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*EFFECT OF METABOLIC ACTIVITY ON THE GLUCOSE PERMEASE OF BACTERIAL CELLS**

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Specific, enzyme-like transport systems, called permeases, mediate the uptake by bacterial cells of β-galactosides,[1-3] galactose,[4-7] maltose,[8] glucose,[2, 9, 10] and probably other metabolites. The compound in question or a nonmetabolizable analogue of it may be accumulated within the cell against a concentration gradient to a level of internal concentration that is hundreds of times greater than the external one. The accumulation of substrates by the β-galactoside, galactose, and maltose transport systems is known to be inhibited by sodium azide and dinitrophenol,[1-8] which are inhibitors of oxidative phosphorylation. The accumulation of the nonmetabolizable substrate, α-methyl glucoside (αMG) by the glucose transport system of *Salmonella typhimurium*, on the other hand, has been shown to be stimulated several fold by these poisons.[10] This has been interpreted as an indication of the existence of an energy-requiring "glucose exit reaction" which serves as a pumping-out mechanism for glucose and αMG. The present studies extend further the observations on the relationship between metabolic activity and the accumulation of αMG in both *S. typhimurium* and *Escherichia coli*.

Materials and Methods.—The procedures followed are as previously described[10, 11] with certain modifications. *S. typhimurium* LT2 and *E. coli*, strains B/r and K12, were grown at 37° in a synthetic medium as previously described,[11] but with the addition of $FeSO_4 \cdot 7H_2O$ at 5×10^{-5} per cent and with different carbon sources as indicated. Cells were harvested in the exponential phase of growth, centrifuged in the cold and resuspended in a mineral base (synthetic medium lacking a carbon

and nitrogen source) to a turbidity equivalent to 290 μg of dry weight per ml. In a few experiments, as will be indicated, the cells were washed in the mineral base.

Uniformly labeled C^{14} alpha methyl D-glucopyranoside (αMG), Nichem Inc., was employed as a measure of the glucose permease.[2, 10] This material was further purified by paper chromatography.[10] It was diluted with recrystallized, unlabeled αMG so that one μmole gave 9.4×10^5 count/min with the counter utilized (see below).

Accumulation of C^{14} αMG was determined as follows. To cells resuspended in cold mineral base, chloramphenicol was added to a final concentration of 50 μg/ml, and a carbon source, when employed, was added to a final concentration of 0.1 per cent. This reaction mixture was incubated for 5 min at the desired temperature on a shaker water bath, and $C^{14}\alpha$MG was added at concentrations to be specified. One-ml samples were taken at various intervals after the addition of the radioactive substrate and were vacuum-filtered onto Millipore HA filters or S & S Type A coarse filters. (The two types of filters gave similar results.) The filters were washed with one ml of ice-cold medium lacking αMG, air-dried, and counted in a Micromil gas-flow Geiger counter. At least one thousand counts were taken for each sample. Results are expressed as count/min/ml or as μmoles of αMG accumulated per gram dry weight of cells.

