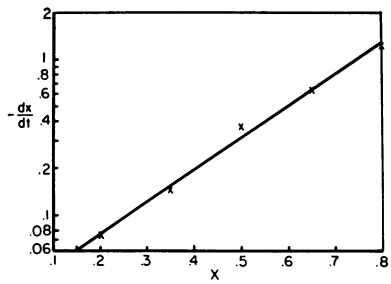

FIG. 1.—Graphical test of conformity to the Elovich equation of a photogenerated chromatophore free radical decay curve. Data are taken from Figure 2 of Ruby, Kuntz, and Calvin.[5] The electron spin resonance signal (x) (which is presumably proportioned to free radical concentration) expressed in normalized units is plotted against the logarithm of the negative of reaction rate ($-dx/dt$) expressed in normalized units divided by 1.6 second time units. Rates were determined by estimating slopes of the curve of Figure 2 of Ruby, Kuntz, and Calvin.[5] Linearity in this plot indicates conformity of the data to the Elovich equation.

prior to the recognition of the wide occurrence and possible significance of the Elovich equation.

In conclusion, it seems reasonable to presume that the conformity of photogenerated free radical decay in chromatophores to the Elovich equation is probably a manifestation of electron conduction governed by the laws of solid-state and surface physics.

Summary.—The Elovich equation describes well the data of Ruby, Kuntz, and Calvin on decay of photogenerated free radicals in chromatophores of *R. rubrum*. This constitutes kinetic evidence for the participation of solid-state and surface physical processes in photosynthesis.

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*THE STABILITY OF LIVER MESSENGER RNA**

BY MICHEL REVEL† AND HOWARD H. HIATT

DEPARTMENTS OF MEDICINE, BETH ISRAEL HOSPITAL AND THE HARVARD MEDICAL SCHOOL, BOSTON, MASSACHUSETTS

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The average cell of the adult rat liver has been estimated to divide less often than once a year.[1, 2] On the other hand, this cell synthesizes an amount of protein equivalent to its own content in less than 6 days.[3, 4] These characteristics differ sharply from those of bacterial cultures, where the time required to double the protein content normally approximates the generation time of the cells.[5] In bacteria, messenger RNA has been demonstrated to turn over rapidly.[6] The combination of a long "generation time" and a rapid rate of protein synthesis in rat liver prompted an investigation of the stability of messenger RNA in this tissue.

Published reports on this subject conflict. Thus, Guidice and Novelli[7] found that actinomycin D, which is known to inhibit DNA-dependent RNA synthesis, had little effect on total amino acid incorporation into regenerating liver, although the antibiotic interfered with the synthesis of a new enzyme. Staehelin *et al.*,[8] on the other hand, with higher levels of the antibiotic observed effects which they ascribed to rapid turnover of messenger RNA. Our observations in normal and actinomycin D-treated rats, which include studies of isotope incorporation into RNA, of template activity of purified RNA, and of amino acid incorporation *in vivo* and *in vitro*, provide strong evidence that most messenger RNA from rat liver cytoplasm is stable.

Materials and Methods.—Male rats of the Charles River strain weighing from 200 to 250 gm and fasted overnight were used. A 0.5% solution of actinomycin D in propylene glycol was diluted with 1 or 2 vol of 0.9% sodium chloride and injected intraperitoneally in the doses indicated. Control animals received a propylene glycol–sodium chloride solution.

For studies of RNA synthesis, orotic acid-6-C^{14}, 6.5 mc/mmole, was injected intraperitoneally in a dose of 10 μc/100 gm of body weight. For studies of amino acid incorporation, rats were fasted 24 hr prior to administration of leucine-C^{14}, 246 mc/mmole, which was given in three intraperitoneal injections 1 hr apart in a total dose of 5 μc/100 gm body weight. The animals were sacrificed by decapitation 1 hr following the last injection. Fractionation of liver was carried out as described previously.[12] Nuclei were purified by the procedure of Chauveau *et al.*[9]

