Rabinowitz, J. L., and Gurin, S. (1953), *J. Am. Chem. Soc. 75*, 5758.
Scannell, J. P., Crestfield, A. M., and Allen, F. W. (1959), *Biochim. Biophys. Acta 32*, 409.
Shugar, D., and Fox, J. J. (1952), *Biochim. Biophys. Acta 9*, 199.
Spector, L. B., and Keller, E. B. (1958), *J. Biol. Chem. 232*, 185.
Szer, W., and Shugar, D. (1961), *Acta Biochim. Polon. 8*, 235.
Witzel, H. (1960), *Ann. 635*, 182, 191.

Effects of Light in the Presence of Iron Salts on Ribonucleic Acid and Model Compounds*

B. Singer and H. Fraenkel-Conrat

ABSTRACT: Under the influence of visible light in the presence of small amounts of ferric iron, tobacco mosaic virus ribonucleic acid becomes degraded and loses infectivity at 0° and neutral *p*H. Ferrous iron has the same catalytic effect at a somewhat lesser initial rate. The same reactions seem to proceed very much more slowly in the dark. Experiments with nucleotides and other model compounds show that bases are destroyed and released from glycosidic linkage under the influence of iron plus visible light. It appears probable that these are the primary events also with ribonucleic acid, and the diester bond breakage occurs secondarily. Hydrogen peroxide potentiates the photoeffects, particularly of Fe^{2+}, on both tobacco mosaic virus ribonucleic acid and model compounds, and catalase suppresses them.

Previous studies of the interaction of TMV-RNA[1] with certain metal ions have shown that some metal complexes were of decreased infectivity while others were fully infectious. Certain metals of both types rendered the RNA more enzyme resistant. Removal of the inactivating metals restored the original infectivity of the RNA (Singer and Fraenkel-Conrat, 1962).

Later studies in which radioactive metals were used led to the conclusion that alkali earth metals were bound primarily to the phosphate groups, while silver and mercury were bound each to a specific site on two of the four bases. Greater amounts of bound silver and mercury were displaced by trivalent metals (In^{3+}, Al^{3+}, Fe^{3+}), although the use of ^{59}Fe indicated that the RNA bound Fe^{3+} less firmly than Ag^{+}, Hg^{2+}, or Ca^{2+} (Singer, 1964).

The effect of iron salts on the infectivity of TMV-RNA differs from those of other metals in that iron causes progressive and irreversible inactivation. Upon exposure of the iron complexes (Fe^{3+} and Fe^{2+}) to visible light this inactivation, which is not reversed by removal of the metal, proceeds very much more rapidly. The present report deals with the mechanism of this photosensitization of RNA by iron and the effect of EDTA and hydrogen peroxide on this system, as studied with both the intact RNA and its small molecular components.

Methods and Materials

TMV-RNA was prepared as previously described (Fraenkel-Conrat *et al.*, 1961). ^{14}C-Labeled TMV was isolated by the method of Sugiyama and Fraenkel-Conrat (1963). $FeCl_3$ and $FeSO_4$ were analytical grade reagents. Solutions of each in H_2O were prepared daily.

The RNA was diluted to 100 μg/ml in glass-distilled H_2O and kept at 0°. Amounts of iron ranging from 6400 to 3 moles per mole TMV-RNA (or from about 1 to 0.0005 mole per mole nucleotide) were added to 1 ml of the diluted RNA. The reaction mixtures were kept in an ice bath either in the dark or under the light from a Hanovia fluoro lamp (31300, 125 w) placed 10 cm from the solution (behind 0.5 cm of a 2% $CuSO_4$ solution serving as a filter for wavelengths below 3500 A). Aliquots were taken at various times and assayed for infectivity (Fraenkel-Conrat and Singer, 1959). They were applied directly to test plants after suitable dilution with either 0.1 M (*p*H 7) sodium phosphate buffer, or with the same buffer containing EDTA to complex the metal. More frequently the aliquots were reconstituted before assay (Fraenkel-Conrat and Singer, 1959).

* From the Virus Laboratary, University of California, Berkeley. *Received September 29, 1964.* This investigation was aided by a grant (G 24236) from the National Science Foundation.

[1] Abbreviation used in this work: TMV, tobacco mosaic virus.

