Preliminary Notes

Inactivation of T-group bacteriophages by ascorbic acid and some poly-phenol derivatives, and the catalytic effects of cupric ion and photo-excited riboflavin

While studying the photodynamic inactivation of bacteriophage, we have found that T-group bacteriophages are irreversibly inactivated by ascorbic acid and some poly-phenol derivatives such as hydroquinone, adrenaline, *p*-phenylenediamine and pyrogallol. In further experiments it has also been revealed that the rate of the inactivation markedly increases in the presence of traces of Cu^{++} or photo-excited riboflavin.

Routine methods of phage technology described by ADAMS[1] were used throughout the study. The saline diluent containing 0.85 % NaCl and 0.025 % $MgSO_4$ was adjusted to pH 7.0 with appropriate amounts of 0.067 *M* Na_2HPO_4 and 0.067 *M* KH_2PO_4.

Using seven strains of T-group bacteriophages and their host, strain B of *Escherichia coli*, experiments were carried out on the inactivation of the phages by 10^{-4} *M* ascorbic acid, $5 \cdot 10^{-3}$ *M* hydroquinone, $5 \cdot 10^{-4}$ *M* adrenaline and $5 \cdot 10^{-5}$ *M* pyrogallol. Kinetic results clearly showed that, in all cases, the inactivation, after a short initial lag, proceeded as a first-order reaction. The rates of the inactivation of seven phage strains by these agents are illustrated in Table I.

Although neither Cu^{++} nor photo-excited riboflavin itself showed any inactivating effect, the presence of one of the agents, as is shown in Tables I and II, markedly accelerates the rates of the inactivation by ascorbic acid and poly-phenol derivatives. Other divalent ions, for instance, 10^{-3} *M* Fe^{++} and 10^{-3} *M* Mg^{++} had no such effect. As is well known [2,3,4], autoxidations of ascorbic acid and poly-phenol derivatives are catalyzed by traces of Cu^{++} and also photochemically catalyzed in the presence of riboflavin.

TABLE I

THE INACTIVATION OF T-GROUP BACTERIOPHAGES BY ASCORBIC ACID AND SOME POLY-PHENOL DERIVATIVES

The velocity constants were measured in 0.067 *M* phosphate-buffer saline (pH 7.0) at 28°. The phage titre used was about $2 \cdot 10^8$ particles/ml. The effect of Cu^{++} was tested only for phage T5 using 10^{-3} *M* $CuSO_4$.

Compounds	*Velocity constants (min^{-1})*							
	T1	*T2*	*T3*	*T4*	*T5*		*T6*	*T7*
					without Cu++	*with Cu++*		
Ascorbic acid (10^{-4} *M*)	0.409	0.498	0.560	0.135	0.885	3.566	0.507	1.090
Hydroquinone ($5 \cdot 10^{-3}$ *M*)	0.054	0.143	0.338	0.141	0.464	2.797	0.407	0.195
Adrenaline ($5 \cdot 10^{-4}$ *M*)	—	0.111	—	—	0.124	1.220	—	—
Pyrogallol ($5 \cdot 10^{-5}$ *M*)	—	0.248	—	—	0.103	0.493	—	—

Moreover, we have observed that the inactivating effect of 10^{-4} *M* ascorbic acid on phage T5 was completely inhibited by 10^{-3} *M* cysteine during a 3 h incubation at 28°. Since it has been generally accepted that, in the presence of O_2, H_2O_2 is formed during the autoxidation of ascorbic acid, catechol, hydroquinone etc.[2,3,4,5], it appeared possible that H_2O_2 might play a crucial part in the inactivation of the phages by these agents. In order to test this point, the effects of H_2O_2 and of catalase were tested on phage T5. Preliminary experiment showed that 10^{-2} *M* H_2O_2 inactivated phage T5 at a rapid rate ($k = 1.171$). It was also found that the inactivation of phage T5 by ascorbic acid was completely inhibited by a small amount of crystalline catalase (0.01 mg/ml of Kat.f. $2.5 \cdot 10^4$).

It must be noted here that the phage particles inactivated by means of ascorbic acid and poly-phenol derivatives, still preserve the capacity of being adsorbed to the host cell.

