STUDIES ON THE FORMATION OF TOBACCO MOSAIC VIRUS RIBONUCLEIC ACID, VI. MODE OF DEGRADATION OF HOST RIBONUCLEIC ACID TO RIBONUCLEOSIDES AND THEIR CONVERSION TO RIBONUCLEOSIDE 5'-PHOSPHATES*

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The formation of TMV in the cell involves the synthesis of RNA and protein,[1] both of which are foreign to the host cell. Since the synthesis of considerable amounts of nucleic acid endowed with the properties of TMV-RNA is not the normal function of the cell, the prerequisite for its formation is the availability of the adequate amounts of building blocks, the purine and pyrimidine components, which can be obtained either by *de novo* synthesis using the cell (host) metabolic machinery or by the degradation of host RNA. Our findings show that the latter is the case. Following infection with TMV, the host RNA is rapidly degraded and its ribonucleoside moieties are utilized in the synthesis of TMV-RNA.[2, 3]

The present paper is concerned with the mode of degradation of host RNA to ribonucleosides and their conversion to ribonucleoside 5'-phosphates.

Experimental Procedures.—Preparation of microsomal extract: Tobacco plants, *Nicotiana tabacum* var. Turkish, used in these studies were grown in a greenhouse. They were 12–14 weeks old. The old bottom leaves, which were turning yellow, and the young top leaves were discarded. The remaining leaves were used for the preparation of microsomal extract.

Unless otherwise stated, all operations were conducted at 0–4°. Immediately after harvesting, the leaves were washed in ice-cold tap water, and excess moisture was removed by spreading them on a cheesecloth. Their midribs were quickly removed. Fifty gm of leaf blades were chilled for 1 hr and homogenized in a Waring Blendor with 100 ml of 0.5 M sucrose containing 0.01 M Tris-HCl buffer at pH 8.0 and 0.002 M $MgCl_2$ for 4 min at a low speed. The homogenate was filtered through four layers of cheesecloth which was previously rinsed with the medium. The filtrate was centrifuged for 20 min at 20,200 g in the refrigerated Servall centrifuge. The supernatant was centrifuged for 2 hr at 105,000 g in the no. 40 rotor of the Spinco model L ultracentrifuge. After draining the supernatant thoroughly, the pellets were suspended in 2 ml of 0.1 M sodium acetate at pH 6.0. The suspension was homogenized for 1 min in a glass homogenizer fitted with a Teflon pestle. The homogenate was centrifuged for 20 min at 12,100 g in the refrigerated Servall centrifuge. The residue was extracted twice with 2-ml portions of 0.1 M sodium acetate at pH 6.0. The combined supernatants, which will be referred to as "microsomal extract," were examined for ribonuclease, phosphatase, and nucleoside phosphokinase activities. The average protein and ribonucleic acid contents of six preparations were 965 μg and 153 μg, respectively, per 0.1 ml extract.

RNAase activity of "microsomal extract" as affected by pH: For the determination of RNAase activity at different pH values, citrate-phosphate buffer (McIlvain) was used. The reaction mixtures, consisting of 0.2 ml of microsomal extract and 0.8 ml of each of the buffers having different pH, were incubated at 37° for 15 min. At the end of the reaction time, the tubes were transferred to an ice bath and to each was added 0.3 ml of cold 2 N HCl with mixing. After 15 min at 0°, they were centrifuged for 20 min at 12,100 g in the refrigerated Servall centrifuge. The supernatants were drawn out with capillary pipettes. Two-tenths ml of each of the supernatants was made up to 3 ml with H_2O and their absorptions were measured at 260 mμ in a Beckman spectrophotometer. The results are presented in Figure 1.