Purification of RNA: Nuclear, cytoplasmic, and microsomal RNA fractions were purified with sodium dodecyl sulfate (SDS) and phenol as previously described.[10] Nuclear RNA preparations were treated with deoxyribonuclease, 5 μg/ml, at 0° for 10 min and oligodeoxyribonucleotides were removed as previously reported.[12] The sucrose gradient analyses were carried out as described earlier.[10] Measurements of ultraviolet absorption were made and recorded with a Gilford Multisample Absorbance Recorder. The trichloracetic acid (TCA) precipitable radioactivity was measured with a low background, gas flow, Tracerlab counting system with an efficiency of 20%. The analytical procedures employed have been described elsewhere.[10, 12]

Assay of stimulatory activity of purified RNA: The cell-free, amino acid incorporating system from *E. coli* described by Nirenberg and Matthaei[11] was used to measure stimulatory activity. Valine-C^{14} incorporation was studied according to procedures described previously.[12]

Studies of in vitro protein synthesis by a cell-free system derived from liver: The system described by Hoagland and Askonas[13] was used to study the incorporation of leucine-C^{14} in microsomes incubated in the presence of a pH 5 fraction. In all experiments 19 C^{12}-amino acids were present in a concentration of 10^{-5} *M*, in addition to the C^{14}-leucine or C^{14}-phenylalanine being studied.

Materials: The deoxyribonuclease, 1 × recrystallized, was obtained from Worthington Biochemicals. Orotic acid-6-C^{14}, 6.5 mc/mmole; L-valine-C^{14}, 117 mc/mmole; L-phenylalanine, 165 mc/mmole; and L-leucine-C^{14}, 246 mc/mmole, were purchased from New England Nuclear Corporation. Actinomycin D was a gift from Merck, Sharp and Dohme.

Results.—Effect of actinomycin D on RNA synthesis: Levels of actinomycin which interfere markedly with rat liver RNA synthesis are lethal, and the interval between administration of the antibiotic and death is dose-dependent (Table 1). In order to carry out studies after at least 18 hr of diminished liver RNA synthesis, a dose of actinomycin D of 1.5 mg/kg of body weight was selected for these experiments. Inhibition of orotic acid-C^{14} incorporation into RNA occurred within 2 hr of injection and was far greater in the microsomal fraction than in the nuclear or the 100,000 × *g* supernatant fraction (Table 1). Higher levels of the antibiotic further reduced orotic acid-C^{14} incorporation into all fractions, but produced additional effects which will be described elsewhere.[15] A decrease in RNA concentration of nuclei but not of cytoplasm was observed after actinomycin injection (Table 2).

The species of RNA labeled in the presence of the antibiotic were examined by

TABLE 1

RELATION OF DOSE OF ACTINOMYCIN D TO C^{14} INCORPORATION INTO RAT LIVER RNA AND TO TIME OF DEATH

Actinomycin D, mg/kg	RNA Specific Activity, cpm/μg			Interval, drug to death, hr
	Nuclear	Microsomal	Supernatant	
0	153.0	30.3	31.7	—
0.5	—	—	—	55–60
1.0	—	—	—	42–50
1.5	81 (47)*	2.6 (91)	11.3 (64)	24–36
3.0	—	—	—	18
5	20.5 (84)	0.4 (99)	2.9 (91)	12

Orotic acid-C^{14} was injected intraperitoneally 30 min following actinomycin D, and the rats were sacrificed 11 hr thereafter.
* Figures in parentheses are per cent inhibition.

TABLE 2

CHANGES IN LIVER RNA CONCENTRATION AFTER ACTINOMYCIN D ADMINISTRATION

Actinomycin D, mg/kg	RNA, μg/μg DNA	
	Total cell	Nuclei
0	7.03	0.43
1.5	6.67 (5)*	0.35 (19)
3	6.60 (6)	0.30 (30)
5	7.13 (0)	0.26 (40)

Animals were sacrificed 12 hr after actinomycin D injection.
* Figures in parentheses represent per cent decrease.

sucrose gradient analysis in two series of studies. In the first, rapidly labeled RNA was studied by injecting orotic acid-C^{14} 17 hr after actinomycin and isolating the labeled RNA 30 min later. In the second, to study those RNA fractions which were labeled more slowly, or in which isotope was retained for a longer period, a pulse of isotope precursor was given 4 hr following actinomycin, and the RNA was examined 13 hr later.