Occasionally EDTA was added to RNA after reaction with iron, and the metal-free RNA was precipitated at 0° with 2.5 volumes of ethanol, prior to assay or chemical or physicochemical tests.

Conditions for study of model compounds (bases, nucleosides, nucleotides and dinucleoside monophosphates) were variable with respect to concentration and *p*H and are given on the respective tables.

Identification of products formed with the simple models was by one-dimensional chromatography on Whatman No. 1 paper in either 86% butanol–H_2O or in 75 ml ethanol–30 ml M (*p*H 7.5) ammonium acetate followed by elution in 0.1 N HCl. Products from the reaction of dinucleoside monophosphates and iron were separated two-dimensionally according to Felix *et al.* (1960).

Determination of free bases split from ^{14}C-labeled RNA was by repeated chromatography. After reaction with iron, the RNA was precipitated with ethanol and the supernatant, with added bases as markers, was chromatographed in 86% butanol–H_2O to achieve separation of most of the iron from the bases. The entire ultraviolet-absorbing area was eluted with 0.1 N HCl, lyophilized, and chromatographed in 65 ml 2-propanol–35 ml 2 N HCl. The well-separated base areas were individually rechromatographed in 86% butanol–H_2O, and finally rechromatographed in 70 ml ethanol–35 ml M (*p*H 7.5) ammonium acetate to separate them from any possible contaminating nucleosides or nucleotides.

Results

Illumination of TMV-RNA containing 3–6400 equivalents of either ferrous or ferric iron at 0° caused progressive inactivation, the rate being dependent on the iron concentration (Table I). Similar reaction mixtures stored in the dark at 0° showed very slow losses of infectivity, i.e., 3–4 orders of magnitude slower than upon illumination and detectable only at the highest iron level. Unexpectedly, inactivation in the dark with the highest amounts of iron seemed to proceed faster at −60° than at 0°. The addition of EDTA markedly suppressed this rate and no continuing loss of infectivity was observed after reconstituting virus from the RNA and added protein in the dark. Illumination of TMV-RNA without added iron also caused a slow infectivity loss which was further retarded by the addition of EDTA (see Figure 1).

The initial rate of inactivation was slower in the presence of Fe^{2+} than of Fe^{3+} but this difference disappeared with extensive inactivation. However, a strict comparison of the effectiveness of Fe^{2+} and Fe^{3+} is impossible since Fe^{2+} appears to be most effective about *p*H 6–7, but Fe^{3+} at *p*H values below 4 which are per se harmful to RNA. Most experiments were performed without *p*H adjustment by adding the acid $FeCl_3$ or the neutral $FeSO_4$ solution to the RNA, but these additions had no noticeable effect on the *p*H of the reaction mixtures which were generally of *p*H 6–7.5 at levels of 1:20 or less of iron per nucleotide.

The sedimentation pattern of TMV-RNA was altered as inactivation proceeded, the typical 30 S component of TMV-RNA diminishing and disappearing within an hour with 300 iron atoms/mole. At the very early stages of the reaction, however, there appeared to be little decrease in 30 S material when the infectivity loss suggested of the order of one to two lethal events (37–14% remaining infectivity) (Table II). Also, prolonged treatment of the RNA with much

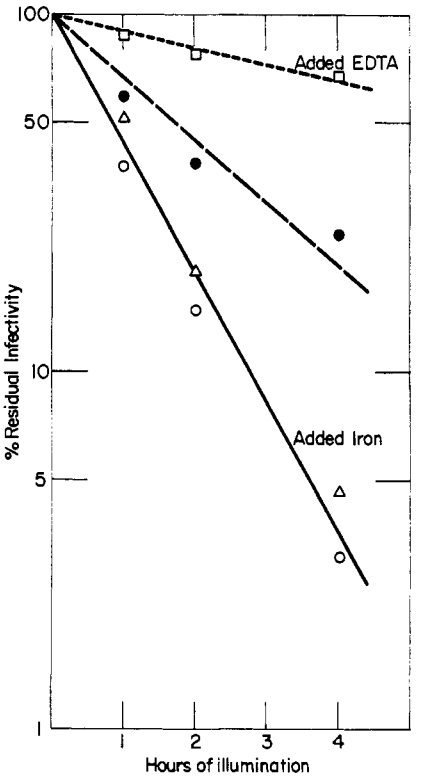

FIGURE 1: Light sensitivity of TMV-RNA. The photoinactivation rate of purified TMV-RNA (●–●–●) is increased by the addition of 3 equivalents of Fe^{2+} (△) or Fe^{3+} (○) per mole of RNA, and decreased by the addition of 10^{-3} M EDTA (□).

