Turnover of Messenger Ribonucleic Acid and Protein Biosynthesis in the Mouse Mammary Adenocarcinoma H2712*

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ABSTRACT: Administration of actinomycin D to C3H/HeJ mice bearing the mammary adenocarcinoma H2712 produced a breakdown of the polysomes (ergosomes) of this tumor. Degradation was evidenced 2 hours after administration of actinomycin and increased progressively with time. An 80–90% breakdown of polysomes was observed 12 hours after actinomycin injection. Messenger ribonucleic acid of tumor labeled with [^{3}H]uridine decayed in a similar fashion after actinomycin administration. Total activity of this component decreased from 6.35×10^3 cpm to 1.39×10^3 cpm after 12 hours. Ribosomal preparations from this tumor tissue also showed a decreased capacity to incorporate L-[U-^{14}C]leucine *in vitro* in a cell-free system following actinomycin administration. The effects of actinomycin upon these three processes varied exponentially with time after administration. *In vivo* incorporation of L-[U-^{14}C]-leucine into tumor tissue was also inhibited by actinomycin. However, the time pattern of this inhibition differed from that observed in the other cases. Finally, calculation of the metabolic turnover of m-RNA of this tumor was made from experiments in which specific activities of m-RNA were determined at varying times after injection of [2-^{14}C]uridine. An average half-life of 30 minutes (or less) for tumor m-RNA was obtained. Breakdown of m-RNA following actinomycin exhibited a different time pattern and was considerably slower than that observed under normal steady-state conditions.

Rates of synthesis and breakdown of ribonucleic acids from various sources, with particular emphasis on messenger and ribosomal RNA, have been investigated intensively in recent years. Thus it has been shown that bacterial m-RNA has an extremely rapid metabolic turnover and that its rate of synthesis is faster than that of s-RNA or r-RNA[1] (Levinthal *et al.*, 1962). Further investigations showed that m-RNA of rat and mouse liver also possessed a rapid metabolic turnover (Staehelin *et al.*, 1963a; Korner and Munro, 1963; Staehelin *et al.*, 1964; Trakatellis *et al.*, 1964a,b; Villa-Trevino *et al.*, 1964). In the present paper, we report studies on the metabolic turnover of m-RNA and metabolic properties of r-RNA of a tumor tissue, mouse mammary adenocarcinoma H2712. These studies have been extended to include effects of administration of actinomycin D upon breakdown of polysomes (ergosomes), decay of labeled RNA, and *in vivo* incorporation of labeled leucine by tumor tissue. These phenomena have been correlated with the ability of ribosomal preparations to incorporate amino acids in an *in vitro* cell-free system at varying times following administration of actinomycin D. Possible regulatory mechanisms of protein biosynthesis and m-RNA breakdown will be discussed.

Materials and Methods

Animals. Male mice of the C3H/HeJ strain with a mammary adenocarcinoma H2712 (Dunham and Stewart, 1953) were purchased from the Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Maine. The tumor was transplanted to C3H/HeJ male mice by subcutaneous injection with a 13-gauge needle 7 days after the original inoculation. Animals were fed a commercial stock ration ad libitum and were always used for experimentation 7 days after transplantation.

Tracer Compounds. [2-^{14}C]Uridine (specific activity 30 mc/mmole), [^{3}H]uridine (specific activity 7.87 c/mmole), and L-[U-^{14}C]leucine (specific activity 223 mc/mmole) were purchased from New England Nuclear Corp., Boston, Mass.

Actinomycin D (C_1 in the new nomenclature). This compound was a generous gift from Dr. Elmer Alpert of Merck Sharp & Dohme, West Point, Pa.

Preparation of Standard Ribosomes. Tumors were excised immediately after killing the animals by decapitation and homogenized in Hoagland's medium A (0.005 M Tris, 0.025 M KCl, 0.005 M $MgCl_2$, and 0.25 M sucrose) with a motor-driven Potter-Elvehjem tissue grinder fitted with a Teflon pestle. All isolation procedures were performed at 0–2°. The postmitochondrial supernatant obtained by centrifuging the homogenate

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[1] Abbreviation used in this work: r-RNA, ribosomal nucleic acids.

