MUTANT OF *AEROBACTER AEROGENES* LACKING GLUCOSE REPRESSION[1]

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Glucose exerts a marked inhibitory effect on the induced biosynthesis of many microbial enzymes (for a partial bibliography, *see* Neidhardt and Magasanik, 1957). This general inhibition is not due to (a) failure of the inducing agents to penetrate the cell in the presence of glucose, (b) the high growth rate of the cells in a glucose-containing medium, or (c) a glucose-caused deficit of the substances known to be essential for protein formation (Neidhardt and Magasanik, 1956*a*). Neidhardt and Magasanik (1956*b*) have proposed a "feedback" hypothesis to account for the glucose effect. This hypothesis, which is best expressed in the "repressor" terminology of Vogel (1957), states that the rapid rate of glucose metabolism leads to high intracellular concentrations of the repressors which regulate the synthesis of inducible, catabolic enzymes.

Since 1956 this view of the glucose effect has been supported by (a) the development of the repressor theory of the control of enzyme biosynthesis (Vogel, 1957; Pardee, Jacob, and Monod, 1959), (b) the discovery of the nutritional conditions necessary to reverse the glucose inhibition (Neidhardt and Magasanik, 1957) and those under which other substances can mimic glucose in inhibiting certain enzyme biosyntheses (Magasanik, Neidhardt, and Levin, 1958), and (c) the results of the extensive study of the glucose effect on β-galactosidase synthesis in *Escherichia coli* (Cohn and Horibata, 1959*a*, *b*, *c*).

A mutant of *Aerobacter aerogenes* has recently been isolated in which glucose no longer exerts its customary inhibitory effect on induced enzyme synthesis. In the present paper the results of studies on this strain will be presented and the repressor theory will be reassessed in the light of this and other recent work.

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MATERIALS AND METHODS

Organism. Two strains of *A. aerogenes* have been used in these experiments: the wild strain, 1033, and a mutant derived from it, JF-4. The method of isolation and the properties of the mutant are described under Experimental Results.

Media. The composition of the basal medium has been described previously (Neidhardt and Magasanik, 1960). The particular compound that was to serve as the major source of carbon and energy was prepared in a 5 per cent or a 10 per cent solution and autoclaved separately. Sufficient amounts were then added to the basal salts solution to give a final concentration of 0.4 per cent. Substances employed in this manner included glucose, glycerol, L-histidine, gluconic acid (neutralized with NaOH), lactose, and galactose.

Solid media were prepared by the inclusion of 2 per cent agar in whatever liquid medium is specified in the text.

Conditions of growth. Cells were grown aerobically with shaking at 37 C with the equipment described elsewhere (Neidhardt and Magasanik, 1960). The details of cultivation are specified in each experiment.

Measurement of growth. Growth was usually followed by measuring the turbidity of the culture in a Klett-Summerson photoelectric colorimeter (filter no. 42) which had been calibrated and standardized with exponential-phase cells of this organism dried to constant weight. The measurements are expressed as dry weight of cells (μg) per ml of culture.

Growth rates were expressed as k, the number of doublings per hour, as described previously (Neidhardt and Magasanik, 1956*a*).

Reagents. Glucose 6-phosphate dehydrogenase and triphosphopyridine nucleotide (TPN) were obtained from the Sigma Chemical Company. Adenosine triphosphate (ATP) was obtained from

the Pabst Laboratories. *o*-Nitrophenyl-β-D-galactoside (ONPG) was obtained from the California Biochemical Corporation. All other chemicals are readily available and of reagent grade.

Chemical determinations. Glucose was measured enzymatically using the commercial preparation, Glucostat (Worthington Biochemical Corporation), which contains glucose oxidase, peroxidase, and a chromogenic hydrogen donor. Samples were diluted to contain from 40 to 400 μg/ml of glucose. To 0.5 ml of such diluted samples were added 2.5 ml of the phosphate buffer (0.1 M, pH 7.0) and 2.0 ml of the Glucostat preparation. The mixture was left at room temperature for exactly 10 min. One drop of 6 N HCl was then added, followed by 5.0 ml of distilled water. The optical density of the resulting solution was measured in a Klett-Summerson photoelectric colorimeter (filter no. 42), and the results expressed as μg of glucose with the aid of a standard curve constructed with each assay.