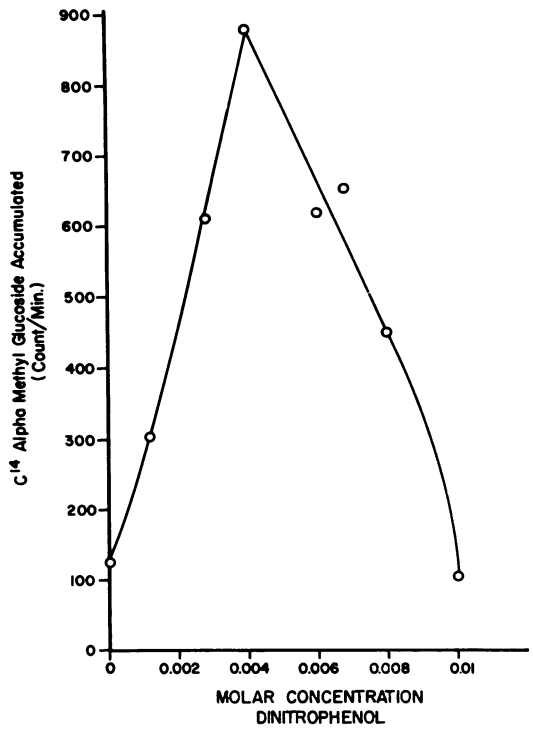

FIG. 1.—The effect of various concentrations of dinitrophenol on the accumulation of αMG in *S. typhimurium*. The reaction mixtures consisted of a mineral base suspension of fructose grown cells (see *Materials and Methods*) at a final concentration of 230 μg dry wt/ml; fructose, 0.1%; chloramphenicol, 50 μg/ml; C^{14} αMG, 2×10^{-5} *M*; and various concentrations of dinitrophenol. The reaction mixtures were incubated at 37° for 15 min after addition of radioactive substrate and duplicate samples were counted.

Oxidation of substrates was determined in a conventional Warburg apparatus at 37°.[12]

Fructose disappearance was determined by the cysteine carbazole method[13] at 540 mμ in a Klett colorimeter, 24 hr after the addition of reagents.

Results and Discussion.—The effects of azide and dinitrophenol: The addition of azide or dinitrophenol to a reaction mixture containing the organic substrate upon which the cells have been previously grown causes an increase in the extent of accumulation of αMG in *S. typhimurium*. An optimal concentration of azide, previously shown to be about 5×10^{-2} *M*, effects nearly a 9-fold increase in the internal concentration of αMG.[10] Further analysis of the azide effect has shown that the optimal concentration of azide varies from experiment to experiment between

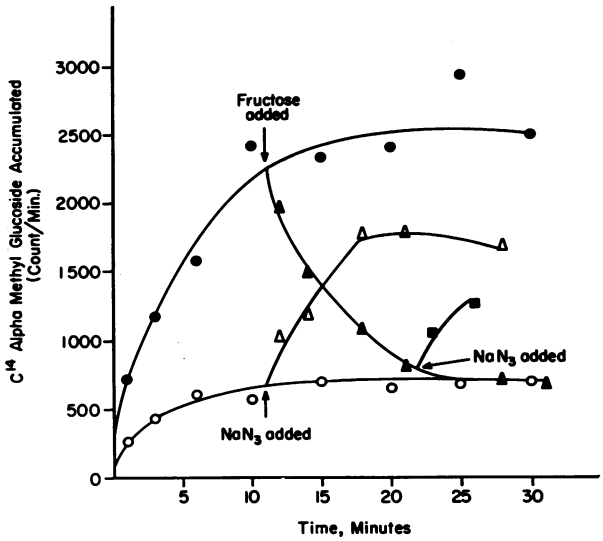

FIG. 2.—Accumulation of αMG by *S. typhimurium* in the presence and absence of an oxidizable substrate. Reaction mixtures were as previously described except for the carbon source (see legend, Table 1). (●—●) indicates accumulation without a carbon and energy source. At the first arrow (11 min), fructose (final concentration, 0.1%) was added (▲—▲); at the second arrow (22 min), azide was added (■—■). The control mixture (○—○) had fructose (final concentration, 0.1%) present at zero time. At the arrow (11 min), azide was added(△—△). (Final concentration of azide in both cases was 8×10^{-2} *M*.) Reaction was run at 25°.

4×10^{-2} *M* to 8×10^{-2} *M*, but the internal concentration of αMG at the optimal azide concentration is in all cases approximately 9-fold greater than that observed in the absence of azide. Similar results were obtained with increasing concentrations of dinitrophenol in the reaction mixture (Fig. 1). The maximal internal concentration of αMG in the presence of the optimal concentration of dinitrophenol, which varied from 4×10^{-3} *M* to 6×10^{-3} *M* in different experiments, was 7-fold that found in the absence of dinitrophenol. Further increases in dinitrophenol concentration above the optimum result in a very steep decline in the internal concentration of αMG (Fig. 1).

