Laboratory Exercises

Solving an Ethical Issue Involved in Experimentation with Animals in a Brazilian Teaching Laboratory*

Received for publication, January 30, 2004, and in revised form, April 26, 2004

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Changes are occurring within Brazilian institutes of higher education; currently several universities are reviewing their course offerings and teaching approaches to determine if they meet the needs of today’s undergraduate students. When changes are made to the curriculum of experimental courses, there should be an understood guarantee that all efforts to avoid ethical and biosafety issues have been diligently considered. Ethical considerations lead us to create an alternative experimental session to be conducted that eliminated the use of rats, the conventional in vivo model employed for learning metabolism of glycogen in our university. To avoid possible biosafety issues, we prepared an alternative sample to simulate human urine, which we called guarurine. Using our new method, it is possible to verify positive results imitating a diabetic and starving people samples for detection of glucose and ketone. The alternative tool described herein is not only particularly suited to bypass the ethics of using animals for teaching, but also permits the discussion of significant aspects of pathological and physiological situations such as diabetics and starvation in a simple, safe, and interesting way.

Keywords: Education, biochemistry, guarana, undergraduate student.

The use of animals for research and teaching is an issue of great concern worldwide. In contrast to the legislative systems in United States, Britain, Germany, Scandinavia, and many other countries [1–6], Brazilian scientists and teachers still can pursue research projects and teaching class with relative freedom [7]. In Brazil, animal research ethical committees were created only in the 1990s. The Federal Law 6638, which provides for the didactic-scientific practice of animal vivisection, was approved in May 1979 but still waits for regulation. In addition, some drafts that provide regulation for the use of animals for teaching and research purposes are still being analyzed by the Brazilian Congress [8, 9].

In the Brazilian Fluminense Federal University, the study of Biochemistry in the Department of Molecular and Cell Biology includes laboratory activities, which mainly allow students to actively explore key concepts in biochemistry in greater depth and acquire skill in scientific reasoning [10–12]. Animals (rats) have been widely used as models for teaching the catabolism of glycogen (glycogenolysis) in this discipline. However, in the last 2 years, the student teaching assistants and some students of several different undergraduate courses that attend this class had considered this experimental session as inappropriate. According to them, there was an “unnecessary sacrifice” of rats to demonstrate the use of glycogen as a fuel by an organism. Thus, the refusal to attend this specific session by some of these undergraduate students, including those from the Faculty of Veterinary School, became common. Because ethics is not a matter of adhering to absolute rules, but rather of doing what will have best consequences, given the constraints under which we act, the ethics of using a specific experimentation will depend on if the goal can be reached while causing the animal less suffering, using fewer animals, or without using animals at all. Anyway, due to ethical and others concerns, we were facing the challenge of changing this teaching session.

In resolving this issue, understanding the reasons why students object to animal laboratories was an important step. In their point of view, this experimental class represented a serious religious/ethical dilemma because the life and death of laboratory animals becomes trivialized in the process of exemplifying only one topic of metabolism. Therefore, based on their opinion and using the principle of our self-regulation, rules were set up to conduct the selection of a future practical laboratory experiment. These were i) avoid the use of laboratory animals that would be
sacrificed; ii) the inclusion of other topics of metabolism such as glycolysis, citric acid cycle, fatty acid and amino acid synthesis and catabolism, and ketogenesis in the experimental discussion; iii) the experiment should have low cost and be performed after the relevant theory material is studied; and finally iv) it also should be easy and fast, due to the limited time of the practical class. Consequently, the whole laboratory experience should not last more than 2 h, according to the scheduled time available for the class. Given these limitations, a previous laboratory class was considered to be an ideal candidate for replacement of the class in question. “Urinalysis” is a nonanimal laboratory experiment basically consisting of assays to determine glucose and ketone levels in the urine from a diabetic and a healthy person. Its experimental work does not last more than 2 h, and all necessary reagents were already part of the stockroom, avoiding any further expenses. This class permits the discussion of several topics of metabolism including those previous established; and, most of all, it allows aligning classroom practices with professional practices. Hence “Urinalysis” met the main criteria that had been determined. However, despite passing these rules, “Urinalysis” raises a biosafety issue, e.g. the risk of using body fluids at the undergraduate level. According to the World Health Organization, the body fluids and substances of all persons should be considered to contain potentially infectious agents [13]. No distinction may be made between body fluids and substances from individuals with a known disease or infection and those from asymptomatic or undiagnosed individuals [13]. Consequently, infection control practices shall be present in the urinalysis class including the use of gloves and masks, frequent hand washing, proper cleaning, and good disinfectant practice. These procedures greatly raise the expense of the exercise, which could be a problem in less economically developed countries such as Brazil [14]. But an extreme example of the necessity for these protocols is the SARS virus, which can survive in urine for at least 24 h [15]. Another virus that can also be transmitted by this body fluid is the cytomegalovirus [16]. In fact, the risk factors due to using body fluids were the reason for the replacement of the “Urinalysis” session in this discipline in the first place. However, due to the several advantages of the “Urinalysis” session theme, we decided to keep it, creating a harmless substitute for urine, averting the original animal ethical and biosafety issues. Additionally, we also planned a different way of class presentation using a problem-based learning-like approach, stimulating the student to be more active in the laboratory class [17, 18]. In this article, we will present the protocol and approach used in this practice class, also including the evaluation by student teaching assistants and undergraduate students from nine different courses (“Biological Science,” “Pharmacy,” “Medicine,” “Veterinary Medicine,” “Nutrition,” “Nursing,” “Odontolgy,” “Chemistry,” and “Industrial Chemistry”).

