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ISOPYCNOGRAPHY OF INTACT CELLS - III: CONCENTRATION OF RIBOSOMES IN *Escherichia coli* VERSUS GROWTH RATE

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SUMMARY:

The relative quantification of the ribosomal content in a population of cells permits correlation between growth rate and available protein synthesis machinery. Isopycnometry of living cells indicates that this correlation is linear in E. coli B. Additionally, the narrow band-widths imply great homogeneity of response by the population at any one time. The precision of this method appears to surpass that of previous determinations.

The technique of density gradient centrifugation in the study of intact, living cells was reported in 1959 by Church and Halvorson (1). The procedure with variations was used in a number of studies until 1967 when work by Pollard and Grady (2) enumerated several ill-defined characteristics of cells such as osmotic effects, and cell wall structure and growth that cast doubts that good understanding could be made of results obtained by this method. After more than a decade in eclipse, during which time the needed cellular information accrued, the technique was reintroduced by Patterson, et al (3) and was applied to the RNA concentration in E. coli B cells over the growth curve. Since cellular specific gravity§ in these cells has been shown to be an index of the concentration of ribosomal RNA (r-RNA), the method is useful in determining the relationship between the cell's complement of protein synthesis apparatus and growth rate. Such a determination has already been reported earlier, but it entailed the use of destructive analysis (4). The

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§ So as to minimize confusion, we are substituting "specific gravity" in lieu of the more proper "density" since the latter term has a different connotation for bacteriologists.

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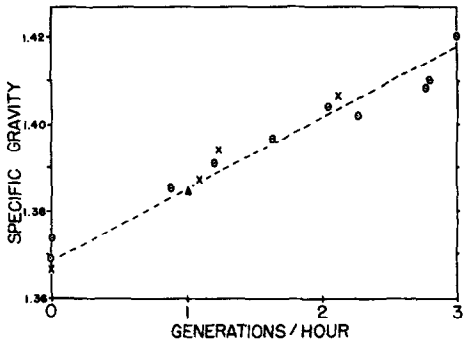

Fig. 1: Cellular Specific Gravity of E. coli B versus Growth Rate. Cells were grown at 37°C in a constant influx chemostat which allowed the imposition of submaximal exponential growth rates. Standard nutrient broth (o), glucose-salts (Δ) and glucose-casamino acids (×) media were used.

method which we report here is by a vastly different approach having intrinsically a high degree of precision. This should add not only further exactitude to former conclusions but also insights into the homogeneity of the behavior of cells because the forte of isopycnography is the detection of types of heterogeneity that affect cellular specific gravity.

MATERIALS AND METHODS

Escherichia coli B were grown in media of three levels of enrichment: standard nutrient broth, casamino acids broth (0.5% casamino acids, 0.7% glucose, in a standard mineral salts elixir), and a standard glucose-salts medium (0.7% glucose). The cultures were grown in a constant influx chemostat and thus variable control was maintained over the rates of exponential growth. As all experimental runs were of relatively short duration, only the minimal care of keeping the broth reservoir at 70C was needed to minimize significant contamination. The duct from this reservoir to the growth chamber was passed through a coolant to lower the temperature of the influent to that of the growth chamber, which was 37C. Our basic working formula for this chemostat was

$$g = T \ln 2,$$

where g is the desired cellular doubling time, and T is the volume of the growth chamber divided by the volume of influent per minute.

Density gradients of aqueous sodium bromide were made to range from 1.2 to 1.5 g/cc. Water saturated with NaBr at room temperature is approximately 1.5 g/cc, and for the sake of brevity is abbreviated 1.5 NaBr. In the construction of a gradient, the distal chamber of the linear gradient maker is filled with a bacterial sample that has been quenched by the addition of 0.6 volume of 1.5 NaBr. This results, therefore, in a cell suspension that approximates 1.2 NaBr. The density gradients were then centri-

fuged at 17,000 x G for 10 minutes by which time isopycnotic equilibrium has been attained. Fractions may be taken by bottom-puncture of the tube, or, as was done here, samples of the highly visible bands were extracted by a capillary pipet from the top of the tube. The specific gravities of these samples were then determined refractometrically.

RESULTS

The data-points closely form a linear relationship between the growth rate and the cellular specific gravity regardless of the type of medium (Fig. 1).

A further observation was that in all cases the bacteria formed single bands of 3 mm or less in the 8 cm deep gradients.

DISCUSSION

The investigation of the kinetics of r-RNA synthesis has two levels of approach. One may elect to study the synthetic rates of its transcription from the genome, or one may study the accumulation of the r-RNA in the ribosome pool. The subtle difference between these two avenues is that the former method measures the production of both the stable molecules as well as those made in excess to be soon broken down, while the latter method measures only the eventually active molecules. The transcriptional rate perspective centers around the work of Kirsten Gausing (5), while the senior experimentalist of the accumulation of stable forms is Sir Cyril Hinshelwood (6). This paper, of course, concerns itself with the stable form approach, which we feel has special importance since it deals with ribosomes, which, in turn, are the foci of protein synthesis.

Our data therefore shows that linearity exists between the growth rate and the concentration of the protein synthesis machinery as determined in *E. coli* cells. Because this non-destructive physical method inherently possesses very good precision, the data points do fall more closely into a line than previously derived chemical data. Thus these data strongly support the early speculation posed by Maaløe and Kjeldgaard (7) that the number of ribosomes should be proportional to the growth rate, and Hinshelwood's oft-cited, albeit rather scattered, data to that effect regardless of the type of medium.

Because of statistical mechanical considerations, systems having very small numbers of operative components might be expected to exhibit considerable deviation about the mean in their activities. Such a case would be an E. coli cell. Although simplistic, the calculation of even the number of hydrogen ions expected in its cell volume should facilitate the reader's perspective in this micro-realm. With a cell volume of about 5×10^{-13} ml, there should be on average only one H^+ per cell presuming a neutral pH throughout that volume. Of course the reader realizes that the notion of pH breaks down when such small volumes are considered because of the transcient nature of any given H^+ OR OH^-.

However such considerations are quite valid when it comes to various species of "permanent" molecules such as a cell's macromolecules, many of which are well known to have very low numbers per cell. Among such extreme cases may be numbered, for example, the DNA-polymerases and even the DNA replicative origin, ori, on the genetic map. Yet, despite the extremely low numbers of these components, the cells manage to march closely in step as the narrowness of the bands in the centrifuge runs invariably indicated. For the ribosome synthesis system, which is comprised of so many parts, many of which are predicated upon species of templates and catalysts in ultra-low concentrations, the organization in these "simple" cells must be truly remarkable.

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