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Advan in Physiol Edu 36:313-318, 2012. ;
doi: 10.1152/advan.00132.2011

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Teaching nutritional biochemistry: an experimental approach using yeast

Manuel Alonso[1] and Carlos A. Stella[2]

[1]*Departamento de Ciencias Biológicas, Ciclo Básico Común, Universidad de Buenos Aires, Buenos Aires, Argentina; and* [2]*Departamento de Bioquímica Humana, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina*

Submitted 3 January 2012; accepted in final form 10 August 2012

Alonso M, Stella CA. Teaching nutritional biochemistry: an experimental approach using yeast. *Adv Physiol Educ* 36: 313–318, 2012; doi:10.1152/advan.00132.2011.—In this report, we present a practical approach to teaching several topics in nutrition to science students at the high school and college freshmen levels. This approach uses baker's yeast (*Saccharomyces cerevisiae*) as a biological system model. The diameters of yeast colonies, which vary according to the nutrients present in the medium, can be observed, compared, and used to teach metabolic requirements. The experiments described in this report show simple macroscopic evidence of submicroscopic nutritional events. This can serve as a useful base for an analogy of heterotrophic human cell nutrition.

nutrition; chemical digestion; essential nutrients; digestive enzymes; *Saccharomyces cerevisiae*

TEACHING NUTRITION comes with an important challenge: how to clearly explain macroscopic events, such as feeding, and submicroscopic processes, such as the extracellular chemical digestion of food, as well as body cell metabolism (9). In textbooks and classroom explanations, nutrition is often defined as the process of obtaining and processing nutrients to convert them into a usable form (20). Nutrition in animals is clearly biochemical. To study this process, one must understand that it is the cells of living organisms that use the nutrients. This relationship between the body and cells is often quite difficult for students to grasp (5, 21), since this systemic interpretation of biochemistry and biology involves the integration of various scientific models to explain complex processes (2).

In teaching these topics, students often ask questions such as:

- What is the importance of ingesting certain nutrients, such as carbohydrates, amino acids, or vitamins?
- Which part(s) of our body really uses these nutrients?
- What happens when "low-calorie" sweeteners are ingested?
- What would happen if food were not chemically broken down?

Numerous studies of high school students have shown that they have difficulty relating the various processes involved in human nutrition to each other. They do not tend to have a clear idea of how cells use nutrients or that nutrients are destined for the cells of the body's organs (3, 16). Moreover, they tend to have a better understanding of the role that energy plays in nutrition than that of matter (13). More recent studies (11, 17) have shown that students' comprehension of the role played by carbohydrates, proteins, and lipids in energy production is unclear; they are unable to make a connection between these compounds, energy, and respiration. Likewise, they cannot explain or positively identify the importance of different compounds in the diet, such as proteins and vitamins (13), nor are they able to comprehend changes or chemical transformations in foods, although they may understand physical ones.

In summary, students have difficulties relating food intake to its subsequent use in biochemical processes on a cellular level, once it has been converted into simple molecules. This relationship is essential to understanding the processes of chemical digestion and human nutrition.

With this in mind, we propose a practical approach to teaching these topics.

This approach involves the following:

- Baker's yeast (*Saccharomyces cerevisiae*), as a biological system model;
- Foods from the human diet, as a source of nutrients;
- Hydrolytic enzymes, involved in chemical digestion.

Here, we present a series of experiments in which yeast is the stand in for human cells.

We propose cultivating this unicellular microorganism in solid media supplemented with different nutrients commonly found in the human diet. The diameter of the colonies that form is used as an indicator of cellular growth and division. Students can therefore infer that the use of these nutrients causes colony growth, since they are required for cell division and growth.

Students will therefore be able to understand the importance that certain nutrients have in their own diet and the need for prior chemical enzymatic digestion before cells can assimilate nutrients.

MATERIALS AND METHODS

Cells

Commercial dry yeast (*S. cerevisiae*) was used in all of the experiments. Cells were suspended in sterile distilled water, and 0.1-ml aliquots containing 50 colony-forming units were plated on solid culture medium (pH 5.5) to obtain single colonies and incubated for 2–3 days at 30°C.[1] To calculate the number of cells per plate, it was deemed that 50% of the weight of the yeast packet corresponded to cells, and 1 mg of cells is equal to 5×10^7 cells.