Fe^{2+} or Fe^{3+} in the dark gave loss of infectivity in excess of loss in 30 S material. This discrepancy suggests that phosphodiester bond breakage was not the primary result of the photoreaction nor the primary cause for inactivation. To study the nature of this breakage samples of ^{14}C-labeled Fe-photoinactivated TMV-RNA were subjected to degradation by alkali and by snake venom phosphodiesterase by the methods recently described (Sugiyama and Fraenkel-Conrat, 1961; Sugiyama and Fraenkel-Conrat, 1963; Singer and Fraenkel-Conrat, 1963) (Table III). The numbers of nucleoside 3′5′-diphosphates found in such digests did not accord with the numbers of nucleoside ends in a manner indicative of simple 3′- or 5′-phosphoester bond breakages, suggesting that the reaction was com-

TABLE I: Inactivation of TMV-RNA upon Illumination in Presence of Iron Salts.[a]

Moles Iron/ Mole TMV-RNA	Per Cent Infectivity[b] in (illumination time):						
	1 min	5 min	15 min	30 min	60 min	2 hr	4 hr
3 Fe^{3+}					38	15	3
30 Fe^{3+}			87		30	15	
300 Fe^{3+}		33	11	1.1	0.16	< 0.006	
6400 Fe^{3+}	18	3	0.03	0			
3 Fe^{2+}					52	19	4.6
30 Fe^{2+}			67		14	4	
300 Fe^{2+}		80	20	8	0.3	< 0.003	
6400 Fe^{2+}	119	63	18	14			
None			80		59	38	24
None, plus 10^{-3} M EDTA					89	77	68

[a] RNA (100 μg/ml) in the presence of varying amounts of $FeCl_3$ or $FeSO_4$ at 0° (1 ml in 5-ml beakers) was illuminated 10 cm from the light source. Aliquots (0.1 ml) were taken for reconstitution. The data represent averages of two to ten experiments, each time period assayed on twelve to thirty-six half-leaves. For reasons not understood, the reproducibility of the rate of inactivation was quite variable from one experiment to the next. [b] Calculated as percentage of nonilluminated control samples containing the same amount of iron, held in the dark at 0° for the duration of the experiment, and reconstituted together with the illuminated samples. The controls for Fe^{3+}-containing samples showed no loss of infectivity when compared with iron-free RNA, but when 6400 moles Fe^{2+} were present, the control reconstitutions averaged 15% of non-Fe^{2+}-containing reconstitutions.

TABLE II: Effect of Iron on Infectivity and Sedimentation Behavior of TMV-RNA.[a]

Infectivity (compared to untreated control) (%)	$s_{20,w}$ = 30 Component (compared to untreated control) (%)	Lethal Events/ Mole (approximate range from infectivity)	Nonfragmented Molecules (average from sedimentation) (%)
68[b]	110		
57	64		
53[c]	81[c]	0.5–1	89
42	105		
31	87		
22[c]	72[c]		
19	51		
15[b]	74	1.5–2.5	68
10	73		
6	21		
2	22	3–4	22

[a] The amount of iron and the time of illumination were varied to obtain a range of infectivities. Each line represents a separate experimental sample. [b] Fe^{3+} inactivation. All others were Fe^{2+} treated. [c] Samples which were not illuminated, but were treated with much Fe^{2+} (650 moles/mole RNA) in the dark for 6 and 24 hours.

plex. It appeared that a splitting off of purine or pyrimidine bases might account for some of the complexities, and this hypothesis was tested by the customary isotope dilution technique with ^{14}C-labeled RNA and added unlabeled bases. Small amounts of all four bases were found in the 70% alcohol supernatant of Fe-illuminated RNA, but the total amounts were distinctly less than the number of lethal events, as judged from inactivation (Table IV). Since model experiments, to be discussed later, showed that iron caused both the release and destruction of bases, this discrepancy is not surprising.