In cytoplasm from normal rats 30 min after administration of orotic acid-C^{14} (Fig. 1*A*), the 18 *S* RNA had a higher specific activity than the 28 *S*,[12] but most of the radioactivity appeared in the 4 *S* area. Isotope in cytoplasmic RNA from treated animals was reduced to approximately 20 per cent of that in the control and was found largely in the 4 *S* component (Fig. 1*B*). Labeled nuclear RNA from control rats was heterogeneous (Fig. 2*A*), with most of the isotope in the 20–40 *S* area and very little in the 4 *S* region. Nuclear RNA from rats injected with actinomycin contained only 40 per cent of the radioactivity observed in the control material; no peak of isotope was evident in the 30–40 *S* area (Fig. 2*B*).

When orotic acid-C^{14} was injected 13 hr before the rats were killed, the isotope distribution in the control cytoplasmic RNA (Fig. 3*A*) was virtually identical with the pattern of optical density. Thus, the control animals presumably synthesized both ribosomal and transfer RNA. In sharp contrast, after actinomycin D treatment the cytoplasmic high-molecular-weight RNA contained almost no radioactivity (Fig. 3*B*), but in the 4 *S* RNA there was 32 per cent of the isotope observed under control conditions.[14] Evidence that the radioactive 4 *S* material is transfer RNA will be presented elsewhere.[15] In nuclear RNA from control animals a large fraction of radioactivity was in the 28 *S* area, with relatively much less in the 18 *S* and 4 *S* areas (Fig. 4*A*). Incorporation of isotope in nuclear RNA from actinomycin D-treated animals was reduced by approximately 50 per cent, but the sedimentation pattern was similar to that in the control (Fig. 4*B*).

Stability of messenger RNA in liver cytoplasm: (1) *Stimulatory activity of purified RNA from actinomycin-treated rats:* We have previously reported[12] that both nuclear and cytoplasmic RNA fractions from rat liver stimulate amino acid incorporation in a protein-synthesizing system derived from *E. coli*,[11] and that the concentration of stimulatory activity in nuclear RNA is 10-fold higher. Further,

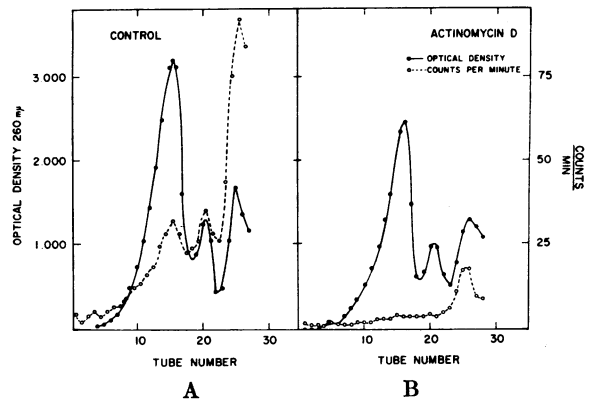

FIG. 1.—Sucrose density gradient analysis of liver cytoplasmic RNA from normal and actinomycin D-treated rats killed 30 min after orotic acid-C^{14}. The actinomycin D-treated rats were injected with 1.5 μg/gm 18 hr prior to orotic acid-C^{14}. Cytoplasmic RNA was purified with phenol and SDS, and was centrifuged through 25 ml of a 5–20% sucrose gradient at 2–4° for 13 hr at 20,000 rpm. Optical density was recorded automatically, and 10-drop samples were analyzed for TCA-precipitable radioactivity.

