 3.5). Radioactivity in areas localized under UV light was extracted with 0.1 *M* HCl and measured as described above.

Results.—Effect of infection on the incorporation of uridine-2-C^{14} into nuclear and cytoplasmic fractions: When HeLa cells are exposed to a 10-min pulse of uridine-2-C^{14}, over 95 per cent of the label is found in the nuclear fraction. In infected cells (Fig. 1), there is a large increase in the amount of labeled RNA in the cytoplasm, detectable as early as $^1/_2$ hr after infection. Incorporation into the cytoplasm reaches a maximum at 4–5 hr after infection, after which it declines. Labeling of

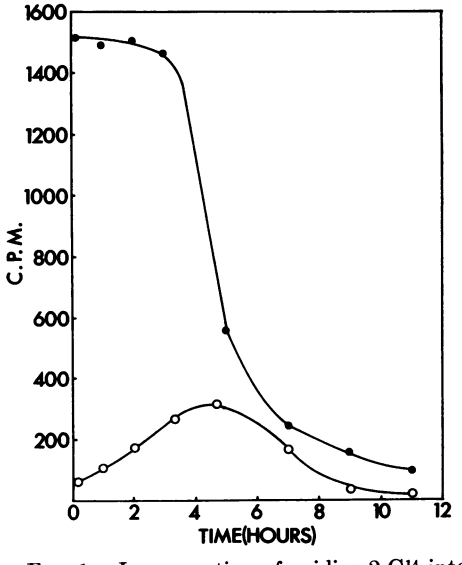

FIG. 1.—Incorporation of uridine-2-C^{14} into nuclei and cytoplasm of cells infected with vaccinia virus. HeLa cells were infected according to Procedure A. At hourly intervals after dilution 6 × 10^7 cells were pulse-labeled for 10 min with 5 μc of uridine-2-C^{14}. The nuclear and cytoplasmic fractions were prepared, analyzed in gradients, and radioactive material precipitable by TCA was determined in each fraction. Incorporation into uninfected control cell fractions remained constant. Closed circles: nuclear fraction. Open circles: cytoplasmic cell fraction.

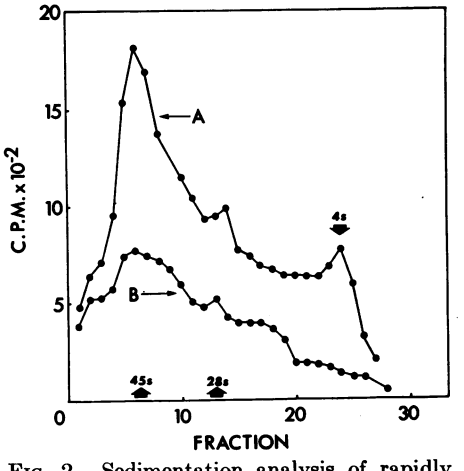

FIG. 2.—Sedimentation analysis of rapidly labeled nuclear RNA. 100 ml of HeLa cells, still growing rapidly at a concentration of 11 × 10^5 cells/ml, were infected according to Procedure B. At 1, 3, and 5 hr after infection samples of 33 ml were pulse-labeled for 15 min with 5 μc uridine-2-C^{14}. 33 ml of uninfected cells were also pulse-labeled. The nuclear fractions were analyzed in gradients as described above. Closed circles: radioactive label insoluble in TCA. Curve *A:* 0, 1, or 3 hr after infection; curve *B:* 5 hr after infection.

