METABOLISM OF INTRACELLULAR POLYSACCHARIDE BY *STREPTOCOCCUS MITIS* AND ITS RELATION TO INDUCIBLE ENZYME FORMATION

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ABSTRACT

GIBBONS, R. J. (Forsyth Dental Center, Boston Mass.). Metabolism of intracellular polysaccharide by *Streptococcus mitis* and its relation to inducible enzyme formation. J. Bacteriol. **87**:1512–1520. 1964.—The synthesis and catabolism of an intracellular iodine staining polysaccharide produced from glucose by *Streptococcus mitis* was investigated. Approximately 15% of the total glucose metabolized by buffered suspensions of *S. mitis* was assimilated. Over 90% of the assimilated glucose was converted into a polysaccharide of the glycogen-amylopectin type. Use of uniformly labeled C^{14}-glucose provided a convenient method for determining polysaccharide accumulation in this organism. Glucose assimilation occurred at a rate of over 80 μg of glucose per hr per 100 μg of starting dry cell weight. Prolonged assimilation produced cells containing over 50% polysaccharide on a dry weight basis. Accumulated polysaccharide was catabolized at the same rate when the organism was suspended in buffer, sugar-free broth, or sugar-free broth containing thiomethyl galactoside. Metabolic intermediates produced from polysaccharide catabolism did not markedly repress inducible enzyme synthesis. The last glucose molecules incorporated into polysaccharide were among the first molecules to be removed during catabolism. Catabolism of polysaccharide provides *S. mitis* with energy in a utilizable form, for cells containing polysaccharide increased in β-galactosidase activity when induced with thiomethyl galactoside in the absence of an exogenous energy source. Cells devoid of polysaccharide, and a polysaccharide-negative variant of *S. mitis* did not increase in β-galactosidase activity when induced in a similar manner. It appears that the intracellular polysaccharide is the sole substrate for the endogenous metabolism of *S. mitis*.

A variety of bacteria have been shown to synthesize intracellular polysaccharides of the glycogen-amylopectin type. These are often assumed to function as energy reserves, for they are synthesized when organisms are provided with excess exogenous carbohydrate and are generally utilized when the organisms are deprived of an exogenous energy source. However, Wilkinson (1959) cautioned that a substance should not be considered an energy-storage compound unless it is shown to provide the organism with energy in a form which is utilizable by the cell. Most investigations have failed to fulfill this criterion. The investigations of Holme and Palmstierna (1956b) established the role of a glycogen-like polysaccharide as a reserve substance in *Escherichia coli*. These investigators demonstrated that, when cells containing C^{14}-glycogen were incubated with a nitrogen source in the absence of a carbon source, C^{14} passed from the glycogen to cell protein. However, because *E. coli* can gain energy from the metabolism of a variety of organic compounds, it may only be concluded that the glycogen functioned as a source of carbon for protein synthesis, although it may also have served as the energy source.

Recently *Streptococcus mitis* was observed to synthesize an intracellular glycogen-amylopectin type polysaccharide when provided with excess glucose. In the absence of environmental glucose, *S. mitis* catabolizes stored polysaccharide and forms lactic acid (Gibbons and Kapsimalis, 1963). As carbohydrates are the only substances which can be utilized as a source of energy for growth of these homofermentative organisms, it would appear that polysaccharide would serve as the major, if not sole, substrate for the organism's endogenous metabolism. The present investigation was undertaken to study the catabolism of intracellular polysaccharide by *S. mitis*, and to determine whether it functioned as an energy reserve.

MATERIALS AND METHODS

Organisms and media. The isolation and biochemical characteristics of the strain of *S. mitis* used in this investigation were described pre-

