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SHORT COMMUNICATION

Some observations on the use of absorbance measurements in bacteriology

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ABSORBANCE measurements are commonly used in bacteriology to follow the growth of cultures and for standardizing cell suspensions. This communication reports some investigations into the relation between absorbance and dry weight of washed cell suspensions and growing cultures of *Escherichia coli*.

EXPERIMENTAL

The organism used was *Escherichia coli* (NCTC 1013). The media, conditions of cultivation and methods of measuring absorbance and radioactivity have previously been described (Rye & Wiseman, 1966). All experiments were made on cultures obtained by diluting exponentially growing cells with medium at 37° to an absorbance of about 0·050 (650 mμ). Uniformly labelled cells were obtained by replacing the glucose in the growth medium with ^{14}C-labelled glucose (specific activity 0·01 μc/mg) and allowing growth through at least eight generations.

Dry weight determinations. Membrane filters (8 cm, Oxoid) were washed with water, dried to constant weight by heating at 95° for 10 min and stored in a calcium chloride desiccator. 100 ml volumes of bacterial suspensions were filtered through these membranes, the cells washed with 10 ml distilled water and their weight determined by difference after redrying the membranes as above.

RESULTS AND DISCUSSION

Fig. 1A shows the absorbance of dilutions of a washed cell suspension of *E. coli* in glucose-free medium as a function of relative bacterial concentration. The relation is almost linear showing that in these washed cell suspensions where growth has been arrested an approximate proportionality exists between absorbance and cell concentration, whether this is measured in terms of cell number or total cell mass. The correction for deviation from linearity, calculated using the Longsworth equation (Longsworth, 1936; Kavanagh, 1963) is only 2% for an observed absorbance of 0·500.

With exponentially growing cells the relation between absorbance and total cell mass was investigated by making dry weight measurements on samples from exponentially growing cultures after different periods of growth when the absorbances were between 0·100 and 0·700.

The graph of these weights against absorbance was markedly curved showing that absorbance measurements made on growing cultures are

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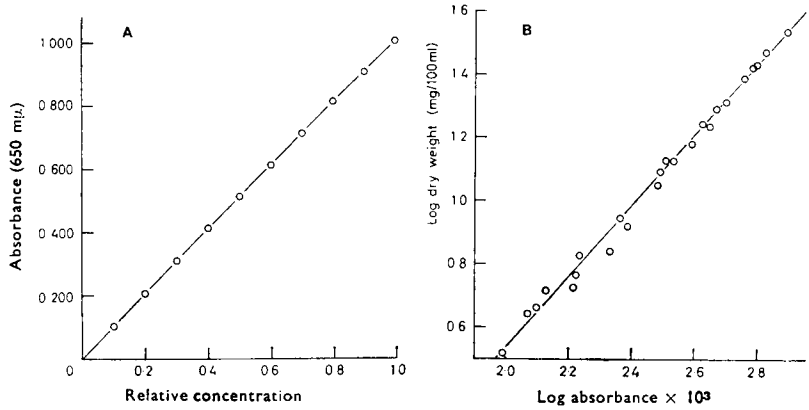

FIG. 1A. The absorbance of dilutions of a washed cell suspension of *E. coli*.

B. The relation between the absorbance and dry weight of exponentially growing cultures of *E. coli*.

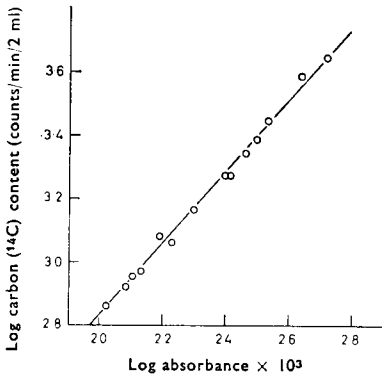

FIG. 2. The relation between absorbance and total cellular carbon[^{14}C] content of cultures of *E. coli*. The line has been drawn with a slope of 1·12 which is equivalent to the equation derived from the results of Fig. 1A.

not proportional to total cell mass. These results are presented in Fig. 1B as a log-log plot and the relation observed can be represented by the equation

$$M = 0{\cdot}0195A^{1.12} \quad .. \quad .. \quad .. \quad (1)$$

where M is dry weight mg/100 ml and A is absorbance $\times 10^3$.

From this equation the dry wt/ml of a growing culture at an absorbance of 0·500 will be 20% greater than would be expected from its dry weight when at an absorbance of 0·100 if the relation shown in Fig. 1A applied during growth.

Fig. 2 shows a log-log plot of absorbance with the total cell carbon [^{14}C] content for exponentially growing cultures of *E. coli* together with a line of slope 1·12 representing the relation between dry weight and absorbance given by equation (1). The total cell carbon content increases

at the same rate as dry weight showing that the cell carbon/dry weight ratio remains constant during growth and that measurements of carbon content can be used as a measure of relative cell mass.

The apparent contradiction between the results obtained using washed cells with those using exponentially growing cells may be attributed to changes in cell size, shape, density or relative refractive index occurring during growth. Koch (1961) discussed the effects of such changes and suggested that "absorbancy measurements are more nearly a measure of bacterial mass than of bacterial numbers". A direct proportionality between dry weight and absorbance which was independent of variations in cell size and shape has been observed with *Salmonella typhimurium* (Schaechter, Maaloe and Kjeldgaard, 1958) and similar results have been reported for a species of lactobacillus (Burns, 1959).

The conclusions to be drawn from our results are (*a*) that suspensions of equal absorbance prepared by harvesting cells from growing cultures of *E. coli* will not necessarily contain the same total cell mass, and (*b*) that the dry weights of suspensions prepared during the early stages of exponential growth will be lower than those of suspensions of the same absorbance prepared from cells harvested at a later time. These conclusions are confirmed by the results of measurements of the carbon [^{14}C] content of four suspensions of equal absorbance prepared from cells harvested at different times during exponential growth (Table 1).

TABLE 1. THE CARBON [^{14}C] CONTENT OF WASHED CELL SUSPENSIONS PREPARED BY HARVESTING CELLS AT DIFFERENT TIMES FROM AN EXPONENTIALLY GROWING CULTURE AND ADJUSTING THE ABSORBANCE TO 0·100

Time of harvesting (min)	81	119	137	153
Absorbance of culture at harvesting	0·142	0·225	0·281	0·340
^{14}C content as counts/min/2 ml	646 ± 2·2	690 ± 2·4	703 ± 2·4	741 ± 2·5

Clearly in quantitative investigations where suspensions of *E. coli* are standardized by absorbance measurements, good correlation between replicate experiments cannot be expected unless the cells are harvested after the same extent of exponential growth has occurred.

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