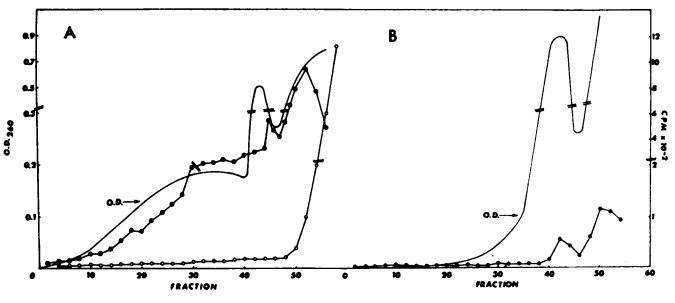

FIG. 3.—The association of rapidly labeled cytoplasmic RNA in infected cells with polyribosomes. HeLa cells were infected according to Procedure A. At $4^1/_2$ hr after infection 1.2×10^8 infected and 6×10^7 uninfected cells were pulse-labeled for 10 min with 20 and 10 μc uridine-2-C^{14}, respectively. The cells were harvested, and the cytoplasmic fractions prepared as described above. Before sedimentation analysis in sucrose gradients, one half of the cytoplasmic fraction of infected cells was treated with 10 μg crystalline pancreatic ribonuclease (Worthington) for 5 min at 0° (*B*). The other half and the cytoplasmic fraction prepared from uninfected cells were centrifuged in the same way (*A*). Closed circles: radioactive label insoluble in TCA, infected cells. Open circles: uninfected cells.

nuclear RNA is unaffected for the first 3 hr after infection; thereafter it declines rapidly, and after 6 hr is only about one fifth of that in control cells. At all times 100% of the incorporated label was rendered acid-soluble by digestion with alkali, indicating that it was localized exclusively in RNA.

The effect of viral infection on the synthesis of host cell RNA: Rapidly labeled RNA of uninfected cells has a sedimentation coefficient of about 45 S, in agreement with Scherrer *et al.*;[1] there is a minor peak of 4 S RNA (Fig. 2). There is little change in this pattern for the first 3 hr after infection. However, thereafter the incorporation of label into the 45 S RNA species decreases, and has virtually ceased after 7 hr.

It should be emphasized that the pattern of labeling by uninfected cells depends largely upon their immediate past history. If the cells are first concentrated to 10^7 per ml in adsorption medium, then the 45 S RNA species is a minor component, and the major portion of incorporated radioactive label is in an RNA fraction with a sedimentation coefficient of about 28 S. However, under these conditions also, the effect of infection is the same; transcription of RNA from the host cell genome is inhibited starting at 3 hr after infection and virtually ceases 7 hr after infection.

Characterization of cytoplasmic RNA of infected cells: (a) *It is associated with polyribosomes.* Polyribosomes from uninfected cells pulse-labeled with uridine-2-C^{14} for 10 min are unlabeled (Fig. 3*A*). On the other hand, polyribosomes derived from infected cells are labeled. The UV absorbancy and radioactivity profiles do not coincide entirely; the radioactivity profile is shifted toward the light region

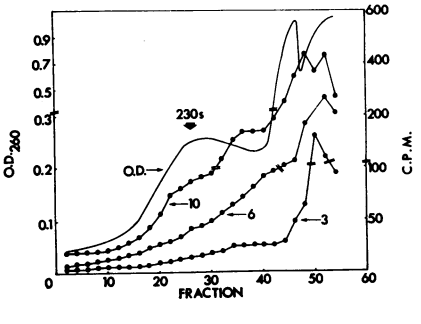

FIG. 4.—Rate of formation of virus-specific polyribosomes. HeLa cells were infected according to Procedure A. At 4 hr after infection 1.8×10^8 cells were pulse-labeled with 30 μc uridine-2-C^{14}. Samples were taken at 3, 6, and 10 min. Cytoplasmic fractions were analyzed in 15–30% sucrose gradients as described above. Closed circles: radioactive label insoluble in TCA.

indicating that virus-specific polyribosomes are slightly smaller than those formed by host cell RNA. If such polyribosome preparations are treated briefly with RNase, both UV and radioactivity profiles are changed drastically (Fig. 3*B*); polyribosomes are broken down to single ribosomes, and over 95 per cent of the label is rendered acid-soluble. The newly labeled RNA associates with ribosomes very soon after its formation. Figure 4 shows the UV absorbancy as well as the radioactivity profiles obtained with pulses of 3, 6, and 10 min. Label first appears in very light polyribosomes within 3–6 min; within 10 min even large polyribosomes are labeled. There is thus no doubt that the newly labeled RNA is messenger RNA.

