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22. Takashi Uemura and Tomomichi Yanagita : An Attempt to Classify the Growth Responses in Bacteria to Various B Vitamins.

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It has been revealed that the mode of growth inhibition of various antibacterial substances could be classified mainly into four groups with respect to the effect on normal growth curve of bacterial culture.[1] In connection with the observation, it may be valuable to disclose the mode of growth-promoting action of various B vitamins on bacteria. Much work on the effect of vitamins on bacterial growth has been published, but only a few described the qualitative aspects on the mode of growth-promoting action of the vitamins with special reference to their effect on bacterial growth curve. Present report deals with this phase of investigation to reveal the types of growth response to B vitamins in bacteria.

Methods

Methods of preparation of test bacterial cultures and culture media were principally based on the description by Barton-Wright.[2] Organisms used were *Micrococcus pyogenes* var. *aureus* TERASHIMA, *Lactobacillus casei* ε, *Streptococcus faecalis* R, *Lactobacillus arabinosus* 17-5, and *Euglena gracilis* var. *bacillalis*. Vitamins used were of commercial origin. A vitamin tested was depleted of a medium and

TABLE I. Growth-promoting Action of Various B Vitamins on Microörganisms

B Vitamin	Organism	Medium*	Type	Growth response		
				lag	log	stationary
Niacin	*M. pyogenes*	(3)	I	+	−	−
	S. faecalis	(3)	—	−	−	−
	L. arabinosus	(4)	II	−	+	+
Biotin	*M. pyogenes*	(3)	—	−	−	−
	S. faecalis	(3)	II	+	+	+
PGA	*M. pyogenes*	(3)	—	−	−	−
	S. faecalis	(3)	II	+	+	+
	L. casei	(6)-a	II	+	+	+
	L. casei	(6)-b	—	−	−	−
Pyridoxamine	*S. faecalis*	(3)	III	−	−	+
Pyridoxine	*M. pyogenes*	(3)	—	−	−	−
	S. faecalis	(3)	III	−	−	+
Riboflavin	*M. pyogenes*	(3)	—	−	−	−
	S. faecalis	(3)	II-I	+	+	+
	L. casei	(5)	III	−	−	+
Thiamine	*M. pyogenes*	(3)	II	−	+	+
	S. faecalis	(3)	mod. II	−	+	−
Vitamin B_{12}	*E. gracilis*	(7)	II	−	+	+
Pantothenate	*M. pyogenes*	(3)	—	−	−	−
	S. faecalis	(3)	—	−	−	−
PABA	*M. pyogenes*	(3)	—	−	−	−
	S. faecalis	(3)	—	−	−	−

* Reference numbers are listed. (6)-a and -b represent Snell and Peterson's media supplemented with yeast supplement and pyridoxine, respectively.

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1) H. Tamiya, T. Yanagita : J. Antibiotics (Japan), **1**, 257(1947); T. Yanagita : Advances in Enzyme Chemistry (Japan), **1**, 285(1949).
2) E.C. Barton-Wright : "The Microbiological Assay of the Vitamin B-Complex and Amino Acids," Pitman Publ., New York (1952).

graded concentrations of the vitamin added into the deficient media. After the inoculation of test organism, the cultural development was followed nephelometrically at time intervals.

Results and Discussion

On investigating the mode of growth-promoting action of vitamins, there should be a large number of combinations in test system among vitamins, organisms, and culture media. First of all, in the present experiment, the effect of various vitamins on two kinds of bacteria cultured in the same medium was examined. Thus, *M. pyogenes* and *S. faecalis* were cultured in Teply and Elvehjem's medium.[3] The results are listed in Table I and the typical growth curves obtained for some vitamins are shown in Fig. 1. Characteristic is the fact that there are three types of growth response to a vitamin. The growth of *M. pyogenes* was found to be promoted by niacin and niacinamide only in shortening the duration of lag phase of cultural development. This type of growth promotion will be designated as Type I in this text. Type II