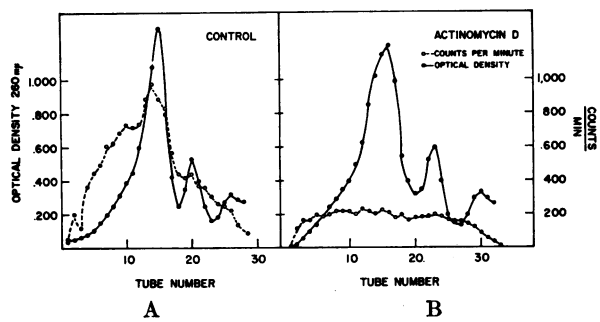

FIG. 2.—Sucrose density gradient analysis of liver nuclear RNA from normal and actinomycin-treated rats killed 30 min after orotic acid-C^{14}. Conditions were as described for Fig. 1, except that RNA was prepared from purified nuclei.

we have observed that much of the stimulatory activity in cytoplasmic RNA sediments in the 18 *S* region.[12] Since actinomycin D led to almost complete inhibition of labeling of this RNA fraction, parallel measurements of stimulatory activity were undertaken in order to obtain information concerning the stability of liver messenger RNA. Microsomal RNA from rats injected 17 hr earlier with actinomycin D showed no decrease in stimulatory activity as compared with the control (Fig. 5). If stimulatory activity does, in fact, reflect the level of messenger RNA, then these data indicate that no decrease occurred in this fraction of RNA for at least 17 hr after actinomycin D, although during this period little cytoplasmic RNA was labeled other than transfer RNA.

(2) *Amino acid incorporation in liver in vivo:* The apparent stability of cytoplasmic messenger RNA suggested that liver protein synthesis would be unaffected by actinomycin D. This prediction was realized in experiments in which isotope incorporation was measured in liver subcellular fractions following leucine-C^{14} administration to normal and actinomycin-treated rats (Table 3). In all cytoplasmic fractions isotope incorporation 17 hr after actinomycin D injection was

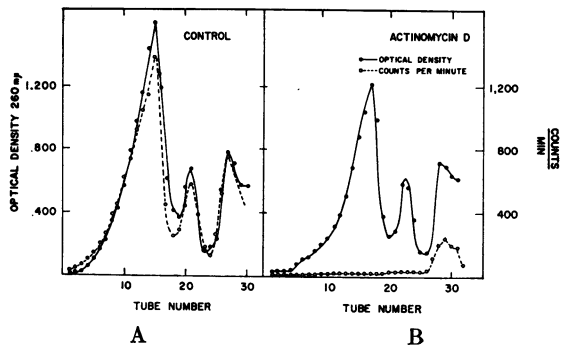

FIG. 3.—Sucrose density gradient analysis of liver cytoplasmic RNA from normal and actinomycin-treated rats killed 13 hr after orotic acid-C^{14}. Actinomycin D, 1.5 μg/gm, and orotic acid-C^{14} were injected 17 and 13 hr, respectively, before sacrifice.

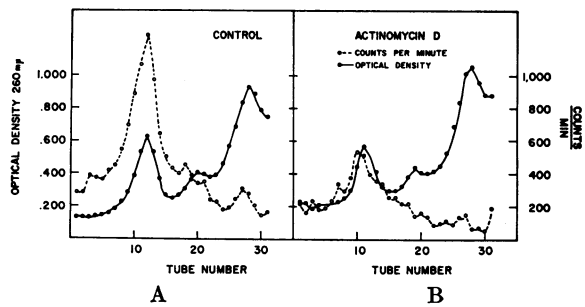

FIG. 4.—Sucrose density gradient analysis of liver nuclear RNA from normal and actinomycin-treated rats killed 13 hr after orotic acid-C^{14}. RNA was from the nuclei of the liver preparations from which the cytoplasmic RNA described in Fig. 3 was obtained. The large amount of ultraviolet-absorbing material close to the top of the gradient has been shown to be oligodeoxynucleotides.[10] In the material pictured in Fig. 2, these nucleotides were removed by repeated treatment with 2 *M* KCl and 20% ethanol. The RNA in this experiment was not subjected to this procedure.

comparable to control values. Even 40 hr following administration of 1 mg of actinomycin per kg body weight, there was no diminution of amino acid incorporation.