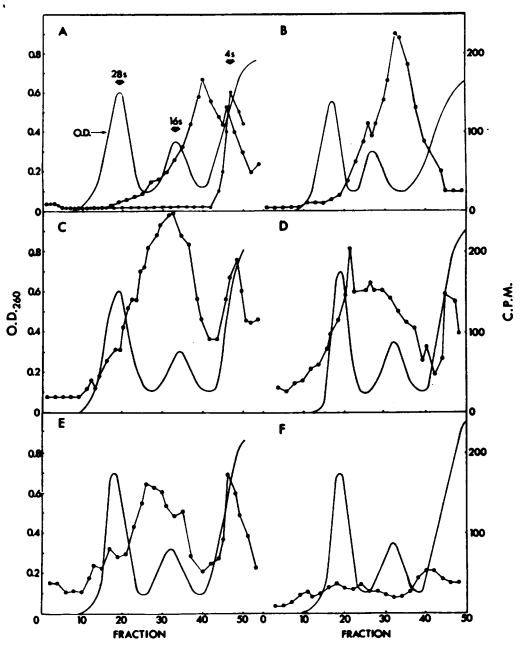

FIG. 5.—Size distribution of the rapidly labeled cytoplasmic RNA. HeLa cells were infected according to Procedure A. Samples of 6×10^7 cells were pulse-labeled for 10 min with 10 μc uridine-2-C^{14} at $^1/_2$ hr (*A*), 1 hr (*B*), 3 hr (*C*), 5 hr (*D*), 7 hr (*E*), and 9 hr (*F*) after infection. 6×10^7 uninfected cells were similarly labeled (*A*). Radioactive material insoluble in 18% TCA was measured. Closed circles: infected cells. Open circles: uninfected cells.

(b) *Size of the newly synthesized mRNA:* The size distributions of cytoplasmic RNA labeled by 10-min pulses of uridine-2-C^{14} at various stages of the infection cycle are shown in Figure 5. The following points emerge: (1) UV absorption reveals three peaks, the 28 S and 16 S ribosomal RNA's, and the 4 S tRNA, the relative amounts remaining unchanged for 9 hr after infection. Infection thus does not induce breakdown of ribosomal or tRNA. (2) In uninfected cells, the only label in the cytoplasm is in tRNA, as discussed above. (3) In the cytoplasm of infected cells there is other labeled RNA besides tRNA. Although some is formed within $^1/_2$ hr after infection, it is synthesized most rapidly at about 4 hr postinfection and to a negligible extent after 7 hr after infection, just after the first mature viral progeny is detectable. (4) The radioactivity profiles reveal a changing pattern as infection progresses. Although this pattern differs somewhat from experiment to experiment, the following generalizations can be made: between $^1/_2$ and 1 hr after infection the predominant RNA species formed is small, with a sedimentation coefficient of 8–12 S. After 1 hr postinfection larger RNA species predominate: the peaks are at about 14 S at 2 hr, 16 S at 3 hr, 18 S at 4 hr, 20 S at 5 hr. There is thus a trend toward larger molecules predominating as infection progresses. There is always some spread; for instance, there are RNA molecules considerably larger than 18 S present at 4 hr, as well as small molecules.

(c) *Specificity of the newly synthesized cytoplasmic RNA:* (1) *Base composition:* As shown in Table 1, the base composition of the RNA appearing on the polyribo-

TABLE 1

BASE RATIOS OF VARIOUS DNA AND RNA SPECIES

Nucleic acid	Ratio $\frac{A+T}{G+C}$ or $\frac{A+U}{G+C}$
HeLa cell DNA	1.285[9]
Vaccinia virus DNA	1.70[15]
HeLa cell ribosomal RNA	0.75[9]
HeLa cell mRNA	1.24
mRNA from cells infected with vaccinia virus	1.75

somes of infected cells closely mirrors the base composition of viral DNA, while in noninfected cells it mirrors the cellular DNA. Both RNA's are different from ribosomal RNA, which apparently contributes negligible amounts of radioactivity in either system. The results clearly indicate that mRNA synthesized between $2^1/_2$ and $3^1/_2$ hr after infection is transcribed from viral DNA. If host cell mRNA is still synthesized at this time, it evidently no longer becomes associated with ribosomes, since in that case the base composition of the mRNA isolated from the polyribosomes of infected cells would have had a base composition intermediate between that of viral and host cell DNA.

(2) *Hybridization with denatured viral DNA:* The RNA newly synthesized in the cytoplasm 1, 3, and 5 hr after infection hybridized with viral DNA, not with HeLa cell DNA (Table 2). 45 S RNA from uninfected cells hybridized with HeLa cell DNA; tRNA could not be detectably hybridized with either HeLa cell or viral DNA.