Protein was measured by the phenol method of Lowry et al. (1951).

Measurements of enzymatic activities. (1) Hexokinase-TPN method:—The ability of cell-free extracts to form glucose 6-phosphate from glucose and ATP was determined by measuring the rate of reduction of TPN in the presence of added glucose 6-phosphate dehydrogenase. The extracts to be assayed were added to a system containing TPN (0.8 μmole), ATP (5.0 μmoles), glucose 6-phosphate dehydrogenase (0.06 mg), glucose (10.0 μmoles), and $MgCl_2$ (5.0 μmoles) in 3.0 ml of 0.2 per cent $NaHCO_3$ buffer, pH 7.5. The reaction was initiated by addition of the glucose, and the rate of reduction of TPN was measured by observing the increase in optical density at 340 mμ in a spectrophotometer at 25 C. Results are expressed as units/mg protein, in which 1 unit reduces 1 μmole of TPN per minute.

(2) Hexokinase and glucose oxidase by glucose disappearance:—The ability of a crude cell-free extract to cause the disappearance of glucose under aerobic conditions without added ATP was assumed to be due to a direct oxidation of glucose without phosphorylation. The enzyme responsible for this activity in *A. aerogenes* resembles glucose oxidase (Magasanik and Bojarska, 1960). The ability of the same extract to cause the disappearance of glucose under aerobic conditions with an excess of added ATP was assumed to be due to the action of hexokinase together with that of glucose oxidase. Hence, by measuring the glucose disappearance in the presence and absence of ATP one is able to estimate the amounts of these two activities present in the extracts. The validity of the assumptions underlying this practice has been established by independent assay methods (see the hexokinase assay method described above, as well as the work of Magasanik and Bojarska (1960)).

In practice, the assay was carried out in a 2.0-ml volume containing phosphate buffer (100 μmoles, pH 6.5), glucose (1.1 μmoles), $MgSO_4$ (1.0 μmole), and 0.2 ml of a cell-free extract containing about 5 mg of protein per ml. The assay was run in the presence and absence of added ATP (1.0 μmole). The amount of glucose present at 0 time and after 20 min incubation at 37 C with shaking was determined by the Glucostat method. The results are expressed as units/mg protein, in which 1 unit of activity causes the disappearance of 1 μmole of glucose per minute.

(3) Histidase:—This activity was measured in cell-free extracts by the method described by Tabor and Mehler (1955). One unit of activity causes the formation of 1 μmole of urocanic acid per minute.

(4) Urocanase:—This activity was measured in cell-free extracts by the method described by Tabor and Mehler (1955). One unit of activity causes the disappearance of 1 μmole of urocanic acid per minute.

(5) β-Galactosidase:—Cell suspensions in 0.1 M sodium phosphate buffer (pH 7.0) were treated with toluene (0.02 ml per 10 ml of cell suspension) with shaking at 37 C for 30 min. To 2.0 ml of a suitable dilution of this suspension was added 1.0 ml of 3×10^{-3} M *o*-nitrophenyl-β-D-galactoside and the mixture was incubated for 15 min at 30 C. Two ml of 1.0 M K_2CO_3 were then added and the yellow color was measured in the Klett-Summerson colorimeter (filter no. 42). Zero time served as the control. One unit of activity is defined as that which causes the hydrolysis of 1 μmole of *o*-nitrophenyl-β-D-galactoside per minute under the conditions of the assay.

Preparation of cell-free extracts. Cultures in the exponential phase of growth were rapidly chilled and centrifuged and the resulting pellet resuspended in distilled water and centrifuged. Then

the washed pellet was either suspended in 11 ml of distilled water and subjected to sonic oscillation (Raytheon 10 kc sonic oscillator) for 4 min at 0 C, or alternatively, the pellet was transferred to a chilled mortar, ground with three times its weight of levigated alumina, and extracted in 10 ml of distilled water. In either case, the resulting extract was freed of unbroken cells and large debris by centrifugation at 10,000 $\times$ *g* for 10 min.

