RESPIRATION AND LIGHT-SCATTERING CHANGES IN ESCHERICHIA COLI [1]

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This article reports on light-scattering (LS) changes related to respiration in intact, resting cells of Escherichia coli. The evidence indicates that LS affords a direct assay for an energy-linked, potassium dependent, facilitated transport phenomenon. The description of this phenomenon appears to relate a system of metabolic swelling observed in protoplasts by Sistrom (1958) and Abrams (1959), with the permease system (cf. Cohen and Monod, 1957) of the intact cell.

Fig. 1 is a typical recording of the kinetics of LS and respiratory changes in a cell suspension incubated with K^+ ions. As illustrated on the left, little change in LS occurs during endogenous oxygen utilization (0.07 μM/sec.). Glutamate initiated a decrease in LS (-14.5%/min.) after a 17 second delay, and respiration was augmented 10-fold above the endogenous value following a 74 second lag period. The total decline in LS brought about by the addition of glutamate was 37.5%; this value is corrected for a 3.5% decrease after diluting the reaction mixture with the addition of glutamate. The experiment was terminated on the exhaustion of oxygen; however, the condition of decreased LS is maintained for a considerable period of time.

The LS decrease and acceleration of respiration initiated by glutamate are K^+ dependent (Fig.2). The maximal effect of K^+ was observed in the

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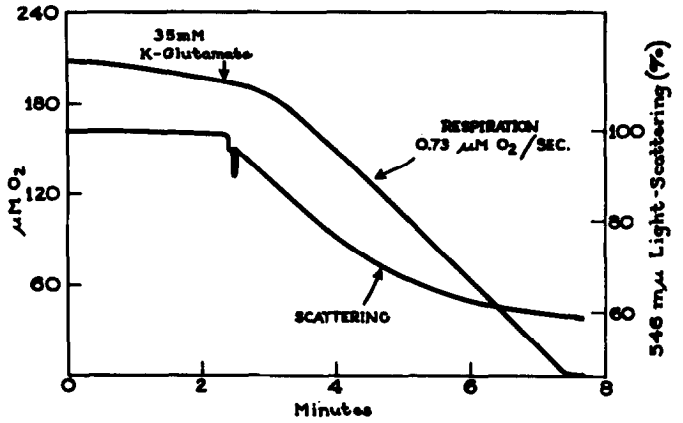

Figure 1. Kinetics of light-scattering and respiration changes. The system (4.0 ml) contained tris(hydroxymethyl)aminomethane (0.03 M, pH 7.6), K^+ (as Cl, 0.20M), and twice water-washed E. coli suspension (about 1 mg dry weight/ml). Glutamate (35 mM) was added as the K^+ salt as indicated. Temperature: 26°C. Light-scattering at 546 mμ was measured at 90° in the Brice-Phoenix light-scattering photometer. Respiration was determined with a vibrating electrode inserted into the light-scattering cuvette. Scattering and oxygen consumption were followed simultaneously on strip chart recorders.

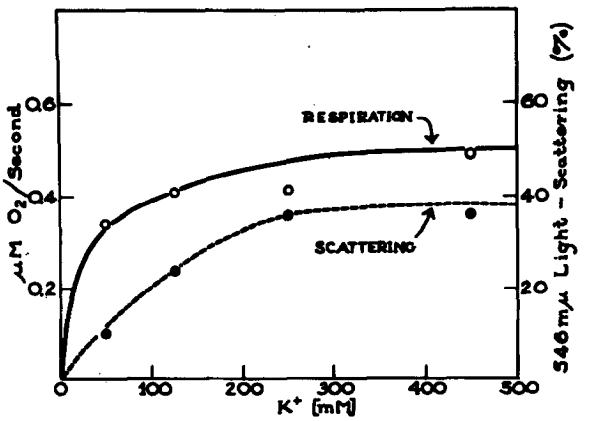

Figure 2. Potassium-dependence of light-scattering and respiration. System and conditions as in Fig. 1 except that the initial reaction mixture contained 25 mM glutamate and no KCl. The value for K^+ = 0 was obtained by employing Na-glutamate instead of the K-salt. Ordinate for LS refers to % decrease.

range of 250-350 mM and these changes parallel one another in response to K^+ concentration with glutamate as in Fig. 2, and with other substrates. Table I shows that Na^+ ions inhibit the rate and extent of the LS change and respiration.

