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Laboratory Exercises

Teaching Lactose Metabolism

A COMPLEX CHALLENGE FACED WITH A SIMPLE KIT

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Andrea Monti-Hughes, Manuel Alonso, Judith Garófalo, Hilda Isabel Burgos, and Carlos Alberto Stella‡

From the School of Medicine, Department of Biochemistry, University of Buenos Aires, Buenos Aires, Argentina

We developed an experimental didactic proposal to teach both carbohydrate metabolism and lactose intolerance as the disease related to that metabolism. Therefore, we implemented an empirical strategy consisting of inexpensive and nontoxic components for which students do not need to know any of the laboratory techniques. The fact that students were able to discuss their own results obtained from the experiments performed in their classroom gave them an additional motivation to learn the subject.

Keywords: Lactose intolerance, carbohydrate metabolism, kit, biological models.

Because of its structural characteristics, cellular metabolism is a fundamental issue for the comprehension of the essential processes that occur in organisms. In introductory biology courses, we have noticed that learning biochemistry and cellular basis of an organism and relation between cellular metabolism and the way the multicellular organisms work are difficult tasks for the students. Essentially, they cannot understand, in biochemical terms, the relation between what they see macroscopically and how the cell uses different nutrients [1].

We firmly believe that these topics could be more attractive to the students if they were explained with relation to the metabolic pathway of a particular disease. If so, students would be able to analyze the normal metabolic pathway of the disease and hypothesize the functional consequences that can take place when one of the steps of the metabolism is altered.

In addition, we consider that if these topics were taught in an empirical way, they would be learnt more easily. However, we are aware that in courses with lots of students, it is really difficult to develop an empirical kind of work due to the lack of laboratories and students' knowledge about the laboratory techniques.

Taking these difficulties into consideration, our purpose was to develop an experimental didactic proposal to teach both carbohydrate metabolism (particularly lactose metabolism) and lactose intolerance as the disease related to that metabolism. This proposal is suitable for students in the secondary school and those taking up courses in medicine or nutrition.

In normal conditions, mucous cells of the intestine synthesize lactase [2, 3], an enzyme that degrades lactose, the sugar present in milk, to its two monomers: glucose and galactose. A number of individuals stop synthesizing lactase when they are 4 years old and can therefore not assimilate lactose [3]. When this happens, lactose reaches the large intestine, where bacteria hydrolyze lactose, producing glucose. As a result of the accumulation and fermentation of the lactose, the person suffers from flatulence and diarrhea [3, 4]. In our experience, students tend to confuse lactose intolerance with the other digestive diseases such as Celiac disease.

This capacity of intestinal bacteria to hydrolyze and metabolize lactose is shared by *Lactobacillus bulgaricus*, a bacterium of the *Lactobacillus* genus, which is used in the milk industry for the production of yoghurt [4]. On the other hand, there is a common eukaryotic microorganism, the yeast *Saccharomyces cerevisiae*, mainly used in the bakery, which like the intestinal cells of people with this disorder, cannot hydrolyze lactose. The metabolic characteristics of both the microorganisms make the baker's yeast and the yoghurt bacterium appropriate models to be used as analogs of the mucous cells of the intestine and the bacteria present in the large intestine, respectively.

Therefore, we implemented an empirical strategy consisting of inexpensive and nontoxic components for which the students do not need to know any of the laboratory techniques, and with which the students can work by themselves in their own classroom.

‡To whom correspondence should be addressed. Tel.: 4508-3672 int 32; Fax: 4508-3672 int 31; E-mail: cstella@fmed.uba.ar.

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MATERIALS AND METHODS

MEF Medium with Either Normal or Reduced Lactose Content

MEF[1] medium (Milk, yeast Extract, Fertilizer) was developed comparing the components of the following media: LB (used for common bacteria) [5], AC (used for growing anaerobic microorganisms) [6], and ROGOSA (used for growing *Lactobacillus*) [7].

