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The Growth of Spirillum volutans Ehrenberg in Mixed and pure cultures* **

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With 2 Figures in the Text

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*Spirillum volutans* Ehrenberg is one of the largest and one of the oldest recognizable species of bacteria. It was first observed by EHRENBERG in 1830 and was described, with illustrations, in his famous treatise *Die Infusionstiere* als vollkommene Organismen (1838). Because of its size, shape, distinctive motility and the existence of the excellent drawings of both EHRENBERG (1838) and COHN (1872), a modern investigator should have no difficulty in concluding that he is observing the same creature as was observed by these early microbiologists. The possible existence of more than one distinct physiological type of the same morphology is, of course, not excluded.

Despite the antiquity of our knowledge of this species and the efforts of several prominent investigators of the genus *Spirillum*, pure cultures of *S. volutans* have never been achieved although there are claims to the contrary. The history of this organism has recently been reviewed (WILLIAMS and RITTENBERG 1957; WILLIAMS 1959), making a discussion of the older literature on this point unnecessary. To quote LEWIS (1940), "Review of the literature affords convincing evidence that this species has not yet been cultivated in pure culture."

We are acquainted with only two articles since 1940 in which the growth of *S. volutans* in pure culture is implied. BELOZERSKY (1941) studied the nucleic acid composition of an organism he referred to as *S. volutans*. No description or pictures of the organism was provided. The spirillum was cultured on meat extract peptone agar supplemented with 5 per cent yeast autolysate. Although this medium will grow some of the small spirilla, there is ample evidence to show that it is not suitable for *S. volutans* culture (WILLIAMS and RITTENBERG 1957; WILLIAMS 1959).

* Dedicated to Professor E. G. PRINGSHEIM on his 80th birthday.
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BÜNNING and GOSSEL (1955) studied the effect of chloral hydrate on a culture labeled *S. volutans* which they grew in a dilute yeast autolysate medium. The culture employed was obtained from the Hygiene Institute of the University of Tübingen. A photomicrograph of the organism is presented in the article and reveals cells of the general size of *S. serpens* or smaller which could not possibly be *S. volutans*. In both instances it is probable that the investigators received mislabeled cultures from a collection and had no interest in the validity of the identification.

Our introduction to *S. volutans* came a decade ago when in the course of a taxonomic study of the genus *Spirillum* we received a mixed culture containing this organism. Its general morphology has been described and illustrated elsewhere (WILLIAMS and RITTENBERG 1957); this paper deals with our efforts to grow it in pure culture.

**Materials and Methods**

1. **Culture source.** The initial mixed culture was obtained from Dr. E. G. PRINGSHEIM in 1951. He originally found the spirillum in water from the cooling tower of a sugar beet refinery in England.

2. **Observational and counting techniques.** For routine qualitative observations about 0.05 ml of culture was placed on a microscope slide and examined wet without the use of coverslips or stains at 100 magnifications. Such drops could be scanned rapidly and as few as a single spirillum detected. Growths observed in this manner were judged on a scale of 0 (less than 20/ml) to 4+ (more than about 200,000/ml).

   For quantitative growth measurements a tiny drop of 1% mercuric chloride and a drop of culture were mixed on the surface of a hemacytometer and immediately covered with a cover glass. The mercuric chloride instantaneously immobilized the spirilla which, for the most part, were fixed to the hemacytometer surface. The cells in the entire ruled area of the hemacytometer (3 mm²) were counted using 100 magnifications and the results were converted to a per ml basis.

3. **Preparation of extracts.** Soil and wheat extracts were components of many of the media employed. Soil extract was prepared by suspending the desired amount of air-dry soil in tap water and heating the suspension to boiling. After cooling and settling, the turbid supernatant liquid was clarified by high speed centrifugation and the resulting clear extract was sterilized at 121°C for 20 min. Unless otherwise specified, the initial proportion of soil to water was 100 g per 100 ml.

   Wheat extract was prepared by heating to boiling 10 g of whole wheat grains in 100 ml of water, cooling and settling, filtering through paper, and sterilizing as above. Unless otherwise specified, 10 ml of the extract were employed per 100 ml of medium (10% wheat extract).

   Freshly prepared yeast autolysate rather than the dehydrated commercial product was used.

   The casein hydrolysate employed was an acid hydrolyzed commercial product (N-Z Case, Sheffield Chemicals).

4. **Media.** Many different media were tested during the course of the investigations, including commercially available dehydrated preparations and synthetic and complex media compounded in the laboratory. Recipes of those of significance are included in the experimental section. The mineral salts base of the synthetic media employed had the following composition: $K_2HPO_4 \cdot 3H_2O$, 0.5 g; $MgSO_4$,