REVERSAL BY FOLIC ACID OF PENICILLIN ACTION ON GROWTH AND ON PENTOSE NUCLEIC ACID SYNTHESIS IN *LACTOBACILLUS CASEI*

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BELLAMY AND KLIMEK (1948) reported that *Staphylococcus aureus*, when made resistant to high concentrations of penicillin, gave a negative Gram reaction. Gale and Rodwell (1948) confirmed this observation and showed that, parallel to development of resistance, the organism acquired an ability to synthesize its amino acid requirement. The Gram complex has been recognized to be a magnesium pentosenucleoprotein (Henry and Stacey, 1943; Bartholomew and Umbreit, 1945). Attainment of resistance to penicillin in this organism was in fact shown to be associated with a change in the general nucleic acid pattern in the direction of Gram negative organism (Gale, 1949). More directly, Mitchell (1949) has observed a disturbance in the nucleic acid-nucleotide balance as a result of penicillin action. George and Pandalai (1948) reported that penicillin inhibition could be reversed with nucleic acid although this was attributed to a lowering of pH on addition of nucleic acid to the growth medium (Ganapathi *et al.*, 1948). Cultural conditions favouring increased pentose nucleic acid (PNA) content of bacterial cells are known to enhance the resistance of *Hemophilus pertussis* cells to various antibiotics (Smolens and Vogt, 1953).

It was reported by Rege and Sreenivasan (1954 *a*), that the synthesis of both PNA and Deoxypentose nucleic acid (DNA) in micro-organisms is controlled by folic acid and vitamin B_{12}. These authors also showed (1954 *b*) that another antibiotic, aureomycin, inhibits synthesis of both PNA and DNA and that folic acid (PGA) exerts a protection against this effect; a higher concentration of vitamin B_{12} is required to exert similar adequate protection. The present report relates to a study of the interrelationship between these vitamins and penicillin with respect to their influence on growth and on nucleic acid synthesis.

EXPERIMENTAL

The organism used was *Lactobacillus casei* A.T.C.C. 7469 maintained by fortnightly transfer on a medium consisting of (percentages): yeast

extract 1, glucose 1, sodium acetate 1 and agar agar 2·5. The basal medium was that of Tepley and Elvehjem (1945) devoid of peptone and purines.

In growth experiments, 5 ml. of double strength basal medium was taken in bacteriological test tubes, the various additions were made and the volume adjusted with glass-distilled water so that, on subsequent addition of penicillin solution after sterilisation, the volume would be 10 ml. The tubes were capped and sterilised at 15 lb. steam pressure for 12 minutes. After cooling, penicillin solution was added aseptically and the tubes were inoculated dropwise using a fifty-fold dilution of a 24-hour culture of *L. casei* in the basal medium containing 0·2 μg. PGA/ml. Growth was measured after 24 hours' incubation at 37° using a Klett Summerson photo-electric colorimeter at 660 mμ and is expressed in terms of galvanometer deflections.

The penicillin used was the crystalline sodium salt of penicillin G (Glaxo).

For studying the nucleic acid make-up, the organism was grown in Erlenmeyer flask containing 100 ml. double strength basal medium diluted after various additions to 200 ml. All additions except penicillin were made before sterilisation as in the growth experiments. The flasks were sterilised by autoclaving at 15 lb. steam pressure for 12 minutes. Each flask was seeded with 1 ml. of 18 to 24-hour inoculum.

After incubation at 37° for 24 hours, the cells were harvested by centrifugation, washed twice with ice-cold glass-distilled water, resuspended in water and made to a definite volume. Aliquots of this were taken for estimation of nucleic acids and for dry weight determinations.

PNA and DNA were extracted and estimated as described by Rege and Sreenivasan (1954 *a*) and expressed as averages of at least three replicates.

Since these studies relate to an organism requiring PGA for growth, the effects of B_{12} were ascertained only in presence of PGA.

RESULTS

Growth.—The concentration of penicillin to bring about approximately 50 per cent. grow h inhibition in presence of optimal concentration of PGA (0·3 mμg./ml.) was found to be 0·2 units/ml. Using this concentration of the antibiotic, the influence on growth inhibition of varying concentrations of PGA, with and without vitamin B_{12} was studied. Results given in Table I are averages of four different sets.

