citrate buffer, pH 6.0, containing 0.15 *M* Na^+ has been utilised for the chromatographic study. Hemoglobin solutions of chick, pigeon (*Columba livia*), duck (*Anas anas*) were resolved into two zones and of Little Cormorant into three zones. It seems rather interesting to note that, although the hemoglobins from white-breasted water hen (*Amaurornis phoenicurus* Pennant), crow (*Corvus splendens splendens*) and black-headed oriole (*Oriolus xanthornus* Linn.) could be resolved into three components by paper electrophoresis, under the present experimental conditions only two zones could be observed.

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Nutrition Research Unit, Indian Council of Medical Research, Department of Applied Chemistry, University College of Science and Technology, Calcutta (India) ANIL SAHA

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Nephelometric determination of cell count in synchronously dividing cultures of bacteria

Synchronization of cellular division of bacteria induced by chilling has been described in several recent papers[1-3]. Although this method is simple, the frequent estimation of the number of bacterial cells at short intervals by plate counts is tedious and time-consuming. Determination of direct cell count in a bacterial counting chamber suffers from the same disadvantage. Standard turbidity measurements in the spectrophotometer do not discriminate between relatively small changes in highly diluted, synchronously dividing populations. The same difficulties arise when other methods of synchronization are used.

In our experiments a Pulfrich photometer-nephelometer (C. Zeiss, Jena) has been chosen for these determinations. This highly sensitive instrument measures the turbidity of a bacterial suspension by means of the light scattered at a given angle and gives reliable readings of the increase in cell number of relatively small concentrations of bacteria, while the experiment is in progress.

An actively growing streptomycin-resistant strain of *Escherichia coli* B (3-h culture at 37°) was inoculated in 150 ml tryptose medium (0.5 % Bacto-Tryptose and

0.5 % NaCl), passed through a fine sintered-glass filter (Jena G4). All glassware was scrupulously free of dust and other extraneous material. Shortly after inoculation growth of the population was synchronized by chilling to 10° in a water bath under continuous shaking. After 30 min the temperature was instantaneously changed to 34°. Samples for turbidity measurements were taken at 10-min intervals. 0.1 ml formalin was added to each 5-ml sample. The plotted values give a typical step-wise growth curve with 40-min lag periods where practically no increase in turbidity occurs. The same samples were read in the Jean & Constant spectrophotometer at 450 and 600 mμ but here the logarithm of the increase in absorbance was linear. The growth rate of a control culture grown under the same conditions but without synchronization treatment was $\mu_{exp} = 1.515$.

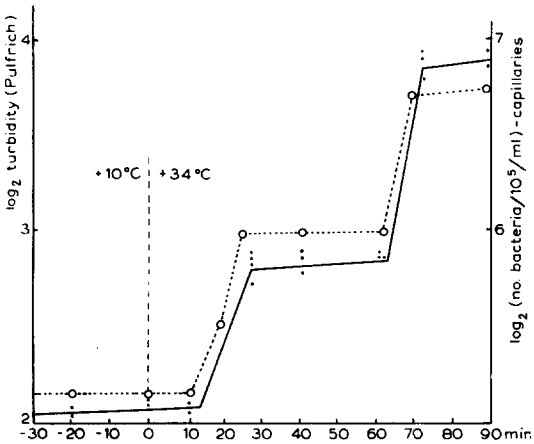

Fig. 1. Growth curve of synchronously dividing *Escherichia coli* B measured in the Pulfrich nephelometer and by direct count in capillaries. From —30 to 0 min, chilling at 10°; from 0 min at 34°.

·····, turbidity (Pulfrich);
———, direct count (capillaries).

Turbidity measurements in the nephelometer were compared with direct cell counts in capillaries by the modified method of GABE[4] and with plate counts. In the range from $5 \cdot 10^5$ to $5 \cdot 10^7$ cells/ml all three methods gave satisfactory parallel results, as shown in Fig. 1. When the system of filters, 4:E:L2h, of the Pulfrich nephelometer were used and turbidity determined by the right diaphragm (with the left diaphragm constantly on 100), 1 division represented $5 \cdot 10^5$ viable cells or $7 \cdot 10^5$ cells/ml calibrated with direct count in capillaries. Thus the smallest suitable initial concentration of bacteria in a synchronization experiment is $8 \cdot 10^5$ cells/ml. The capillaries with the bacterial suspension were fixed on glass slides by means of paraffin, mounted in immersion oil or water and covered with a cover-slip. Total count of cells in one field of the phase-contrast microscope was determined and the inner volume of the capillary measured. Analogous results were obtained with *Bacillus megaterium*.

The nephelometric method enables rapid cell count estimations in synchronization experiments and it is useful in biochemical studies of synchronously dividing bacteria.

Department of General Microbiology, Charles University, Viničná 5, Prague 2 (Czechoslovakia) J. STÁRKA J. KOZA

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