viously (Gibbons and Kapsimalis, 1963). The medium utilized throughout this study had a base of the following composition: 2% Trypticase (BBL), 0.4% K_2HPO_4, 0.1% KH_2PO_4, 0.2% NaCl, 10^{-3} M $MgSO_4$, and 10^{-4} M $MnSO_4$. Stock solutions of glucose were autoclaved separately and aseptically added to this base to complete the medium, for no growth occurred in the absence of a fermentable carbohydrate. Stock cultures were maintained in broth containing 0.1% glucose, and on plates containing 2% glucose. Incubation was performed anaerobically in brewer jars filled with 95% hydrogen and 5% carbon dioxide at 35 C unless noted otherwise. Colonies of the organism were regularly examined for ability to store iodophilic polysaccharide by flooding the plates with iodine solution (0.2% I_2 in 0.4% KI). On several occasions, it was observed that polysaccharide-negative colonies of the organism appeared (Fig. 1). When isolated in pure culture, these variants continued to give rise to colonies which did not stain with iodine, although they were morphologically and biochemically identical to the parent strain in other respects. The appearance of these negative variants in stock cultures necessitated frequent purification of the cultures to keep them homogenous with respect to polysaccharide formation.

Assimilation of C^{14} glucose. The assimilation of uniformly labeled C^{14}-glucose was studied with 18-hr cultures of *S. mitis* grown in 0.1% glucose broth. When grown in this manner, the organisms contained little or no intracellular polysaccharide (Gibbons and Kapsimalis, 1963). Cells were harvested by centrifugation and washed once with buffer of the following composition: 0.4% $K_2H \cdot PO_4$, 0.1% KH_2PO_4, 0.2% NaCl, 10^{-2} M 2-mercaptoethanol, 10^{-3} M $MgSO_4$, and 10^{-4} M $MnSO_4$. The organisms were suspended in sufficient buffer to obtain a suspension containing approximately 200 μg (dry weight) of cells per ml. Samples of the suspension were dispensed into culture tubes which were sealed with vaccine caps. Two syringe needles were pushed through each cap, and nitrogen was introduced through one needle to obtain anaerobiosis. The needles were then withdrawn, and the tubes were incubated in a water bath at 35 C. After 10 min of thermal equilibration, sufficient uniformly labeled C^{14}-glucose (specific activity, 100 counts per min per μg) was introduced with a syringe to give a final concentration of 0.5%. Samples of approximately 1 ml were removed periodically with a syringe and placed

in a small culture tube. Triplicate samples of 0.2 ml were immediately added to tubes containing 4 ml of 95% ethanol to inactivate the organisms and extract compounds of low molecular weight. The cells were then analyzed for radioactivity as described below.

Polysaccharide catabolism. To study polysaccharide catabolism, organisms were permitted to synthesize polysaccharide from C^{14}-glucose for 20 to 30 min, as described above. The cells were then washed three times to free them from residual glucose, and suspended in buffer or sugar-free broth. Suspensions were incubated under a nitro-
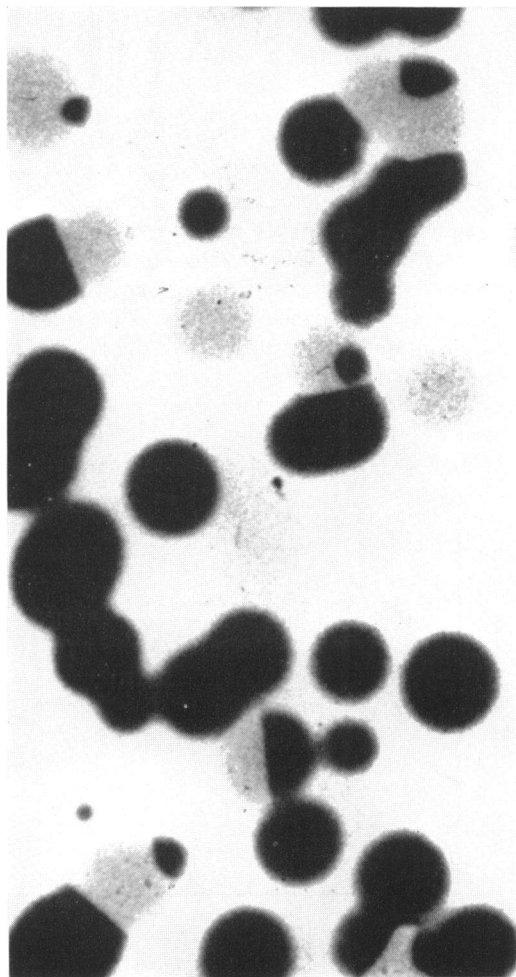

FIG. 1. *Colonies of Streptococcus mitis grown on 2% glucose agar stained with iodine showing the presence of polysaccharide-negative sectors and colonies.*

gen atmosphere in tubes sealed with vaccine caps at 35 C. Samples were removed periodically and 0.2-ml portions were added to 4 ml of 95% ethanol to inactivate the organisms. The inactivated organisms were assayed for radioactivity as described below. Loss of radioactivity from the cells was indicative of polysaccharide catabolism.