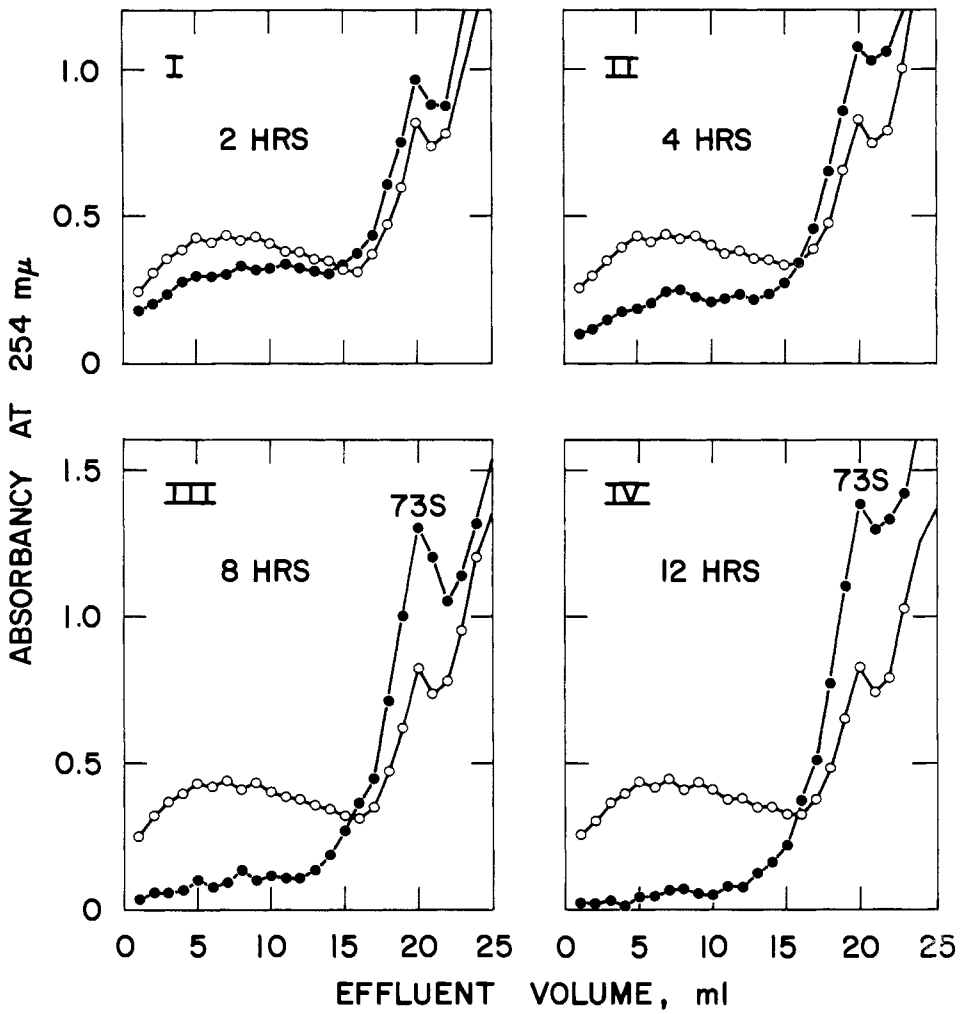

FIGURE 1: Zone centrifugation analysis of ribosomes from tumors of control and actinomycin-treated mice. The procedure has been described in the text. Gradients were prepared by dissolving sucrose in Hoagland's salt buffer. C3H/HeJ mice bearing the mammary adenocarcinoma H2712 were injected intraperitoneally with actinomycin (200 μg/ml of 0.9% NaCl) at a level of 200 μg/100 g body weight and sacrificed by decapitation at zero time (controls) and 2, 4, 8, and 12 hours later. Ribosomes obtained from 0.9 g of tumor tissue were utilized in all experiments, and resulting ribosomal patterns obtained 2, 4, 8, and 12 hours after injection of actinomycin are presented in I–IV as indicated. Each pattern is plotted together with that of a zero time control animal. ○—○, control animal; ●—●, actinomycin-injected animal.

for 20 minutes at 6590 × *g* was treated with deoxycholate (Korner, 1961), and ribosomes were obtained by subsequent centrifugation for 2 hours at 105,000 × *g*.

Preparation of Purified Ribosomes. Purified ribosomes were obtained by centrifugation of the deoxycholate-treated postmitochondrial fraction through two layers of sucrose (0.5 and 1.8 M) (Wettstein *et al.*, 1963).

Fractionation of Ribosomal Preparations into Polysomes of Varying Aggregate Size. The procedure of Wettstein *et al.* (1963) was utilized. Three ml of the deoxycholate-treated postmitochondrial supernatant was layered over 27 ml of a cold linear sucrose gradient (0.3–1.0 M). After centrifugation for 3 hours and 15 minutes at 25,000 rpm and 1° in the Spinco rotor SW25, fractions were collected and their absorbancies at 254 mμ determined (Trakatellis *et al.*, 1964c).

Extraction and Resolution of RNA from Purified Ribosomes. This procedure has been described and discussed extensively in previous publications (Staehelin *et al.*, 1964; Trakatellis *et al.*, 1964b). Measurement of the radioactivity of the RNA fractions was performed by precipitation with $HClO_4$ according to the method of Staehelin *et al.* (1964) with minor modifications (Trakatellis *et al.*, 1964b).

Cell-free Amino Acid-Incorporating System. Ribosomes were suspended carefully in 0.5 ml of an ice-cold buffer solution (0.045 M Tris, 0.0075 M $MgCl_2$, 0.12 M KCl, and 0.075 M NaCl, *p*H 7.2); 0.2 ml of this suspension was utilized per incorporation assay and 0.1 ml