Time course of degradation of microsomal RNA: Time factor of degradation of RNA present in the "microsomal extract" was investigated by incubating at 37° 5 ml of the extract (containing 7.2 mg RNA and 44.6 mg protein) in 0.1 M sodium acetate at pH 6.0. At intervals of time (Fig. 2) 0.1 ml was pipetted into test tubes, placed in an ice bath containing 0.4 ml of 0.1 M sodium acetate at pH 6.0 and 0.15 ml of 2 N HCl. After 15 min at 0°, the precipitates were removed by cen-

trifugation for 20 min at 12,100 *g* in the refrigerated Servall centrifuge. Two-tenths ml of each of the supernatants was made up to 3 ml with H_2O and their absorptions were measured at 260 mμ. The results are presented in Figure 2.

Phosphatase activity of the "microsomal extract": Four ml of the microsomal extract (protein, 37.6 mg; RNA, 5.15 mg; phosphorus, 0.506 mg) in 0.1 *M* sodium acetate at pH 6.0, was incubated at 37°. At intervals of time (Fig. 3) 0.2-ml aliquots were pipetted into test tubes, placed in an ice bath containing 0.2 ml of 20% TCA. After 15 min at 0°, the precipitates were removed by centrifugation for 20 min at 12,100 *g* in the refrigerated Servall centrifuge. The inorganic orthophosphate (Pi) present in the supernatants was determined as follows:[4] 0.2 ml of supernatant was diluted to 4.0 ml with H_2O and to this were added 0.33 ml of 70% $HClO_4$, 0.33 ml of 5% ammonium molybdate, and 0.17 ml of 0.2% aminonaphtholsulfonic acid. The final volume was made up to 5 ml with H_2O and mixed. After 5 min at room temperature, the absorption was measured at 700 mμ in the Beckman spectrophotometer. The results are presented in Figure 3.

Nature of the products formed by the combined actions of RNAase and phosphatase on the RNA present in the microsomal extract: To 4 ml of the microsomal extract in 0.1 *M* sodium acetate at pH 6.0, 3 drops of chloroform were added to prevent microbial growth. This was incubated at 37° for 24 hr. The heavy precipitate that was formed during incubation was removed by centrifugation for 15 min at 30,900 *g*. Aliquots of the supernatant were placed on Whatman no. 3 MM filter paper and dried in a current of air at room temperature. The chromatograms were developed in the isopropanol-water-ammonia solvent system[5] for 24 hr, dried at room temperature, and examined under ultraviolet light. There was no ultraviolet-absorbing material at the origin or in the region of nucleotides. However, there were two bands, one corresponding to the reference guanosine and the other corresponding to the reference mixture containing adenosine, cytidine, and uridine. The two bands were eluted by running H_2O through them. The substances present in the eluates were further identified using procedures described earlier.[6]

Removal of RNA in the "microsomal extract": Six ml of the microsomal extract (containing 53.3 mg protein and 9.6 mg RNA) in 0.1 *M* sodium acetate at pH 6.0 was incubated at 37° for 3 hr. At the end of the incubation period, the solution was transferred to a dialysis bag and dialyzed at 0° with continuous stirring for 24 hr against 2 changes of 2 liters of 0.1 *M* sodium acetate at pH 6.0. The contents of the dialysis bag were mixed and centrifuged at 17,300 *g* for 15 min in the refrigerated Servall centrifuge. The supernatant had an absorption maximum at 270 mμ in 0.02 *M* Tris-HCl buffer at pH 8.0 and contained 1.64 mg protein and 34 μg RNA per ml. This procedure renders about 98% of the RNA present in the microsomal extract dialyzable. As is evident from the experiment described below, this preparation, which will be referred to as "dialyzed preparation," possesses both the RNAase and phosphatase activities.

