CHANGES IN THE COMPOSITION OF AN ACTINOMYCIN COMPLEX DURING GROWTH OF A STREPTOMYCES CULTURE

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The actinomycins thus far isolated from the culture media of certain species of *Streptomyces* have been shown to consist of a group of closely related chemical compounds composed of a quinonoid chromophore linked to a peptide.[1, 2] Physical and chemical differences observed among the various actinomycins so far described are considered to be due to variations in the number, arrangement, and kinds of amino acids present in the peptide;[2] the chromophoric moiety is believed to be identical in each of these substances.[3] The actinomycins reported in the literature have been designated as A, B, C, D, I, J, and X.[4] Paper chromatography and countercurrent distribution of these actinomycins have revealed that they are generally composed of a mixture of several components present in different proportions.[2, 4]

Whereas a number of investigations have been carried out on the isolation and characterization of the actinomycin complexes and on the classification and identification of the microörganisms producing these substances, insufficient attention has been given to the influence of nutrition and conditions of cultivation on the nature of the complex formed by a given organism. Brockmann observed[5] that the proportion of various components of an actinomycin complex was modified to some extent by changes in the nitrogen source of the organism. For example, when KNO_3 was supplied to actinomycin X–producing strains, production of actinomycin X_1 was slight, whereas with glycine the level of actinomycin X_1 in the complex increased considerably. However, in none of Brockmann's studies did a variation of cultural conditions cause a minor component to become a major one. Such a reversal in the ratio of the components of an actinomycin complex has been found during growth of a *Streptomyces* culture and is the subject of the present communication.

Streptomyces 3720, isolated from garden soil, initially was found to produce an actinomycin complex of the B type during growth on a soya peptone–glucose medium. In a subsequent experiment conducted under comparable environmental conditions, the actinomycin complex formed was identified as the I type. The sole difference between the two experiments was the length of incubation of the culture. In the first case the material was extracted from 5-day-old cultures and in the second from 7-day-old cultures. To resolve this discrepancy, a more comprehensive investigation was carried out.

Experimental.—Five milliliters of a 48-hour-old shake culture of *Streptomyces* 3720 was inoculated into 2-liter Erlenmeyer flasks each containing 500 ml. of soya peptone medium.[6] The flasks were then incubated at 28° C. on a rotary shaker. At 12-hour intervals aliquots of the broth were taken for the extraction of actinomycin. Using circular paper chromatography (solvent system: 5 per cent sodium orthocresotinate and ethyl acetate–*n*-butyl ether [2:1]), a qualitative determination of the complex was achieved. The individual zones on a paper chromatogram

were then cut out, the actinomycin eluted with acetone-water (9:1), and the relative percentage of each component in a complex calculated after photometric determination of the eluate with a Beckman DU spectrophotometer.

It can be seen (Fig. 1) that with an increase in time of incubation there is a considerable change in the relative percentage of the components found in the actinomycin complex. After 2 days' incubation the complex was of the B type; thereafter, a gradual transition took place, and by 5 days the complex was definitely of the I type.

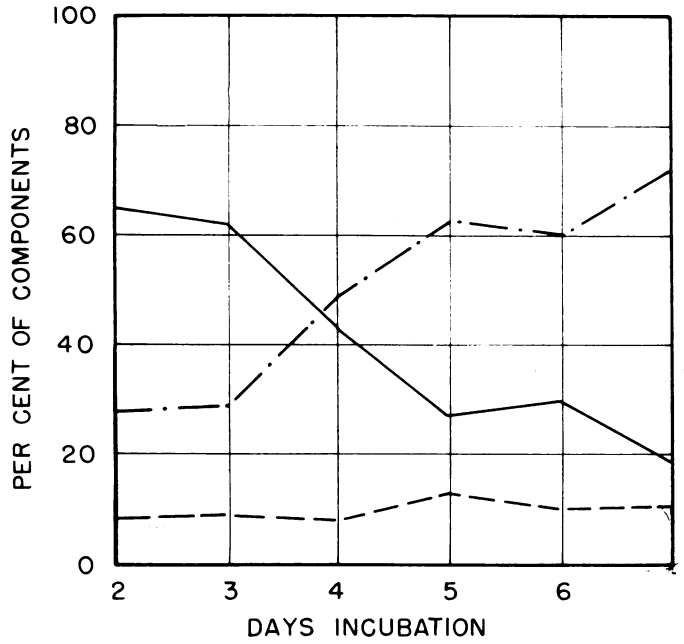

FIG. 1.—Relative per cent of the components in the actinomycin complex produced by *Streptomyces* 3720. The dashed line represents the slow-moving component, the dot-dashed line represents the intermediate component, and the solid line represents the fast-moving component on a circular paper chromatogram using a 5 per cent sodium orthocresotinate and ethyl acetate/*n*-butyl ether system.