Radioactivity measurements. Water (4 ml) was added to the inactivated alcoholic cell suspensions, and the organisms were collected on 25-mm membrane filters (pore size, 0.45 μ). They were washed three times with 2-ml volumes of 50% ethanol, and the filter containing the organisms was immediately cemented to a planchet (Millipore filter cement). These were dired under infrared light, and counted with a Micro-mil flow counter (Nuclear Chicago Corp., Des Plaines, Ill.). All planchets were assumed to be at infinite thinness.

β-Galactosidase assay. Cells to be assayed for β-galactosidase were washed twice with sugar-free broth and suspended in buffer containing 20 μg/ml of chloramphenicol. The cell density was adjusted between 100 and 150 μg (dry weight) of cells per ml. o-Nitrophenylgalactoside at a concentration of 10^{-3} M was utilized as the substrate, as described by Lederberg (1950). Samples (2 ml) of the reaction mixture were removed periodically and inactivated by the addition of 4 ml of 1 M K_2CO_3. The organisms were removed by

FIG. 2. *Assimilation and catabolism of C^{14}-glucose by resting cells of Streptococcus mitis. The organisms were incubated with 0.5% C^{14}-glucose for 45 min to permit assimilation. They were then washed, and suspended in sugar-free buffer to permit polysaccharide catabolism.*

centrifugation, and the o-nitrophenol liberated was determined at 420 mμ.

It was necessary to use whole untreated cells for the assay, as preliminary experiments indicated that toluene treatment completely inactivated the organisms, as did lysis by sonic oscillation. Evidently the enzyme is more labile in this organism than in *E. coli* (Lederberg, 1950), for even lactose-grown cells rapidly lost activity when suspended in buffer. Similar findings have been reported in *S. faecium* by Buecher and Brock (1962). The inclusion of magnesium and manganese ions in the buffer helped stabilize the enzyme in intact cells.

Polysaccharide extraction and chemical methods. Cells containing polysaccharide were digested with 30% KOH, and the polysaccharide was precipitated with ethanol as described by Holme, Laurent, and Palmstierna (1957). Other cell carbohydrate, presumably of capsular or cell wall origin, evidently was present in these preparations, for only 70% of the total carbohydrate present was glucose.

Total carbohydrate was determined with anthrone reagent as described by Seifter et al. (1950). Glucose was determined with glucose oxidase (Glucostat, Worthington Biochemical Corp., Freehold, N.J.). Cell dry weight was determined from a standard curve of optical density plotted against dry weight of cells per milliliter. It was found that the dry weight of the organisms was proportional to their optical density only when they contained little or no polysaccharide, and when the optical density of the suspension was less than 0.8.

RESULTS

Assimilation and catabolism of C^{14}-glucose by resting cells. Cells of *S. mitis* suspended in buffer rapidly assimilated exogenous C^{14}-glucose, as determined by their increase in radioactivity (Fig. 2). Assimilation was linear for over 30 min, and occurred at a rate of more than 80 μg of glucose per hr per 100 μg of starting dry cell weight. The increase in radioactivity of the cells was accompanied by an increase in the turbidity of the suspension which was presumably due to the increase of cell mass due to the assimilation. Cells incubated for over 1 hr doubled their original starting weight. If the cells were washed free of exogenous glucose and suspended in buffer, they slowly lost radioactivity through catabolism (Fig. 2).
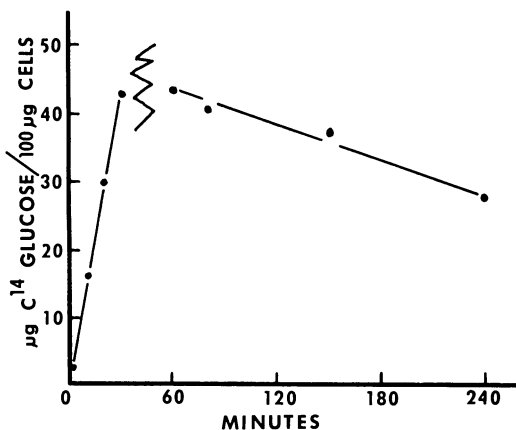
To determine whether the assimilated C^{14} was present in the cells as carbohydrate, the following experiment was performed. Cells of *S. mitis* were harvested from a 0.1% glucose broth culture by centrifugation, washed once, and suspended in buffer at a concentration of 200 μg (dry weight) per ml. A sample of the suspension was heat-inactivated and analyzed for total carbohydrate. The remainder of the suspension was incubated with 0.5% C^{14}-glucose for 30 min. The organisms were heat-inactivated and washed three times to remove exogenous glucose. The cells were then chemically analyzed for total carbohydrate as well as for the quantity of C^{14} incorporated. The results indicated that the assimilated C^{14}-glucose remained as carbohydrate, for the increase in C^{14} in the organisms corresponded to their increase in total carbohydrate.