To test for destruction of bases in illuminated TMV-RNA, changes in its ultraviolet absorption spectrum were sought. After 18 hours of illumination at 5° with 3000 equivalents of Fe^{3+} per mole no loss of absorbancy was detected. However, it appeared possible that this was the result of several compensating changes, such as the change in absorbance of the iron salt upon illumination, the hyperchromicity of released bases or oligonucleotides, and the destruction of bases. Therefore, to ascertain the extent of the last, Fe^{3+}-illuminated and control RNA samples were hydrolyzed with alkali, the iron hydroxide was removed by centrifugation, and the absorbance of the completely hyperchromed nucleotide mixtures was compared. Under these conditions and after illumination at 30° for 6 hours with 3000 equivalents of Fe^{3+}, 15% of the absorbancy was destroyed compared to the sample illuminated without added iron. Chromatographic separation of the four 3′(2′)-nucleotides indicated that the loss was mainly caused by destruction of pyrimidines.

TABLE III: Terminal Nucleosides Found with Alkali and Snake Venom Phosphodiesterase Degradation of Photoinactivated TMV-RNA.

Conditions of Inactivation with $FeCl_3$[a]	Digestion	Moles Nucleoside/Mole RNA[b]				
		A	G	U	C	Total
650 equiv/mole RNA, 0°, 1 hr.	KOH	1.5	0.27	0.19	0.06	2.0
400 equiv/mole RNA, 0°, 1 hr	KOH	0.9	0.2	0.04	0.24	1.3
None (average of many experiments[c])	KOH	1.1	0.11	0.18	0.1	1.5
800 equiv/mole RNA, 0°, 1 hr	SV-PDE[d]	1.1	0.3	0.3	0.2	1.9
650 equiv/mole RNA, 0°, 1 hr	SV-PDE	3.2	0.4	0.4	0.3	4.3
None (average of many experiments[e])	SV-PDE	2.2	0.55	0.4	0.65	3.8

[a] All samples were inactivated to 1–5% of the control, which represents 3–5 lethal events. [b] Breaks of the 5′-phosphoester bond would produce additional nucleosides upon SV-PDE digestion. Breaks of the 3′-phosphoester bond would produce additional nucleosides upon KOH digestion. The appearance of new nucleosides, under both conditions, should be accompanied by an equal number of nucleoside 3′,5′-diphosphates. The amounts of the latter were erratic and not interpretable in the above experiments. [c] Sugiyama and Fraenkel-Conrat (1963). [d] SV-PDE = snake venom phosphodiesterase. [e] Singer and Fraenkel-Conrat (1963).

TABLE IV: Liberated Bases Found after Fe-Photoinactivation of ^{14}C-labeled TMV-RNA.

Conditions of Fe-Light Treatment	Infectivity (% of untreated control)	Moles Base Found/Mole RNA[a]				
		A	G	U	C	Total
600 equiv Fe^{3+}/mole RNA, 0°, 1 hr	2 (4 lethal events)	0.4	0.4	0.11	0.14	1.05
6000 equiv Fe^{2+}/mole RNA, 0°, 1 hr	0 (>12 lethal events)	1.5	0.4	1.4	1.4	4.7
6000 equiv Fe^{2+}/mole RNA, 0° 3 hrs	0 (>12 lethal events)	2.5	0.7	2.6	2.3	8.1

[a] See Methods and Materials for technique of isolation of ^{14}C-labeled bases from 70% alcohol supernatant of iron-treated RNA.

The Fe^{2+}- and the Fe^{3+}-catalyzed photoinactivation of TMV-RNA proceeded more rapidly than usual in both N_2- and O_2-saturated solutions, and the comparative levels of inactivation differed in unpredictable manner from one experiment to the other, possibly owing to impurities in the gases.

A survey of the effects of several other mono-, di-, and trivalent metals (Ag^+, Hg^{2+}, Ni^{2+}, Cu^{2+}, In^{3+}, Al^{3+}, Cr^{3+}) indicated that these neither sensitized RNA toward light nor caused inactivation in the dark at a ratio of 300 moles of metal per mole of RNA, with Ni^{2+} and Cu^{2+} showing some protecting action, compared to the metal-free illuminated control, in a single experiment.

