A Profile of Ribonucleic Acid Synthesis in *Corynebacterium Diphtheriae* Treated with Actinomycin S

It has been reported that actinomycin inhibits DNA dependent cellular RNA synthesis (Reich et al., 1962, Hurwitz et al., 1962, Levinthal et al., 1962), presumably by interacting with DNA molecules (Kawamata et al., 1960), whereby the synthesis of various cellular RNA species may be suppressed equally by this antibiotic (Reich et al., 1962). However, while studying the action of actinomycin S on RNA synthesis in a strain of *Corynebacterium diphtheriae*, we have recently observed that the synthesis of the cellular RNA of larger molecular size appears to be preferentially inhibited by low concentrations of this antibiotic.

A toxinogenic strain, Park Williams No. 8 (Biken) was used in this study. The organism was grown at 35°C with shaking in a modification of Mueller and Miller's casamino-acid medium (1941) by the method described by Yoneda (1957). When the O.D. at 590 mμ of the culture in a Bausch & Lomb spectrophotometer reached 8, it was quickly added with ^{14}C uracil (0.02 μc/O.D.) and, after 2 minutes incubation at 35°C with shaking, the whole culture was then divided into four parts. To these four parts actinomycin S was added immediately at final concentrations of 0, 1, 5 and 10 μgs per ml., respectively and they were incubated at 35°C for a further 30 minutes. After incubation, these cultures were quickly chilled with ice and the cells were collected by centrifugation. The packed cells thus obtained

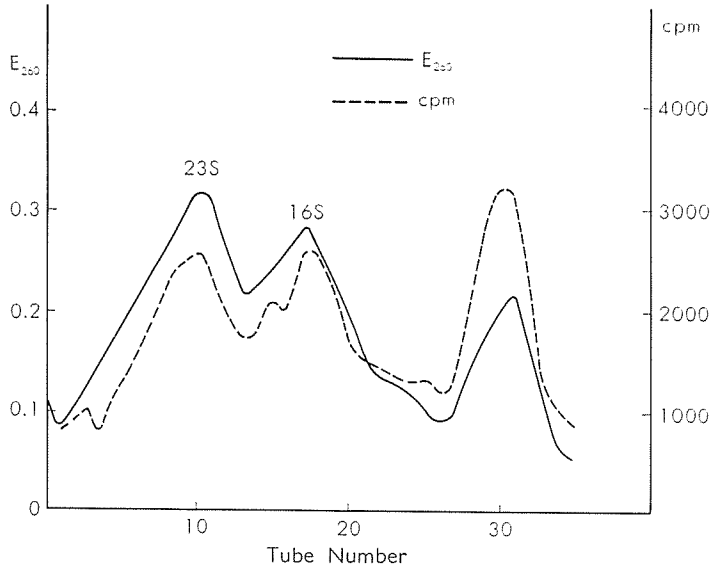

Fig. 1. Sucrose Density Gradient Pattern of Nucleic Acid. Labelled for 32 minutes

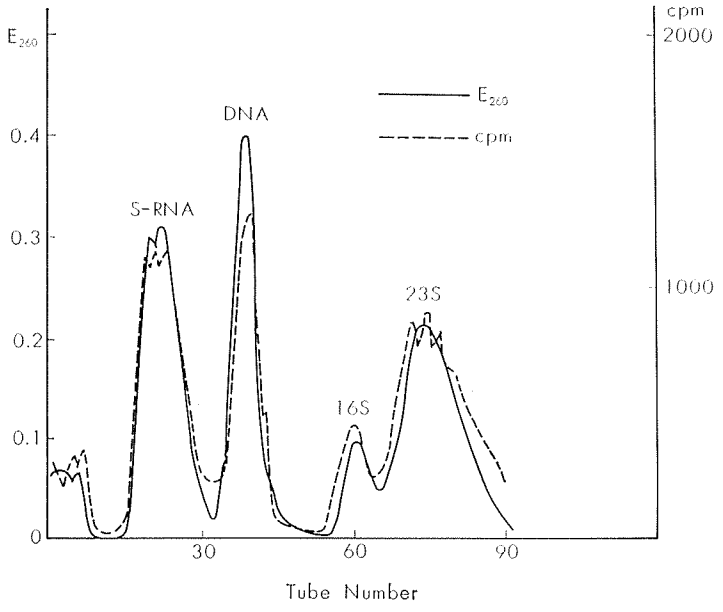

Fig. 2. MAC Chromatographical Pattern of Nucleic Acid. Labelled for 32 minutes.