Action of "dialyzed preparation" on 2′, 3′-cyclic phosphates of adenosine, guanosine, cytidine, and uridine: Reaction mixtures containing one mg of each of the cyclic nucleotides dissolved in 0.2 ml of 0.1 *M* sodium acetate at pH 6.0, 0.2 ml of "dialyzed preparation" containing 328 μg protein, and a drop of chloroform, were incubated at 37° for 18 hr. Aliquots of the reaction mixtures were placed on Whatman no. 3 MM filter paper and developed in isopropanol-water-ammonia solvent system[5] as described above. The chromatograms were dried at room temperature, the ultraviolet-absorbing regions were marked with a pencil and eluted by running H_2O through them. The identity of the substances present in the eluates was established using procedures described earlier.[6] The results are presented in Table 1.

Nucleoside phosphokinase activity of microsomal extract: The enzyme, which catalyzes the synthesis of ribonucleoside 5′-phosphates by transference of phosphate from ATP to ribonucleosides, has been observed in the microsomal extract and will be called nucleoside phosphokinase.

The conversion of ribonucleosides to ribonucleotides was determined by incubating "microsomal extract" or partially purified enzyme with C^{14}-ribonucleosides, precipitating the proteins with HCl, isolating the acid-soluble nucleotides by paper chromatography, and measuring the radioactivities of the nucleotides present in the eluates. Incubation mixture (0.1 ml) contained 0.01 ml of C^{14}-ribonucleoside (0.01 *M*), 0.005 ml $MgCl_2$ (0.1 *M*), 0.01 ml of ATP (0.01 *M*), 0.025 ml of sodium acetate (1 *M*, pH 5.6), and 0.05 ml of enzyme (microsomal extract, 0.5 mg protein or partially purified enzyme, 16 μg protein). The mixture was incubated for 30 min at 37°. The tubes were transferred to an ice bath and then were added 6 μl of cold HCl (2 *N*) and 5 μl of unlabeled ribonucleoside 5′-phosphate (0.02 *M*). After 15 min at 0°, the precipitate was removed

by centrifugation for 20 min at 12,350 g in the refrigerated Servall centrifuge. An aliquot of the supernatant was placed on Whatman no. 3 MM filter paper and dried in a current of air at room temperature. The chromatograms were developed in the isopropanol-water-ammonia solvent system[5] for 24 hr at room temperature and examined under ultraviolet light. The ultraviolet-absorbing bands corresponding to the reference nucleotide markers were marked with a pencil, cut out, and eluted by running H_2O through them. Aliquots of the eluates were dried on metal planchets, and their radioactivities were measured in a gas-flow counter.

Effect of pH: The maximal activity of nucleoside phosphokinase was at pH 5.6 in sodium acetate buffer.

Fractionation of microsomal extract with ammonium sulfate: The microsomal extract was prepared as described above. Before the addition of ammonium sulfate, the protein content of the microsomal extract was brought to 8–10 mg per ml by diluting it with 0.1 M sodium acetate at pH 6.0. Forty-three ml of the diluted extract was fractionated by the addition of solid ammonium sulfate. The precipitates that were formed at 15, 30, 45, and 60% ammonium sulfate saturation were dissolved in 3 ml of 0.1 M sodium acetate at pH 6.0 and dialyzed at 4° for 2 hr with continuous stirring against 2 liters of 0.1 M sodium acetate at pH 6.0. The contents of dialysis bags were mixed and centrifuged at 31,550 g for 15 min in the refrigerated Servall centrifuge. The supernatants were tested for nucleoside phosphokinase activity as described above. All the fractions possessed the activity. However, the precipitate, formed at 45–60% ammonium sulfate saturation, had the highest activity. The results reported in this paper were obtained using this fraction.

Conversion of adenosine, guanosine, cytidine, and uridine to their corresponding ribonucleoside 5′-phosphates as affected by different phosphate donors: Comparison of ATP, ADP, and AMP as phosphate donors was made using the assay conditions described above. The results are presented in Table 2.