To determine whether the assimilated carbohydrate was present as a glycogen-like polymer, cells of *S. mitis* were permitted to assimilate C^{14}-glucose for 30 min, as described above. The organisms were washed twice with buffer, and a sample was removed to determine the quantity of C^{14} assimilated. The polysaccharide was extracted from the remaining cells by digestion with 30% KOH followed by alcohol precipitation. It was found in two separate experiments that the polysaccharide fraction contained 91.5 and 94.5% of the total radioactivity of the organisms. One of the precipitates was dissolved in buffer and treated with 200 μg/ml of α-amylase for 30 min at 35 C. Before treatment with amylase, samples stained reddish purple with iodine. Afterwards, the solution failed to develop color upon addition of iodine solution. Two volumes of ethanol were added, and, after chilling in an ice bath, the precipitate was removed by centrifugation. It was found that less than 10% of the radioactivity was precipitable by alcohol after α-amylase hydrolysis.

To compare glucose assimilation by *S. mitis* and a polysaccharide-negative variant derived from this strain, buffered suspensions of each organism were incubated with 0.5% C^{14}-glucose for 60 min. Samples were removed periodically, and the organisms were assayed for radioactivity. It was found (Fig. 3) that the polysaccharide-negative variant assimilated only 8% as much C^{14}-glucose as did the parent strain.

To determine the proportion of the total glucose metabolized which was assimilated into polysaccharide by *S. mitis*, a suspension was in-
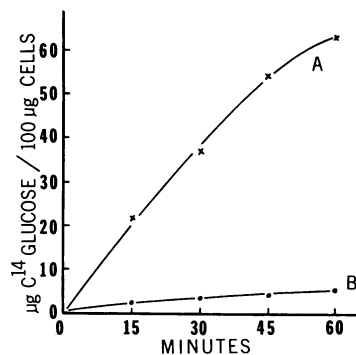

FIG. 3. *Comparative assimilation of C^{14}-glucose by Streptococcus mitis (curve A) and a polysaccharide-negative variant (curve B) derived from this strain.*

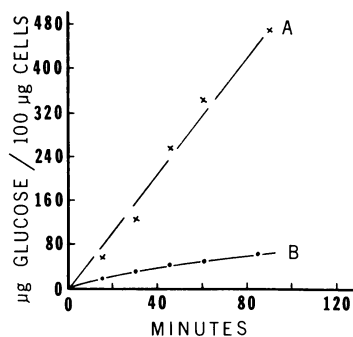FIG. 4. *Glucose metabolism and assimilation by resting cells of Streptococcus mitis. (A) Total glucose metabolized. (B) The quantity of glucose assimilated.*

cubated with 0.5% C^{14}-glucose. Samples were withdrawn periodically. One portion of each sample was used to determine the amount of C^{14}-glucose assimilated; the remainder was heat-inactivated, centrifuged to remove the organisms, and analyzed for glucose with glucose oxidase (Fig. 4). Of the total glucose metabolized by the organism, approximately 15% was assimilated into polysaccharide.