RESULTS

Isolation of JF-4. Although biosynthesis of the L-histidine-degrading enzymes of *A. aerogenes* is strongly inhibited by the presence of glucose in the growth medium, this effect can be specifically reversed by the exclusion of nitrogen sources other than L-histidine (Neidhardt and Magasanik, 1957). Since the reversal is only partial, growth in such a glucose-L-histidine medium tends to select any variant capable of producing more histidase. It was with the hope of isolating a mutant which would exhibit a constitutive formation of the histidase series of enzymes that serial transfers of the wild type, 1033, were carried out in a medium consisting of the basal salts solution minus the $(NH_4)_2SO_4$ and containing 0.4 per cent L-histidine and 0.4 per cent glucose.

After about 40 generations in this medium, small samples of the culture were spread on agar plates of the same medium. After 12 hr incubation the plates were found to have many small colonies and a few large ones. Several of the large colonies were isolated and one of them, named JF-4, was studied further.

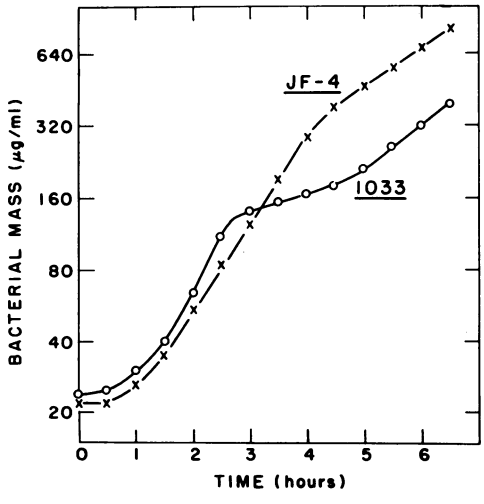

Figure 1. Comparison of the growth of *Aerobacter aerogenes* strains 1033 and JF-4 on a mixture of glucose and L-histidine.

Growth of JF-4 in a mixture of glucose and L-histidine. It seemed reasonable that the rapid growth of this mutant in a glucose medium containing L-histidine as the sole source of nitrogen was due to an increased rate of production of the L-histidine-degrading enzymes. The first question to be considered, then, was whether this strain could accomplish the same production if the customary nitrogen source of the basal medium, ammonium sulfate, were again added to the glucose-L-histidine medium. If JF-4 could produce histidase in such a medium then it would not be expected to show a diauxic lag following the exhaustion of glucose. For this reason a culture of the wild type and one of the mutant strain were grown overnight in the basal medium (which from this point on always contained ammonium sulfate)containing an excess (0.4 per cent) of both glucose and L-histidine. The following morning the cultures were centrifuged and the cells washed once with cold basal medium. The two strains were then each inoculated at a density of 25 μg dry weight per ml into a separate flask containing basal medium supplemented with 0.2 per cent L-histidine and a small amount of glucose, 300 μg per ml. The flasks were incubated with shaking and the growth of the cultures followed spectrophotometrically (figure 1).

The customary diauxic lag during the transition from growth on glucose to growth on L-histidine was exhibited by the wild strain but not by the mutant. Rather, there was a smooth change in the growth rate from that characteristic of growth on glucose (plus L-histidine) to that characteristic of L-histidine alone. In addition, the growth rate transition of the mutant occurred at a much higher cell density than did that of the wild strain. The simplest hypothesis to account for these observations would be that the mutant was capable of degrading L-histidine during the first phase of growth as well as the second. The next experiment confirmed this idea.

Capacity of JF-4 to make inducible enzymes in the presence of glucose. Measurements were made of the levels of two enzymes of the L-histidine-degrading series, histidase and urocanase, in cells of the mutant and of the wild type grown in media containing (a) L-histidine alone, (b)

TABLE 1

Effect of glucose on the levels of histidase and urocanase in cultures of Aerobacter aerogenes strains 1033 and JF-4 growing in the presence and absence of L-*histidine*