Position of phosphate in the nucleotide: The phosphate of ATP could be transferred to the 2′, or 3′ or 5′-carbon of the ribonucleoside. To obtain information on this point, reaction mixture (0.3 ml) containing 0.03 ml of C^{14}-ribonucleoside (0.01 M), 0.015 ml of $MgCl_2$ (0.1 M), 0.03 ml of ATP (0.01 M), 0.075 ml of sodium acetate (1 M, pH 5.6) and 0.15 ml of partially purified enzyme (48 μg protein) was incubated at 37° for 30 min. It was transferred to an ice bath and 15 μl of cold HCl (2 N) was added. After 15 min, the precipitate was removed by centrifugation for 20 min at 12,500 g in the refrigerated Servall centrifuge. The supernatant was subjected to chromatography as described above. Markers of nucleotides were run on the chromatograms side by side with the experimental sample. The band containing the nucleotide was cut out and eluted with water. The eluate was dried *in vacuo* over anhydrous $CaCl_2$. The dried residue was taken up in 0.1 M borate buffer at pH 8.6 containing 0.005 M $MgCl_2$. To this was added 100 μg of snake venom and it was incubated at 37° for 1 hr. Under these conditions all the nucleotide was converted to nucleoside, suggesting that the ribonucleosides were phosphorylated at the 5′-carbon by the nucleoside phosphokinase.

Analytical methods: Protein and RNA were determined spectrophotometrically.[7] In some experiments protein was determined according to the colorimetric procedure[8] and RNA according to the chromatographic procedure described earlier.[2] Total phosphorus content of the microsomal extract was determined according to the colorimetric procedure[4] with some modification in the digestion.[9]

Reagents: 1,2,4-Aminonaphtholsulfonic acid was purchased from Eastman Kodak Co. and purified according to the procedure of Fiske and Subba-Row.[10] Dried *Crotalus adamanteus* venom was purchased from Ross Allen's Reptile Institute, Silver Springs, Florida. ATP, ADP, AMP, GMP, CMP, and UMP were purchased from the Pabst Laboratories, Milwaukee, Wisconsin. C^{14}-labeled ribonucleosides and 2′,3′-cyclic phosphates of adenosine, guanosine, cytidine, and uridine were purchased from Schwarz BioResearch, Inc., Orangeburg, N. Y. These were further purified by paper chromatography in the isopropanol-water-ammonia solvent system.[5]

Results.—Properties of RNAase present in the microsomal extract of tobacco leaf: pH optimum of RNAase present in the microsomal extract is 5.8–6.0 (Fig. 1). In this respect this enzyme differs from the RNAase, isolated earlier from tobacco leaves, which has a pH optimum of 5.1.[11]

Exhaustive digestion of microsomal RNA by the RNAase resulted in its com-

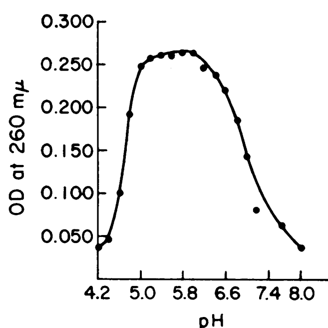


FIG. 1.—Effect of pH on the activity of ribonuclease, associated with the microsomes of tobacco leaf.

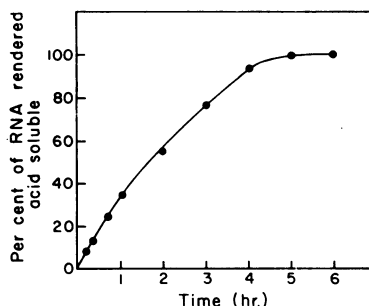


FIG. 2.—Time course of degradation of RNA by the ribonuclease, associated with the microsomes of tobacco leaf.