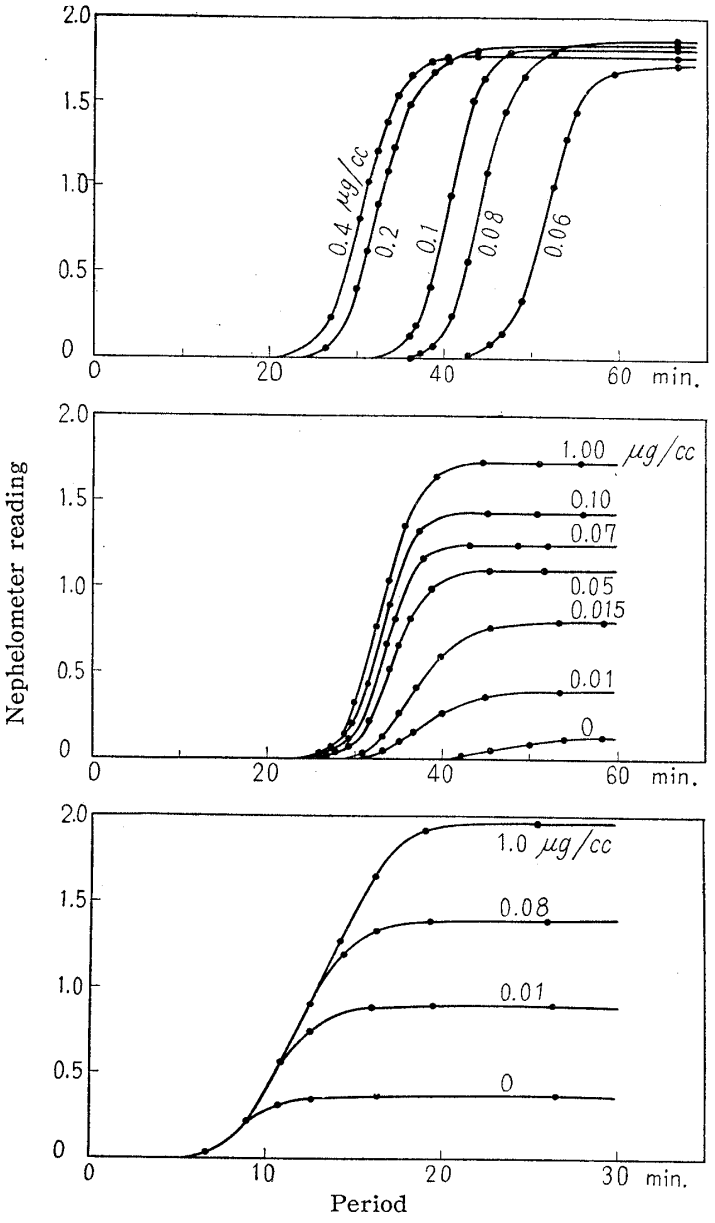

Fig. 1. Characteristic Types of Growth Promotion by Three Different B Vitamins

3) L. J. Teply, C. A. Elvehjem : J. Biol. Chem., **157**, 303(1945).

growth promotion was represented by pteroylglutamic acid (PGA) as added into *S. faecalis* culture. This vitamin shortened the lag phase, accelerated the rate of logarithmic growth, and enhanced the final yield of the population. In this case, however, the effect on the lag phase may be only seeming, since the size of inoculum was so small that the initial growth could not be detected by the nephelometrical determination. Therefore, if the viable count of the culture is followed, it is probable that the shortening of the real lag phase may not be so conspicuous as revealed nephelometrically. In recapitulation the PGA seems to promote the growth to *S. faecalis* in growth rate and yield. Either pyridoxamine or pyridoxine was found to affect the growth of