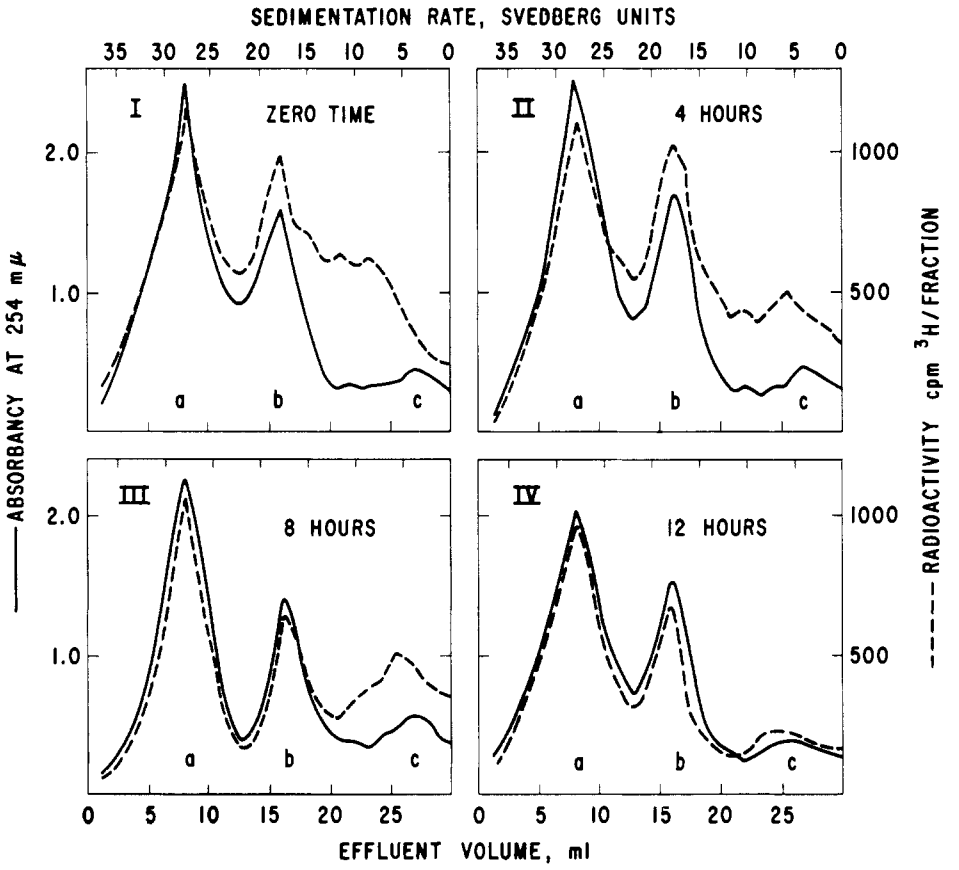

FIGURE 2: Sedimentation patterns of pulse-labeled RNA from C3H/HeJ mammary adenocarcinoma H2712 after actinomycin injection. Each mouse received 750 μc of [^{3}H]uridine intraperitoneally and, 2 hours later, 200 μg of actinomycin D/100 g of body weight by intraperitoneal injection. Animals were sacrificed by decapitation at 0, 4, 8, and 12 hours after actinomycin injection. Preparation of standard ribosomes from tumor tissue and extraction and resolution of RNA by centrifugation at 25,000 rpm for 40 hours at 1° through a sucrose density gradient have been described in the text. Sedimentation patterns of pulse-labeled tumor RNA at 0, 4, 8, and 12 hours after actinomycin injection are shown in I–IV, respectively.

for determination of RNA content. To this ribosomal suspension (0.2 ml) were added 0.3 ml of a mouse liver supernatant and 0.5 ml of a buffer solution (0.02 M Tris, 0.005 M $MgCl_2$, 0.080 M KCl, and 0.050 M NaCl, *p*H 7.2) containing 0.825 μmole of β-mercaptoethanol, 10.0 μmoles of phosphoenolpyruvic acid (tricyclohexylamine salt purchased from the Sigma Chemical Co.), 30 μg of pyruvate kinase (purchased from the Sigma Chemical Co.), 1.0 μmole of ATP, 0.6 μmole of GTP, and 0.005 mmole of L-[U-^{14}C]leucine. The liver supernatant was prepared from a 10% homogenate of normal mouse liver in Hoagland's salt buffer containing 0.44 M sucrose. All operations were conducted at 1–2°. The supernatant obtained by centrifugation of the homogenate at 4000 rpm for 10 minutes was centrifuged in a Spinco rotor No. 40 at 20,000 rpm for 20 minutes. The resulting postmitochondrial supernatant was centrifuged at 40,000 rpm in a Spinco No. 40 rotor for 150 minutes to yield the postmicrosomal supernatant utilized in the incorporation assay. Methods employed for the precipitation of protein and measurement of radioactivity after incubation have been described (Wettstein *et al.*, 1963).

In Vivo Incorporation Experiments. L-[U-^{14}C]Leucine (20 μc/100 g body wt) was injected intraperitoneally at varying times following administration of actinomycin, and the animals were sacrificed by decapitation 30 minutes after injection of the labeled amino acid. Tumors were excised immediately and homogenates were prepared as described above in ice-cold 0.25 M sucrose at a concentration of 20 mg of tissue/ml. One ml of 1 M NaOH was added to 1 ml of homogenate. The mixture was kept at room temperature for 1 hour to remove radioactive leucine bound to s-RNA, and 20 mg of Celite and 1 ml of 10% trichloracetic acid were added. The precipitates were collected by suction on filter paper disks (Whatman No. 540) layered with 20 mg of Celite in a stainless steel filtration apparatus,[2] washed successively twice with 5 ml of 5% trichloracetic

[2] E-8B filtration apparatus (Tracerlab, Inc.).

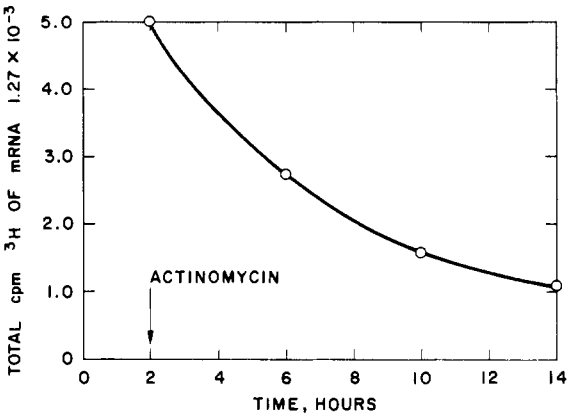

FIGURE 3: Decay in radioactivity of m-RNA calculated from data of Figure 2. [^{3}H]Uridine was administered at time zero.