MATERIALS AND METHODS
Preparation of Guarurine

Normal urine is a clear straw-colored liquid, which under normal conditions does not contain sugars, yeast cells, protein, ketones, bacteria, or parasitic organisms. In order to select a substance that imitates perfectly the visual aspect of urine, the famous Brazilian guarana (*Paullinia spp.*) was tested. The guarana seeds are used mainly to produce a soft drink that present similar

![Fig. 1. Schematic representation of the preparation of guarurine samples (upper panel) and comparison of results using guarurines and urines in a typical urinalysis session (lower panel). The positive (+) or negative (–) results mean the presence or absence of the molecules (glucose or ketone) tested in the samples.](image)
visual appearance of urine [19]. Hence the soft drink in its diet version was utilized to prepare samples to be tested on the urine diagnostic assays. These assays include the detection of glucose by the classic Benedict’s copper reduction reaction and of acetoacetic acid (the physiological ketone) by the reaction with sodium nitroprusside in a strongly basic medium.

In order to prepare the guarurine samples, a 150-ml cup of diet guarana was diluted twice with distilled and deionized water and three samples of 100 ml were obtained, which we named guarurines (Fig. 1). On the first sample, glucose (5% w/v) and acetone (10% v/v) were added, representing the diabetic urine (Fig. 1). To the second sample, only acetone (10% v/v) was used to reproduce the urine from a starving person with prolonged diarrhea and vomiting (Fig. 1). The last 100 ml was considered the normal urine, therefore no glucose or acetone were added (Fig. 1). Usually the urine specimens should not remain unrefrigerated for longer than 2 h, but in the case of guarurine samples they resisted several weeks out of the fridge, probably due to the presence of preservative in its formula. This aspect permits the storage of guarurine samples for longer periods than urine.

Detection of Glucose and Ketone Using Guarurine Samples

**Classical Benedict’s Copper Reaction (Glucose)—** In the glass tubes, 3 ml of Benedict’s reagent was added to 5 ml of the guarurine samples. The test tubes were immediately placed into a beaker of boiling water and left for 3 min. When heated with Benedict’s reagent, guarurine sample containing glucose (sample 1) formed an orange-brown precipitate similar to the diabetic urine (Fig. 1).

**Sodium Nitroprusside Reaction in a Strongly Basic Medium (Ketone)—** Two drops of sodium nitroprusside reagent were added in 5 ml of the guarurine samples in a glass tube. The test tube was mixed and tilted to about a 30° angle, and the samples were slowly overlaid with 15% ammonium hydroxide. A pink ring appeared at the junction of the two liquids in the guarurine samples containing acetone (samples 1 and 2) after 15 min, similar to that observed for starving and diabetic people’s urine.

Therefore, guarurine samples showed to be adequate as a harmless substitute for human urine to be used in the “Urinalysis” session, because they act similar to the respective human samples (Fig. 1).

**RESULTS AND DISCUSSION**

**Evaluation of the Practical Experimental Session by Student Teaching Assistants**

After the preparation of the protocol and arranging all necessary laboratory material including the guarurine, it was possible to evaluate this new practical class with the group of student teaching assistants from the Biochemistry discipline (n = 6). The new problem-based learning-like approach used consisted of the presence of an initial situation where three unlabeled “urine” samples from three different people (a diabetic, a starving, and a healthy person) needed to be identified (Fig. 2). The main purpose was to correctly identify this “biological” material according to the testing results that would be obtained during the experimental class. This approach would lead to the discussion about the initial expected results and those finally found.