(3) *Amino acid incorporation by liver microsomes in vitro:* Because *in vivo* incorporation experiments may be affected by a variety of factors in addition to the integrity of the protein-synthesizing apparatus, related experiments were carried out *in vitro*. When liver microsomal fractions from actinomycin D-treated rats were

TABLE 3

AMINO ACID INCORPORATION *in vivo* INTO LIVERS OF CONTROL AND ACTINOMYCIN-TREATED RATS

Experiment		Fraction, cpm/mg protein			
		Nuclear	Mitochondrial	Microsomal	Supernatant
A	Control	1,387	2,250	3,401	1,022
	Actinomycin D	1,229	2,284	3,491	986
B	Control	1,013	1,807	2,393	989
	Actinomycin D	1,241	1,856	3,186	1,029
C	Control	1,344 (8,300)*	—	2,746 (10,200)*	1,456
	Actinomycin D	1,880 (13,200)*	—	3,064 (14,300)*	1,516

Actinomycin D, 1.5 μg/gm, was injected intraperitoneally 17 hr (Experiments A and B) or 1.0 μg/gm 40 hr (Experiment C) before sacrifice. Leucine-C^{14}, 5 μc/100 gm, was injected in equally divided doses 3, 2, and 1 hr before killing.

* Cpm/mg of RNA.

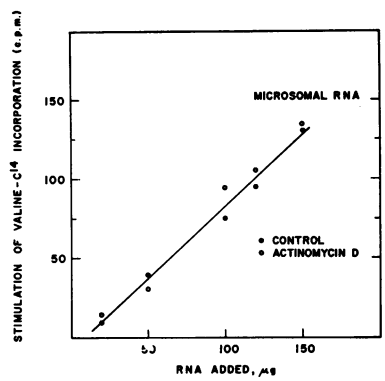

FIG. 5.—Stimulation of valine-C^{14} incorporation in an *E. coli* extract by liver microsomal RNA from normal and actinomycin-treated rats. Microsomal RNA was assayed in the 0.25-ml system containing 0.9 mg bacterial protein described previously.[12] 100 cpm represents 2 μμmoles. The figures presented are for cpm in excess of background incorporation of 120 cpm.

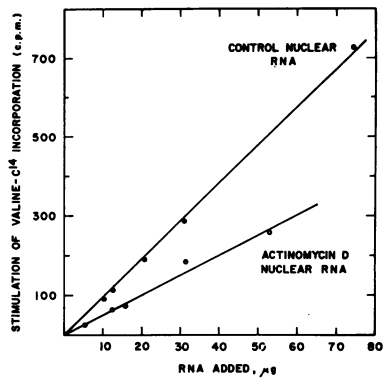

FIG. 6.—Stimulation of valine-C^{14} incorporation in an *E. coli* extract by liver nuclear RNA from normal and actinomycin-treated rats. Nuclear RNA was assayed in a system increased to a final volume of 1.0 ml (2.4 mg bacterial protein) in order to measure maximal activity of large amounts of RNA. However, the markedly increased stimulatory capacity of nuclear RNA as compared to cytoplasmic RNA has also been observed when the assay conditions were those described for Fig. 5.[12] 100 cpm represent 2 μμmoles. The figures are for cpm in excess of background incorporation of 175 cpm.

incubated with a pH 5 fraction from control rat liver and leucine-C^{14}, no effect on incorporation was detected (Table 4). Thus, the microsomal fraction remained fully active for at least 17 hr after administration of actinomycin D. (Parallel experiments revealed no effect on the pH 5 fraction.)

Effects of actinomycin D on messenger RNA in liver nuclei: As noted above, the inhibitory effect of actinomycin on isotope incorporation in RNA was more pronounced in cytoplasm than in nuclei. However, in contrast to the stability of the cytoplasmic material, stimulatory activity was decreased by 50 per cent in liver nuclear RNA from rats treated 17 hr earlier with actinomycin D, 1.5 mg/kg (Fig. 6). This reduction approximated the extent of inhibition of labeling of nuclear RNA. Nuclear RNA preparations from control and treated animals were additive when introduced into the same system, thus demonstrating that the diminished stimulatory activity in material from treated animals was not attributable to the presence of an inhibitor. Despite the reduction of *in vitro* stimulatory activity of purified nuclear RNA, amino acid incorporation *in vivo* into TCA-precipitable material was not reduced in liver nuclei of treated animals (Table 3).