Alternately, if measuring the optical density is a possibility, colonies can be isolated from the suspension of dry yeast and used as a source of cells in all of the experiments. The number of colonies per plate is calculated, assuming that 0.5 optical densiometry units are equal to 1 mg/ml of cells.

Culture Media

Solid culture media were prepared as follows: 2% (wt/vol) agar (cat. no. 214530, Difco, Detroit, MI) with beef bouillon extract and commercial sucrose at different concentrations. A 10% (wt/vol) stock solution of

Address for reprint requests and other correspondence: M. Alonso, Departamento de Ciencias Biológicas, Ciclo Básico Común, Universidad de Buenos Aires, Pab. III, P. B., Av. Intendente Cantilo s/n, Ciudad Universitaria, Buenos Aires C1428EGA, Argentina (e-mail: m_alonso@live.com.ar).

[1] If a 30°C heater is not available, the cultures can be left at room temperature over a longer period of time.

beef bouillon was prepared beforehand, and the fat was removed by filtering the cooled mixture through a cloth filter. Commercial yeast powder [1% (wt/vol)] can be used instead of beef bouillon as a source of nitrogen and cofactors. In some cases, the medium was supplemented with a 2 mg/ml multivitamin made up of 12 vitamins and 8 minerals (Supradyn, Bayer). In other cases, the medium was supplemented with a commercial dietary supplement of amino acids (Lafarmen) at a final concentration of 100 mg/ml. Finally, in other experiments, the medium was supplemented with both vitamins and amino acids.

For the sweetener assay, cells were grown at 30°C for 3 days on solid media containing a 10% (vol/vol) stock solution of beef bouillon supplemented with 2% sucrose (wt/vol) or with 3.2% (wt/vol) sweetener containing cyclamate and saccharin.

Commercial nonfat dry milk and flour were used in the digestion experiments. Human saliva was used as an amylase source. Flour (0.5 g) was incubated with 4 ml of saliva for 2 h. In other experiments, the supernatant of a kiwifruit (*Actinidia deliciosa*) shake, centrifuged in a clinical centrifuge at maximum speed for 5 min, was used as a source of protease (3 ml/sample) and incubated with 0.5 g of milk for 2 h. Subsequent autoclaving of the media led to the inactivation of enzymatic activity.

Development of the Proposal

Day 1. On *day 1*, the teacher explains the MATERIALS AND METHODS to students, going over the method for preparing and inoculating the culture media. It is important that students note the absence of growth in the colonies.

Day 2. Three days after yeast cultivation, students are given the plates to observe the diameters of colonies. Later, the teacher discusses the results with students.

RESULTS AND DISCUSSION

The yeast colonies consist of cells that grow and divide, causing the diameters of colonies to expand. Similarly, when the body of a multicellular organism grows, it is due to the growth and division of its cells.

Cells in rich media grow more than those in poor media. These differences can be seen macroscopically in colony growth. These experiments lead students to associate their understanding of the importance of nutrients in cellular division with actual visual proof (i.e., colony diameters) (6).

Role of Carbohydrates and Nitrogen in Cell Growth

Cells need a source of energy and nitrogen to grow. In these experiments, we propose to show that carbohydrates and amino acids are two key nutrients in cell growth. Sucrose will supply the carbohydrates, as a source of energy, whereas beef bouillon will provide amino acids, as a nitrogen source as well as necessary quantities of other nutrients as cofactors. The results of these experiments are shown in Figs. 1 and 2.

Figure 1 shows colonies plated on solid medium containing a 10% (vol/vol) stock solution of beef bouillon and 0%, 0.25%, 0.5%, 1%, or 2% (wt/vol) sucrose for 2 days at 30°C, as described in MATERIALS AND METHODS. The colonies grown with greater quantities of sucrose are greater in diameter. These results show that carbohydrates are an important component in cell growth. The concentration of beef bouillon is fixed in this experiment, and the diameter of colonies is measured at different concentrations of sucrose in the medium. Colonies reach a maximum diameter in the medium with 1–2% sucrose. Therefore, we chose to use a 2% sucrose concentration in the rest of the experiments.

By way of this experiment, students can deduce that the greater the amount of sucrose in the medium, the greater the colony growth. The results of this experiment will be discussed again when students observe colonies grown in the medium with sweetener.

However, cells do not only require carbohydrates. It is also important to look at how the different concentrations of beef bouillon might affect the diameter of colonies.

