BIOCHEMISTRY 101

AUTUMN QUARTER 1960 -61

In recognition of and in the spirit of the Honor Code, I certify that I will neither receive nor give unpermitted aid and that I will report to the best of my ability all Honor Code violations observed by me. _____

NOTE: All questions have the same value, with the exception of question 8, which will count for double credit. Place the letter representing your answer to each question in the blank space provided at the end of the question.

A medical student, B. R. Tunemorse, as part of his BMS research project, was investigating the growth properties of Pseudomonas lagunitae, an organism he had isolated from Lake Lagunita mud the year before on a biology field trip. In particular he was trying to characterize this organism with respect to its sugar metabolism and he carried out the following experiments.

He inoculated a suitable number of cells into a synthetic medium (one containing $PO_4^{\equiv}$, $SO_4^{\equiv}$, NH_4^+, and trace metals) containing 1 gm per liter of the sugars shown in Table I, and determined the exponential rate of growth and the final yield of cells obtained. The results are summarized in Table I:

Table I

Sugar	Cell doublings per hour	Cell yield in mg of N
Glucose	2.1	65.0
Fructose	2.0	65.1
Galactose	1.9	64.6
Sucrose	1.9	63.9
Lactose	1.9	64.1

On further investigation he noted that the cells grown on galactose, sucrose and lactose displayed an interesting phenomenon. During the early phases of the growth period the cells accumulated an intracellular polysaccharide. When the carbohydrate component in the medium had disappeared the amount of intracellular polysaccharide began to decrease and when it had completely disappeared growth stopped. There was no accumulation of polysaccharide when glucose or fructose was the carbon source. Analysis of the polysaccharide showed it to be identical in structure to glycogen.

Duly noting this phenomenon, he attempted the isolation of mutants of this organism which might be deficient in their ability to utilize certain of the sugars.

After UV irradiation, and other appropriate manipulations, he was able to obtain a cell* (and a culture therefrom) which when tested in the experiment described above gave the following results. In the experiments with glucose, sucrose, and lactose all the substrate in the culture was consumed by the time the cell yields were determined. These results are given in Table II.

Table II

Sugar	Cell doublings per hour	Cell yield in mg of N
Glucose	2.1	64.8
Fructose	2.0	65.0
Galactose	no growth	no growth
Sucrose	2.0	32.8
Lactose	2.0	32.4

He also reinvestigated the phenomenon of glycogen accumulation and found that when the cells were grown in the presence of sucrose and lactose (but not with the other sugars), he obtained the same kind of polysaccharide accumulation but in this instance the polysaccharide never disappeared.

By this time Mr. Tunemorse was pretty muddled and so he gathered his colleagues in the Biochemistry course and asked them to help him find out and clear up what was going on. He felt that if they reviewed what they had learned in Dr. Berg's lectures then his observations would become meaningful. The following are the questions he asked. You are to provide the appropriate answers.

1. We know that the first step in the utilization of glucose is:

   a) oxidation to gluconic acid
   b) phosphorylation with ATP to form glucose 6-phosphate
   c) phosphorylation with ATP to form glucose 1-phosphate
   d) cleavage of the glucose to two trioses

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2. We also know that the first step in the utilization of galactose is:

   a) oxidation to galactonic acid
   b) phosphorylation with ATP to form galactose 6-phosphate
   c) phosphorylation with ATP to form galactose 1-phosphate
   d) cleavage of galactose to two trioses

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3. Furthermore we learned that the first step in the utilization of lactose is:

   a) cleavage to galactose and glucose
   b) phosphorolytic cleavage to glucose 1-phosphate and galactose
   c) phosphorolytic cleavage to galactose 1-phosphate and glucose
   d) phosphorylation with ATP to form lactose-phosphate

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* By appropriate genetic tests he could show that this mutant was the result of a single genetic alteration.

4. Since in the mutant sucrose gives only half the yield of cells as obtained with glucose or fructose alone we can assume that in this organism the first step in sucrose utilization:

a) gives rise to free glucose and fructose
b) gives rise to glucose 1-phosphate and free fructose
c) gives rise to fructose 2-phosphate and free glucose
d) gives rise to sucrose-phosphate

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5. You may remember that glycogen synthesis very likely occurs from glucose 1-phosphate:

a) by transferring the glucosyl group from the phosphate to pre-existing glycogen
b) via UDPG
c) by first cleaving off the phosphate and then linking the free glucose to pre-existing glycogen
d) which is first converted to maltose and then the disaccharide is linked to pre-existing glycogen

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6. And we know that the utilization of the glycogen later in the growth period is very likely initiated by :

a) hydrolytic cleavage to free glucose
b) hydrolytic cleavage to yield free maltose which is then cleaved to 2 glucose units
c) phosphorolysis of the glycogen to yield glucose 1-phosphate
d) reaction of the glycogen with UDP to reform UDPG

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7. The utilization of glucose 1-phosphate by phosphoglucomutase results in the formation of :

a) fructose 1-phosphate
b) glucose 6-phosphate
c) sedoheptulose 1-phosphate
d) fructose 1, 6 diphosphate

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8. If all of our answers are correct then it seems most likely that the genetically controlled enzyme defect in the mutant is :

a) a loss of UDPG-glycogen synthetase activity
b) a loss of glycogen phosphorylase activity
c) a loss of hexokinase activity
d) a loss of phosphoglucomutase activity
e) a loss of one of the enzyme activities converting galactose to glucose 1-phosphate

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Question 8 will count for double credit