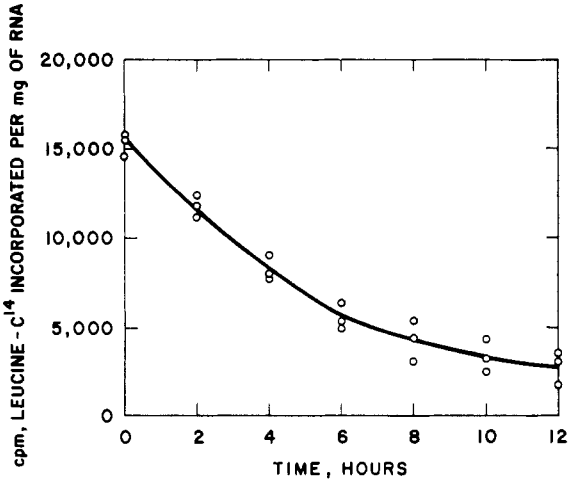

FIGURE 4: *In vitro* incorporation of L-[U-^{14}C]leucine by standard ribosomal preparations prepared from mouse mammary tumor. C3H/HeJ mice with mammary adenocarcinoma H2712 were injected intraperitoneally with 200 μg of actinomycin/100 g body weight and sacrificed by decapitation 2, 4, 6, 8, 10, and 12 hours later. Zero time controls which did not receive actinomycin were also employed. Procedure described in the text was utilized. RNA was estimated from the absorbancy at 254 mμ (20 absorbancy units = 1 mg). Assay samples containing 0.400–0.500 mg of RNA were incubated at 37° for 30 minutes.

acid containing 5 mg of nonradioactive leucine per ml, three times with 5 ml of 2-propanol–diethyl ether (1 : 2), twice with 5 ml of 2-propanol–diethyl ether–chloroform (2:2:1), and finally three times with small portions of diethyl ether. Dried precipitates were transferred to glass-counting vials, and 1 ml of hydroxide of hyamine 10-X solution added. After 15 minutes at room temperature, the closed vials were placed in a water bath at 70° for 10 minutes to effect complete solubilization of protein. Nine ml of a 0.5% solution of 2,5-diphenyloxazole in toluene was then added and radioactivity measured in a Packard Tri-Carb liquid scintillation spectrometer. Results obtained with this procedure were very similar to those obtained by a previous method (Trakatellis and Axelrod, 1964), particularly when the total quantity of protein per assay did not exceed 2 mg. Protein content of the homogenates was determined by the method of Lowry *et al.*, 1951.

Results

Breakdown of Polysomes after Actinomycin Injection. It has been reported (Staehelin *et al.*, 1963a) that the injection of actinomycin produced a breakdown of rat liver polysomes (ergosomes) which the authors concluded was due to degradation of m-RNA and inhibition by actinomycin of the synthesis of new m-RNA. This effect was accompanied by a corresponding loss of ability of ribosomes to incorporate amino acids *in vitro*. We (Trakatellis *et al.*, 1964a) have noted that the inhibitory effects of actinomycin D upon the ability of mouse liver ribosomes to incorporate DL-[1-^{14}C]-leucine paralleled the rate of decay of m-RNA subsequent to administration of actinomycin. In these experiments, m-RNA was labeled by prior administration of [6-^{14}C]orotic acid. In the present experiments, we have studied the breakdown of polysomes of the C3H/HeJ mouse mammary adenocarcinoma H2712 at varying times after actinomycin injection (Figure 1, I–IV). This tissue is more sensitive to actinomycin than liver, and the breakdown of polysomes was already evidenced 2 hours after administration of actinomycin (Figure 1, I). This breakdown was very clear at 4 hours (Figure 1, II) and increased progressively with time. Thus, the degree of polysome breakdown was approximately 20% after 2 hours, 43–70% at 4 hours, 56–80% at 8 hours, and 79–90% at 12 hours. It can also be seen that the extent of breakdown was more pronounced in the area of the heavy aggregates. Data presented in Figure 1 are representative of a series of 30 animals. Although minor variations have been observed, breakdown of polysomes occurred in all animals in the typical pattern shown in Figure 1. In similar experiments performed with liver tissue of the tumor-bearing mice, polysomal breakdown was not observed 2 hours following actinomycin administration and only slight changes were noted after 4 hours. Definite breakdown of polysomes (35–55%) occurred 8 hours after actinomycin injection.

Decay of m-RNA after Actinomycin Injection. Results of a typical experiment determining the decay of radioactively labeled m-RNA are presented in Figure 2 (I-IV). C3H/HeJ mice with the mammary adenocarcinoma H2712 received an intraperitoneal injection of [^{3}H]uridine followed after 2 hours by an intraperitoneal injection of actinomycin D. Animals were sacrificed by decapitation at varying times after injection of actinomycin. Sedimentation patterns of pulse-labeled tumor RNA are shown in Figure 2 (I–IV).