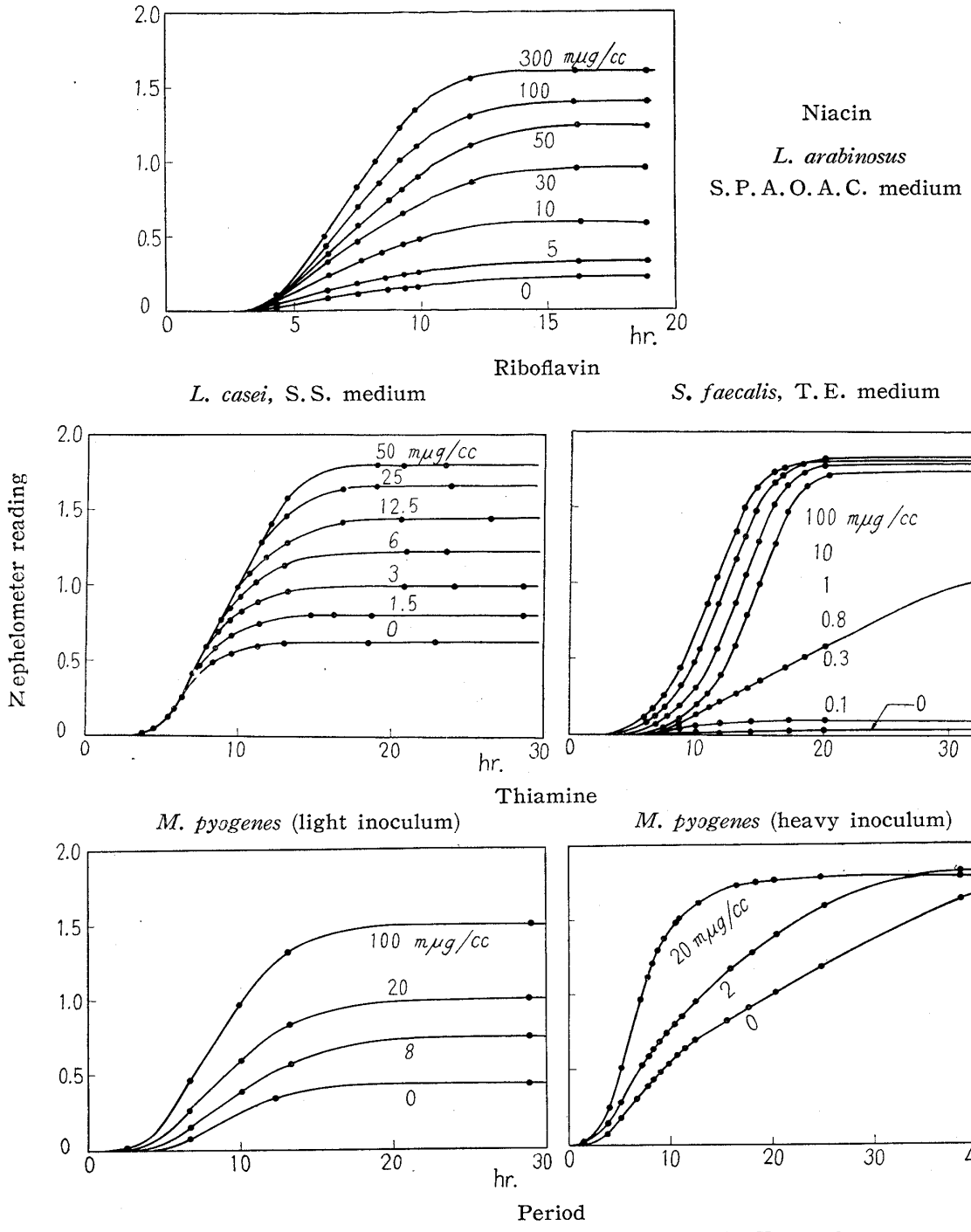

Fig. 2. Several Examples of the Variation in Type of Growth Promotion by Certain Vitamins

S. faecalis in the other mode, which was classified into Type III. This vitamin affected only the yield of final population leaving other phases of growth unaffected. The result of the classification for each vitamin is listed in Table I.

Noteworthy is the observation that these types of growth promotion are not necessarily a fixed character of a vitamin. As shown in Fig. 2, in some instances the same vitamin manifested different types of growth promotion as the organism and medium tested varied. As shown in Fig. 1 (top), niacin manifested typical Type I growth promotion on *M. pyogenes*, while the same vitamin was found to exert Type II growth promotion on *L. arabinosus* in a certain medium[4] (Fig. 2, top). Riboflavin, when added to *L. casei* cultured in Snell and Strong's medium,[5] showed a characteristic Type III growth promotion, while it showed peculiar type on *S. faecalis* cultured in Teply and Elvehjem's medium (Fig. 2, middle). In the latter combination, when the concentration of riboflavin was low, the mode of growth response of the organism seemed to be of Type II, whereas it appeared to shift to Type I as the concentration increased; this type being temporarily designated as Type II–I. The reason for the exhibition of such a complex mode of growth promotion is yet unsolved. Thiamine, on the other hand, exerted its effect on *M. pyogenes* by the mode of Type II, while somewhat differently on *S. faecalis*. In the latter organism, if the size of inoculum was small enough, the same type (Type II) of growth response to thiamine was observed. However, when the size of inoculum was larger, only the rate of logarithmic growth was accelerated leaving other phases unaffected as indicated in Fig. 2 (bottom, right). This type will be called modified Type II. It may be considered that in interpreting these observations on thiamine action there are at least two possibilities : (1) With larger size of inoculum, there is a higher probability to carry over a small number of mutants which are capable of synthesizing the thiamine, and (2) although the inoculum cells are washed thoroughly* prior to inoculation, the heavier the inoculum, the larger the amount of thiamine adsorbed tightly onto the cells is carried into the test medium. In the case of PGA no such difference in the type of growth promotion was observed even though the test organism and medium[6] were varied.

Finally, the growth promoting action of vitamin B_{12} on *Euglena gracilis* cultured in a synthetic medium[7] under illumination should be mentioned briefly. As shown in Fig. 3, it showed a typical Type II effect on the growth. In this instance, however, the level of stationary phase began to decrease as growth proceeded. The decrease in nephelometrical reading may be attributed either to the diminution in cell size or to the partial lysis of cells at the stationary phase.