MEF medium consisted of yeast extract 1% (w/v) (Calsa™), gardening fertilizer (powder) 0.6% (w/v) (ammonium phosphate), powdered milk 2% (w/v), and agar 2% (w/v). For the acidification experiment, powdered milk concentration was 4% (w/v). In this case, the medium for each Petri dish (25 mL) was supplemented with 85 μL of bromocresol purple (BCP) dissolved in ethanol (2.4 mg/mL).

MEF medium with low concentration of lactose consisted of yeast extract 1% (w/v) (Calsa), gardening fertilizer (powder) 0.6% (w/v) (ammonium phosphate), powdered milk reduced in lactose 2% (w/v), and agar 2% (w/v).

In both the media, milk constituted the carbon and nitrogen source. In standard milk, lactose is the main carbon source, whereas milk reduced in lactose contains mainly of glucose, galactose, and a small amount of lactose. The yeast extract and the crushed gardening fertilizer provide vitamin B [8] and phosphate ions, respectively.

Microorganisms Used as Biological Models

Yeast–Pressed baker's yeast (0.1 g) (*Saccharomyces cerevisiae*) (Dánica™) was diluted in 10 mL of sterile water. From this solution, a 1/10 dilution was prepared to inoculate the Petri dishes.

Bacteria–Yoghurt (0.1 mL), previously incubated by students, was diluted in 10 mL of sterile water. A teaspoon of any kind of yoghurt in 100 mL of skimmed milk was incubated at 30 °C for 45 min. Before this incubation, skimmed milk was boiled to 42 °C before adding the yoghurt.

Use of the Kit

We consider the kit as the group of devices and compounds needed for the experiment. The kit consisted of Petri dishes, each containing MEF medium, pressed bakery yeast and yoghurt dilutions, glass balls (3 mm in diameter), 15-mL tubes with twist-off lids, and a trash container (Fig. 1).

One advantage of our proposal is that the students can safely prepare both the media and the dilutions of yeast and yoghurt, because every component of the kit is nontoxic.

Sterilization of the medium and the tubes was done with a pressure pot for 1 h from the moment it released steam.

To inoculate the medium, each student received a tube with a ball soaked in the corresponding dilution. The advantage of this technique is that the use of pipettes or plating under laminar flow is not necessary. The teacher indicated the students to open the Petri dish just before inoculating the medium. Then, the students placed the glass sphere on the Petri dish lid, closed it, inverted it, and moved the dish gently so as to make the sphere move around the medium for 2 or 3 min, and finally inverted the Petri dish again to be able to remove the sphere and throw it away. The incubation of the inoculated plate can be carried out at room temperature or in a laboratory stove at 30 °C.

[1]The abbreviations used are: MEF, Milk, yeast Extract, Fertilizer; BCP, bromocresol purple.

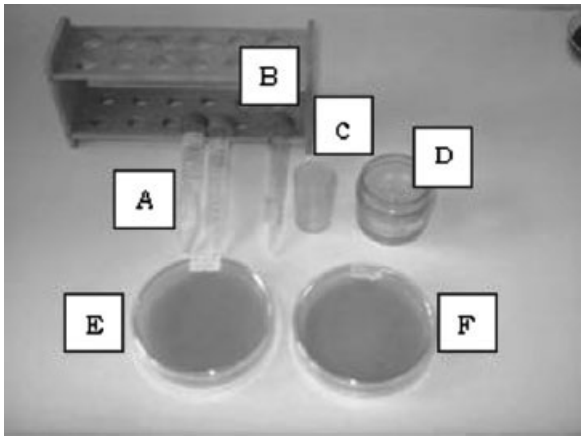

FIG. 1. **Components of the kit.** *A*) Dilutions from baker's yeast and yoghurt, *B*) 15-mL tubes with twist-off lids, *C*) flasks to discharge small spheres, *D*) glass spheres 3 mm in diameter, and *E*) and *F*) dishes with MEF medium (either standard milk or milk reduced in lactose, yeast extract, and fertilizer).

Teacher Activities

We developed a 5-day scheme as summarized in Table I.