Beef bouillon contains a high concentration of nitrogen compounds, such as peptides and amino acids, and several cofactors for enzymatic activity, such as vitamins. Figure 2 shows how the diameter of colonies increases as the concentration of beef bouillon increases in the medium. In these tests, cells were plated on solid medium containing 2% (wt/vol) sucrose and a 0%, 2%, 4%, 6%, 8%, or 10% (vol/vol) stock solution of beef bouillon for 2 days at 30°C. As proposed in MATERIALS AND METHODS, beef bouillon can be replaced in both experiments (Figs. 1 and 2) with yeast, yielding similar results (data not shown). In this case, the results of this experiment should be discussed again when students observe colonies grown on medium supplemented with vitamins and amino acids.

Through these two experiments, students are able to macroscopically observe the importance of carbohydrates and nitrogen in cell growth. At this point, students can discuss what role each of the compounds used in cell growth and division might have. Students are familiar with both sucrose and beef bouillon, and the nutritional importance of these substances can be discussed based on the results of the experiments. At this point, it is interesting to ask what would happen to colony diameters if the standard growth medium were altered by adding other nutrients to it.

Role of Vitamins and Amino Acids in Cell Growth

In these experiments, the standard medium with sucrose and beef bouillon was now supplemented with vitamins, amino acids, or both.

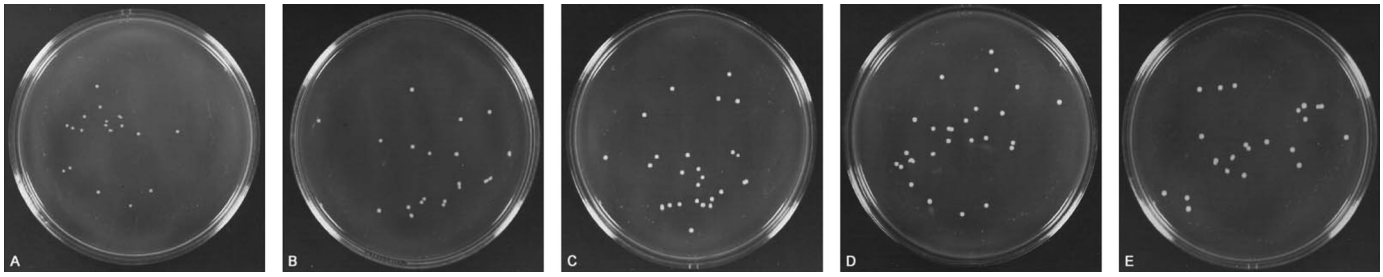

Fig. 1. Role of carbohydrates in cell growth. Cells were plated on solid medium containing 10% (vol/vol) stock solution of beef bouillon and 0.0% (wt/vol) (*A*), 0.25% (wt/vol) (*B*), 0.5% (wt/vol) (*C*), 1% (wt/vol) (*D*), and 2% (wt/vol) (*E*) sucrose. Incubation for 2–3 days was performed at 30°C.