Strain	Organic Constituents of Medium	Enzyme Levels	
		Histidase	Urocanase
		unit/mg protein	
1033 (wild)	Glucose	<0.010	<0.010
	L-Histidine	0.900	0.153
	L-Histidine + glucose	0.102	0.024
JF-4 (mutant)	Glucose	<0.010	<0.010
	L-Histidine	0.878	0.156
	L-Histidine + glucose	0.835	0.152

Exponential-phase cultures of the two strains were prepared by overnight growth in media of the content listed above (each organic substrate at 0.2 per cent) followed by subculture in media of the same composition. The cultures were chilled and harvested, and the cells disrupted by sonic oscillation. Measurements were made of the enzyme activities and the protein content of the cell-free extracts (*see* Materials and Methods).

glucose alone, or (c) glucose plus L-histidine. The results of these measurements (table 1) show clearly that in the mutant strain the synthesis of these enzymes is still inducible, but no longer inhibited by glucose.

To test whether this loss of the glucose effect was unique for the formation of the histidase series of enzymes, measurements were made of the synthesis of another inducible system, β-galactosidase, in this strain. The results presented in table 2 show that in the mutant the induced formation of this enzyme is likewise no longer sensitive to glucose.

Effect of various mixtures of substrates on the capacity of JF-4 to form inducible enzymes. The heritable alteration in the mutant which permits it to form inducible enzymes in the presence of glucose must be due either to some alteration in the general enzyme-forming capacity of this organism or to some alteration in the specific physiology of the glucose effect. The choice is readily made by considering the results of an experiment in which the effect of glycerol and of gluconic acid on the formation of the L-histidine-degrading enzymes was investigated (table 3).

TABLE 2

Effect of glucose on the level of β-galactosidase in cultures of Aerobacter aerogenes strains 1033 and JF-4 growing in the presence and absence of lactose

Strain	Organic Constituents of Medium	β-Galactosidase
		unit/mg protein
1033 (wild)	Glucose	<0.010
	Lactose	0.352
	Lactose + glucose	<0.010
JF-4 (mutant)	Glucose	<0.010
	Lactose	0.348
	Lactose + glucose	0.302

Exponential-phase cultures of the two strains were prepared by overnight growth in media of the content listed above (each organic substrate at 0.2 per cent) followed by subculture in media of the same composition. The cultures were chilled and harvested, and the cells treated with toluene. Measurements were made of the enzyme activity and the protein content of these suspensions (*see* Materials and Methods).

TABLE 3

Effect of glycerol and of gluconic acid on the level of histidase in cultures of Aerobacter aerogenes strains 1033 and JF-4 growing in the presence of L-*histidine*

Strain	Organic Constituents of Medium	Histidase Level
		unit/mg protein
1033 (wild)	L-Histidine	1.008
	L-Histidine + glycerol	0.321
	L-Histidine + gluconic acid	0.023
JF-4 (mutant)	L-Histidine	0.924
	L-Histidine + glycerol	0.345
	L-Histidine + gluconic acid	0.023

Exponential-phase cultures of the two strains were prepared by overnight growth in media of the content listed above (each organic substrate at 0.2 per cent) followed by subculture in media of the same composition. The cultures were chilled and harvested, and the cells disrupted by sonic oscillation. Measurements were made of the enzyme activity and the protein content of the cell-free extracts (*see* Materials and Methods).

TABLE 4

Growth rate of Aerobacter aerogenes strains 1033 and JF-4 growing on various single sources of carbon and energy

Organic Constituents of Medium	Growth Rate Constant, k (doublings/hr)		$\frac{k_{JF-4}}{k_{1033}}$
	Wild strain (1033)	Mutant (JF-4)	
Lactose	1.12	1.00	0.89
Glycerol	1.01	0.99	0.98
L-Histidine	0.62	0.60	0.97
Galactose	0.93	1.04	1.12
Gluconic acid	1.04	1.04	1.00
Glucose	1.10	0.73	0.66
Glucose (2%)	1.09	0.70	0.64
Glucose (0.02%)	1.10	0.72	0.65

Cultures of the two strains were grown overnight in media of the content listed above (each organic substrate at 0.2 per cent concentration except where specified otherwise). The cultures were then inoculated at a very low cell density (less than 30 μg bacterial dry weight/ml) into 10 ml of media in a 250-ml Erlenmeyer flask equipped with a side arm enabling direct readings in the Klett colorimeter at 20-min intervals without altering the volume of the culture. The flasks were incubated at 37 C with shaking and the turbidity was observed for a 20-fold increase.