The effect of exogenous oxidizable substrates: An increase in internal concentration of αMG similar to that found with azide and dinitrophenol occurs in *S. typhimurium* if an oxidizable substrate is omitted from the reaction mixture or, in general, if the oxidizable substrate used in the reaction mixture was not previously employed in the growth of the culture (Table 1, Fig. 2). For example, when cells grown with glycerol are provided with no exogenous substrate or with fructose or malate in the reaction mixture, their internal concentration of αMG is two to three times greater than that found when they are provided with glycerol. On the other hand, when glycerol or malate is added to the reaction mixture containing cells grown with fructose, or if an exogenous substrate is omitted, the internal concentration of αMG is four to five times greater than that found when fructose is added (Table 1, Fig. 2). After a steady-state level of accumulation of αMG is reached in the absence of fructose, the addition of fructose causes an immediate decrease in the amount of internal αMG until a new steady-state level is attained, the level normally found in the presence of fructose.

TABLE 1

THE RELATIONSHIP BETWEEN THE CARBON SOURCE EMPLOYED FOR GROWTH AND ITS EFFECT ON THE INTERNAL CONCENTRATION OF αMG IN *S. typhimurium*

Carbon source employed in growth medium	Carbon Source in the Reaction Mixture: Glycerol + Malate	Glycerol	Malate	Fructose	Citrate	None
	(μmoles of alpha methyl glucoside/gm dry wt bacteria)					
Glycerol + malate	2.3	3.6	7.5	9.0	12.3	9.4
Glycerol	3.8	3.8	8.8	8.1	—*	9.2
Malate	2.1	10.1	3.2	11.2	—*	21.6
Fructose	9.0	9.3	9.7	1.7	—*	9.2
Citrate	8.3	8.9	3.6	7.8	2.7	9.3
Glucose	8.1	6.7	8.5	7.2	—*	7.0

* Not tested.

Reaction mixtures consisted of the following: carbon source, 0.1%; chloramphenicol, 50 μg/ml; C^{14} αMG, 4×10^{-5} *M*; KH_2PO_4, 0.3%; K_2HPO_4, 0.7%; $MgSO_4 \cdot 7H_2O$, 0.01%; and $FeSO_4 \cdot 7H_2O$, 5×10^{-5}%. Washed cells grown with the indicated carbon source were added to a final concentration equivalent to 270 μg dry wt/ml. Mixtures were incubated 10 min at 37° after the addition of labeled αMG.

TABLE 2

OXIDATION OF VARIOUS CARBON SOURCES BY *S. typhimurium*

Carbon source employed in growth medium	Carbon Source in the Respirometer Reaction Mixture: Glycerol	Fructose	Citrate	Malate	None
	(μl O_2 consumed/first 10 min interval)				
Glycerol	85	13	5	—*	2
Fructose	7	70	3	10	3
Citrate	—*	—*	95	63	3

* Not tested.

Warburg vessels contained in the main compartment, 2 ml cells in *M*/30 potassium phosphate buffer pH 7.0 (equivalent to 680 μg dry wt/ml) and chloramphenicol (final concentration, 50 μg/ml). At zero time, 4 μmoles of the indicated carbon source were tipped into the mixture.

Prior growth in the presence of a given substrate determines the effectiveness of the same substrate in depressing αMG accumulation, presumably because during such growth the specific permease and/or catabolic enzymes required for the rapid utilization of the compound are fully induced. Respirometric experiments described in Table 2 support this conclusion. Fructose and glycerol, for instance, are only oxidized at a high rate by cells previously grown with the respective substrate. The fact that both citrate and malate are oxidized by citrate-grown cells and depress their αMG accumulation may be attributed to the induction by citrate of a permeation system that is shared by both acids.[2, 11, 14, 15] On the other hand, cells grown under our conditions with a mixture of glycerol and malate do not readily oxidize malate, nor does malate have a strong effect on their αMG accumulation. This may reflect a repression by glycerol on induction to malate utilization similar to the "glucose effect." A similar effect by glycerol has been previously shown in the repression of histidase synthesis in this organism.[10]

Since low accumulations of αMG are obtained with different organic substrates, some of which are structurally very different from αMG (see Table 1), it is unlikely that the effects observed are the result of competition by these substrates for the glucose permease.

The effect of anaerobiosis: Cells grown aerobically with fructose were allowed to accumulate αMG under aerobic conditions with fructose as the oxidizable substrate. After a steady-state level was attained, nitrogen was bubbled into a sample of the mixture (Fig. 3). It can be seen that an increase in internal concentration of αMG, similar to that obtained with the addition of azide (Fig. 2) or dinitrophenol, oc-

curred. However, as shown in the figure, the disappearance of fructose from the medium was not inhibited under anaerobic conditions.