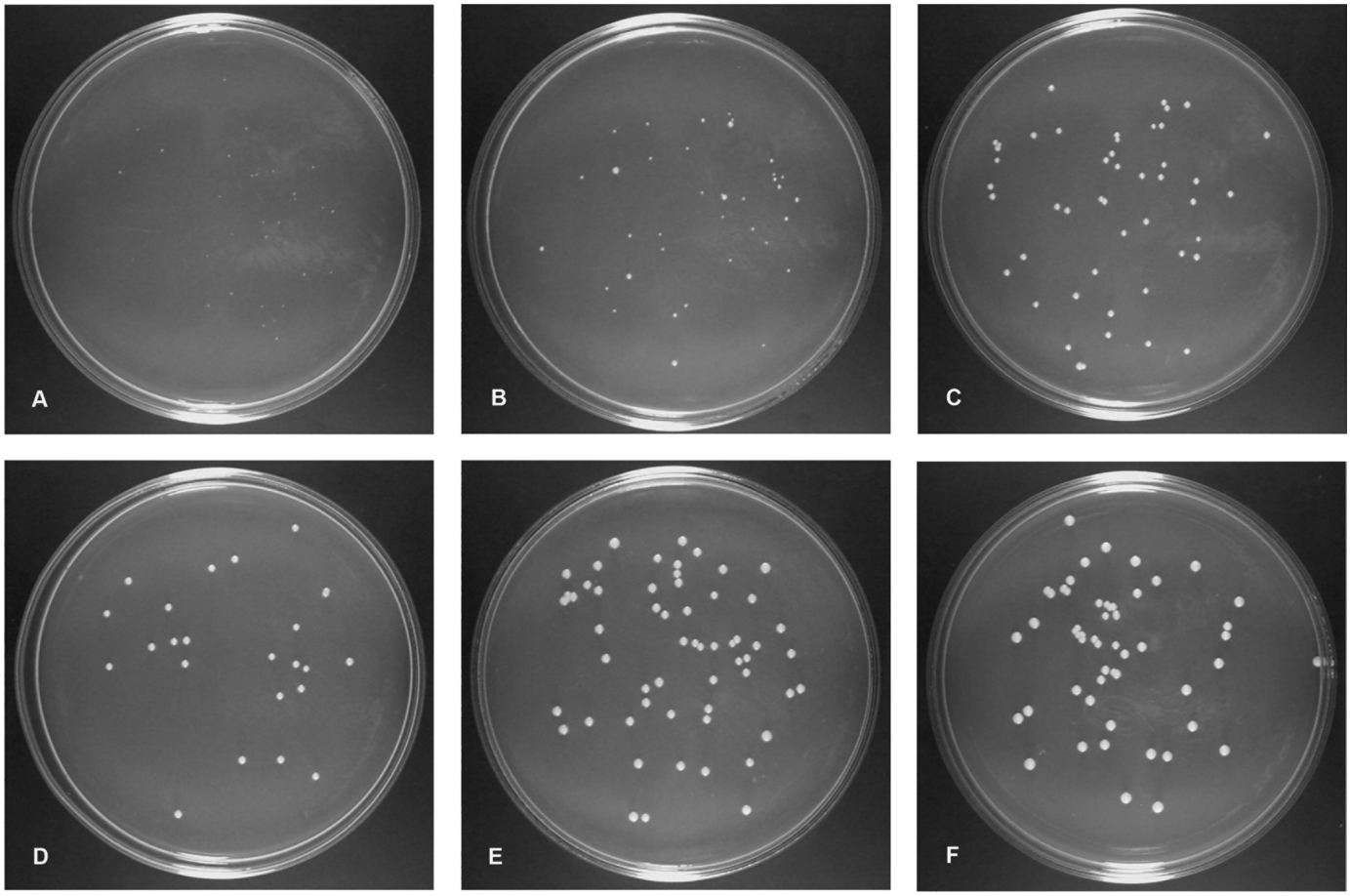

Fig. 2. Role of nitrogen source in cell growth. Cells were plated on solid medium containing 2% sucrose and 0% (vol/vol) (*A*), 2% (vol/vol) (*B*), 4% (vol/vol) (*C*), 6% (vol/vol) (*D*), 8% (vol/vol) (*E*), and 10% (vol/vol) (*F*) stock solution of beef bouillon. Incubation over 2–3 days was performed at 30°C. Standard culture media were adjusted to 2% sucrose and a 10% stock solution of beef bouillon.

Figure 3 shows the growth of colonies in three different media. Since beef bouillon consists of various components, such as amino acids and cofactors, in this experiment the minimum concentration of beef bouillon tested in Fig. 2 was used as the base medium. The control medium contained only 2% sucrose and a 2% (vol/vol) stock solution of beef bouillon (Fig. 3*A*). An extra 2 mg/ml of vitamins (Fig. 3*B*) or 100 mg/ml of amino acids (Fig. 3*C*) enabled further cell growth. When both components were added to the medium, the colonies reached an even greater diameter (Fig. 3*D*).

In summary, each of the supplements caused colony diameter to expand, but when both were present in the medium, the effect was even greater. It is therefore possible to conclude that these nutrients are required in cell growth and division.