In attempts to elucidate the mechanism of iron photosensitization of TMV-RNA, the effect of hydrogen peroxide on the reaction of TMV-RNA with iron was studied. The presence of 0.01 or 0.1 μg/ml of hydrogen peroxide alone caused variable inactivation upon illumination but no effect was observed in the dark. This inactivation was prevented by the addition of EDTA and is attributed to contaminating traces of metals. As was expected, the inactivation rate of TMV-RNA was consistently found to be markedly greater, in the light as well as in the dark, if both Fe^{2+} and H_2O_2 were present. The action of Fe^{3+} was not similarly potentiated by H_2O_2 (Table V).

Since hydrogen peroxide potentiated iron in its ability to photosensitize TMV-RNA, the possibility was considered that H_2O_2 represented an intermediate in the photoreaction with iron and water. Since catalase decomposes peroxides and thus prevents the effect of added H_2O_2, the effect of this enzyme on the iron reaction was investigated. The addition of catalase decreased the rate of inactivation of TMV-RNA by Fe^{2+} and light, while showing a doubtful effect on the Fe^{3+}-catalyzed reaction (Table VI) and none on the photoinactivation of TMV-RNA without added iron.

Attempts to determine the action spectrum of the Fe^{3+}-catalyzed inactivation of TMV-RNA were not successful. No significant loss of infectivity was observed upon illumination (up to 15 minutes) of the reaction

TABLE V: Effect of H_2O_2 Combined with Iron on the Inactivation of TMV-RNA.

Conditions	Per Cent Infectivity (of untreated control)[a]					
	5 min light	15 min light	1 hr light	30 min dark	1 hr dark	2 hr dark
0.1 μg/ml H_2O_2[a]	48	20	4	80		86
0.01 μg/ml H_2O_2[a]	91	76	50	99		120
30 moles Fe^{2+}		49	24		51	
30 moles Fe^{2+} + 0.1 μg/ml H_2O_2				0.17		
30 moles Fe^{2+} + 0.01 μg/ml H_2O_2		11	4		12	
30 moles Fe^{2+} + 0.001 μg/ml H_2O_2		55	9		35	
300 moles Fe^{2+}	85	21	0.3	80	35	
300 moles Fe^{2+} + 0.1 μg/ml H_2O_2[b]	0	0				
300 moles Fe^{2+} + 0.01 μg/ml H_2O_2	0.1	0.05	0		0.35	
300 moles Fe^{3+}	41	2	0.4			
300 moles Fe^{3+} + 0.1 μg/ml H_2O_2	29	1	0.12	82		

[a] Representative experiments. The effect of H_2O_2 alone was in several other experiments appreciably less pronounced, and is attributed to the presence of varying traces of iron. [b] No infectivity was detected even on immediate reconstitution without illumination.

TABLE VI: Effect of Catalase on the Photoinactivation of Fe-treated TMV-RNA.

Conditions and Length of Illumination	Per Cent of Infectivity		
	15 min	30 min	60 min
650 moles Fe^{2+}	30	14	4
650 moles Fe^{2+} + 2 μg/ml catalase	100	64	56
650 moles Fe^{3+}	70		51
650 moles Fe^{3+} + 2 μg/ml catalase	79		63

mixture containing one iron per nucleotide at the selected wavelengths of 5780, 5461, 4358, 4045, and 3660 A (the main output wavelengths of the lamp usually used) by means of a General Electric 1185-C-3 lamp used in connection with a Beckman DU spectrophotometer. While the longer light path (1 cm) and the lesser light intensity available at any one wavelength might account for a lesser inactivation rate under these as compared to standard conditions, the observed level of resistance of the RNA to selected wavelengths suggests that a cooperative effect requiring more than one range of the spectrum is required for the reaction under investigation.