Polysaccharide catabolism. To determine the proportion of assimilated C^{14}-glucose that could be lost through endogenous metabolism, cells of *S. mitis* were permitted to assimilate C^{14}-glucose for 15 min. The organisms were washed three times and suspended in sugar-free broth. A sample was removed and the quantity of C^{14}-glucose assimilated was determined. The remainder of the suspension was incubated anaerobically for 18 hr at 35 C. The organisms were
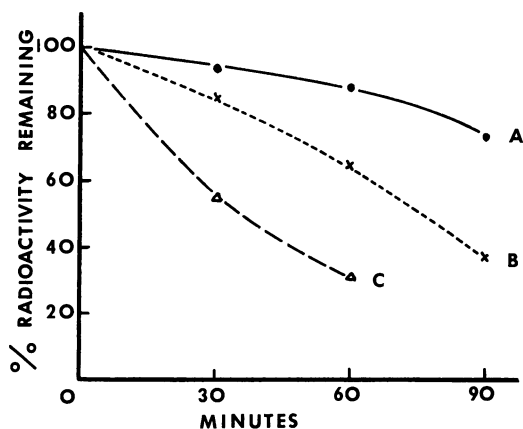
FIG. 5. *Loss of radioactivity by Streptococcus mitis cells catabolizing differentially labeled polysaccharide. All cells were incubated with glucose to permit synthesis of polysaccharide for 30 min. (A) Organisms incubated with labeled glucose only during the initial 5 min of synthesis. (B) Cells incubated with labeled glucose throughout. (C) Cells incubated with labeled glucose only for the last 5 min.*

then analyzed for their residual C^{14} content. It was found in two experiments that 10.2 and 12.7% of the initial assimilated C^{14} remained. For comparison, cells of *S. mitis* grown in a medium containing C^{14} algal protein hydrolysate were utilized. One sample of these protein-labeled organisms was incubated with glucose for 15 min. The cells were washed and permitted to catabolize polysaccharide as described above. A second sample of protein-labeled cells was incubated in sugar-free broth without having assimilated glucose. Samples were analyzed for their C^{14} content before and after incubation. It was found in two separate experiments that 92.5 and 90.7% of the C^{14} remained in the organisms after 18 hr of incubation under conditions insufficient for growth. No difference was noted between the cells which had assimilated glucose and those which had not.

Holme et al. (1957) demonstrated that, when glycogen is synthesized by *E. coli*, there is an increase in the molecular weight of already existing glycogen molecules in the organism. To determine whether polysaccharide synthesis by *S. mitis* occurred in an analogous manner, a suspension of the organism in buffer was divided into three parts. One portion was incubated with 0.5% C^{14}-glucose for 30 min. A second portion was incubated with 0.5% C^{14} glucose for 5 min, and

the organisms were then washed three times to remove exogenous glucose and reincubated with unlabeled glucose for 25 min. The third sample was incubated with unlabeled glucose for 25 min, washed, and incubated with C^{14}-glucose for 5 additional min. At this time, the organisms in each suspension were washed three times and suspended in sugar-free broth. The suspensions were incubated at 35 C anaerobically to permit catabolism of assimilated polysaccharide. Samples were removed periodically, and the organisms were analyzed for radioactivity. It was found (Fig. 5) that organisms which were exposed to C^{14}-glucose initially lost their radioactivity slowly in comparison with cells which were continuously exposed to C^{14}-glucose. Organisms which were exposed to C^{14}-glucose only at the end of the assimilation period lost radioactivity rapidly. These data indicate that the last glucose molecules incorporated into intracellular polysaccharide are among the first to be removed during catabolism.