Again, at this point, students can discuss the importance of amino acids and vitamins in cell proliferation. These are everyday products that the students are familiar with. The amino acids were derived from a dietary supplement often used by athletes, and vitamins are used by many students. Therefore, it will be easy for students to relate these products to their prior

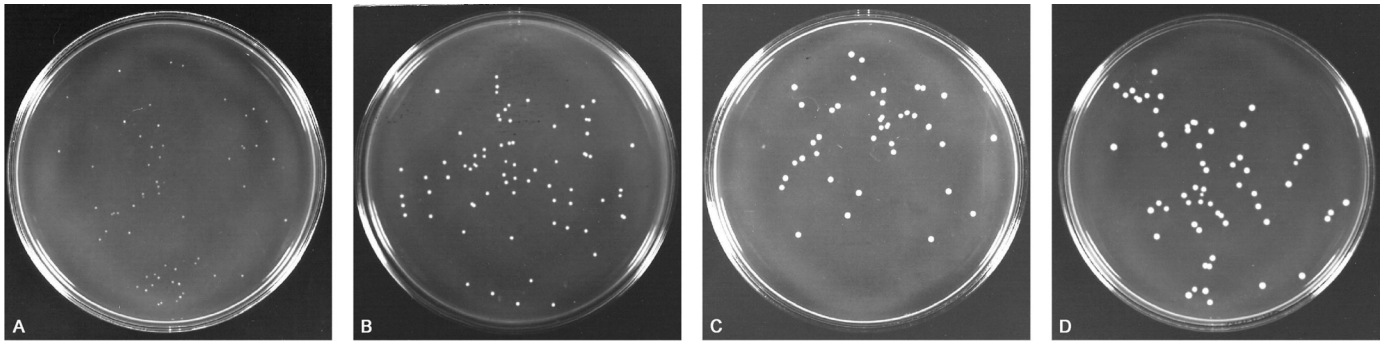

Fig. 3. Role of amino acids and vitamins in cell growth. Cells were grown at 30°C over 3 days on solid media containing 2% (wt/vol) sucrose and a 2% (vol/vol) stock solution of beef bouillon (*A*) and supplemented with 2 mg/ml vitamins (*B*), a 100-mg/ml amino acid dietary supplement (*C*), or both vitamins and amino acids (*D*).

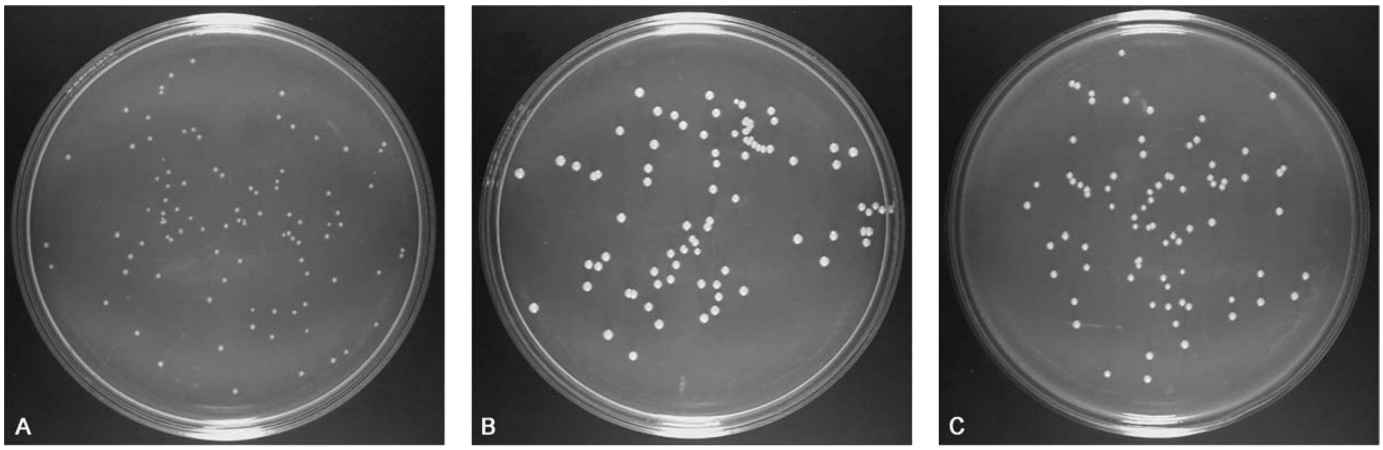

Fig. 4. Sweetener and low-calorie foods. Cells were grown at 30°C for 3 days on solid media containing a 10% (vol/vol) stock solution of beef bouillon (*A*) and supplemented with 2% (wt/vol) sucrose (*B*) or with 3.2% (wt/vol) sweetener (*C*).

knowledge of them. The teacher should guide the discussion, to assign the correct metabolic role to each of these substances.