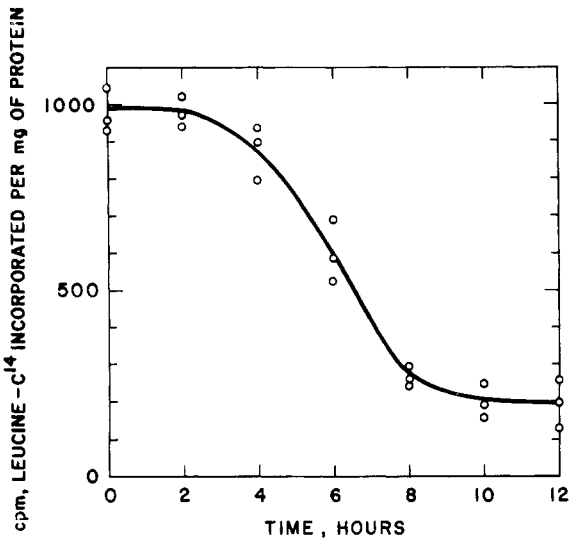

FIGURE 5: *In vivo* incorporation of L-[U-^{14}C]leucine into tumor tissue. C3H/HeJ mice with mammary adenocarcinoma H2712 were injected intraperitoneally with 200 μg of actinomycin D/100 g body weight and sacrificed by decapitation 2, 4, 6, 8, 10, and 12 hours later. Zero time controls which did not receive actinomycin were also employed. L-[U-^{14}C]Leucine (20 μc/100 g body weight) was injected intraperitoneally 30 minutes prior to sacrifice. Procedure for determining degree of incorporation has been described in the text.

The sedimentation pattern of pulse-labeled liver RNA has been analyzed extensively in recent investigations (Staehelin *et al.*, 1964; Munro and Korner, 1964; Trakatellis *et al.*, 1964b) and is comparable to that of tumor RNA of the control animal (no actinomycin, 2-hour labeling; Figure 2, I). RNA of this tumor tissue shows 3 peaks, a, b, and c, and an area between peaks b and c with high radioactivity. Radioactive peaks a and b coincide with the 29 S and 18 S ribosomal RNA, respectively. Peak c, which corresponds to an RNA component of approximately 4.5–5.0 S, consists of labeled s-RNA. The radioactive area b–c has a high specific activity and is heterogeneous with components possessing sedimentation rates corresponding to 4.5–20 S. Accumulated evidence supports the view that this area consists of a spectrum of m-RNA molecules (Staehelin *et al.*, 1964; Munro and Korner, 1964; Trakatellis *et al.*, 1964b).

Decay of m-RNA was apparent 4 hours after injection of actinomycin (Figure 2, II) and became more pronounced at 8 hours (Figure 2, III) and 12 hours (Figure 2, IV) after actinomycin. The decay of m-RNA calculated from the data of Figure 2 is presented in Figure 3. Total radioactivity of m-RNA fell from 6.35 $\times$ 10^3 cpm to 1.39 $\times$ 10^3 cpm. These changes correspond approximately to a 45% breakdown of m-RNA 4 hours after actinomycin injection and 69 and 79% degradation at 8 and 12 hours, respectively. Similar patterns of m-RNA breakdown have been observed in two additional experiments conducted in identical fashion. These time relationships of m-RNA decay correlate well with those reported above for polysome breakdown after actinomycin injection. It can also be noted that m-RNA decay was more pronounced in the area comprising components with greater sedimentation values (Figure 2). This observation also correlates with the more pronounced polysome breakdown among the heavier aggregates (Figure 1).

Studies with a Cell-free Amino Acid-Incorporating System. It has been demonstrated previously (Staehelin *et al.*, 1963a; Korner and Munro, 1964; Trakatellis *et al.*, 1964a) that rat and mouse liver ribosomal preparations derived from actinomycin-treated animals possessed a decreased ability to incorporate amino acids *in vitro* in a cell-free system. These results were in agreement with the observed breakdown of polysomes and decay of m-RNA. In the present experiments, ribosomal preparations from C3H/HeJ mouse mammary adenocarcinoma H2712 were tested for their capacity to incorporate L-[U-^{14}C]leucine in a cell-free system at varying times after actinomycin D injection (Figure 4). A progressive decrease in the incorporating ability was observed. Of the original incorporating capacity, only 54% was present 4 hours after actinomycin injection, and approximately 30 and 20% at 8 and 12 hours, respectively. These results are in close agreement with those on polysome breakdown and m-RNA decay of this tumor tissue reported above.

In Vivo Incorporation of Labeled Leucine. Previous studies with regenerating liver (Guidice and Novelli, 1963), liver or liver slices (Revel and Hiatt, 1964; Revel *et al.*, 1964) failed to demonstrate an effect of actinomycin D upon incorporation of amino acids into protein *in vivo*. In our experiments dealing with the *in vivo* incorporation of labeled leucine into protein of tumor tissue (Figure 5), we did not observe any decrease 2 hours after actinomycin injection and noted only a slight decrease of approximately 12% at 4 hours. However, a dramatic decrease in incorporation was apparent at 6 and 8 hours after actinomycin injection with an inhibition of incorporation of 80% at 12 hours. This time effect of actinomycin upon amino acid incorporation *in vivo* differed markedly from its effect on *in vitro* incorporation by ribosomal preparations in a cell-free system. This difference which was particularly evident in the first 4 hours following actinomycin injection will be discussed.