plete conversion to acid-soluble products (Fig. 2). Furthermore, the combined actions of RNAase and phosphatase resulted in the conversion of microsomal RNA to ribonucleosides (Fig. 3 and also see section on the nature of degradation products). In order to determine the mode of degradation of RNA by the RNAase associated with the microsomes, the action of RNA-free "dialyzed preparation" on 2′,3′-cyclic nucleotides was studied. The results presented in Table 1 show that both the purine and pyrimidine cyclic nucleotides were hydrolyzed to their corresponding nucleoside 3′-phosphates, which were further converted to ribonucleosides by the phosphatase present in the "dialyzed preparation." From these results it can be concluded that the RNAase, associated with the microsomes, acts on RNA in two stages. First is an intramolecular transphosphorylation to form ribonucleoside 2′,3′-cyclic phosphates, and the second is a hydrolysis of purine and pyrimidine cyclic nucleotides to their corresponding nucleoside 3′-phosphates. Thus, the RNAase associated with the microsomes differs from the RNAase, isolated earlier[6] in hydrolyzing both the purine and pyrimidine cyclic nucleotides to their corresponding nucleoside 3′-phosphates. The RNAase associated with the microsomes of tobacco leaf appears to be similar to the one present in the nucleoprotein particles of *Escherichia coli* in its hydrolytic properties. However, the RNAase present in the nucleoprotein particles of *Escherichia coli* is in a latent form, and its activity is not revealed until the particles are treated with EDTA or urea.[12, 13] In the present case no such treatment was necessary, and hence the RNAase might be loosely bound to the microsomes of the tobacco leaf.

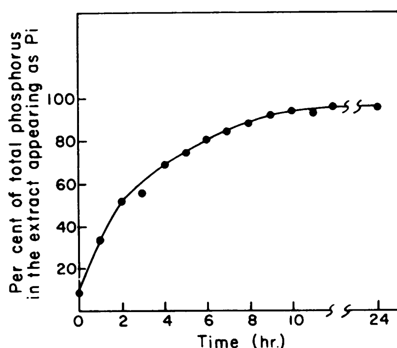


FIG. 3.—Time course of dephosphorylation by the phosphatase, associated with the microsomes of tobacco leaf, as measured by the appearance of orthophosphate.

These studies revealed the existence of two types of RNAases in the tobacco leaf cells and these will be called TLRNAase-I[6, 11] and TLRNAase-II (enzyme described in this paper). The numbering is done in the order of their discovery.

Properties of nucleoside phosphokinase present in the microsomal extract of tobacco leaf:

TABLE 1

HYDROLYSIS OF 2′,3′-CYCLIC PHOSPHATES OF ADENOSINE, GUANOSINE, CYTIDINE, AND URIDINE BY THE DIALYZED PREPARATION

Nucleotide	Per cent of Total Cyclic Nucleotide Hydrolyzed to Corresponding: Ribonucleoside	Ribonucleoside 3′-phosphate	Ribonucleoside 2′,3′-cyclic phosphate
Adenosine 2′,3′-cyclic phosphate	87	13	
Guanosine 2′,3′-cyclic phosphate	92	8	
Cytidine 2′,3′-cyclic phosphate	48	34	18
Uridine 2′,3′-cyclic phosphate	70	30	

Experimental details are described in the text.

pH optimum of nucleoside phosphokinase present in the microsomal extract is 5.6. Results presented in Table 2 show that it catalyzes the phosphorylation of both purine and pyrimidine nucleosides by the transfer of phosphate of ATP or ADP or AMP. The efficiency of phosphate transfer reaction was dependent on the nature of phosphate donor. Under the experimental conditions ADP was found to be the best phosphate donor. However, *in vivo* ATP might be the preferred phosphate donor. The nature of nucleotide synthesized in each case was nucleoside 5′-phosphate (see section on the position of phosphate in the nucleotide). The enzyme preparation used in these studies might consist of a single enzyme or a family of enzymes, each catalyzing the phosphorylation of one type of nucleoside. The answer to this question will have to await further purification.