To determine the rate of polysaccharide catabolism by *S. mitis* in various chemical environments, cells were permitted to assimilate C^{14}-glucose for 30 min at 35 C. The organisms were washed three times with buffer to free them of glucose, and samples were suspended in the following: (i) buffer, (ii) sugar-free broth, (iii) sugar-free broth containing 10^{-3} M thiomethyl galactoside (TMG), (iv) 0.1% glucose broth, (v) 0.1% lactose broth, (vi) buffer containing 0.1% glucose. Each suspension was incubated anaerobically at 35 C. Samples were removed periodically, and the organisms were assayed for residual radioactive polysaccharide. It was found (Fig. 6) that the organisms catabolized polysaccharide at approximately the same rate irrespective of whether they were suspended in buffer, sugar-free broth, or sugar-free broth containing TMG. Cells suspended in lactose broth catabolized polysaccharide at a rate comparable to buffer for approximately 40 min, and then their radioactive content increased slightly. Evidently, β-galactosidase (which is inducible in this organism) was synthesized during the first 40 min, permitting the organisms to utilize lactose. The increase in cell radioactivity is likely due to reincorporation of labeled intermediates produced during polysaccharide catabolism. Cells incubated in either buffer or broth containing glucose lost radioactivity slowly. Under these conditions, the organisms were synthesizing
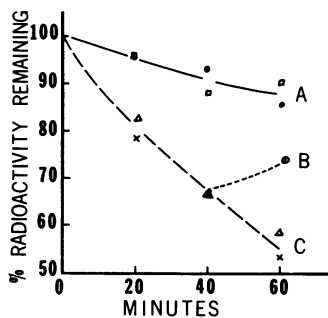
FIG. 6. *Loss of radioactivity through catabolism of labeled polysaccharide by Streptococcus mitis in different environments. (A) Organisms suspended in either broth or buffer containing glucose. (B) Organisms suspended in lactose broth. (C) Organisms suspended in either sugar-free buffer, broth, or broth containing TMG.*
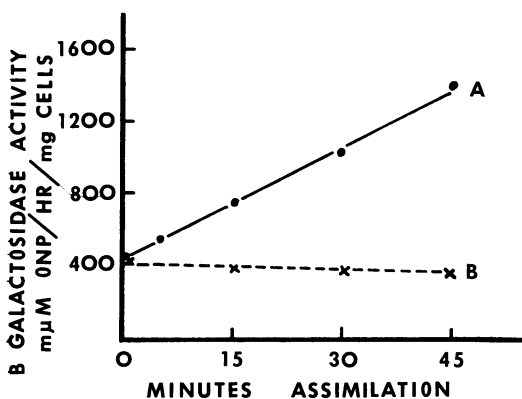

FIG. 7. *Effect of time of glucose assimilation on the β-galactosidase activity of (A) Streptococcus mitis and (B) a polysaccharide-negative variant of S. mitis.*

additional polysaccharide (Gibbons and Kapsimalis, 1963) and the turbidity of each suspension increased. The loss of C^{14}-polysaccharide from these cells is probably not due to lysis, for previous experiments with this organism have indicated that their nitrogen content is not altered under comparable conditions.

Polysaccharide catabolism and β-galactosidase synthesis. To determine whether catabolism of polysaccharide could provide *S. mitis* with energy utilizable for the synthesis of an inducible enzyme, buffered suspensions of *S. mitis* were permitted to assimilate glucose for varying periods of time. As the accumulation of polysaccharide is linear for at least 30 min (Fig. 2 and 3), it was

possible to obtain cells with varying amounts of polysaccharide. The organisms were washed three times to free them of exogenous glucose and suspended in sugar-free broth containing 10^{-3} M TMG. The suspensions were incubated anaerobically at 35 C for 18 hr to permit maximal polysaccharide catabolism and β-galactosidase synthesis. Cells containing polysaccharide incubated in sugar-free broth in the absence of TMG were used as a control. For comparison, the polysaccharide-negative variant strain was treated in a similar manner. After incubation, the organisms were washed twice with sugar-free broth and analyzed for β-galactosidase activity. It was found (Fig. 7) that cells of *S. mitis* which contained polysaccharide increased in β-galactosidase activity when induced with TMG. Control cells containing polysaccharide but incubated in the absence of TMG, exhibited only base-line β-galactosidase activity. This was comparable to the activity of cells containing no polysaccharide, but induced with TMG. The increase in β-galactosidase activity of cells with polysaccharide was proportional to the time the organisms synthesized polysaccharide. In contrast, the polysaccharide-negative variant strain did not increase in β-galactosidase activity, irrespective of the time it was incubated with glucose.