Discussion.—Actinomycin D is known to inhibit DNA-dependent RNA synthesis in both mammalian[16-18] and bacterial[19] systems. Measurements of the half-life

TABLE 4

EFFECT OF ACTINOMYCIN D ON AMINO ACID INCORPORATION BY LIVER MICROSOMAL FRACTIONS

	Leucine-C^{14}			Phenylalanine-C^{14}	
Expt. no.*	1	2	3	3	4
	(μμmole/mg protein†)				
Control	27.8	21.4	22.2	8.2	11.9
Actinomycin D 1.5 μg/gm	32.0	20.6	22.7	8.2	9.0

* Actinomycin D was given 17 hr prior to sacrifice.
† Incubation for 45 min at 37°. In all experiments a pH 5 fraction from control rat liver was used.

of messenger RNA have been made by determining the interval between the cessation of RNA synthesis caused by the antibiotic and a decrease in the rate of protein synthesis.[6, 17, 20] The observation that labeling of liver cytoplasmic high-molecular-weight RNA, including a fraction shown to stimulate amino acid incorporation *in vitro*,[12] was almost completely stopped after actinomycin administration permitted an inquiry into the longevity of liver messenger RNA. Considerable stability of at least most of this fraction was suggested by three series of experiments. First, purified liver cytoplasmic RNA from control and treated rats produced equal stimulatory effects in an *in vitro* protein-synthesizing system derived from *E. coli*.[11] Further, amino acid incorporation into liver protein *in vivo* was unaffected for as long as 40 hr following injection of the antibiotic. Finally, microsomal fractions from treated rats showed no impairment in *in vitro* amino acid incorporation studies. Guidice and Novelli[7] have observed that actinomycin leads to no effect, or only a slight one, on amino acid incorporation into regenerating liver. These workers have shown that the antibiotic does, however, interfere with the appearance of a new enzyme in regenerating liver. Inhibition of synthesis of an induced enzyme by actinomycin has also been shown by Greengard and Acs.[21] Very high levels of actinomycin have been demonstrated to cause diminished amino acid incorporation in deoxycholate purified ribosomes,[8, 22] and a decrease in polyribosome content in rat liver extracts.[8] However, studies to be presented elsewhere indicate that while such effects are demonstrable in cell-free preparations from rats treated with the very high levels of the antibiotic, amino acid incorporation is unimpaired[15] and polyribosomes are present (Revel, J. P., and M. Revel, unpublished) in liver not subjected to cell fractionation. Further, stimulatory activity of purified microsomal RNA from the livers of such animals approximates control levels, when assayed in the *E. coli* amino acid incorporating system.[15]

The effects of actinomycin D on liver nuclear RNA are of great interest. The reduction of isotope incorporation of 50 per cent approximated the diminution in stimulatory activity of the purified nuclear RNA in the *in vitro* amino acid incorporating system. However, there was no concomitant reduction of *in vivo* amino acid incorporation into liver nuclei of treated animals. This apparent paradox may be related to certain characteristics of nuclear RNA. It has been shown[12, 23, 24] that the nucleus contains more than 10 times the stimulatory activity per unit of RNA found in cytoplasm. Since nuclear protein synthesis per unit of RNA does not exceed cytoplasmic (Table 3), it seems unlikely that the level of messenger RNA limits protein synthesis in this cell fraction. Thus, it is reasonable that a reduction of even 50 per cent in nuclear messenger RNA might be unaccompanied by a diminution in nuclear protein synthesis. Recently, Georgiev *et al.*[25] have reported changes in the base composition of liver RNA from rats treated with actinomycin D. They have interpreted their data to indicate that the antibiotic preferentially inhibits the synthesis of liver ribosomal RNA. Our demonstration of a decrease in nuclear stimulatory activity approximating the reduction in nuclear labeling suggests that the level of actinomycin used by us does inhibit the synthesis of messenger RNA.