Turnover of m-RNA. Calculation of the metabolic turnover time of m-RNA of C3H/HeJ mouse mammary adenocarcinoma H2712 was made from experiments in which specific activities of m-RNA were determined at varying times after injection of [2-^{14}C]uridine. Results of a typical experiment are shown in Figure 6 (I–VI), in which the labeling time was varied from 30 minutes to 6 hours. Labeled m-RNA appeared within 30 minutes in the polysome population (II) and reached a maximum level at 1 hour (III) which remained constant until termination of the experiment at 6 hours (VI). This is clearly shown in Figure 7,

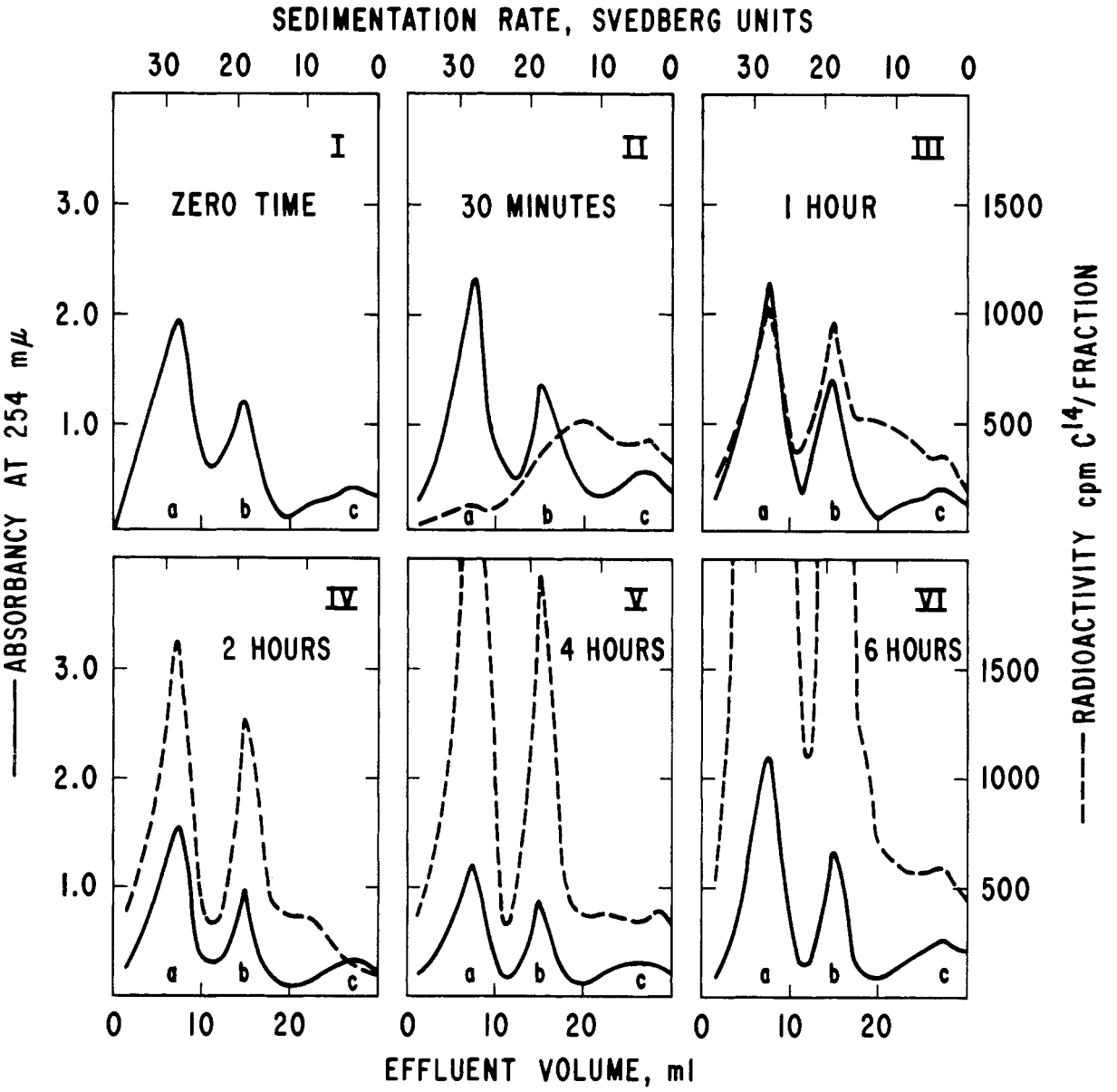

FIGURE 6: Sedimentation patterns of pulse-labeled RNA from purified ribosomes of mouse mammary adenocarcinoma H2712. Each mouse received 300 μc of [2-^{14}C]uridine/100 g body weight by intraperitoneal injection and was sacrificed by decapitation at the indicated times. The animal utilized to obtain the pattern of I (zero time) did not receive any labeled uridine. Preparation of purified ribosomes from tumor tissue and extraction and resolution of RNA by centrifugation at 25,000 rpm for 40 hours at 1° through a sucrose density gradient have been described in the text.

which records the specific activities of m-RNA. With the assumption that a steady state of m-RNA exists through equivalence of m-RNA breakdown to its synthesis and release into the cytoplasm, our results can be interpreted to indicate that the m-RNA of the entire polysomal population is completely renewed approximately every hour. An average half-life of approximately 30 minutes, or less, for the spectrum of m-RNA molecules of C3H/HeJ mouse mammary adenocarcinoma H2712 can, therefore, be derived. This value is considerably less than that of 4–6 hours obtained from our studies reported above on the effect of actinomycin upon polysome breakdown, decay of labeled m-RNA, and incorporation of labeled leucine by ribosomal preparations. Similar differences in the estimated turnover of liver m-RNA have been noted previously (Villa-Trevino, *et al.*, 1964; Trakatellis, *et al.*, 1964a,b).