Low-Calorie Foods

In the colonies shown in Fig. 4, the yeast was grown in a medium with a 10% (vol/vol) stock solution of beef bouillon, and sucrose was replaced with sweetener. In this case, it is possible to compare the growth of colonies in media without sucrose (Fig. 4*A*) with those with 2% sucrose (Fig. 4*B*) and with 3.2% sweetener instead of sucrose (Fig. 4*C*). The diameter of colonies was smaller when cells were plated in medium with sweetener. The light growth observed could be the result of other components present in the sweetener that may replace sucrose. The discussion of the experiment can focus on the importance of energy in cell growth, keeping in mind the association of sweeteners with low calories. The next question is whether any source of carbohydrates or amino acids can serve as a nutrient for cells.

Role of Extracellular Chemical Digestion

In the following experiments, cells were grown in media that contain commonly consumed foods. Students may assume that the yeast colonies will grow in a medium containing milk- or flour-based products, since they themselves ingest them for nutrients. The goal of this experiment is to show that this is not possible.

Starch Breakdown

Starch is a carbohydrate present in flour. Given that it is a macromolecule, flour cannot be directly assimilated as such by cells, and cells are not able to hydrolyze it extracellularly.

Sucrose, used as a source of carbohydrates in the previous experiments, was replaced with flour. Figure 5 shows cells plated in solid media containing flour previously incubated with (*B*) or without (*C*) saliva, as described in MATERIALS AND METHODS. Saliva contains the enzyme amylase, which catalyzes starch hydrolysis into glucose, which can easily be used by cells. Therefore, the colonies reached a greater diameter when starch treated with saliva was added to the culture medium. In all cases, the medium was supplemented with a 10% (vol/vol) stock solution of beef bouillon, as a source of amino acids and cofactors. In the control experiment (Fig. 5*A*), the standard medium with sucrose and beef bouillon was used.

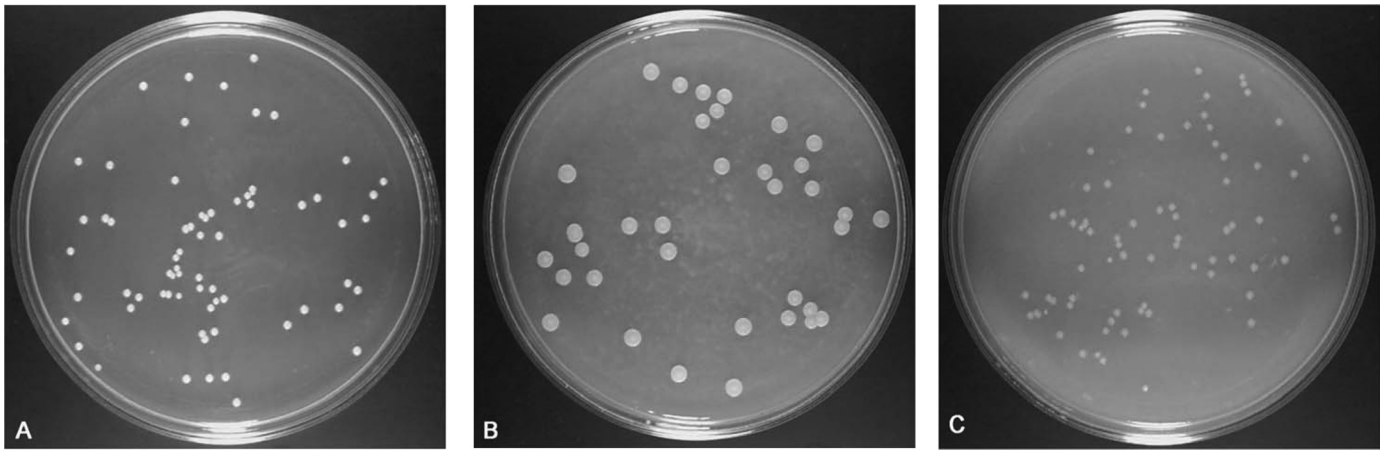

Fig. 5. Extracellular breakdown of starch is necessary for cell growth. Cells were plated on solid medium containing a 10% (vol/vol) stock solution of beef bouillon and 2% sucrose (wt/vol) as a control (*A*), a 10% (vol/vol) stock solution of beef bouillon and 2% (wt/vol) flour digested during 2 h at 37°C with 4 ml of saliva (an amylase source; *B*), or a 10% (vol/vol) stock solution of beef bouillon and 2% flour (*C*). Incubation of cultures for 3 days was performed at 30°C.