By providing specific reagents and the three guarurine samples, the students were invited to use the laboratory reagents in order to find out about the origin of these samples. The goal of this practical class was mainly to provide to these students the opportunity to develop their own reasoning and acquire knowledge from the experimental results obtained. The support given during this experimental session at this moment consists of aiding in clarifying the objectives of the experiments, organizing the content, and in the “diagnosing” the clinical cases. Consequently, these student teaching assistants were also being prepared to coordinate future “guarurinalysis” session for undergraduate students. In the end, all six students were able to complete the whole session on time and also identify correctly the physiological “source” of the given material.

An interview at the end of the experimental session revealed that all six student teaching assistants voted...
unanimously to the inclusion of “guarurinalysis” in the discipline. Aspects such as students safety; the nonobligatory use of gloves and mask but keeping other safety procedures such as frequent hand washing, which would permit the discussion about biosafety issues; and most of all, the opportunity of simulating a “body fluid” clinical analysis in a safer way, were pointed as the main advantages in this experimental session during their interview.

**Evaluation of the Practical Experimental Session by Undergraduate Students**

This experimental protocol was also tested with 206 students from different undergraduate courses (“Biological Science,” “Pharmacy,” “Medicine,” “Veterinary Medicine,” “Nutrition,” “Odontology,” “Chemistry,” and “Industrial Chemistry”) using the help of the students teaching assistants. At the beginning, undergraduate students were divided into groups for the organization of the experiments. However, everyone was stimulated to participate of the discussion during the whole teaching session. At the end, most of students could correctly identify the samples, increasing their knowledge about the biochemistry topics discussed.

In case of the students, this practical session was evaluated by a questionnaire consisting of simple and objective questions raised about four important issues; the acceptance level of the practical session; its permanence in the discipline for the next years; its direct correlation with biochemical topics discussed on the lectures; and the clear applicability of this knowledge on their future professional career (Table I). The survey results indicated that nearly all students find this experimental session interesting because all of them attributed good (51%) or very good (46%) acceptance level for it. In accordance with this data, 99% of the students voted for the permanence of this practical session in the Biochemistry discipline for the next years (Table I). The undergraduate students noticed the direct correlation with biochemical topics discussed on the lecture part; and the clear applicability of this knowledge on their future professional career (Table I). The survey results indicated that nearly all students find this experimental session interesting because all of them attributed good (51%) or very good (46%) acceptance level for it. In accordance with this data, 99% of the students voted for the permanence of this practical session in the Biochemistry discipline for the next years (Table I).

The undergraduate students noticed the direct correlations of this experimental simulation with the biochemical theoretical topics (94%) and their future professional life (81%) (Table I). Although these are positive results, the opinion about the professional-related topic radically changes if the “Odontology” undergraduate course is analyzed individually. Surprisingly, “Odontology” students did not agree (38%) or partially agree (31%) that the knowledge offered by this practical session will be useful on their professional career, seeing no direct correlation between them (not shown). This result emphasizes the necessity of an improvement of the lectures, including professional themes in the Biochemistry discipline to the understanding of the undergraduate student [20]. In case of the “Odontology” class, clinical cases involving topics such as the problems with the healing process in teeth extraction procedures on diabetic persons could be a good example on how important is the knowledge about this disease and consequently of this experiment for the dentist.

It is important to emphasize the major improvement in the student participation during the learning process using this specific practical approach. In fact, some students asked during the class for testing their own urine (10–20%), which was not allowed. This specific students behavior indicate a direct interpretation about the applicability of those assays in quotidian. This is probably due to the fact that they were more required to do critical analysis of experiment’s result when a problem-based learning-like approach is used.

**CONCLUSION**

Various pressures are driving changes on Brazilian higher education. They are from government, students, professional bodies, and employers; from changes in teaching styles and in the discipline itself; and from ethical/animal rights considerations. As these demanded changes occur, an alteration in the knowledge, skills, and attitudes of academic professor will be required, which includes the way teaching is delivered and learning is facilitated [21, 22]. Herein we described a laboratory class experiment that can be applied in any biochemistry course avoiding ethical and biosafety issues. Through the correct application of our established alternative tool and the understanding of the technical and clinical details of this practical session, the students could acquire knowledge not only about biochemistry but also about specific problems related to handling of the samples and reagents and how clinical results are obtained and interpreted. Other advantages of this practical class are that it is fast, reasonably safe, and have low cost, requiring fewer resources to simulate clinical cases. This economical advantage is important to a country that struggles to maintain its own public academic institutions such as Brazil.
Acknowledgments—We thank the Fluminense Federal University for the Honorable Mention for practical and teaching approach development in the 2003 Coordinators Awards. We also thank Professor Cicero Carlos de Freitas, Mr. Hugo Rodolfo de Oliveira Barbosa Filho and Mrs. Dione M. Silva for their help and technical assistance.

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