INCORPORATION OF SARCOSINE-1-C^{14} INTO ACTINOMYCINS*

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Actinomycins are chromopeptides with antibiotic and cytostatic activities produced by species of Streptymycetes (Waksman and Woodruff, 1940). Chemically, actinomycins are characterized by the presence of a chromophoric phenoxazinone nucleus linked to two peptide chains containing five amino acids each. The amino acid composition of the peptide chain may vary with the strain of Streptomyces used or by changing the cultural conditions of the microorganisms. In all the actinomycins so far isolated sarcosine is always present. In general, two moles of sarcosine per mole of antibiotic have been found but the relative proportion of the amino acid may be increased by growing the microorganisms in the presence of sarcosine (Schmidt-Kastner, 1956).

Evidence is now presented for the incorporation of sarcosine-1-C^{14} in the sarcosine moiety of the actinomycins synthesized by Streptomyces antibioticus 1692 and S. parvus N.R.R.L. B-1455.

Experimental

18.1 micromoles of sarcosine-1-C^{14} (specific activity 8.1 mC/mM, obtained from the Radiochemical Centre, Amersham, England) were added to each 100 ml of galactose-glutamic acid medium (Katz and Goss, 1959) 12 hours after inoculation of the medium with mycelium from 48 hours old culture of the Streptomycetes. The cultures were incubated on an alternating shaker at 27°C until antibiotic production, as microbiologically determined, reached the max-

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imum (5 to 7 days). After centrifugation to remove the mycelium, the radioactive actinomycins were extracted thrice with one-half volume of n-butanol, the n-butanol fractions combined and evaporated to dryness. The residue was taken up in acetone, centrifuged and the supernatant solution taken again to dryness. The residue was dissolved in benzene and applied to a column of silicic acid equilibrated with the same solvent. After washing with benzene, the orange-red band of the actinomycins was eluted as a single peak with 2% methanol in ethyl acetate. The fractions containing the radioactive antibiotic, as determined by microbiological assay, radioactive counts and absorbancy at 435 mμ, were pooled and evaporated. Depending on the experimental conditions, 0.6 to 5.2% of the total radioactivity was incorporated in the antibiotic. The free amino acids from the peptide moiety of the antibiotic were obtained by hydrolyzing with 9 N HCl at 110°C for 24 hours; a melanin-like substance, from the chromophoric moiety, was separated by centrifugation. The amino acids were separated by chromatography on a Whatman no. 1 cellulose column using as solvent the top phase of a n-butanol/acetic acid/water mixture (4:1:5, v/v) (Johnson et al., 1952). The order of emergence from the column, as determined by paper chromatography, was: methyl-valine $\geq$ valine > alloisoleucine (when present) > proline > sarcosine > threonine. Sometimes, threonine was present only in traces, possibly for decomposition during hydrolysis (Johnson and Mauger, 1959). Purity of the isolated sarcosine was checked by paper chromatography in two different solvent systems.

The despeptidoactinomycin (actinomycinol B) was obtained by hydrolizing the antibiotic with 2 N $Ba(OH)_2$ and purified according to the procedure of Dagliesh et al. (1950).

Radioactivity was determined on quadruplicate samples on a S.E.L.O. thin mica window counter (counting efficiency 12.5%). The samples were counted to infinite thinness and corrected for background activity.

Results and Conclusions

The results (table) show that practically all the radioactivity, incorporated into the actinomycins produced by the tested strains, is located in the sarcosine portion of the antibiotic. Furthermore, preliminary results on the

degradation of the sarcosine obtained in the above reported experiments showed that all the radioactivity of the amino acid is located in the carboxyl group of the molecule, that is in the position in which the amino acid was originally labelled.

The present data, hence, suggest that sarcosine may be directly incorporated as such in the molecule of the actinomycins.

The absence of a mechanism for the activation of sarcosine (Ciferri et al., 1961) together with the reported incorporation of the amino acid as such in the antibiotic, suggest that the biosynthesis of these peptides takes place through a different mechanism than that involved in the biosynthesis of proteins. It may be added that, from research now in progress, no transfer to soluble RNA seems to take place in the case of sarcosine. A "stepwise addition" of amino acids (Ito and Strominger, 1960) to form the peptide chain could be operative in the biosynthesis of the antibiotic. The peptide chains could then react with the phenoxazinone moiety, independently synthesized (Weissbach and Katz, 1961), to give the complete molecule of the antibiotic. Alternatively, the peptide chains may link to 3-hydroxy-methyl-anthranilic acid residues to give anthranilic acid peptides (Brockmann, 1960). The condensation of such two units would give rise to the complete antibiotic.

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Radioactivity
(c.p.m.)

Actinomycins from	Exp.	Purified antibiotic	Hydrolized peptide	Melanine residue	Despeptido-actinomycin	Amino acids from peptide chain				
						Me-Val+Val	Allo	Pro	Thr	Sar*
S. antibioticus	I	2139	1952	3	-	39	6	2	0	1498 (71)
	II	9728	9960	12	-	105	79	83	-	8114 (83)
	III	4000	-	-	60	-	-	-	-	-
S. parvus	I	6732	6723	7	-	19	-	46	2	6540 (98)
	II	1488	1209	3	-	3	-	6	-	1192 (80)

* In brackets the percent of radioactivity recovered in the sarcosine portion of the antibiotic.
Me-Val = N-methyl-valine; Val = valine; Allo = alloisoleucine; Pro = proline; Thr = threonine; Sar = sarcosine