Day 1–The teacher explained how to prepare the medium and then discussed why they needed to use these components, which were the biological models involved, what was necessary for these cells to grow, which of these compounds could be energy sources, and why the medium had to be sterilized. After that, the students inoculated the Petri dishes containing different concentrations of lactose (Table II), and the teacher discussed the possible results to be obtained from a qualitative–quantitative point of view.

Days 2–4–Incubation of plates.

Day 5–After the dishes were grown for 3 days in Days 2–4, each student received the dish he/she had inoculated and analyzed the obtained results. Finally, the work was completed with an integrative discussion.

RESULTS AND DISCUSSION

Pilot Test and Utilization of the Kit

We decided to establish the degree of complexity of the technique to be used. We were aware that if the students had any trouble or resistance with the experimental proceedings, they could easily get diverted from the main objective of the work, which was to learn the metabolism of carbohydrates.

With this idea in mind, we performed a pilot test with a group of 10 students of biological chemistry from the School of Medicine of Buenos Aires University, who had not yet received any training in microbiological techniques. This subject is studied in the second year of medicine.

We gave each student two Petri dishes with MEF medium reduced in lactose, and two 15-mL tubes with twist-off lids. Each tube contained a glass sphere soaked with the yeast suspension. Then, we explained to the students the steps involved in inoculating the Petri dish, and to find out the difficulties they had encountered, students were given a questionnaire. Also, we examined the extent of contamination and the number of colonies

TABLE I
Five-day scheme of activities developed by students and teacher

Day 1	First: The teacher explained how to prepare the medium and discussed why they used those components and biological models. The teacher made sure that the students understood the conceptual leap from bacteria and yeast cells to human intestinal cells. Second: Students inoculated the Petri dishes containing different concentrations of lactose (Table II) as it is explained in "Use of the Kit." Students are divided into four groups: one student inoculated MEF with yeast, the second one MEF without lactose with yeast, the third one MEF with yoghurt, and the fourth MEF without lactose with yoghurt. Also, they inoculated plates with bromocresol purple. Third: Each group expressed what they thought would happen based on the explanation made by the teacher before starting the experiment (1st). After that, the teacher made the final hypothesis based on what the students had hypothesized.
Days 2–4	Incubation of the Petri dishes inoculated by the students on *Day 1*.
Day 5	First: Each student received the Petri dish he/she had inoculated and compared it with what he/she had hypothesised on *Day 1*. In each group, each member told his/her classmate what had happened in his/her Petri dish and why. After that they drew a conclusion, taking into account the result that each member had obtained. Second: Each group expressed its conclusion in class and together with the teacher drew a final conclusion of the experiment made.

TABLE II
Inoculation performed on the first day of the proposal

	MEF	MEF reduced in lactose	MEF with bromocresol purple
Baker's yeast	One Petri dish	One Petri dish	–
Bacteria from yoghurt	One Petri dish	One Petri dish	One Petri dish (control) One Petri dish (2% w/v milk) One Petri dish (4% w/v milk)

obtained (data not shown). Figure 2 presents the growth obtained in three dishes inoculated independently by three different students from the group.

This preliminary test showed that the kit was easy to manipulate even though the students had had no previous experience in the laboratory work. The questions asked by the students during the activity reflected their interest and also the fact that they had worked at ease. For example, they asked what would happen after finishing the activity; what would they see after the incubation of the Petri dishes; and how the medium was made. The number of colonies obtained was nearly the number we had expected. Finally, it could have been thought that the absence of conditions of sterility in the classroom have induced contamination in the dishes. However, we observed only three colonies of contaminants (data not shown).

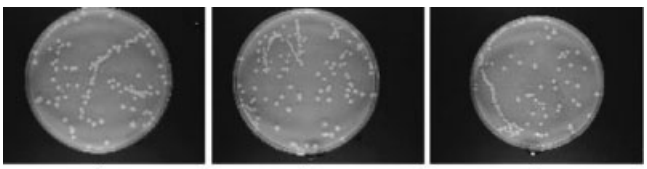

FIG. 2. **Pilot test to evaluate the inoculation methodology and cell growth in MEF medium.** Each student received a tube containing a sphere soaked with the suspension of yeast to be used to inoculate the dish. After transferring the sphere to the dish, which was loaded with low-lactose MEF medium, the dish was rotated in order to make the sphere cover the entire surface of the dish. Dishes *A*), *B*), and *C*) came from the inoculation performed independently by three different students. The average for the number of colonies obtained by 20 students was 97 ± 34 colonies.

