NOTES
NUCLEIC ACID SYNTHESIS IN PENICILLIN-TREATED ALCALIGENES FAECALIS

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Studies of the effects of penicillin on gram-positive bacteria (Gale, Symposia Soc. Exptl. Biol., No. 3, 232, 1949; Strominger, J. Biol. Chem., 224, 525, 1957), have shown that, in addition to blocking cell wall synthesis, this antibiotic inhibits synthesis of ribonucleic acid. Although it is known that penicillin has an effect on the synthesis of the cell walls of gram-negative bacteria, (Liebermeister and Kellenberger, Z. Naturforsch., 11b, 200, 1954; Lederberg, Proc. Natl. Acad. Sci. U.S., 42, 574, 1956), quantitative studies of nucleic acid synthesis in the presence of penicillin have not been carried out.

Penicillin transforms Alcaligenes faecalis strain LB into a protoplast-like globular form which multiplies as such when the transformation is carried out in tryptone broth. In defined medium a nonmultiplying form (crescent) is produced (Lark, Can. J. Microbiol., 4, 165, 1958). We have carried out determinations of the ribonucleic acid, deoxyribonucleic acid, and protein content of cultures of A. faecalis undergoing transformation into these protoplast-like structures under the influence of 50 units per ml of penicillin. The transformation was carried out as described previously (Lark, Can. J. Microbiol., 4, 165, 1958). Cultures to be transformed in tryptone or synthetic medium were grown in these respective media prior to the addition of penicillin and 35-ml aliquots of the culture being transformed were removed at intervals, precipitated and washed with cold 5 per cent trichloracetic acid, and extracted three times with alcohol and ether. The residual precipitate was extracted twice at 90 C with 10 per cent trichloracetic acid and the supernatant examined for deoxyribonucleic acid using diphenylamine (Dische, Z. Microchem., 8, 4, 1930) and for ribonucleic acid using orcinol (Mejaun, Z. Physiol. Chem. Hoppe-Seyler's, 258, 117, 1939). The hot trichloracetic acid precipitate was suspended in 0.1 n NaOH and protein was estimated by use of the modified Folin reagent (Lowry, Rosebrough, Farr, and Randall, J. Biol. Chem., 193, 266, 1951).

The experiment in figure 1A represents the transformation of cells into globular forms, that in figure 1B into crescents. The ordinates in both figures are given in arbitrary units and do not express the absolute values of ribonucleic and deoxyribonucleic acids or protein found. In both experiments, the protein, ribonucleic and deoxyribonucleic acids content for normal cells (i. e., the 0 min aliquots) were the same:

| Ribonucleic acid/cell equivalent to 1.12 × 10⁻¹⁰ mg of Na nucleate (Nutritional Biochemical Corporation). |
| Deoxyribonucleic acid/cell equivalent to 2.48 × 10⁻¹¹ mg of Na deoxynucleate (Nutritional Biochemical Corporation). |
| Protein/cell equivalent to 1.42 × 10⁻¹⁰ mg of crystalline bovine serum albumin (Armour and Company). |

It may be seen in figure 1A that, under conditions in which the protoplast-like structures continue to multiply exponentially, deoxyribonucleic and ribonucleic acids and protein continue to be synthesized at the new and decreased exponential rate of growth. On the other hand, these syntheses eventually cease in the nonmultiplying forms, figure 1B. In both cultures, ribonucleic acid and protein synthesis are affected somewhat earlier by penicillin than is deoxyribonucleic acid synthesis. This finding is similar to that reported for gram-positive organisms although the degree to which ribonucleic acid synthesis is reduced in A. faecalis is not so drastic.

From the data in figure 1A it appears that
under appropriate conditions, penicillin is capable of altering the deoxyribonucleic and ribonucleic acid and protein content of *A. faecalis* without destroying the ability of this organism to multiply. This experiment shows a transition from balanced growth in tryptone medium to balanced growth in tryptone plus penicillin medium. When this transition takes place there is a simultaneous decrease in the rate of ribonucleic acid and protein synthesis followed by a later decrease in deoxyribonucleic acid synthesis. A similar observation has been reported in studies which were made of the forward and reverse transitions between balanced growth of *Salmonella typhimurium* in glucose-minimal salts medium and in broth (Kjeldgaard, Maaløe, and Schaechter, J. Gen. Microbiol., accepted for publication). In this case, changes in ribonucleic acid, protein, and deoxyribonucleic acid synthesis of a similar nature were observed on changing the nutritional content of the medium. These findings are consistent with the idea (Lark, Can. J. Microbiol., 4, 165, 1958) that penicillin imposes a new growth requirement in *A. faecalis* which is supplied by tryptone.