DISCUSSION

Several methods have been used to measure the accumulation of polysaccharides of the starch-glycogen type by bacteria. Staining with iodine affords a simple qualitative or semiquantitative procedure, and has been used for a variety of bacteria (Carlson and Hehre, 1949; Gibbons, Doetsch, and Shaw, 1955; Doetsch et al., 1957; Carrier and McCleskey, 1962). The accumulation of glycogen-like polysaccharides has been quantitatively determined by measuring increases in cell carbohydrate by chemical procedures (Dagley and Dawes, 1949; Duguid and Wilkinson, 1953; Gibbons and Kapsimalis, 1963), and by infrared spectrophotometry (Levine et al., 1953). Perhaps the most reliable procedure was used by Palmstiernia (1956) and Holme et al. (1957). These investigators quantitatively studied the accumulation of glycogen in *E. coli* by extracting the polysaccharide by KOH digestion followed by alcohol precipitation. This procedure was effective in removing other cellular carbohydrates of *E. coli*, although it required comparatively large samples for analysis. This technique

was attempted with *S. mitis* in the present investigation. However, it was found that the organism contained alkali-stable polysaccharides in addition to the glycogen-amylopectin polymer, because only 60 to 70% of the total carbohydrate present in the extracts was glucose.

Use of uniformly labeled C^{14}-glucose afforded a convenient procedure for studying the synthesis and catabolism of intracellular polysaccharide by resting cells of *S. mitis*. This method appears valid under the conditions utilized for the following reasons. (i) Measurement of the increase in cell total carbohydrate as determined with anthrone reagent was in good agreement with measurements made of C^{14}-glucose. (ii) Over 90% of the incorporated radioactivity could be extracted from the organisms by alkali digestion and alcohol precipitation. Almost all of the extracted radioactive polysaccharide was digested with α-amylase. (iii) A polysaccharide-negative variant derived from *S. mitis* was found to assimilate only 8% as much C^{14}-glucose as the parent strain. (iv) Approximately 90% of the assimilated C^{14}-glucose is lost from the cells after 18 hr of endogenous metabolism. The loss is not attributable to cell lysis, for organisms grown in the presence of C^{14}-amino acids lost less than 10% of their radioactivity under the same conditions. These data indicate that approximately 90% of the glucose assimilated by resting cells of *S. mitis* is incorporated into a glycogen-amylopectin type polymer. The remaining 10% probably is incorporated into other cell carbohydrates such as capsular or cell wall material.

Previous experiments indicated that *S. mitis* accumulated the largest quantities of polysaccharide when presented with excess exogenous carbohydrate under conditions which were unsuitable for rapid growth. This occurred when cultures entered the stationary phase of growth or when protein synthesis was impaired by the addition of chloramphenicol or by incubation in the absence of a nitrogen source (Gibbons and Kapsimalis, 1963). Comparable results have been obtained by other investigators with *E. coli* (Dagley and Dawes, 1949; Holme and Palmstiernia, 1956a) and *Aerobacter aerogenes* (Duguid and Wilkinson, 1953). The conditions used in the present investigation were well suited for polysaccharide accumulation, for, in all instances, the starting cells were grown under conditions of glucose limitation and contained little or no polysaccharide as judged by their iodine staining

properties. In addition, they were provided with exogenous glucose in the absence of a nitrogen source. Assimilation for over 1 hr resulted in cells in which polysaccharide comprised more than 50% of the dry weight. In *E. coli* and *Ruminococcus albus*, glycogen-like polysaccharides have been observed to comprise 25 and 35% of the dry weight of these organisms, respectively (Holme and Palmstiernia, 1956a; Hungate, 1963). The magnitude of accumulation in *S. mitis* is of significance when one considers that carbohydrates are the only substances which serve as energy sources for this organism. In contrast, *E. coli* can metabolize a variety of organic compounds for energy; lipids, protein, ribonucleic acid, and glycogen have been suggested as substrates for its endogenous metabolism (Dagley and Johnson, 1953; Dawes and Ribbons, 1962).