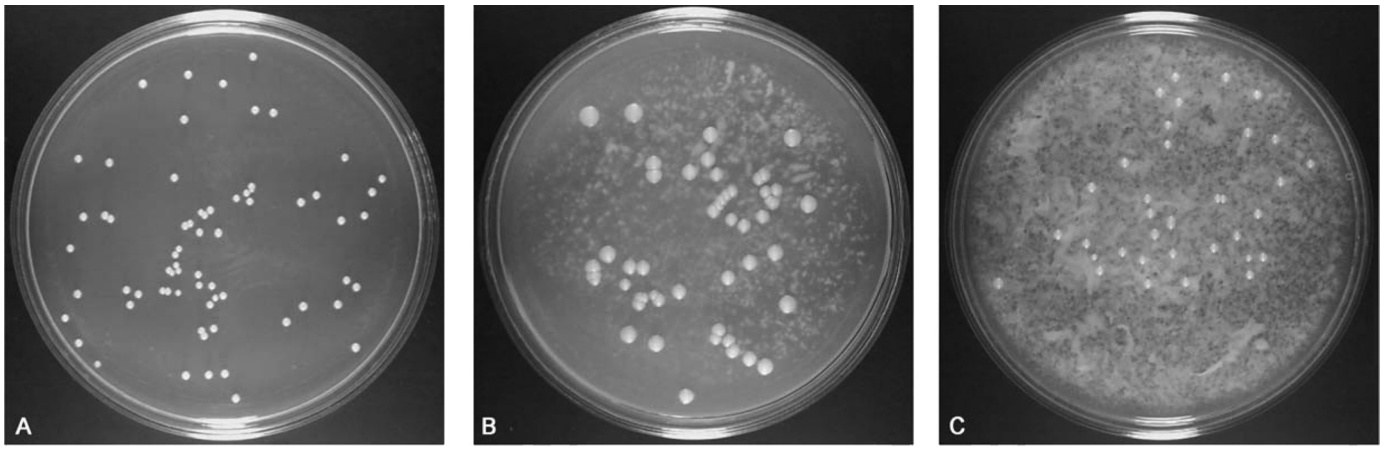

Fig. 6. Extracellular breakdown of protein is necessary for cell growth. Cells were plated on solid medium containing a 10% (vol/vol) stock solution of beef bouillon and 2% (wt/vol) sucrose as a control (*A*), 2% (wt/vol) sucrose and 2% (wt/vol) nonfat dry milk digested during 2 h at 37°C with 3 ml of kiwifruit shake supernatant (a protease source; *B*), or 2% (wt/vol) sucrose and 2% (wt/vol) nonfat dry milk (*C*). Incubation of cultures for 3 days was performed at 30°C. (Background of plates in *B* and *C* is due to the precipitation of milk protein.)

Protein Breakdown

Milk is high in protein [~34% (wt/wt)]. These proteins are a source of amino acids, but they must first be hydrolyzed in the extracellular medium.

As shown in Fig. 6, cells were plated in the standard solid medium (2% sucrose and a 10% stock solution of beef bouillon) as a control (*A*) and in a medium with sucrose and incubated milk with (*B*) or without (*C*) kiwifruit extract, as described in MATERIALS AND METHODS. Beef bouillon was not added to the medium to avoid the addition of amino acids. Kiwifruit extract is high in proteolytic enzymes that hydrolyze proteins into amino acids. The colonies were greater in diameter when milk was incubated with kiwifruit extract, since the amino acids were freed through proteolysis. As a further control, a plate with medium containing sucrose, kiwifruit extract, and milk, not previously incubated, was cultured to show that kiwifruit extract does not make colonies grow on their own (data not shown).

These experiments show that cells require an extracellular digestion of macromolecules, such as starch and protein, into glucose and amino acids, respectively, in order for them to be incorporated and used by cells.

Crossed Experiments

A variation of the experiments described in Figs. 5 and 6 consists of incubating milk with saliva or starch with protease. The diameter of colonies was similar to those of the controls without saliva or kiwifruit extract (data not shown). In other words, proteins and starch must be digested by specific enzymes. The enzymes that hydrolyze starch do not hydrolyze proteins, and vice versa. This is an important aspect of metabolism that should be noted.