TABLE I

Cation dependence of respiration and light-scattering. System and conditions as in Fig. 1 except that glutamate (K^+ or Na^+ salt as indicated) was 25 mM.

Added cation (mM)	Respiration $\mu M\ O_2$/ sec	Light-scattering	
		% Decrease first minute	Total % Decrease
250 K^+	0.42	8	36
250 Na^+	0.13	2	9

The effect of substrate is illustrated in Fig. 3; saturation occurs near 50 mM. The time required to establish a linear rate of glutamate respiration (cf. Fig. 1) was also concentration dependent, the lag times being 0, 43, 74 and 91 seconds for 5, 15, 35 and 50 mM glutamate, respectively.

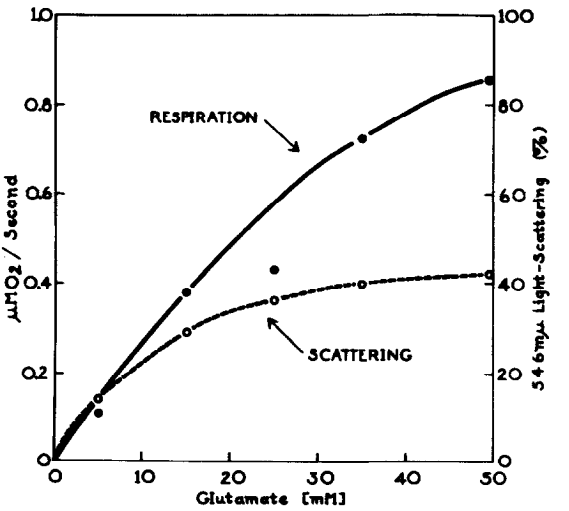

Figure 3. Glutamate dependence of light-scattering and respiration. Conditions as in Fig. 1. Ordinate for LS refers to % decrease.

The influence of glutamate on LS changes is dependent on its oxidation, as decreases in LS cease on anaerobiosis. Similar findings were obtained with other substrates; however, glucose was also capable of eliciting LS changes under anaerobic conditions, but at a reduced rate and extent. LS changes are completely blocked by 0.01 M azide but this amount of azide did not appreciably affect respiration. It was concluded that the LS changes were related to metabolic energy derived from aerobic or anaerobic processes.

Since LS changes often reached levels of 40 to 50% below their initial values, cells were tested for viability. There was no change in colony counts obtained on aliquots from suspension maintained in their normal scattering state and the state of decreased scattering. Thus decreased scattering is not interpreted as being a measure of cell lysis.

A turbidity (or LS) decrease dependent upon K^+ ions and anaerobic glycolysis has been reported for intact cells of Streptococcus fecalis suspended in (and also dependent on) a high sucrose medium (Abrams, 1959). It was concluded that this turbidity decrease was a measure of "metabolic lysis" (Abrams, 1959) brought about by a system for accumulating sucrose (Abrams, 1960), notwithstanding that turbidity decreases in protoplasts of this organism were correlated with swelling (cf. also Mager et al., 1956). Earlier, Sistrom (1958) had followed the intracellular accumulation of B-galactosides in E. coli protoplasts by measuring the decline in turbidity which accompanies osmotic swelling. In agreement with the characteristics of permease catalysis of galactoside accumulation, it was found that turbidity did not decrease in protoplasts from non-induced cells. Kepes (1957) has reported an enhancement of oxygen utilization during the accumulation of B-galactosides in induced but not in uninduced E. coli. In other experiments, not cited here, it was observed that large LS decreases not accompanied by lysis can be brought about by low concentrations of K^+ and substrate if E. coli suspension are incubated in media containing high sucrose. These considerations point to general requirements of K^+ ions and metabolic energy for the entry of metabolites into the bacterial cell. It appears that the system described affords a direct measure of the rate and extent of intra-

cellular accumulation and utilization of the transported substance.

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