Polyribosomes in infected cells: In order to ascertain the size distribution of the polyribosomes as infection progressed, cells at various stages of the infection cycle were pulse-labeled with uridine-2-C^{14} for 15 min, and the cytoplasmic fraction was subjected to centrifugation in sucrose density gradients. The radioactivity profile gives an estimate of the size distribution of newly labeled polyribosomes, that is, polyribosomes formed by mRNA specified by the viral genome, while the absorbancy profile yields an estimate of the over-all size distribution of polyribosomes present at any stage. At first, the polyribosome population is formed predominantly by host cell mRNA; as this mRNA decays it will be replaced by viral mRNA since, as indicated above, beginning 2 hr after infection, host cell mRNA is no longer available for polyribosome formation. It was to be anticipated, therefore, that although the absorbancy and radioactivity profiles could conceivably diverge in the early stages of infection, later in the infection cycle these two profiles could be expected to coincide. As shown in Figure 6, during the early stages of infec-

TABLE 2

HYBRIDIZATION OF RAPIDLY LABELED CYTOPLASMIC RNA WITH VACCINIA AND HELA CELL DNA

Experiment	Hr after infection	Size of RNA	% RNase-resistant-radioactivity After: Hyb. with WR DNA	Hyb. with HeLa cell DNA	Non-hybridized
1	1	12 S	25.5	5.5	6.0
	3	4 S	9.0	12.0	9.0
		16 S	33.5	9.0	8.0
	5	4 S	8.0	9.0	8.0
		16 S	26.5	7.0	6.6
		22 S	29.5	9.0	7.0
2	0	45 S	—	29.5	5.0
	1	12 S	25.5	5.5	6.0
	3	16 S	31.5	11.0	10.0
	5	16 S	28.5	11.0	7.0
		22 S	30.0	8.5	7.0

10^8 HeLa cells, infected according to Procedure A, were pulse-labeled for 15 min with 10 μc uridine-2-C^{14} at 1, 3, and 5 hr after infection. Labeled RNA species of various sizes were isolated and hybridized as described above.

tion many of the newly labeled polyribosomes are appreciably smaller than the bulk of the total population, presumably reflecting the small size of mRNA specified by the viral DNA at that time. Later, when the total number of polyribosomes in the cells has decreased markedly, the over-all size of virus-specific polyribosomes is greater, in conformity to the larger size of the mRNA then transcribed. There is then fairly good coincidence between the absorbancy and radioactivity profiles; and it is likely that an appreciable fraction of the total polyribosomes then present in the infected cell are formed by mRNA specified by viral DNA. Polyribosomes have virtually disappeared 7 hr after infection, which agrees with the finding[16] that protein synthesis has virtually ceased at that time. It is noteworthy that at 7 hr less than 10 per cent of viral progeny has matured. Finally, one can estimate that the half life of host cell mRNA in infected cells is not markedly different from the value of 3 hr calculated from its rate of decay in uninfected cells treated with actinomycin D.[9]

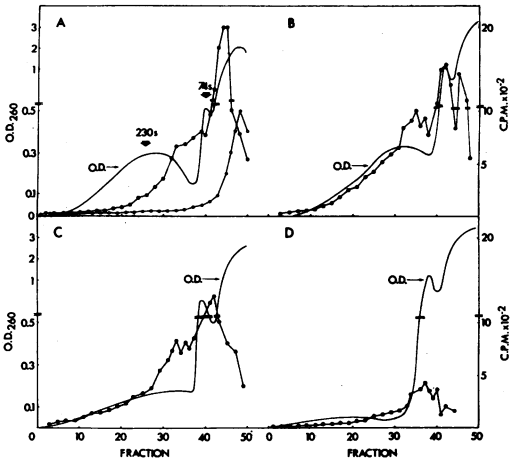

FIG. 6.—Polyribosome profiles of infected cells. HeLa cells were infected according to Procedure A. Samples of 6×10^7 cells were pulse-labeled for 15 min with 10 μc uridine-2-C^{14} at 1 hr (*A*), 3 hr (*B*), 5 hr (*C*), and 7 hr (*D*). Cytoplasmic fractions were analyzed in sucrose density gradients as described above. Uninfected cells were similarly treated (*A*). Closed circles: radioactive label insoluble in TCA, infected cells. Open circles: uninfected cells.