were disintegrated by grinding them with quartz sand in the presence of 2.5 per cent sodium dodecylsulfate. Nucleic acids were extracted and purified from the homogenate by the phenol method (Gierer et al., 1956). The nucleic acid fractions were then subjected to both sucrose gradients and methylated albumin column chromatography. Linear density-gradients of sucrose (5 to 20%) in 0.01M tris-acetate buffer (pH 5.0) containing 0.1M NaCl were prepared according to the procedure of Monier et al. (1962) and the chromatography was done by modification of the method of Mandell & Hershey described by Mizuno (1963). Measurements were made of the radioactivity and absorbancy at 260 mμ of the drop fractions collected after centrifugation of the former in an RPS40-130 rotor (Hitachi 40P ultracentrifuge) at 40,000 rpm for 270 minutes and also the eluates obtained from the latter. The radioactivity was assayed in a Nuclear Chicago gas flow counter. When necessary, the RNA and DNA contents of the fractions were determined by the orcinol and diphenylamine reactions.

Results are shown briefly in the accompanying figures. As can be seen from these figures, there was a remarkable difference among the sucrose-gradients patterns, depending on the concentration of actinomycin S. Thus, in the presence of 10 μgs/ml of actinomycin S, the level of ^{14}C uracil incorporation into all species of RNA were equally inhibited (Fig. 3 and 4), while, in the presence of 1 μg/ml of the antibiotic, only the incorporation into the RNA around 23S region was found inhibited (Fig. 6). A concentration of 5 μgs/ml of antibiotic appeared to have an intermediate effect (Fig. 5). In addition, evidences have been obtained

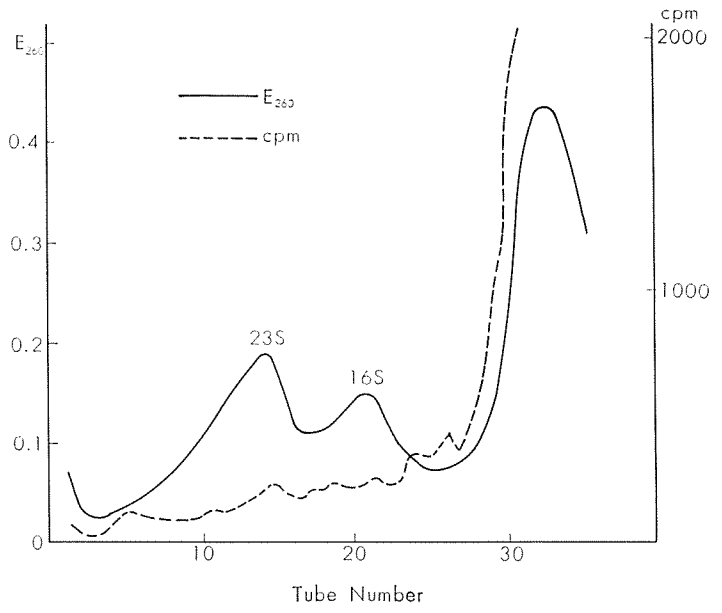

Fig. 3. Sucrose Density Gradient Pattern of Nucleic Acid. Labelled for 32 minutes in the Presence of 10 μgs/ml of Actinomycin S.

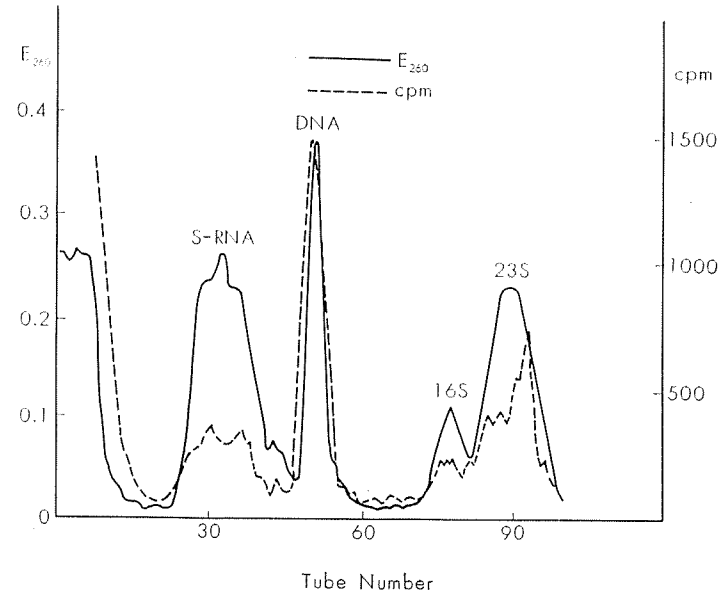

Fig. 4. MAC Chromatographical Pattern of Nucleic Acid. Labelled for 32 minutes in the Presence of 10 μgs/ml of Actinomycin S.