In the wild strain glycerol inhibits histidase biosynthesis about 60 per cent and gluconic acid inhibits it about 95 per cent; in strain JF-4 the same inhibitions were observed. One may conclude, therefore, that the mutant differs from the wild type in possessing some specific interference with glucose repression.

Growth rates of JF-4 and the wild type on glucose and on other carbon sources. As a preliminary investigation of the nature of the physiological alteration in the mutant, its growth rate was compared to that of the wild type on various single sources of carbon and energy. The results (table 4) show that the mutant grows as well as the wild type when presented with any one of a wide selection of carbon and energy sources, with the sole exception of glucose. Growth on glucose is only 66 per cent as fast in JF-4 as in the wild strain. Since this relation was observed over a 100-fold range in glucose concentration, it was thought to be due to a defect in glucose metabolism rather than the entry of glucose into the cell. The subsequent experimental results confirm this finding.

TABLE 5

Glucose metabolism by cell-free extracts of Aerobacter aerogenes strains 1033 and JF-4

Extract Made from Strain	Glucose Degradation			Hexokinase (TPN Method)
	With ATP (hexokinase + "glucose oxidase")	Without ATP ("glucose oxidase")	Difference (hexokinase)	
	μmole/min $\times$ mg protein			*unit/mg protein*
1033 (wild)	0.025	0.013	0.012	0.027
JF-4 (mutant)	0.011	0.001	0.010	0.032

Exponential-phase cultures of the two strains were prepared by overnight growth in the basal medium supplemented with 0.2 per cent glucose followed by subculture in 300 ml of medium of the same composition. The two cultures were chilled and the cells were harvested by centrifugation and washed once with distilled water. Cell-free extracts were prepared by sonic oscillation. Measurements were then made of the protein content and enzymatic activities of the extracts as described under Materials and Methods.

Enzyme studies on cell-free extracts. The ability of cell-free extracts of the mutant and the wild strain to degrade glucose was next investigated. The two strains were grown on glucose and extracts were prepared by sonic oscillation. Under aerobic conditions and in the presence of magnesium ions and ATP (*see* Materials and Methods), the extract prepared from strain JF-4 was able to degrade glucose at only 40 per cent the rate shown by a similar extract of the wild strain (table 5), supporting the conclusion that glucose degradation is in some way impaired in the mutant. The omission of ATP from the system resulted in a 50 per cent reduction in the glucose-degrading ability of the strain 1033 extract, and in a complete abolition of activity in the extract of the mutant strain.

The amount of hexokinase present was measured in the two extracts by the TPN method. The assay showed (table 5) that the hexokinase activities were equivalent in the two strains.

These findings, together with the fact that growth of JF-4 on gluconic acid is as rapid as that of the wild type, point to the biochemical lesion of the mutant as being in the conversion of glucose to gluconic acid. This indication has

been confirmed by Magasanik and Bojarska (1960), who have established that extracts of glucose-grown cultures of JF-4 are unable to convert glucose to gluconic acid whereas extracts of the wild strain perform the conversion readily.

Utilization of glucose during growth of strains JF-4 and 1033. A peculiar feature of the growth on glucose of wild strains of *A. aerogenes* is the rapidity with which glucose disappears from the medium. Under aerobic conditions glucose is metabolized at a rate double that commensurate with the growth rate and the total crop of cells (Neidhardt and Magasanik, 1956*b*). This rapid dissimilation is accompanied by excretion into the medium of various intermediary metabolites which are taken back up by the cells after the glucose has been exhausted. This behavior results in the phenomenon illustrated in figure 2. A culture of strain 1033 which was incubated in a medium initially containing 450 μg glucose per ml increased in bacterial mass from 50 μg per ml to 200 μg per ml with a disappearance of 2.72 μg of glucose for every μg of new protoplasm synthesized. Following the exhaustion of the glucose from the medium the amount of new protoplasm increased by a further 65 per cent. Such a result is typical of wild strains of *A. aerogenes*. In the same figure are presented the results observed when the experiment was done with the mutant, JF-4. A culture of this strain presented with 450 μg of glucose per ml increased from 50 to 300 μg of bacterial mass with a disappearance of only 1.76 μg of substrate for every μg of new protoplasm synthesized. After exhaustion of the glucose from the medium, the cell mass increased further by only an additional 12 per cent.