Discussion.—From the evidence here presented a composite picture can be constructed of the pattern of RNA biosynthesis in HeLa cells infected with vaccinia virus. Soon after infection a small RNA species (8–12 S), transcribed from viral DNA, appears on polyribosomes. These polyribosomes are on the average significantly smaller than those formed by host cell mRNA, which predominate at this time. As infection progresses, the size of mRNA specified by the viral genome increases strikingly: 1 hr after infection only 20 per cent of the virus-specific RNA molecules have a sedimentation coefficient greater than 16 S, while at 5 hr 70 per cent of them are larger than 16 S. Presumably in consequence of the larger mRNA, the polyribosomes formed by virus-specific mRNA in the latter part of the infection cycle are larger than during the early stages. This changing size pattern of mRNA molecules being transcribed from the viral genome is probably reflected in the sequential synthesis of different protein species. It is known that a virus-induced thymidine kinase[17] is formed early in the infection cycle; while Salzman *et al.*[18] have presented evidence that protein synthesis is necessary as late as $^1/_2$ hr before maturation. A sequential synthesis of various viral antigens has also been demonstrated by Loh *et al.*[19]

The present demonstration that a mRNA transcribed from the viral genome is synthesized as early as $^1/_2$ hr after infection probably reflects the high multiplicity used in the present study (about 200 virus particles per cell). Previous studies have indicated that the major portion of infecting vaccinia virus particles

is not uncoated until 1–2 hr after infection.[7] However, lower multiplicities were then used, and it is known that as the multiplicity is increased, the lag before uncoating is decreased.[7]

Introduction of virions into the cell not only causes the appearance of virus-specific mRNA's in the cytoplasm but also profoundly affects the genetic activity of host cell DNA. This is not an immediate effect: transcription of RNA from host cell DNA is not switched off during the first 3 hr after infection. However, during the next 3 hr there is a rapid decrease in the formation of cellular RNA, which virtually ceases at 7 hr. The reason for this powerful block in nuclear RNA synthesis, while transcription of viral mRNA then still proceeds at the maximum rate, is not known. Experiments are in progress to determine whether the inhibition is due to the direct action of a protein coded for by viral DNA.

It is remarkable that although transcription of cellular RNA appears to be unaffected during the first 3 hr of infection, the mRNA associating with polyribosomes between $2^1/_2$ and $3^1/_2$ hr after infection is exclusively of the viral type. A similar conclusion has been reached by Salzman *et al.*[20] It is possible that host cell mRNA synthesis is arrested soon after infection, and this effect is not detected because the absolute amount of mRNA synthesized at any time is small compared with the amount of 45 S ribosomal precursor RNA formed. Alternatively, host cell mRNA is made, but may be unable to associate with ribosomes.

The present experiments shed no light on the source of the mRNA concerned in the synthesis of uncoating protein.[7] No evidence was obtained for the early synthesis in the nucleus of a new RNA species. However, the sensitivity of the technique as described here is not high, and the synthesis of a small amount of such an RNA species is not excluded.

Finally, the evidence presented here indicates that synthesis of mRNA specified by viral DNA has virtually ceased by 7 hr after infection, shortly before polyribosomes disappear from infected cells. Very little synthesis of protein is therefore possible during the time when the major portion of the viral progeny matures. This is not in conflict with the evidence of Salzman *et al.*[18] that some protein synthesis is necessary up to $^1/_2$ to $^3/_4$ hr before maturation. Only a single new protein species may then be necessary; and the possibility cannot be excluded that a small number of polyribosomes containing a relatively stable mRNA is functional throughout most of the maturation phase.

Abbreviations: mRNA, messenger RNA; tRNA, transfer RNA; SDS, sodium dodecyl sulfate; DOC, sodium deoxycholate; O.D., optical density; TCA, trichloroacetic acid; PCA, perchloric acid.

* This work was supported by grant G24263 from the National Science Foundation and by grants AI 04913-02 and GM 876-02 from the U.S. Public Health Service.

† On leave from Hebrew University, Hadassah Medical School, Jerusalem, Israel.