The maximal accumulation of αMG under anaerobic conditions was found to be approximately 18 μmoles per gm dry weight. The addition of glucose to a culture that had accumulated αMG under anaerobic conditions and in the presence of fructose caused a rapid disappearance of the label from the cells.

Stimulation of αMG accumulation by azide and dinitrophenol in E. coli: To determine whether *S. typhimurium* is fundamentally different from *E. coli* with regard to αMG accumulation, accumulation experiments were performed with *E. coli*, strains B/r and K12. As shown in Table 3, an increase in internal concentration of αMG occurs in the absence of an exogenous oxidizable substrate as well as in the presence of azide or dinitrophenol when these are added together with an oxidizable substrate upon which the cells have been previously grown. The failure of previous workers[2, 6] to demonstrate this increase in accumulation of αMG by *E. coli* upon the addition of azide or dinitrophenol was no doubt due to the absence of an effective oxidizable substrate from their reaction mixtures. Results such as they have obtained, in which azide and dinitrophenol are ineffective or slightly inhibitory, can be demonstrated in *S. typhimurium* by using a reaction mixture lacking an oxidizable substrate to which the cells have been fully induced (Table 4). With fructose-

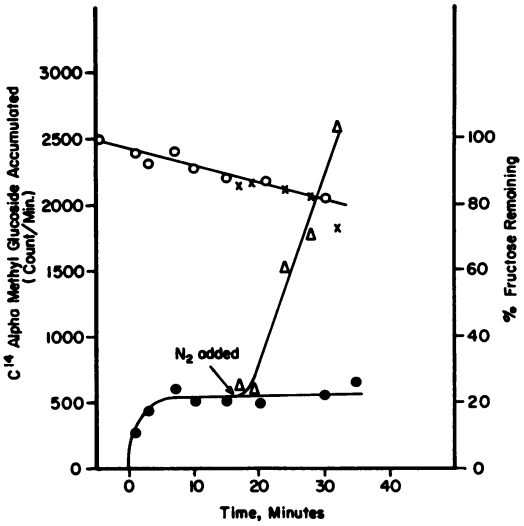

FIG. 3.—The effect of anaerobic conditions on the internal concentration of αMG in *S. typhimurium*. The reaction mixture was the same as that for Figure 1, except that the final concentration of cells was 270 μg dry wt/ml, C^{14} αMG was 4×10^{-5} *M*, and there was no dinitrophenol added. The mixture was incubated at 25°, and samples were removed at the indicated times. At the arrow, N_2 was bubbled into a portion of the mixture and samples removed at the indicated times. In a similar reaction mixture (lacking αMG), samples were removed for an assay of fructose remaining in the reaction mixture. (O) refers to samples from the aerobic culture; (×) refers to samples from the anaerobic culture.

TABLE 3

THE EFFECT OF OXIDIZABLE SUBSTRATE, AZIDE, AND DINITROPHENOL ON THE INTERNAL CONCENTRATION OF αMG IN *E. coli*

Additions to the reaction mixture	μmoles of αMG per gm dry weight cells of *E. coli* Strain K12	Strain B/r
None	29.6	4.8
Substrate(s)*	12.1	1.5
Substrate(s) + 1×10^{-2} *M* azide	33.6	4.4
Substrate(s) + 4×10^{-2} *M* azide	12.6	2.4
Substrate(s) + 8×10^{-2} *M* azide	4.5	2.2
Substrate(s) + 3×10^{-3} *M* dinitrophenol	44.0	6.1
Substrate(s) + 6×10^{-3} *M* dinitrophenol	37.0	7.0

* Glycerol (0.1%) added as oxidizable substrate to suspensions of *E. coli* K12; glycerol + sodium malate (0.1% of each) to *E. coli* B/r.

Reaction mixtures were as previously described in legend to Table 1 except for specified additions. Washed cells of *E. coli* K12 grown with glycerol or of *E. coli* B/r grown with glycerol plus malate were added to a final concentration equivalent to 250 μg dry wt/ml. Suspensions were incubated 10 min at 37° after addition of labeled αMG.