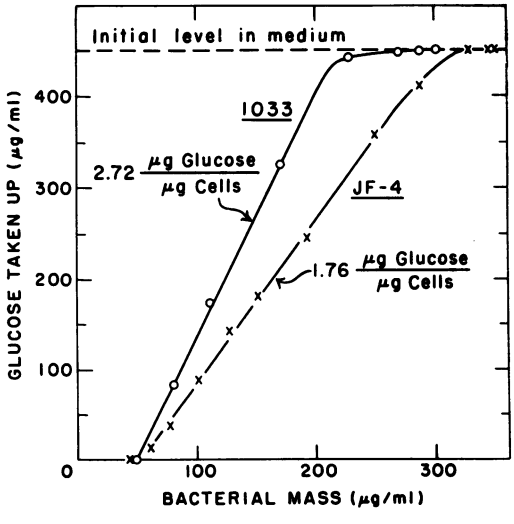

Figure 2. Utilization of glucose during growth of *Aerobacter aerogenes* strains 1033 and JF-4.

In the mutant, therefore, growth on glucose is characterized by the dissimilation of this substance at a rate that is commensurate with the biosynthetic processes.

DISCUSSION

The "feedback" hypothesis of the glucose effect as proposed in 1956 (Neidhardt and Magasanik, 1956*b*) makes the following assertions: (1) the formation of each catabolic enzyme is controlled by the intracellular level of some particular metabolite which is an immediate or an ultimate product of the activity of that enzyme, (2) glucose inhibits the formation of many inducible enzymes because the metabolism of glucose gives rise to the various metabolites which control the synthesis of those enzymes, and (3) the dissimilation of glucose occurs faster than the synthetic capacities of the cell can utilize the metabolites formed; this imbalance leads to high intracellular concentrations of intermediary metabolites.

The first of these assertions requires little elaboration here; the outstanding work of Pardee, Jacob, and Monod (1959) on β-galactosidase synthesis in *Escherichia coli* has led to the extension of Vogel's (1957) repressor theory of enzyme formation to include one of the most reputable of inducible, catabolic enzymes and one which is glucose sensitive. It seems clear from their work as well as that of others (Torriani, 1960) that the synthesis of catabolic enzymes is controlled in much the same way as is that of biosynthetic enzymes: by repressors which are immediate or ultimate products of the activity of these enzymes.

The nature of the enzymes the synthesis of which is inhibited by glucose strongly supports the second assertion of the theory. Indeed, it can be categorically stated that all known instances of the glucose effect involve the formation of enzymes which produce metabolites readily derived from glucose. This generalization is made all the stronger by the fact that dependence of enzyme synthesis on external induction has no bearing on whether the synthesis of a particular enzyme is glucose sensitive. The formation of

some inducible enzymes is not glucose sensitive (e.g., penicillinase (Pollock, 1950)), and the formation of some enzymes not dependent on an external inducing agent is glucose-sensitive (e.g., oxalacetic carboxylase (Amarasingham and Umbarger, 1955)).

The recent work by Magasanik and Bojarska (1960) on the metabolism of glucose in *A. aerogenes* analyzes in detail the finding (Neidhardt and Magasanik, 1956*b*) that glucose dissimilation in this organism (and presumably others) proceeds at a rate far in excess of the synthetic capacities of the cell to utilize the metabolites and energy thus formed.

Thus, each of the major points of the repressor hypothesis has been supported by experimental evidence. In addition, several predictions of the theory have been verified by recent findings:

(1) By inhibiting the formation of a given repressor from glucose, one should obtain a reversal of the glucose effect specific for the appropriate enzyme. The successful accomplishment of this in the case of histidase formation in *A. aerogenes* has been fully described previously (Neidhardt and Magasanik, 1957).