[1] Scherrer, K., H. Latham, and J. E. Darnell, Jr., these PROCEEDINGS, **49**, 240 (1963).
[2] Brenner, S., F. Jacob, and M. Meselson, *Nature*, **190**, 576 (1961).
[3] Cairns, H. J. F., *Virology*, **11**, 603 (1960).
[4] Eagle, H., *Science*, **130**, 432 (1959).
[5] Joklik, W. K., *Biochim. Biophys. Acta*, **61**, 290 (1962).
[6] Marcus, P. I., S. J. Cieciura, and T. T. Puck, *J. Exptl. Med.*, **104**, 655 (1956).
[7] Joklik, W. K., *J. Mol. Biol.*, in press.
[8] Warner, J. R., P. Knopf, and A. Rich, these PROCEEDINGS, **49**, 122 (1963).

[9] Penman, S., K. Scherrer, Y. Becker, and J. E. Darnell, Jr., these PROCEEDINGS, **49**, 654 (1963).
[10] Gilbert, W., *J. Mol. Biol.*, **6**, 389 (1963).
[11] Martin, R. G., and B. N. Ames, *J. Biol. Chem.*, **236**, 1372 (1961).
[12] Joklik, W. K., *J. Mol. Biol.*, **5**, 265 (1962).
[13] Philipson, L., *J. Gen. Physiol.*, **44**, 899 (1961).
[14] Geiduschek, E. P., J. W. Moohr, and S. B. Weiss, these PROCEEDINGS, **48**, 1078 (1962).
[15] Joklik, W. K., *Virology*, **18**, 9 (1962).
[16] Joklik, W. K., and Y. Becker, in preparation.
[17] McAuslan, B. R., *Virology*, **20**, 162 (1963).
[18] Salzman, N. P., A. J. Shatkin, and E. D. Sebring, *Virology*, **19**, 542 (1963).
[19] Loh, P. C., and J. L. Riggs, *J. Exptl. Med.*, **114**, 149 (1961).
[20] Salzman, N. P., A. J. Shatkin, and E. D. Sebring, *J. Mol. Biol.*, in press.

PUROMYCIN INHIBITION OF PROTEIN SYNTHESIS: INCORPORATION OF PUROMYCIN INTO PEPTIDE CHAINS

BY DANIEL NATHANS

DEPARTMENT OF MICROBIOLOGY, THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE

Communicated by Fritz Lipmann, February 24, 1964

The similarity in structure between puromycin and aminoacyl-sRNA (Fig. 1), first noted by Yarmolinsky and de la Haba,[1] led to the hypothesis that the antibiotic inhibits protein synthesis by acting as an analogue of esterified sRNA. Puromycin blocks protein synthesis after aminoacyl-sRNA formation,[1, 2] and at the same time it leads to the accumulation of small peptides.[3] Both of these effects appear to be due to the splitting of ribosome-bound peptidyl-sRNA,[4] which results in release of incomplete peptide chains.[5–7] If puromycin does act as an analogue of "charged" sRNA, one might anticipate the possibility that it could substitute for an incoming aminoacyl-sRNA as the acceptor of the carboxyl-activated peptide, forming peptidyl-puromycin, thus ending growth of the polypeptide.[7, 8] Such a mechanism is supported by the finding of Allen and Zamecnik[7] that when puromycin labeled in the amino acid moiety is incubated with reticulocyte ribosomes, a portion of the radioactivity precipitates with the released polypeptides, the amount being equivalent to the N-terminal valine of these peptides. In order to establish, however, that puromycin replaces aminoacyl-sRNA as an acceptor of the peptide chain it is desirable to show unequivocally that puromycin as such becomes linked to polypeptide via a peptide bond in the course of protein synthesis. The study reported here was undertaken to determine whether or not peptidyl-puromycin is formed when susceptible cells are exposed to the antibiotic.

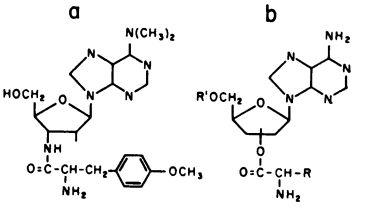

FIG. 1.—The structure of puromycin (*a*) and of aminoacyl-sRNA (*b*).

Materials and Methods.—H^3-puromycin, labeled in the methoxy group, was prepared under the