The specific activities of the 29 S r-RNA of tumor tissue increased linearly with ime of pulse labeling (Figure 8). The metabolic turnover of m-RNA was far more rapid than that of r-RNA, with the differences in

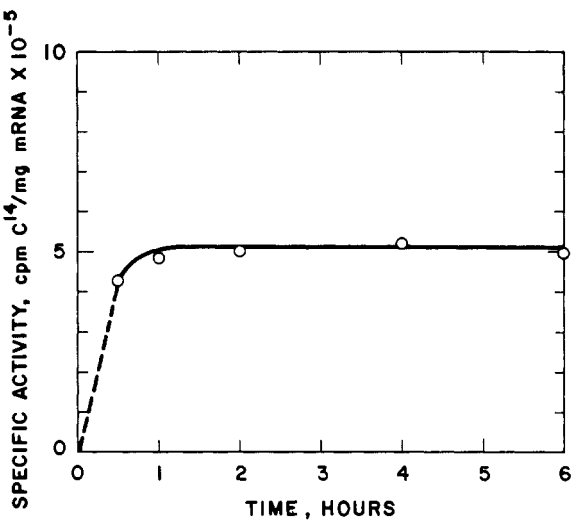

FIGURE 7: Specific activities of m-RNA calculated from data presented in Figure 6. Amount of m-RNA was taken as 1.3% of total ribosomal RNA (Staehelin *et al.*, 1964) determined from the absorbancy values.

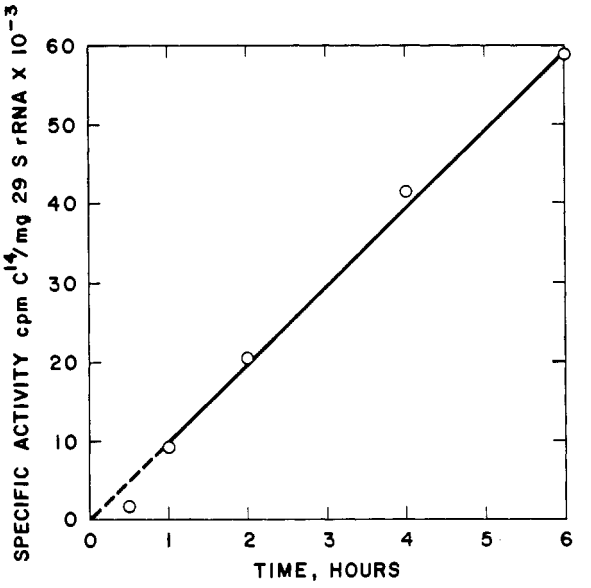

FIGURE 8: Specific activities of 29 S r-RNA calculated from data of Figure 6.

specific activity diminishing with increases in pulse-labeling times. Thus, the ratio of specific activities of m-RNA to r-RNA decreased from 210 at a pulse-labeling time of 30 minutes to 8 after 6 hours.

Discussion

The observations that polysomes of the C3H/HeJ mouse mammary adenocarcinoma break down progressively (as has been observed before in liver tissue after actinomycin injection) and that this phenomenon correlates with the observed decay of labeled m-RNA and decreased incorporating capacity of ribosomal preparations *in vitro* agree with the conception that polysomes are composed of single ribosomes held together by a strand of m-RNA, and that m-RNA possesses a rapid metabolic turnover.

The view that the breakdown of liver polysomes and the accompanying decrease in amino acid incorporation of homogenates following actinomycin injection is not related to m-RNA turnover (Revel *et al.*, 1964) is not compatible with a variety of data demonstrating a short half-life for liver m-RNA. Thus, a study of the kinetics of reassembly of ergosomes and restoration of protein synthesis upon reversal of ethionine action by adenine and methionine has shown that the half-life of m-RNA is 48 minutes (Villa-Trevino *et al.*, 1964). Similarly, a rate of renewal of cytoplasmic m-RNA of approximately 60 minutes has been obtained from studies of the kinetics of ^{32}P incorporation into m-RNA (Noll, Oura, Staehelin, and Wettstein, unpublished observations[3]). Kinetic experiments in our laboratory have indicated that the m-RNA of the entire mouse liver polysome population was renewed approximately every 4 hours (Trakatellis *et al.*, 1964b). Furthermore, in kinetic experiments reported in this paper we have found that the half-life of m-RNA of the mouse mammary adenocarcinoma H2712 is approximately 30 minutes or less. This rapid half-life could well explain the sensitivity of this tumor tissue to actinomycin. Finally, the observation made in the present study that the *in vivo* incorporation of L-[U-^{14}C]leucine into proteins of tumor tissue decreases progressively with time after actinomycin administration does not support the view that changes in polysomes are detectable only after tissue fractionation (Revel, *et al.*, 1964). It should be noted that polysome breakdown after actinomycin injection also occurs in other tissues (spleen and kidney) examined in our laboratory. These accumulated observations constitute strong evidence that the observed breakdown of polysomes following injection of actinomycin is a consequence of the rapid metabolic turnover of m-RNA.