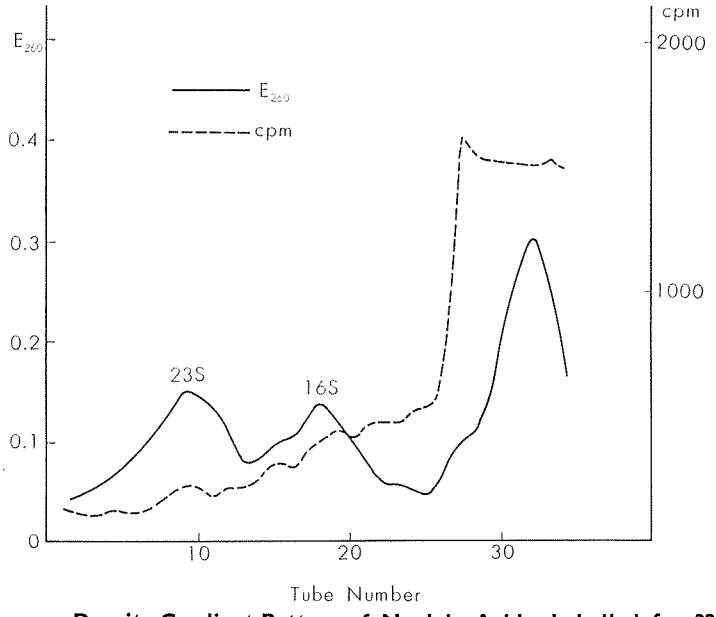

Fig. 5. Sucrose Density Gradient Pattern of Nucleic Acid. Labelled for 32 minutes in the Presence of 5 μgs/ml of Actinomycin S.

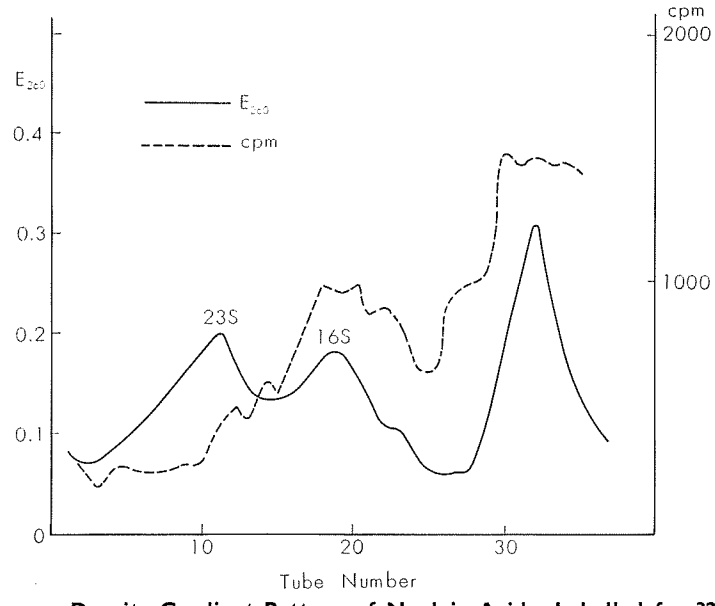

Fig. 6. Sucrose Density Gradient Pattern of Nucleic Acid. Labelled for 32 minutes in the Presence of 1 μg/ml of Actinomycin S.

from our separate experiments indicating the presence of the similar effect of this antibiotic on messenger RNA (Yamada, unpublished data). It therefore seems that there are appreciable differences in the actinomycin S sensitivities of various RNA fractions, mainly dependent on their molecular sizes. Similar results have recently been obtained in *Bacillus subtilis* SB 15, in which the net synthesis of 23S RNA was inhibited by a low concentration of actinomycin S while that of other RNA fractions was not.

The significance of these findings is not yet clear. A possible explanation is, however, that the frequency of binding of the antibiotic molecules to DNA increases in parallel with increase in the size of the cistron, particularly at low concentration of the antibiotic, and this may result in preferential inhibition of DNA dependent synthesis of RNA of large molecular weight. The intrinsic mechanism involved, however, still awaits further study. The details of these experiments will be published elsewhere.

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