(2) By slowing down the anabolic processes of a cell while allowing the catabolic processes to proceed, one should observe a mimicking of the glucose effect by other carbon sources. This phenomenon is actually what happens when a culture is suspended in a medium which for some reason does not permit growth (deficiency of guanine or nitrogen, for example). Under such conditions the presence of *any* utilizable carbon and energy source causes an inhibition of the formation of a large variety of inducible catabolic enzymes (Magasanik, Neidhardt, and Levin, 1958; Pardee, 1955).

(3) Any general interference with glucose metabolism should result in a general reversal of the glucose effect. This prediction of the repressor hypothesis is clearly borne out in the experiments of Cohn and Horibata (1959*c*) who showed that the transfer of a culture from aerobic to anaerobic conditions renders the cells temporarily immune to the glucose effect; following physiological adaptation of the cells to the anaerobic conditions (as shown by a return to the normally rapid growth rate of *E. coli*) the cells once more exhibit the glucose effect. This important finding not only is in accord with the prediction of the repressor hypothesis, but it also shows that aerobic metabolism is not necessary for glucose to exert its inhibitory effect on enzyme synthesis.

(4) Any mutant that has lost sensitivity to glucose for a large number of inducible systems should have either (a) an impaired glucose dissimilation, or (b) an improved over-all anabolic capacity. As far as is known, all mutants that have lost glucose repression have a quantitative impairment in the dissimilation of glucose. Mention has been made by Cohn and Horibata (1959*c*) of the fact that cultures of *E. coli* maintained on succinate medium for many generations gradually lose the glucose effect; simultaneous with such loss is a decreased growth rate on glucose. Englesberg (1959) has described the loss of glucose repression in a strain of Salmonella and has correlated this loss with a 2- to 3-fold increase in the activity of a phosphatase in the cell. In view of the fact that the mutant shows a definite decrease in growth rate on glucose (and not on other compounds) it seems that an impairment of glucose metabolism abolishes glucose repression in this case as in the others cited.

The mutant strain (JF-4) of *A. aerogenes* described in this paper clearly represents an organism which can readily synthesize many inducible, catabolic enzymes while growing in the presence of glucose. The biochemical lesion in this organism seems to be a loss of the ability to metabolize glucose by a direct oxidation. This loss diminishes the over-all rate of glucose dissimilation and, presumably, permits the biosynthetic processes of the cell to maintain lower repressor concentrations in the cell.

Since the three major points of the repressor hypothesis of the nature of the glucose effect on enzyme synthesis have all been strongly supported by experimental evidence, and since four important predictions of the hypothesis have been verified, it seems reasonable to conclude that it accurately describes the observed phenomenon.

ACKNOWLEDGMENT

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SUMMARY

The wild strain (1033) of *Aerobacter aerogenes* is capable of a glucose sensitive, inducible formation of a series of L-histidine-degrading enzymes. An appropriate selection pressure yielded a strain, JF-4, that was still histidase-inducible, but which had gained the ability to make large amounts of the entire histidase series of enzymes despite the

presence of glucose. This heritable reversal of the "glucose effect" proved not unique for the histidase series: other inducible enzymes the synthesis of which was blocked by glucose in the wild strain showed no such block in JF-4.

This phenomenon was shown to be due to a specific alteration in the physiology of the glucose effect rather than a general increase in the enzyme-forming capacity of the mutant, for substances other than glucose, e.g., glycerol and gluconic acid, continued to exert their characteristic inhibitory effects on histidase formation by the mutant.

The mutant appears to have lost the ability to form a major degradative system for glucose during growth on this substance. This lesion has resulted in a decrease in the rate of glucose degradation to a value only 35 per cent that of the wild strain. Growth of the mutant on glucose, however, was shown to occur at 66 per cent the rate of the wild strain, indicating that the mutant degrades glucose at a rate more commensurate with the anabolic capacities of the cell than does the wild strain.

These observations have been shown to support strongly the concept that the "glucose effect" is due to an excessive rate of glucose dissimilation which builds up various repressors of enzyme biosynthesis.

The repressor hypothesis has been examined in the light of other recent observations.

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