Growth of Bacteria and Yeast in Either MEF Medium or MEF Medium Reduced in Lactose

As a first step in the development of the work, the students had to inoculate the dishes with either a normal or a low concentration of lactose.

Results obtained with MEF medium (Fig. 3*A*) showed that the number of yeast colonies was smaller and that their diameter was shorter than that reached with the MEF medium reduced in lactose (Fig. 3*B*). This difference was explained to be due to the incapability of the yeast enzymes to metabolize lactose and that, when metabolized, lactose can be used as a source of carbon and energy by cells. Students were also asked the reasons for this lack of growth. To encourage the discussion, as an example, the teacher mentioned that the level of some nutrients might have been insufficient to hold cell growth.

This result was compared with the metabolism of human cells. When lactose intolerance occurs, epithelial cells such as yeast cells are not able to metabolize lactose. In the condition of this disorder, the nutrient availability of epithelial cells is not affected since they have other sources of energy that allow them to grow normally. Then, the incapability of these cells to hydrolyze lactose allows lactose to move and reach the large intestine, where it is fermented by bacteria. The fermentation produced by the bacteria causes the characteristic symptoms of the disease.

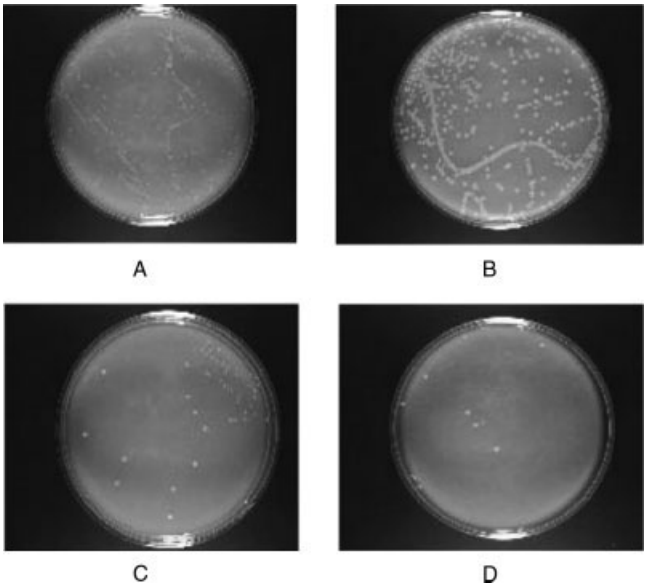

FIG. 3. **Growth of the yeast and bacterial cells in MEF and low-level lactose MEF.** Media were inoculated according to the dilutions and procedures described in Materials and Methods section. The dishes were incubated at 30 °C for 3 days. *A*) MEF medium inoculated with yeast, *B*) MEF reduced in lactose inoculated with yeast, *C*) MEF medium inoculated with bacteria, and *D*) MEF reduced in lactose inoculated with bacteria.

When the yeast cells were provided with a medium with milk reduced in lactose, we observed an increase not only in the colony diameter but also in the number of colonies (Fig. 3*B*). This was discussed and explained by the fact that this milk contained glucose due to the enzymatic hydrolysis of the lactose performed during the industrial processing. As a result, the level of lactose in this milk decreased while the level of glucose increased.

After these conclusions, the teacher questioned on the characteristics of the milk that would render it consumable by people with lactose intolerance. Students deduced that the people with this disorder have to consume milk with lactose that has been previously hydrolyzed. In this way, the symptoms would be prevented, and nutrients from the milk can be dissipated to the cells.