TABLE 2

CONVERSION OF ADENOSINE, GUANOSINE, CYTIDINE, AND URIDINE BY NUCLEOSIDE PHOSPHOKINASE TO THEIR CORRESPONDING RIBONUCLEOSIDE 5′-PHOSPHATES AS AFFECTED BY DIFFERENT PHOSPHATE DONORS

Ribonucleoside	Phosphate donor	mμMoles of Nucleotide Formed (per mg of enzyme) Microsomal extract	Partially purified enzyme
	ATP	8.9	228.9
Adenosine-8-C^{14}	ADP	19.9	523.7
	AMP	17.2	396.6
	ATP	2.0	51.3
Guanosine-8-C^{14}	ADP	6.9	267.9
	AMP	7.0	244.2
	ATP	6.3	123.2
Cytidine-2-C^{14}	ADP	14.7	355.5
	AMP	14.5	327.7
	ATP	3.9	141.3
Uridine-2-C^{14}	ADP	13.8	398.9
	AMP	14.8	363.2

Conditions of standard assay.

Enzymes capable of catalyzing the transfer of phosphate to nucleosides were reported to be present in various plant extracts and these were designated as "nucleoside phosphotransferases."[14–16] Because of the differences in the experimental techniques used by us and the other workers, it is difficult to state whether the enzyme system associated with the microsomes of tobacco leaf is the same as the ones isolated from wheat and carrots, even though both the systems transfer phosphate only to the 5′-carbon of all the nucleosides so far tested.

Discussion.—As shown in this paper, the microsomal extract of tobacco leaves

contains an RNAase which degrades RNA to ribonucleoside 3′-phosphates, a phosphatase which dephosphorylates nucleotides to ribonucleosides, and a nucleoside phosphokinase which catalyzes the transfer of phosphate of ATP to ribonucleosides converting them to ribonucleoside 5′-phosphates. It was shown earlier (see introduction) that host RNA is rapidly degraded following infection with TMV and its ribonucleoside moieties are utilized in the synthesis of TMV-RNA.[2, 3] Thus, the existence of enzymes that degrade RNA to ribonucleosides and a nucleoside phosphokinase which phosphorylates them suggests a pathway for the biosynthesis of TMV-RNA in which ribonucleosides derived from host RNA can serve as intermediates.[3]

Concerning the quantitative changes in the levels of these enzymes following infection with TMV, the activity of TLRNAase-I increases. The extent of TMV formation parallels the TLRNAase-I activity.[17] Similar increases and a correlation between its activity and virus formation were also reported in bean leaves (*Phaseolus vulgaris* L. var. Pinto and Black Valentine) infected with pod mottle virus.[18] As regards the changes in the activity of TLRNAase-II following infection with TMV, out of six experiments performed, three showed 100 per cent increase, two showed 40 per cent increase, and one showed no increase. Thus, the evidence in support of the increase in the activity of TLRNAase-II following infection with TMV is not unequivocal.[19] The quantitative changes in the levels of nucleoside phosphokinase activity following infection with TMV were not determined.

Studies relating to the conversion of ribonucleoside 5′-phosphates to ribonucleoside 5′-triphosphates and the isolation and properties of the enzyme involved in the synthesis of TMV-RNA will be described at a later date.

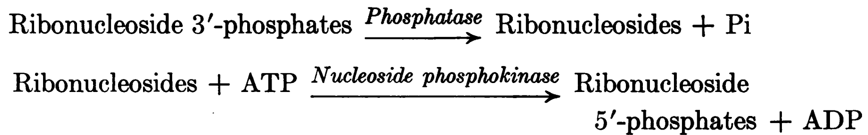
Summary.—Partial purification and properties of tobacco leaf enzymes, involved in the degradation of cellular RNA to ribonucleosides and their conversion to ribonucleoside 5′-phosphates, have been described.

Ribonuclease (TLRNAase-II) associated with the microsomes has a pH optimum of 5.8–6.0 in citrate-phosphate buffer. The degradation of RNA proceeds via transphosphorylation followed by hydrolysis of both the purine and pyrimidine 2′,3′-cyclic nucleotides to their corresponding nucleoside 3′-phosphates. The action of TLRNAase-II differs from that of the ribonuclease (TLRNAase-I), isolated earlier from tobacco leaves, which hydrolyzes only purine 2′,3′-cyclic nucleotides to their corresponding nucleoside 3′-phosphates. TLRNAase-II is loosely bound to the microsomes.

