Sporogen—an 'Inductor' for Bacterial Cell Differentiation

CELLULAR differentiation has proved to be one of the most fascinating subjects for investigations in the field of biology. Since Spemann's[1] discovery of the phenomenon of induction by organizers, numerous investigations have been directed towards the isolation and identification of inductor substances in morphogenesis. Efforts to obtain such genotropic substances and to analyse the mechanisms of their actions have so far been unsuccessful. In my investigations, bacterial sporogenesis has been taken as a model system for studying the biochemical aspects of intracellular differentiation.

My colleagues and I reported earlier[2] that an endogenous factor was present in the early stationary phase of growth of *Bacillus cereus* strain *T*. This factor played an active part in triggering the vegetative cells to the sporulating stage. It has now been fractionated and a biologically active substance isolated by means of the method which we used to detect biological activity in our previous communication[2]. The trivial name of 'sporogen' has been given to the crystalline material.

The procedure for the isolation of sporogen from 5 lb. (wet weight) of *B. cereus* strain *T* cells was as follows. The cells were suspended in 2·5 l. 0·01 M potassium phosphate buffer at *p*H 6·8. The suspension was mixed with an equal volume of glass beads (100μ in diameter obtained from Minnesota Mining and Manufacturing Co.), and the cells disintegrated by passing the mixture through an Eppenbach Colloid mill. The glass beads were removed by decanting off the fluid after allowing the mixture to stand for a while; the cell debris and unbroken cells were obtained by centrifugation. The clear supernatant fluid was adjusted by adding hydrochloric acid so that it had a *p*H of 2·0; at this *p*H a precipitate formed and this was removed by centrifugation. The solution, golden yellow in colour, was adjusted to *p*H 5·5, this time using potassium hydroxide, and left overnight at 4°–5° C. The small amount of precipitate which formed was removed and the solution was evaporated to dryness under reduced pressure; a residue weighing 100 g was obtained. This material was suspended in 5 l. of a mixture of ethanol formic acid and water in the proportion 90 : 5 : 5 and shaken thoroughly at room temperature overnight. The suspension was filtered off and the filtrate once again evaporated to dryness under reduced pressure; 10 g of residue was obtained. This was treated with a minimal volume of 0·2 N acetic acid and fractionated on a column (50 cm × 5 cm) of 'Sephadex' *G*-25 pretreated with 0·2 N acetic acid. The fractions were monitored by measuring the absorption at a wave-length of 249 mμ in a Beckman spectrometer. The active fractions were pooled, and once the solvent had been removed under reduced pressure, the residue was further fractionated on a cellulose column using a mixture of butanol–acetic acid–water (4 : 1 : 1) as the developing solvent. The fractions were analysed by paper chromatography using the same solvent mixture. Biological activity was exhibited by the fraction with an R_F of 0·35. This fraction was collected, the solvent removed by evaporation under reduced pressure and the residue crystallized from aqueous solution (yield 19 mg). The crystalline substance obtained melted between 162° and 165° C and had an elementary composition as follows: C, 42·78 per cent; H, 4·89 per cent; N, 18·63 per cent; O, 33·7 per cent (by difference). It had a characteristic ultra-violet absorption spectrum with an absorption maximum at 249 mμ (Fig. 1). The infra-red spectrum of the compound is presented in Fig. 2.

The chemical nature of the substance is at present under investigation, but little is known about the mechanism of its action. As a working hypothesis, adopting Jacob and Monod's model[3] for induced enzyme synthesis, it may be taken for granted that sporogen derepresses the regulators controlled by the 'sporogenes', thereby releasing the operator gene which in turn switches on the structural genes responsible for the synthesis of macromolecules which is required for the initial steps in sporulation.

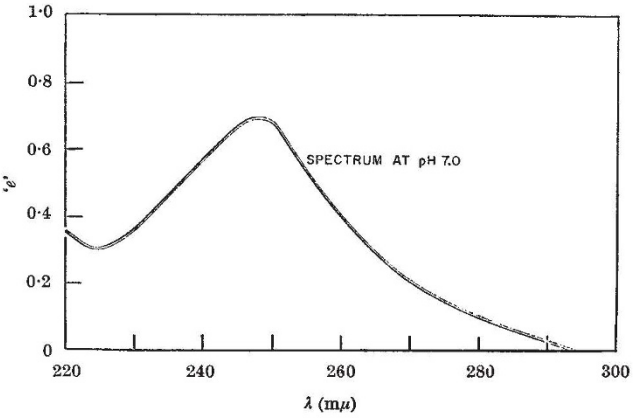

Fig. 1. Ultra-violet absorption spectrum of sporogen

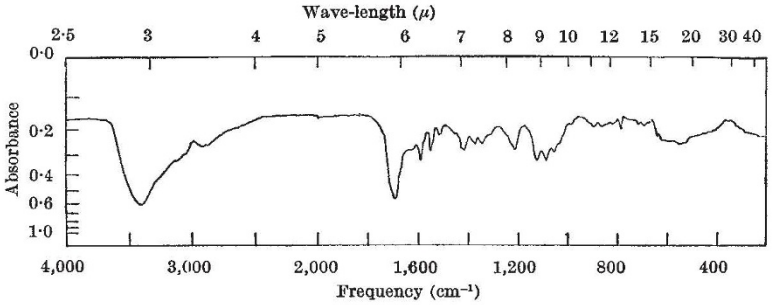

Fig. 2. Infra-red spectrum of sporogen

I thank Dr. H. Orin Halvorson for his advice, and Mr. Francis Engle for his assistance. I also thank Dr. J. G. Garver of the University of Wisconsin for making available his facilities for growing large batches of organisms.

These investigations were supported by grants from the Office of the Naval Research and U.S. Public Health Service.

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GENETICS

Synchrony of Chromosome Duplication

IN some recent publications statements have been made on the marked asynchrony of homologous pairs of autosomes with respect to duplication of the chromosomes—as studied with tritiated thymidine[1–3]. In an earlier publication, in which quantitative analysis of grain counts on chromosomes has been performed, we have reported that no evidence for a marked asynchrony was found[4].

In view of these apparently contradictory reports, the question was re-examined. The results, which are described here, indicate that there is no detectable