The discussion with students may focus on various points. On the one hand, emphasis can be placed on the importance of milk or flour as sources of substances required by our cells. On the other hand, it is interesting to ask students why the components in these foods must be broken down before being used by cells. Finally, the function and need for enzymatic activity and specificity can be explored. As an alternative to the enzyme sources used in this work, we suggest using other enzymes, readily available at pharmacies and supermarkets. This has been done in previous work (8, 12).

Final Remarks

Recently published educational proposals have shown a clear and global concern with regard to teaching metabolism and nutrition.

For instance, to motivate students to learn about metabolic pathways, it has been suggested that analyzing different types of low-carbohydrate diets might be effective (19). Similarly, blood tests could also be given to student volunteers to record glucose and triglyceride levels before and after eating pizza and pasta (18). Other authors (7) have highlighted the importance of teaching the biochemistry of digestion along with the physiology of nutrient absorption, suggesting that the combined approach is more effective and also gives students an opportunity to critically evaluate the claims of companies marketing nutritional supplements.

Another interesting proposal to teach high school students about the digestive system involves the use of computers equipped with a commercially available data-acquisition system and a couple of sensors. This enables students to see the importance of the mechanical breakdown of food, the enzymatic activity of enzymes, the antibacterial activity of hydrochloric acid, and the importance of the villi in absorption (22). While none of these cases analyzed learning outcomes, the emphasis was on the motivation generated by the didactic proposals.

The use of microorganisms as a model for understanding aspects of human metabolism has been previously proposed. In this sense, we can mention the use of yeast to study the breakdown and fermentation of complex carbohydrates in human digestion (4) and our didactic proposal in a previous work (15), to teach the metabolism of lactose, using yogurt bacteria and yeast.

The experiments outlined here provide a useful base for understanding some aspects of nutrition of heterotrophic human cells by mean of simple macroscopic evidence of submicroscopic events. The results may motivate students to answer

questions regarding the importance of ingesting certain nutrients and why macromolecules present in foods must be broken down in their constitutive units by specific enzymes before being used by the body's cells.

Since these experiments present a biochemical and microbiological approach, the teacher must lead a discussion with students, to provide a systemic context for the results. In this way, students should be able to understand the importance that the biochemical processes shown in these experiments have in a multicellular organism. Teachers may also add their own variations to each of these experiments, with the goal of evaluating the requirements of heterotrophic cells for their growth and division. For example, beef extract can be replaced with liquefied meat or beef liver as a source of nitrogen or sucrose can be replaced with honey as a source of carbohydrates. Likewise, instead of using a multivitamin complex, each group of students can test a single vitamin. Wheat flour may be replaced by corn flour, and since salivary amylase does not hydrolyze cellulose, cellulose fiber could be incubated with saliva. In these cases, it is important to discuss with students the different roles of each substance used in these experiments. This could contribute to changing some misconceptions held by high school and college students, such as glucose being the sole metabolic fuel (10, 17).

This proposal was tested in a workshop given to 20 high school biology teachers and 4 university students of biology.[2] The participants, when questioned as to why they had chosen to attend the workshop, answered that they found attractive the possibility of working with living cells, in a simple and nonrisky manner. Once the workshop concluded, we asked participants for a written evaluation of the didactic proposal. All of them were enthusiastic and evaluated the proposal positively. Many commented that this approach facilitated the teaching of biochemical and cellular aspects of such an important subject, many of which are often difficult to address in laboratory experiments. The simple methodology of this proposal is extremely useful for working with freshmen students. Several participants emphasized the possibility of directly observing colony growth and associating such growth with nutrients familiar to any student. In fact, the participants attended the laboratory on several occasions to follow up on colony growth. In summary, the experiments were seen as promoting active learning and as highly motivating.

These considerations lead us to believe that this proposal contributes to existing ones, with respect to the meaningful learning of nutrition. This subject is important not only for biomedicine students but for students in general, as key knowledge for healthy living.

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GRANTS

This work was supported by University of Buenos Aires Grant UBACyT 2008–2010 U011.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.A. and C.A.S. conception and design of research; M.A. and C.A.S. performed experiments; M.A. and C.A.S. analyzed data; M.A. and C.A.S. interpreted results of experiments; M.A. and C.A.S. prepared figures; M.A. and C.A.S. drafted manuscript; M.A. and C.A.S. edited and revised manuscript; M.A. and C.A.S. approved final version of manuscript.

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