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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[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

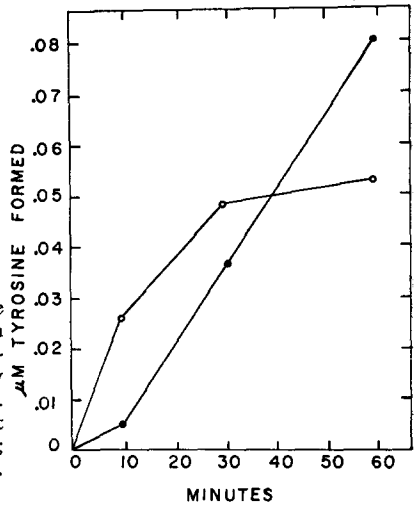

Fig. 1. The stimulation of tyrosine formation by the cofactor isolated from rat liver and by THF. The system contained the same components as used in the experiment described in Table I. Tyrosine was determined by the nitrosonapthol procedure as described previously[2]. The incubations were carried out at 25° in open test tubes, with mechanical shaking. ○, 0.12 μmole THF; ●, about 10 μ of the natural cofactor from rat liver.

TABLE I

THE EFFECT OF TETRAHYDROFOLIC ACID AND THE NATURAL COFACTOR ON THE PHENYLALANINE-DEPENDENT OXIDATION OF TPNH

Additions	*Rate (Optical density × 1000/min)*	
	0–6 min	*24–30 min*
None	0.1	0.1
Natural cofactor	3.6	11.0
Tetrahydrofolic acid	19.8	0.5

The reactions were carried out at 340 mμ in 1.0 cm Beckman cuvettes containing the following components (in μmoles): potassium phosphate buffer, pH 6.8, 100; L-phenylalanine, 2.0; TPNH, 0.12; purified rat enzyme, 0.16 mg protein; purified sheep enzyme, 0.07 mg protein. Where indicated, about 10 μ of the cofactor isolated from rat liver, or 0.12 μmole THF were added. Final volume made up to 1.0 ml with water. Each reported value has been corrected for any TPNH oxidation which occurred in the absence of added phenylalanine.

of TPNH, as shown in Table I. It would appear from these studies that the natural cofactor functions catalytically in the system, while THF does not.

The finding that THF, or a compound derived from it, can stimulate the rate of tyrosine formation suggests the possibility that the folic acid compounds may be involved in biological hydroxylation reactions, in addition to their known role in one-carbon metabolism. As already noted, preliminary physical and chemical studies on the natural cofactor are consistent with a pteridine-containing compound. While such a tentative conclusion regarding the structure of the natural cofactor is strengthened by the activity of THF in the enzymic system under consideration, further work will be required before the relationship of the natural cofactor to the folic acid compounds is elucidated.

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[1] S. KAUFMAN, *Biochim. Biophys. Acta*, 23 (1957) 445.
[2] S. KAUFMAN, *J. Biol. Chem.*, 226 (1957) 511.
[3] S. KAUFMAN, *Federation Proc.*, 16 (1957) 203.

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