Similar studies were performed with *Streptomyces* 3720, employing a chemically defined medium consisting of 1 per cent galactose, 0.2 per cent glutamic acid, 0.1 per cent K_2HPO_4, 0.0025 per cent $MgSO_4 \cdot 7H_2O$, 0.0025 per cent $FeSO_4 \cdot 7H_2O$, 0.0025 per cent $ZnSO_4 \cdot 7H_2O$, and 0.0025 per cent $CaCl_2 \cdot 2H_2O$ in distilled water. Once again the transition from the B- to the I-type complex occurred.

To determine whether different strains within the parent culture were responsible for the formation of the B- and I-type complexes, several colonial types found in the parental culture were isolated and grown separately in soya peptone–glucose medium. Analysis of the actinomycin produced during growth of these isolates revealed that all initially formed the B-type, and subsequently the I-type, complex. Additional studies of the parent culture as well as of the variant types derived from it are in progress. On the other hand, a preliminary investigation of *Streptomyces*

3686, an actinomycin I producer, revealed the fact that only the I-type complex was formed throughout the entire incubation period.

Unpublished data[7] on the physical and chemical properties of pure components of certain actinomycin complexes (B, D, I) have revealed that some of these are identical. Therefore, differences among certain actinomycin complexes may depend solely on the relative proportions of comparable components. As demonstrated in this preliminary report, such proportions can be modified to a considerable extent during prolonged cultivation of an organism. What factors may be operating to bring about this change are unknown at the present time. It remains to be determined whether this modification might involve an effect of the gradually changing cultural environment upon the physiological activities of the organism or whether such changes may influence the selection of specific types with different synthetic abilities.[8]

A detailed study of the culture *Streptomyces* 3720 revealed it to be a typical representative of the *S. antibioticus* group. It is to be recalled that it was a member of this group of actinomycetes that was first found[9] to produce actinomycin, later designated as "actinomycin A."

Summary.—*Streptomyces* 3720, a member of the *S. antibioticus* group, produces a significant change in the relative percentage of the components present in the actinomycin complex during growth on either a complex organic or chemically defined medium. This organism produces in the medium during the early stage of growth the actinomycin B complex. On continued incubation of the culture, the actinomycin B is changed to the actinomycin I-type complex.

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[1] C. E. Dalgliesh, A. W. Johnson, A. R. Todd, and L. C. Vining, *J. Chem. Soc.*, p. 2946, 1950.

[2] H. Brockmann, *Angew. Chem.*, **66**, 1, 1954.

[3] H. Brockmann and K. Vohwinkel, *Naturwissenschaften*, **41**, 257, 1954.

[4] S. A. Waksman, *Antibiotics and Chemotherapy*, **4**, 502, 1954; **5**, 409, 1954.

[5] H. Brockmann and N. Pfennig, *Z. physiol. Chem.*, **292**, 77, 1953; H. Brockmann, H. Linge, and H. Gröne, *Naturwissenschaften*, **40**, 224, 1953; H. Brockmann, and H. Gröne, *Chem. Ber.*, **87**, 1036, 1954.

[6] R. A. Manaker, F. J. Gregory, L. C. Vining, and S. A. Waksman, *Antibiotics Annual, 1954–55* (New York: Medical Encyclopedia, Inc., 1955), p. 853.

[7] L. C. Vining, personal communication.

[8] W. Braun, *Bacterial Genetics* (Philadelphia: W. B. Saunders Co., 1953).

[9] S. A. Waksman and H. B. Woodruff, *Proc. Soc. Exptl. Biol. Med.*, **45**, 609, 1940; *J. Bacteriol.*, **42**, 231, 1941.