The catabolism of polysaccharide by *S. mitis* was observed to occur at approximately the same rate when the organisms were suspended in buffer, sugar-free broth, or sugar-free broth containing TMG. This suggests that the processes of enzyme induction or growth do not place demands upon the organism's energy reserves above those required for cell maintenance. Cells catabolizing polysaccharide in lactose broth readily synthesized β-galactosidase before their polysaccharide reserve was completely depleted. Therefore, the rate of polysaccharide catabolism is sufficiently slow so that metabolic intermediates formed do not accumulate and markedly repress synthesis of inducible enzymes. Cells of *S. mitis* containing C^{14}-labeled polysaccharide incubated in the presence of nonlabeled exogenous glucose were observed to lose about 10% of the label during 1 hr of incubation. During this time, the cells were actually accumulating polysaccharide. However, it cannot be concluded from these data that the endogenous metabolism of the organism is proceeding simultaneously with the metabolism of exogenous glucose, for labeled glucose could be lost as a result of exchange through transglycosylation. Similarly, it cannot be concluded that the endogenous metabolism of the organism is repressed by exogenous glucose. Danforth and Wilson (1961) postulated the existence in *Euglena gracilis* of metabolically labile and stable reserves which are in equilibrium. These investigators proposed that the labile reserves undergo catabolism continuously. The assimilation of exogenous substrate by organisms possessing labeled reserves would dilute the label, and consequently

the quantity of labeled end products resulting would be reduced.

Catabolism of differentially pulse-labeled polysaccharide by *S. mitis* indicated that the last glucose molecules to be incorporated were among the first molecules to be removed during catabolism. These data are consistent with the findings of Holme et al. (1957), who reported that in *E. coli* there is an increase in the molecular weight of glycogen accompanying the accumulation of this compound. The data exclude the possibility that polysaccharide formation in *S. mitis* involves *de novo* synthesis of molecules which constitute part of a metabolically homogeneous polysaccharide pool. They do not exclude the possibility that *S. mitis* contains two or more polysaccharide pools which differ in metabolic availability. The occurrence of metabolically dissimilar glycogen pools has been postulated for mammalian tissues (Stetten, Katzen, and Stetten, 1956), for yeasts (Eaton, 1960), and for *Euglena gracilis* (Danforth and Wilson, 1961).

The catabolism of accumulated intracellular polysaccharide provides *S. mitis* with energy in a utilizable form. Cells containing polysaccharide increased in β-galactosidase activity when induced with TMG in the absence of an exogenous source of energy. Cells devoid of polysaccharide did not increase in β-galactosidase activity when induced in an analogous manner. The failure of the polysaccharide-negative variant to increase in β-galactosidase activity after being given the opportunity to assimilate glucose excludes the possibility that other cell carbohydrates were serving as the required energy reserve. Indeed, the failure of both the negative variant and the parent strain devoid of polysaccharide to increase in β-galactosidase activity implies that no other compounds present in the cell other than the glycogen-amylopectin polysaccharide can function as energy reserves.

Because it was not possible to lyse the organisms either with toluene or by sonic oscillation and retain enzyme activity, it was not possible to distinguish between synthesis of β-galactosidase and galactoside permease, should the organisms possess this means of galactoside transport. It seems likely however, that β-galactosidase itself was synthesized by the organisms, for increasing the concentration of the o-nitrophenyl-galactoside substrate did not increase the apparent β-galactosidase activity of the organism. Since the β-galactosidase activity of the organ-

isms increased in proportion to the time they were permitted to assimilate glucose, and consequently to their polysaccharide content, one may deduce that a given quantity of internal polysaccharide provides the organism with sufficient energy to synthesize a given quantity of protein. The amount of energy made available through polysaccharide catabolism does not appear to be sufficient to permit growth to any marked extent, however. Measurement of the growth yields of *S. mitis* in media containing varying quantities of glucose indicated that 10 μg of glucose were required to produce 1 μg (dry weight) of bacteria. Thus, even cells which contained over 50% of their dry weight of polysaccharide could be expected to increase their protoplasmic constituents by only 5% through the metabolism of polysaccharide. Growth to this limited extent may have important ecological implications, however. *S. mitis* is an indigenous microorganism of man and appears to represent the single most numerous species present in oral debris, (Gibbons et al., 1963). This site receives intermittent exposure to carbohydrates and contains a variety of bacteria which are not dependent upon carbohydrates for growth. The ability of *S. mitis* to store carbohydrate when available exogenously may permit the organism to compete for other required nutrients during periods when exogenous carbohydrate is lacking. In addition, it may favor the survival of this organism, as has been suggested for *A. aerogenes* by Strange, Dark, and Ness (1961). It should be noted that streptococci, presumably of the *S. mitis* type, have been shown to store intracellular iodophilic polysaccharides in vivo in the oral cavity (Gibbons and Socransky, 1962).

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