An attempt will be made in this section to explain the variable effects of actinomycin upon *in vitro* and *in vivo* incorporation of amino acids as well as the observed differences in decay time of m-RNA following actinomycin administration, as contrasted with that noted under steady-state conditions. According to the hypothesis of the tape mechanism of protein biosynthesis (Gierer, 1963; Gilbert, 1963; Watson, 1963) which has been verified in experiments on polysomes or ergosome function (Noll *et al.*, 1963; Goodman and Rich, 1963; Staehelin *et al.*, 1963b; Hardesty *et al.*, 1963a,b; Williamson and Schweet, 1965), the readout of genetic information begins with the attachment of single ribosomes to the beginning of an m-RNA strand and, via a moving process, terminates with their release

[3] Quoted in Villa-Trevino *et al.* (1964).

at the end of the strand. During this process, the m-RNA strand passes through the condensing site of each ribosome with successive exposure of codons. A polypeptide chain grows continuously at that condensing site until the reading of the message is completed. The ribosome now at the end of the m-RNA strand is released with the complete polypeptide chain. The attachment and readout processes *in vivo* are extremely rapid. As a consequence, one m-RNA molecule serves as the template for the synthesis of many polypeptide chains until its breakdown. Strong evidence has been presented by Villa-Trevino *et al.* (1964) in support of this concept, and these authors have calculated from published data (Staehelin *et al.*, 1964; Jungblut and Turba, 1963) that the rate of attachment of single ribosomes to the m-RNA strand is about 0.16 particle/sec and that the speed of the reading process is about 14 nucleotides/sec. Under normal conditions, this results in a distance of 90 nucleotides between ribosomes in the m-RNA strand. Recent investigations from other laboratories (Goldstein, *et al.*, 1964; Villa-Trevino, *et al.*, 1964; Sidransky, *et al.*, 1964) have given special emphasis to the regulatory role on protein biosynthesis of the attachment mechanism.

The nature of the attachment mechanism is not known. However, it is logical to assume that the number of free single ribosomes relative to the available m-RNA molecules could be a significant factor in the over-all attachment process. In animals treated with actinomycin, rapid degradation of m-RNA without compensatory replacement would produce an increase in single ribosomes (verified experimentally) along with a decreased number of m-RNA molecules. This situation would favor an increase in rate of ribosome attachment resulting in the synthesis of more polypeptide chains per m-RNA strand and might explain the failure to observe an effect upon the *in vivo* incorporation of labeled leucine into liver proteins 4–8 hours after actinomycin injection despite the 30–50% breakdown of m-RNA demonstrable at this time by other experimental techniques. The influence of an increased rate of attachment would not be as marked in an *in vitro* cell-free incorporating system since the recycling of ribosomes in this case is limited (Noll *et al.*, 1963). The possibility exists that the over-all process of protein biosynthesis may be dependent upon factors other than the ratio of single free ribosomes to m-RNA molecules. Thus, a direct increase in the rate of activation of the ribosomes as well as an increase in the reading rate of the m-RNA message may also be involved. However, an increase in the rate of attachment is obligatory to explain these data since no net increase in protein biosynthesis per m-RNA strand would occur in the absence of this increase.

A difference between the *in vitro* and *in vivo* incorporation of labeled leucine was also noted in experiments reported in this paper with mammary adenocarcinoma H2712. Indeed, in tumor tissue no effect upon the *in vivo* incorporation of labeled leucine was apparent 2 hours after administration of actinomycin and only a slight inhibitory effect was noted at 4 hours. However, inhibition was pronounced at 6 hours after actinomycin injection and increased progressively with time. These *in vivo* effects were in contrast to the more rapid action of actinomycin upon *in vitro* incorporation of labeled leucine, polysome breakdown, and m-RNA decay. These differences may be explained by an increased rate of attachment of ribosomes to m-RNA as described above. Variations between *in vitro* and *in vivo* effects of actinomycin administration are less pronounced with tumor tissue than with liver, probably because of the faster turnover rate of m-RNA in tumor.

There is no implication in the foregoing discussion that the ratio of various proteins synthesized under normal conditions is the same as that following actinomycin injection. Actually, the converse may be expected since m-RNA molecules are probably heterogeneous with respect to their metabolic turnover.

We would further postulate that m-RNA is degraded *in vivo* by action of nucleases during the time interval between attachment of two ribosomes. Thus, a breakdown at the beginning of the m-RNA strand would be decisive since further attachment of ribosomes would be impossible.[4] Ribosomes existing on the m-RNA strand would move toward the end of the strand, and destruction of this m-RNA molecule would be completed by further action of nucleases. A mechanism for the degradation of m-RNA *in vitro* through action of an exonuclease has been presented by Staehelin *et al.* (1964). An increased rate of attachment of ribosomes to m-RNA would decrease the time interval between successive attachment of ribosomes and, if our postulate is correct, decrease the probability of m-RNA breakdown. The possibility of an increased rate of attachment of ribosomes to m-RNA following actinomycin administration has been discussed above. Its realization could, therefore, afford a rational explanation for the decreased decay time of m-RNA after actinomycin injection as contrasted with its metabolic turnover under steady-state conditions.

Acknowledgments

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[4] After completion of this manuscript, a paper by Eikenberry and Rich (1965) appeared with the suggestion that ribosomes move from the 3′-OH end of the m-RNA strand toward the 5′-OH end and that m-RNA destruction *in vitro* may be carried out by exonucleolytic attack from the 3′-OH end. These authors also suggested that a similar mechanism existed *in vivo*. Although the direction of ribosome movement has not yet been established unequivocally, the suggestion of the authors of a decisive m-RNA breakdown at the beginning of this molecule is similar to ours.

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CORRECTION

In the paper by L. W. Cunningham in Volume 3, No. 11, November 1964, on p. 1630, in the right-hand column, lines 3–5 under Experimental Procedures should read: "... the addition of increasing amounts of a standard solution of 0.02 N iodine in 0.05 M potassium iodide to..."