Differently, bacteria grew well both in the medium with a normal level of lactose (Fig. 3*C*) and that with low concentration of lactose (Fig. 3*D*). The teacher explained that in the first condition, this was due to the presence of the lactase enzyme synthesized by bacteria, and that in the second condition, due to the presence of galactose and glucose and a small amount of lactose, there were many carbon and energy sources for the bacterial growth.

Then, the teacher compared this growth condition with the bacterial conditions in the large intestine of patients with lactose intolerance when they consume milk. The amount of lactose present cannot be hydrolyzed by the intestine epithelial cells, but can be hydrolyzed by bacteria, thus causing the typical symptoms of this disorder.

Therefore, when comparing the yeast and bacteria growth in the two media, students were able to realize that this is a suitable experimental approach to understand the characteristic of lactose metabolization in the human intestine.

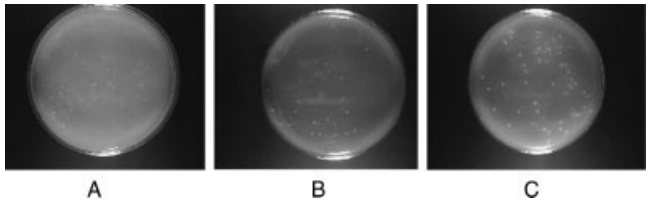

FIG. 4. **Extrusion of acids during the metabolization of the lactose present in milk.** The bacteria were inoculated in MEF medium either with the addition of BCP or without BCP. Dishes were incubated at 30 °C for 3 days. *A*) MEF medium (2% w/v of milk) without BCP, *B*) the same MEF medium with addition of BCP, and *C*) MEF medium (4% w/v of milk) with the addition of BCP.

To sum up, Figs. 3*A* and 3*B* compare the yeast growth with or without lactose, respectively. They showed a representative difference in the cellular growth. The aim of this comparison was to represent the condition of the cells of an ill or a normal intestine in the presence of milk with or without lactose. On the other hand, Figs. 3*C* and 3*D* compare the bacterial growth in MEF medium with or without lactose, respectively, and no difference was seen. This comparison represents what would happen in the presence of milk with or without lactose in bacteria growing in an ill or a normal intestine.

Finally, the teacher dealt with the diagnosis of the disease. As mentioned in the Introduction, one of the main symptoms of the disease is diarrhea, following lactose ingestion. In these cases, especially in infants, the faecal material is evaluated to determine the presence of lactic acid, a product of bacterial metabolization [3, 4]. This result contributes to the diagnosis of the disease.

To teach this important aspect, the teacher asked the students to discuss about the results they expected to obtain in a Petri dish supplemented with an acid–base indicator such as BCP. This dye presents a violet color at pH values higher than 6.8 and changes to yellow when the pH value is lower than 5.2. Figure 4*C* shows that the medium around the colonies became yellow because of the acidification produced by the bacteria. This observation allowed the students to conclude that there had been an extrusion of acids during the metabolization of lactose by bacteria. Apart from that, comparing Fig. 4*C* with Figs. 4*A* and 4*B*, they concluded that the MEF medium without BCP or MEF medium with 2% milk (w/v) was not useful to show the acidification produced by the bacteria, because less growth (Fig. 4*B*) leads to less acid production.

To sum up, we, here, presented an experimental proposal for teaching an important pathway of cellular metabolism, which students can develop in a class under minimal supervision. The kit components can be obtained easily and are not harmful since they are used in home activities. Finally, the growth medium is easy to prepare and permits the growth of not only bacteria but also yeast.

We can assume that the medium would have a potential utilization in other didactic proposals involving these microorganisms. The methodology is not complicated, and the results are obtained in a short period of time. In

this way, the experimental procedure for both the students and the teacher is free of stress.

These characteristics enable the analyses of the results, which are not hindered or forgotten by distraction, making it possible for the students to build up more complex concepts about the metabolic pathway being studied.

Also, the characteristics contribute to the work of teachers with the students with a different degree of knowledge and institutions with limited material resources.

Finally, we observed that students were able to discuss their own results obtained from the experiments, and this gave them the additional motivation to learn the subject.

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