It is observed in Table I that PGA alone and, more effectively, with B_{12} affords protection against the bacteriostatic action of the antibiotic.

TABLE I

Effect of PGA and B_{12} on penicillin growth inhibition of L. casei

Additions to 10 ml. basal medium		Growth at end of 24 hours		
PGA mμg.	B_{12} mμg.	In absence of penicillin	In presence of penicillin	Per cent. growth inhibition
1	..	45	19	58·0
3	..	91	46	49·4
10	..	93	58	37·6
20	..	93	60	35·4
3	10	90	53	41·1
3	20	92	53	42·3
10	10	93	68	26·8
20	20	91	67	26·3

Nucleic acid make-up.—The nucleic acid contents of *L. casei* cells grown in presence of optimal concentration of PGA (0·3 mμg./ml.) with and without penicillin (0·2 units/ml.) are reported in Table II.

TABLE II

Effect of penicillin on nucleic acid content of L. casei

	PNA	DNA
	Per cent. dry weight of cells	
Without penicillin ..	9·54	2·08
With penicillin (0·2 units/ml.)	7·21	2·12

It is observed that *L. casei* cells grown in presence of penicillin contain decreased amounts of PNA, the amount of DNA being unaffected.

As in the growth experiments described earlier, PGA and vitamin B_{12} antagonise the bacteriostatic action of the antibiotic and as both these vitamins are known (Rege and Sreenivasan, 1954 *a*) to be involved in nucleic acid synthesis in micro-organisms, particularly *L. casei*, their effects on nucleic acid synthesis were studied (Table III).

TABLE III

Effect of PGA and vitamin B_{12} on nucleic acid make-up of L. casei *cells grown in presence of penicillin*

Supplements per ml. basal medium	DNA		PNA		
	With penicillin (0·2 units/ml.)	Without penicillin	With penicillin (0·2 units/ml.)	Without penicillin	Per cent. decrease
	Per cent. dry weight of cells				
PGA (0·1 mμg.) ..	1·64	1·47	4·36	7·21	39·0
PGA (0·3 mμg.) ..	2·25	2·20	7·30	9·62	24·1
PGA (2·0 mμg.) ..	2·61	2·75	8·24	9·80	15·9
PGA (0·3 mμg.) + B_{12} (2·0 mμg.) ..	2·90	2·81	8·76	9·73	9·9
PGA (2·0 mμg.) + B_{12} (2·0 mμg.) ..	3·35	3·48	9·29	10·08	7·8

The results indicate that folic acid alone and, more effectively, with B_{12} partially overcomes the antagonistic effect of penicillin on PNA in a manner similar to its protection against growth inhibition by the drug.

DISCUSSION

Penicillin is known to exert its effect during the logarithmic phase of bacterial growth (Hobby *et al.*, 1942; Boivin, 1947). This phase is usually associate with active synthesis of PNA (Boivin, 1947) and hence the growth-inhibitory action might be linked to an inhibition of PNA synthesis. The results outlined here demonstrate this influence of penicillin on PNA make-up of *L. casei* more directly. The work of Gale (1949) has also indicated such an effect in *S. aureus*.

PGA and vitamin B_{12}, which have been shown (Rege and Sreenivasan, 1954 *a* to control the synthesis of PNA and DNA in micro-organisms, are

found to counteract the inhibitory effect of penicillin on PNA. The role of the vitamins in the formation of the nucleic acids is presumably confined to the biosynthesis of the purine and pyrimidine moieties (Rege and Sreenivasan, 1954 *a*). The antibiotic may, in a similar manner, be acting by interfering in the formation of these bases. There are reports of penicillin having an inhibitory effect on pentose nucleases (Krampitz and Werkman, 1947; Massart *et al.*, 1947) and it is less likely therefore that the decreased level of PNA results from an accelerated breakdown consequent on the activation of the degradative mechanism(s) by the antibiotic.

Summary

1. Cells of *L. casei* grown in presence of penicillin are found to contain decreased amounts of PNA, DNA being unaffected.

2. PGA by itself and, more effectively, in presence of vitamin B_{12} exerts protection against the inhibitory action of the antibiotic on growth as well as on PNA formation.

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