In 1945, MCCARTY[6] reported that the transforming substance of pneumococcal types was reversibly inactivated by ascorbic acid in the presence of Cu^{++} and he suggested that the inactivation might possibly be attributed to the action of the H_2O_2 formed in the autoxidation of ascorbic acid. It is conceivable, therefore, that the H_2O_2, formed in the autoxidation of ascorbic acid and

TABLE II

THE EFFECT OF PHOTO-EXCITED RIBOFLAVIN ON THE INACTIVATION OF BACTERIOPHAGE T5 BY ASCORBIC ACID AND POLY-PHENOLS

The velocity constants were measured in 0.067 M phosphate-buffer saline (pH 7.0) at 20°. Just before irradiation, the phage was quickly diluted to $2 \cdot 10^8$ particles/ml in the buffer-saline solution containing riboflavin ($10^{-4} M$) and ascorbic acid or poly-phenols. Samples were irradiated in a layer 1.5 mm thick at a distance of 15 cm from the centre of the lamp. A 20 watt fluorescent daylight lamp (Matsuda) was used as a source of visible light. As a control, samples were kept in the dark chamber under the same conditions.

Compounds	*Velocity constants (min^{-1})*	
	Dark control	*Irradiation*
Ascorbic acid ($10^{-4} M$)	0.421	1.911
Hydroquinone ($10^{-3} M$)	0.196	0.312
Pyrogallol ($5 \cdot 10^{-3} M$)	0.614	1.258

poly-phenol derivatives, is primarily involved in the inactivation of the phage particles by affecting the phage DNA. Because of the irreversibility [6] of the inactivation of the phages by these agents, however, a participation of other mechanisms in the phage inactivation cannot be ruled out.

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Department of Bacteriology, Nagoya University, School of Medicine, Nagoya (Japan) NOBUTO YAMAMOTO

[1] M. H. ADAMS, *Methods of study of bacterial viruses*, in *Methods in Medical Reseach*, Vol. 2, Year Book Publishers, Chicago, Ill., 1950.
[2] A. O. DEKKER AND R. G. DICKINSON, *J. Am. Chem. Soc.*, 62 (1940) 2165.
[3] E. SILVERBLATT, A. L. ROBINSON AND C. G. KING, *J. Am. Chem. Soc.*, 65 (1943) 137.
[4] D. B. HAND AND E. C. GREISEN, *J. Am. Chem. Soc.*, 64 (1942) 358.
[5] M. MCCARTHY, *J. Exptl. Med.*, 81 (1945) 501.
[6] N. YAMAMOTO, in preparation.

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The participation of tetrahydrofolic acid in the enzymic conversion of phenylalanine to tyrosine

The oxidation of phenylalanine to tyrosine, shown in equation (1), is known to require TPNH (reduced triphosphopyridine nucleotide), oxygen and at least two enzymes[1,2].

$$\text{TPNH} + \text{H}^+ + \text{O}_2 + \text{phenylalanine} \longrightarrow \text{TPN}^+ + \text{H}_2\text{O} + \text{tyrosine}$$

Recently it has been found that, in addition to TPNH, a new cofactor isolated from rat liver is involved in the reaction[3].

The ultraviolet absorption spectrum, the fluorescent characteristics of an alkaline-degradation product of the purified rat-liver cofactor as well as its high nitrogen content suggested the possibility that it contained a pteridine moiety. When folic acid and a group of related pteridines were tested for cofactor activity, it was found that THF (tetrahydrofolic acid) was highly active in replacing the natural cofactor. Anhydroleucovorin (5, 10-formyltetrahydrofolic acid) showed slight activity; folic acid, leucovorin, and pteroic acid were completely inactive.

The activity of THF in the system could be demonstrated by measuring the stimulation of either tyrosine formation or of the phenylalanine-dependent oxidation of TPNH. A comparison of the activity of THF and the natural cofactor in the tyrosine assay is shown in Fig. 1. Under the conditions used in this experiment there is no detectable tyrosine formation in the absence of added cofactor. It can be seen that although THF is highly active, the time-course of the reaction is markedly different from that observed with the natural cofactor. In the latter case, there is a lag of several minutes[2] and after this initial period the rate of the reaction is constant. In contrast, with THF there is an initial burst of activity and then the rate falls off. Similar differences in kinetics can be observed when the reaction is followed by measuring the phenylalanine-dependent oxidation