Model Experiments

In view of the complexity of the effects of the iron-catalyzed photoinactivation of TMV-RNA, the indicated course was to study this reaction with simple model compounds. These experiments were performed with equimolar amounts of iron at 25–35° and at various *p*H values, and either at concentrations suitable for direct spectrophotometry or at 200-fold higher concentration. Most of the experiments were performed without *p*H adjustment using $FeCl_3$ (*p*H of 2–3) and $FeSO_4$ (*p*H 6). Adjustment of both types of reaction mixtures over the range of *p*H 2–8 showed that Fe^{3+} became less effective at *p*H 5–8, possibly because of the insolubility of the hydroxide at the concentrations used. Fe^{2+} was ineffective at *p*H 2–5 where it does not tend to become oxidized, which suggests that the active agent was always in a higher oxidation state. Both iron salts showed progressive *p*H drops at *p*H values higher than 5.

Illumination of the bases in presence of Fe^{3+} (*p*H 2–3) caused marked changes in the ultraviolet absorption spectra. Uracil lost its absorption and the guanine spectrum became low and uncharacteristic within 15 minutes, while cytosine lost half its absorption in 30

TABLE VII: Effect of Light on Nucleotides in the Presence of Iron.[a]

	$FeCl_3$ (*p*H 2–3)				$FeSO_4$ (*p*H 5–6)			
	Decrease of OD_{max} 30 min	Formation of Base 30 min	Decrease of OD_{max} 3 hrs	Formation of Base 3 hrs	Formation of Base 30 min	Formation of Base 3 hrs	Decrease[b] of OD_{max} 30 hrs	Formation of Base 30 hrs
Nucleotide	(%)	(%)[c]	(%)	(%)[c]	(%)[c]	(%)[c]	(%)	(%)[c]
pA	14	8	16	15	9	18	16	29
pG	15	5	16	6	2	5		6
pU	33	9	34	17	8	23	18	37
pC	25	6	36	19	10	21	15	32

[a] Solutions of 5′-nucleotides (10^{-4} M) were illuminated, for the times indicated, with equimolar amounts of iron salts at 25–35°. After the absorption spectra (which remained typical for each base constituent) had been plotted, the samples were concentrated *in vacuo* and chromatographed on washed Whatman No. 1 paper in 75 ml ethanol + 30 ml 1 M (*p*H 7.5) ammonium acetate. The products were eluted and their spectra were plotted. Rechromatography in 86% butanol–H_2O identified the products of illumination as bases, rather than as nucleosides or a mixture. The values given are the averages of two to four separate experiments. [b] Absorbancy increased at first upon illumination of Fe^{2+}-RNA reaction mixtures. Since this was the case also for the iron salt alone, changes in absorbancy are not easy to interpret, and the small changes observed after short reaction periods are not listed on the table. [c] Of original nucleotide.

minutes, and adenine less than 10%. Much lesser decreases in absorbancy were noted with Fe^{2+} (about 76% for uracil in 90 minutes).

When the nucleotides were studied in the same manner, similar but somewhat slower losses in absorbance occurred with Fe^{3+} in the light, and little if any change in absorbance with Fe^{2+} except after many hours of illumination (Table VII). However, the absorbancy of nucleotide-free solutions of similar amounts of Fe^{3+} and particularly of Fe^{2+} changed upon illumination, thus rendering minor changes in the absorbancy of the bases difficult to determine. Upon chromatography of all four nucleotides illuminated in the presence of Fe^{3+} or Fe^{2+}, free bases were found present in roughly similar amounts. The amounts of base present increased with the length of illumination, although not proportionately, and in view of the instability of the bases under the reaction conditions, which was discussed above, the actual amount of base formed was probably much greater than the amount found at any one time period. No nucleosides were detected in such reaction mixtures at any time period. The decomposition proceeded considerably faster in the more dilute solutions (10^{-4} M). Illumination of uridylic acid by an ultraviolet lamp rather than by visible light showed no marked difference whether an equimolar amount of Fe^{3+} was present or not. This finding supports the belief that the observed reactions are catalyzed by wavelengths above 3500 A.

The behavior of nucleosides, as exemplified by uridine, was qualitatively similar to that of the corresponding nucleotide, resulting in a loss of absorbance and formation of base. After 30 minutes' illumination with Fe^{3+} 60% of the absorbancy was destroyed, and 20% of the remaining absorbancy was found to be uracil.