Phosphatase, associated with the microsomes, is a nonspecific type and is similar to the one isolated earlier[20] from tobacco leaves. It hydrolyzes ribonucleoside 3′-phosphates, formed as a result of the degradation of RNA, to ribonucleosides.

Nucleoside phosphokinase, associated with the microsomes, has a pH optimum of 5.6 in sodium acetate buffer. It catalyzes the formation of ribonucleoside 5′-phosphates by the transfer of phosphate from ATP or ADP or AMP to ribonucleosides.

Mode of degradation of cellular RNA to ribonucleosides and their conversion to ribonucleoside 5′-phosphates is summarized below:



These reactions provide a pathway for the biosynthesis of TMV-RNA in which ribonucleosides derived from host RNA can serve as intermediates.

Abbreviations: TMV, tobacco mosaic virus; TMV-RNA, tobacco mosaic virus ribonucleic acid; Pi, inorganic orthophosphate; ATP, ADP, and AMP, adenosine tri-, di-, and 5′-monophosphate; GMP, CMP, and UMP, 5′-phosphate of guanosine, cytidine, and uridine; TCA, trichloroacetic acid; TLRNAase, tobacco leaf ribonuclease; EDTA, ethylene-diaminetetraacetate.

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[1] Reddi, K. K., these Proceedings, **51**, 619 (1964).
[2] *Ibid.*, **50**, 75 (1963).
[3] *Ibid.*, 419 (1963).
[4] King, E. J., *Biochem. J.*, **26**, 292 (1932).
[5] Markham, R., and J. D. Smith, *Biochem. J.*, **52**, 558 (1952).
[6] Reddi, K. K., *Biochim. Biophys. Acta*, **28**, 386 (1958).
[7] Warburg, O., and W. Christian, *Biochem. Z.*, **310**, 384 (1941).
[8] Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
[9] Reddi, K. K., *Biochim. Biophys. Acta*, **24**, 238 (1951).
[10] Fiske, C. H., and Y. Subba-Row, *J. Biol. Chem.*, **66**, 375 (1925).
[11] Frisch-Niggemeyer, W., and K. K. Reddi, *Biochim. Biophys. Acta*, **26**, 40 (1957).
[12] Elson, D., *Biochim. Biophys. Acta*, **36**, 372 (1959).
[13] Spahr, P. F., and B. R. Hollingnorth, *J. Biol. Chem.*, **236**, 823 (1961).
[14] Brawerman, G., and E. Chargaff, *Biochim. Biophys. Acta*, **15**, 549 (1954).
[15] Tunis, M., and E. Chargaff, *Biochim. Biophys. Acta*, **37**, 257 (1960).
[16] *Ibid.*, 267 (1960).
[17] Reddi, K. K., *Biochim. Biophys. Acta*, **33**, 164 (1959).
[18] Diener, T. O., *Virology*, **14**, 177 (1961).
[19] Reddi, K. K., and Lj. Mauser, unpublished results.
[20] Reddi, K. K., *Biochim. Biophys. Acta*, **36**, 132 (1959).

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*ACTINOMYCIN D INHIBITION OF MICROSOMAL-BOUND HYDROXYPROLINE FORMATION IN RABBIT EMBRYO SKIN IN VITRO**

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It has been demonstrated both *in vivo* and *in vitro* that collagen synthesis takes place via the microsomal pathway in a manner similar to other proteins. This was shown in chick embryo,[1, 2] in rabbit skin,[3] and in bone fragments.[4] Recently it was reported that collagen is synthesized on ribosomes linked together by messenger RNA (mRNA) in aggregates called polyribosomes.[5] Thus far, except