It is becoming increasingly apparent that the rate of turnover of messenger RNA varies widely in living forms. Thus, Levinthal *et al.*[6] estimated an average half-life of $2^1/_2$ min for total messenger RNA in a culture of *Bacillus subtilis* with a

generation time of 100 min. In marked contrast, messenger RNA in the reticulocyte appears to be stable throughout the entire protein-synthesizing period of the cell.[26, 27] Indeed, evidence is accumulating which indicates variations in the stability of different messengers within the same cell.[7, 28–30] A principal objective of the present work had been to measure the turnover of messenger RNA in rat liver cytoplasm. As noted earlier, the generation time of the *average* hepatocyte[31] from rats of the size used in our experiments has been estimated to be about one year.[1] On the other hand, RNA is renewed about 15 times more rapidly than DNA,[32] or approximately once in 25 days. Our experiments indicate that the bulk of the cytoplasmic messenger fraction is stable for at least 40 hr.[33] Although these data do not permit precise comparison, they do indicate that the rate of turnover of most rat liver cytoplasmic messenger is not appreciably more rapid than that of ribosomal RNA.

Summary.—Levels of actinomycin D which inhibit labeling of rat liver RNA have no effect on cytoplasmic amino acid incorporation *in vivo* or *in vitro*, or on the stimulatory activity of purified microsomal RNA in an *in vitro* amino acid incorporating system.

The collaboration of Dr. Murray L. Levin and the expert technical assistance of Miss Jacqueline Lareau and Mrs. Vera Redl are gratefully acknowledged.

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† Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

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[33] More exact estimates of turnover cannot be made *in vivo* with our present technique. The level of actinomycin D used by us not only inhibits cytoplasmic RNA labeling in rat liver, but also is lethal to the animal. It is apparent that in whole animal experiments measurements of turnover of messenger RNA are possible only in those tissues in which some decay of this fraction occurs before the antibiotic is fatal.

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*USE OF THERMAL DENATURATION STUDIES TO INVESTIGATE THE BASE SEQUENCE OF YEAST SERINE sRNA**

BY G. FELSENFELD AND G. L. CANTONI

LABORATORY OF MOLECULAR BIOLOGY, NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, AND LABORATORY OF CELLULAR PHARMACOLOGY, NATIONAL INSTITUTE OF MENTAL HEALTH

Communicated by Seymour S. Kety, March 16, 1964

An optical method has recently been developed for examining the composition of base-paired regions in DNA and RNA.[1] The method depends upon the measurement of the optical absorbance of the sample, at many wavelengths in the ultraviolet region, as it undergoes thermal denaturation. From the observed dependence of absorbance upon wavelength and temperature, together with some assumptions which will be reviewed below, it is possible to deduce the fraction of A-T or A-U pairs[2] and of G-C pairs denatured as a function of temperature. This provides information about the base composition of independently denaturing regions in the polynucleotide.

This optical method has already been applied to a sample of yeast sRNA heterogeneous with regard to amino acid acceptor activity;[1] the analysis of this material revealed that the longest ordered regions present had a very high G-C content, but it was not possible to determine whether the properties observed arose from intermolecular heterogeneity, or from the heterogeneity of independently denaturing regions within each molecule. In this paper the results of application of the optical denaturation analysis to a purified serine acceptor sRNA from baker's yeast are described. With very few assumptions, the analysis reveals the presence of independently denaturing regions containing about half of all the G-C pairs in the molecule and at most one A-U pair. Information about the base composition of other regions is also obtained.

A tentative base sequence model has recently been proposed[3] for purified serine sRNA from baker's yeast. This model represents an attempt to combine the results of the analysis of oligonucleotide sequence frequencies, obtained by specific enzymatic digestion of sRNA, with other data derived from physical and enzymatic studies of the secondary structure of sRNA. The principal features of this model (Fig. 1) are (*a*) a helical region formed by the doubling back of individual sRNA molecules upon themselves with about 25 contiguous bases of one limb specifically hydrogen-bonded to the 25 complementary bases of the other according to the