TABLE 4

THE EFFECT OF AZIDE ON THE INTERNAL CONCENTRATION OF αMG IN *S. typhimurium* IN THE PRESENCE AND ABSENCE OF THE CARBON SOURCE USED FOR GROWTH

Substrate added	μmoles of αMG per gm dry weight bacteria without azide	with azide
None	9.2	6.9
Glycerol	9.3	5.5
Malate	9.7	6.5
Glycerol + malate	9.0	6.3
Fructose	1.7	8.3

Reaction mixtures were as previously described in legend of Table 1, with the additions noted. Final concentration of azide was 8×10^{-2} *M*. Washed cells grown with fructose were added to a final concentration equivalent to 270 μg dry wt/ml. Mixtures were incubated 10 min at 37° after addition of labeled αMG.

grown *S. typhimurium*, a large internal concentration of αMG is established in the absence of fructose and a slight decrease in its level occurs upon the addition of azide. The stimulatory effect of azide is apparent only when fructose is present. The slight decrease in internal concentration of αMG caused by the addition of azide in the absence of an oxidizable substrate is presumably due to the inhibition of the entrance reaction. Although this inhibition of entry may always occur in the presence of azide, it would be masked by the strong stimulation of the over-all accumulation of αMG by the same poison in the presence of an oxidizable substrate. The inhibitory effect of azide and dinitrophenol on the entrance reaction may be the explanation of the decrease in αMG accumulation with increasing concentrations of these compounds above the optimum.

Summary and Conclusions.—The experiments that have been described all point to a difference between the glucose (α methyl glucoside) accumulating system of both *S. typhimurium* and *E. coli* and the β-galactoside and galactose accumulating systems of *E. coli*. Active respiratory activity accompanied by oxidative phosphorylation depresses rather than enhances the steady-state level of αMG accumulated within the cells. The level attained during the oxidation of an exogenously supplied substrate can be raised by the addition of dinitrophenol or azide at suitable concentrations. Furthermore, the αMG accumulation is greater when the cells depend for their energy supply on endogenous respiration or on the anaerobic fermentation of an exogenous substrate rather than on the active oxidation of such a substrate in the absence of poisons.

Although the observed facts can be explained in some other manner, they do support our original proposal that an energy source is required for the glucose exit reaction.[10] Thus, one might imagine that the agents or sites for the exit reaction require ATP for their activity just as do those for the entry reaction, but that the former become saturated at higher levels of ATP than do the latter. An increase in the level of ATP available to the cell would then be reflected in the relative increase in the rate of exit as compared with that of entry for any given internal and external concentrations of the accumulated substrate. As a result, the steady-state level of accumulation would fall until the two reactions were in balance.

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Some of these studies have been reported in a preliminary communication, Hoffee, P., and E. Englesberg, *Bacteriol. Proc.*, **1962**, p. 120.

Abbreviations: ATP, adenosine triphosphate; αMG, alpha methyl D-glucopyranoside.

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*SOME FUNCTIONAL EFFECTS OF SECTIONING THE CEREBRAL COMMISSURES IN MAN**

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It has been possible in studies of callosum-sectioned cats and monkeys in recent years to obtain consistent demonstration of a variety of interhemispheric integrational functions mediated by the corpus callosum.[1, 2] These animal findings stand in marked contrast to the apparent lack of corresponding functional deficits produced by similar surgery in human patients.[3–9] The general picture of callosal functions based on the animal studies tends to be supported in current early testing of a 48-year-old male war veteran with recent complete section of the corpus callosum, anterior and hippocampal commissures.

The patient (W. J.) had been having grand mal convulsions for fifteen years subsequent to war injuries suffered in 1944. The seizures were refractory to medical management with a frequency, at best, of about 1 per week and, at worst, of 7 to 10 per day culminating in status epilepticus every 2–3 months. The subject was right handed, had an I.Q. of 113, and showed no significant sensory, motor, or associative disturbances in a battery of visual, tactile, and motor tests applied prior to surgery, excepting a mild hypesthesia on the left side.

The commissures were sectioned in a single operation by exposure and retraction of right frontal and occipital lobes. The massa intermedia was judged by the surgeons[10] to be absent and some atrophy of the exposed right frontal pole was ob-