Physiological interpretation of several types of the proposed growth promotion of vitamin has not been made as yet. Unpublished data of the authors showed that nitrogen source (ammonium salt) and carbon source (glucose) for the cultivation of *Escherichia coli* exerted their growth-promoting effect in the mode of Type II and III, respectively. In other words, nitrogen source seemed to limit the rate and yield of cultural development, while carbon source limited the yield only. Although these observations may not be directly related to the effect of vitamins on bacterial growth, they may be worth referring to in considering the mode of growth promoting action of vitamins. Lindegren and Raut[8] observed that a pantothenate-deficient yeast, cultured

* The inoculum cells washed three and six times prior to inoculation showed no differences in growth curves when cultured in the presence of graded concentrations of the said vitamin.

4) Analyst, **71**, 397(1946).
5) E.E. Snell, F.M. Strong : Ind. Eng. Chem., Anal. Ed., **11**, 346(1939).
6) E.E. Snell, W.H. Peterson : J. Bacteriol., **39**, 273(1940).
7) S.H. Hutner, *et al.* : Proc. Soc. Exptl. Biol. Med., **70**, 118(1949).
8) C.C. Lindegren, C. Raut : Ann. Missouri Botan. Garden, **34**, 85(1947).

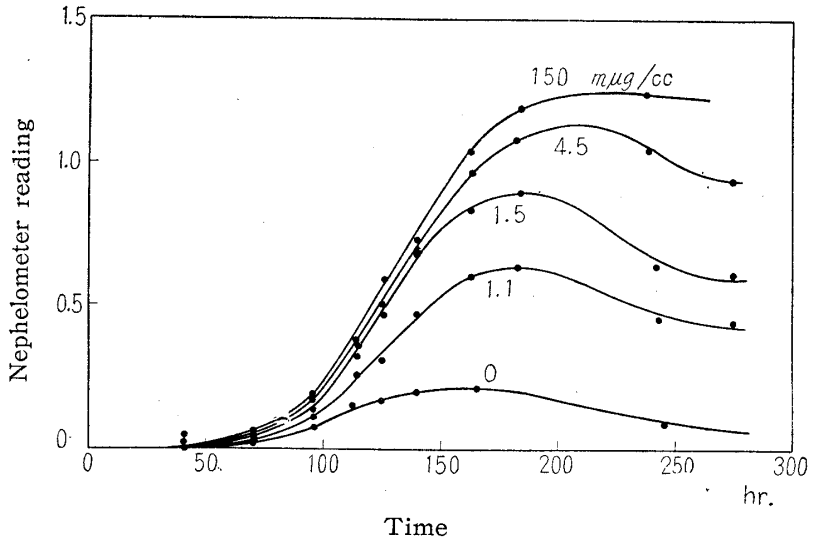

Fig. 3. Growth Curves of *E. gracilis* in the Presence and Absence of Graded Concentrations of Vitamin B_{12}

in a medium containing pantothenate in a small amount, less than the minimum requirement, could eventually grow by long incubation. The growth curves thus observed appears to be in the category of Type I growth promotion. These workers concluded that the growth of the yeast after a long lag was due to the selective growth of mutants which were able to synthesize pantothenate. The said mutation and selection mechanism may probably be involved in the exhibition of Type I growth promotion.

From the present observation it should be emphasized that for the turbidimetric bioassay of vitamins and other growth-promoting substances, the choice of a test system comprising the combination of test substance, organism, and medium should be taken into account especially from the view-point of the type of growth promotion. The combination which results in Type III (or II) growth should be the most desirable. If the combination results in the growth promotion of Type I (or modified Type II), reliable quantitative results may hardly be expected, since the reproducibility of the results will be disturbed by the eventual growth of mutants. In the case of bioassay of vitamin B_{12} using *E. gracilis* as the test organism, the time of reading of results should be carefully determined, since the most reliable results may be obtained only when the culture attained maximum growth.

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Summary

The growth responses of bacteria to various B vitamins were found to be classified into four groups. In Type I the growth is affected only in shortening the lag phase of cultural development, in Type II the rate of logarithmic growth and the yield of final population are promoted, and in Type III only the final yield is enhanced leaving other phases of growth unaffected. These types of growth promotion seemed not to be a fixed character of a vitamin. Several examples showing the variability of the type for a vitamin according to the cultural conditions were presented. Physiological interpretation of these types of growth promotion was discussed. Finally, importance of the choice of test system for the turbidimetric bioassay of vitamin was suggested from the view-point of the proposed types of growth promotion.

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