Four dinucleoside monophosphates were also studied. After extensive illumination with Fe^{3+} (*p*H 2–3) ApA and CpA lost about 30%, and GpC about 65%, of absorbancy. No loss in absorbancy was observed at 37° in the dark with Fe^{3+}, nor when illuminated without Fe^{3+}. As far as reaction products were concerned, all possible bases were found, as well as several other ultraviolet-absorbing products which were not identified.

Many attempts to detect ribose phosphate or other products composed of phosphate and sugar upon chromatographic or electrophoretic resolution of reaction mixtures after extensive iron-catalyzed illumination of dinucleoside monophosphates or nucleotides were unsuccessful. However control experiments indicated that the electrophoretic and chromatographic behavior of such compounds in the presence of iron salts was very complex and their detection was difficult.

The question of the possible role of atmospheric oxygen was attacked by illuminating both 0.01 M uracil and pU, containing equimolar amounts of Fe^{3+} or Fe^{2+}, in oxygen- or nitrogen-flushed stoppered tubes. The loss of absorbancy and the formation of free base were similar in the two tubes when the reaction mixture contained Fe^{3+}, but with Fe^{2+} there was a distinct difference in that only about half as much absorbancy was lost and uracil formed in N_2 than in O_2.

A great excess of hydrogen peroxide potentiated the action of Fe^{2+} on U and pU, but was less effective with Fe^{3+} (Table VII). Bases illuminated in the presence of great amounts of hydrogen peroxide without added iron also showed loss of absorbancy, and nucleotides released bases under these conditions. These effects were not detectable in the presence of EDTA (Table VIII).

TABLE VIII: Degradation of 5′-Uridylic Acid by Iron and H_2O_2.[a]

Moles Reactants/Mole pU	Conditions	Uracil (%)	Uridylic Acid (%)
90 moles H_2O_2			
90 moles H_2O_2	light 30 min	8	51
90 moles H_2O_2	light 1 hr	4	35[b]
90 moles H_2O_2	dark 30 min	0	82
90 moles H_2O_2	dark 1 hr	0.9	83
0.4 mole Fe^{2+}	light 1 hr	6	51
0.4 mole Fe^{2+} + 90 moles H_2O_2	light 1 hr	2	0
0.4 mole Fe^{2+} + 90 moles H_2O_2	dark 1 hr	6	37
0.2 mole Fe^{2+}	dark 1 hr	1	91
0.2 mole Fe^{2+}	light 1 hr	2	96
0.2 mole Fe^{2+} + 90 moles H_2O_2	dark 1 hr	4	46
0.2 mole Fe^{2+} + 90 moles H_2O_2	light 1 hr	3	12
0.2 mole Fe^{3+}	dark 1 hr	0.8	81
0.2 mole Fe^{3+}	light 1 hr	1.2	89
0.2 mole Fe^{3+} + 90 moles H_2O_2	dark 1 hr	2	56
0.2 mole Fe^{3+} + 90 moles H_2O_2	light 1 hr	3.5	27

[a] The reaction mixtures (30 mM uridylic acid) were chromatographed. The uracil and uridylic acid eluted are expressed as percentage of the original uridylic acid used, which was virtually 100% recovered from chromatograms. [b] In presence of EDTA (1.7×10^{-4} M) the loss of absorbancy of pU (1.7×10^{-4} M) was decreased from 16 to 2% in 1 hour and from 39 to 9% in 2 hours of illumination.

Discussion

The reported finding that iron photocatalytically inactivates and decomposes TMV-RNA seems to have several interesting implications. Other metals catalyze the hydrolysis of polyribonucleotides (Huff *et al.*, 1964), but the effect of iron, which in the presence of light is marked even at low iron concentrations and at neutrality and 0°, seems to represent a singular case the elucidation of which would be of intrinsic biochemical interest. The observation that as few as 3 equivalents of iron per mole TMV-RNA (6400 nucleotides) have a definite effect makes it appear likely that the traces of iron which are always found firmly bound to RNA from natural sources (Huff *et al.*, 1964; Wacker and Vallee, 1959) may play a role in rendering RNA to varying extent less stable than its structure would predict. Our long-standing observation that EDTA stabilizes the infectivity of TMV-RNA has thereby received a rational explanation. The comparison of the average half-life upon illumination of preparations of TMV-RNA (1.7 hours), compared to the same preparations with 3 equivalents of added Fe^{2+} or Fe^{3+} (0.8 hours), or in the presence of 10^{-3} M EDTA (6.6 hours) (see Figure 1) permits the estimate that the original iron content corresponded to about 5 equivalents per mole, a figure which is similar to that derived from analytical data by Wacker and Vallee (1959). (We attempted to study the "intrinsic" iron by producing TMV on hydroponically grown plants which had been starved in iron for 25 days prior to being supplied with 2.5 mg Fe, containing 5 mc ^{59}Fe, over a period of 19 days, beginning 1 day before infection. The purified virus after treatment with 0.02 M EDTA for 18 hours at 37° contained of the order of 1 atom ^{59}Fe per particle [115,000 cpm/60 mg virus], and the RNA isolated from it, without bentonite and in presence of EDTA, contained one-fiftieth of this amount per mole RNA. Yet the plants which had a dry weight of the order of 2 g had taken up an appreciable fraction of the ^{59}Fe, which was added as the chelate, as indicated by the presence of 2.4×10^8 cpm in the press juice, of the order of 10% of the amount added to the medium. This would suggest that infective viral RNA containing much less than one atom of iron per mole can be produced, provided that the ^{56}Fe remaining in starved plants [about 0.28 mg/g dry wt, determined in similar studies privately reported to us by M. P. Gordon and W. E. C. Wacker] was not preferentially used and the 5-fold greater amount of added labeled iron was excluded during virus synthesis.)

The study of the effect of iron plus light on model compounds has shown that the ultraviolet absorbancy is diminished, and that bases but not nucleosides are formed. Thus the labilization of the glycoside bond and destruction of the pyrimidine ring appear to be the primary events, and the diester bond breakage seems to be only a secondary consequence of these. This sequence of events is supported by the kinetics of inactivation and of fragmentation of TMV-RNA.

The detailed mechanism of the reactions involved

remains to be elucidated. The finding that the addition of hydrogen peroxide greatly potentiates the action of Fe^{2+} on TMV-RNA and model compounds suggests the occurrence of reactions involving radicals and activated oxygen. This seems to be borne out by the favorable effect of oxygen on the Fe^{2+}-catalyzed decomposition of pU, but the absence of a similar effect with RNA remains unexplained. The observation that the Fe^{2+} effect is counteracted by catalase supports the belief that oxygen radicals are involved, although the possibility that the iron is competitively bound and removed by the great amounts of added protein must also be considered. However, one would in that case expect catalase to suppress both the Fe^{2+} and the Fe^{3+} catalysis of the photodecomposition of RNA or nucleotides, while actually the enzyme has no effect on the Fe^{3+} reaction. Since the effects of Fe^{3+} are not prevented by the presence of catalase, hydrogen peroxide seems not to be an intermediary in that reaction.

The same photoreactions which are catalyzed by added iron salts have been observed in the presence of hydrogen peroxide alone, both in regard to inactivation of TMV-RNA, and to the degradation of model compounds. However, these effects are suppressed by EDTA and are thus attributed to the presence of traces of metals.

References

Felix, F., Potter, J. L., and Laskowski, M. (1960), *J. Biol. Chem. 235*, 1150.

Fraenkel-Conrat, H., and Singer, B. (1959), *Biochim. Biophys. Acta 33*, 359.

Fraenkel-Conrat, H., Singer, B., and Tsugita, A. (1961), *Virology 14*, 59.

Huff, J. W., Sastry, K. S., Gordon, M. P., and Wacker, W. E. C. (1964), *Biochemistry 3*, 501.

Singer, B. (1964), *Biochim. Biophys. Acta 80*, 137.

Singer, B., and Fraenkel-Conrat, H. (1962), *Biochemistry 1*, 852.

Singer, B., and Fraenkel-Conrat, H. (1963), *Biochim. Biophys. Acta 72*, 534.

Sugiyama, T., and Fraenkel-Conrat, H. (1961), *Proc. Natl. Acad. Sci. U. S. 47*, 1393.

Sugiyama, T., and Fraenkel-Conrat, H. (1963), *Biochemistry 2*, 332.

Wacker, W. E. C., and Vallee, B. L. (1959), *J. Biol. Chem. 234*, 3257.