Practical Report

A Biochemical Experiment to Assess Cognitive Skills and Procedural Understanding in High School Students

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Learning of concepts in biology can be made effective by providing historical perspectives, social context and inclusion of laboratory exercises in regular teaching practice. Among laboratory exercises, biochemical experiments prove to be useful tools to promote biology as a subject of logical reasoning requiring analytical skills. One such investigation of an enzyme-catalyzed redox reaction is presented here. This investigation was carried out by a selected group of high school students. At the end of the experiment, the procedural and conceptual understanding of students was probed using specially designed multiple-choice questions (MCQs). Students' responses to MCQs and experimental readings reflected their skills such as understanding about appropriate controls, hands-on skills, and procedural understanding. Analysis indicated that 57% of the students demonstrated Lower Order Cognitive (LOC) skills while only 23% of the students demonstrated Higher Order Cognitive (HOC) skills. Students' responses indicated that their procedural understanding varied between 14 - 50%, while hands-on skills were in the range of 27 - 41%. This shows the need of such laboratory practices in regular biology curriculum. Inclusion of this experiment as a guided inquiry in the regular curriculum can help students enhance their HOC skills.

Keywords: *biochemical exercise, hands-on skills, HOC skills, LOC skills, procedural understanding, succinate dehydrogenase*

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INTRODUCTION

The rapid growth in knowledge in the discipline of biology necessitates a major shift in the approach towards teaching. The focus needs to shift from reciting of facts and knowing of terminologies to understanding concepts, acquiring skills to analyse and applying information to challenging new situations (Fiona, 2014). According to Bloom's taxonomy, observation and recall of facts as well as comparing and contrasting data are considered as Lower Order Cognitive (LOC) skills, while analysing and organising data, applying knowledge in a novel situation and generalisation from facts are considered as Higher Order Cognitive (HOC) skills (Krathwohl, 2002). Further, assessments that are designed to test students' above-mentioned HOC skills and competencies will force students to adopt a 'deeper approach' to learning as against the 'superficial approach' to learning (Momsen *et al*., 2013). The biochemistry experiment reported here was designed as an effort to assess the LOC and HOC skills as well as hands-on skills of students. Since this study was carried out on high-achieving high school students, it was used in a 'test' format. However, the same exercise can be used in a 'guided' format in a regular educational set-up so as to enhance the above-mentioned cognitive skills in students.

In the current high school curriculum, apart from the classical biology subjects, such as plant and animal anatomy, there is a major emphasis on understanding concepts in some areas of modern biology, such as molecular biology and biochemistry. Thus, biomolecules and cells and enzymatic processes form the core concepts in the high school biology course. It is expected that the hands-on activities performed in the laboratory during the course would further strengthen the concepts involved. It is feasible to handle various types of cells and perform experiments using biomolecules in the wet lab. However, when it comes to metabolic processes, students mostly rely on textbooks to learn the basic reactions. Also, several concepts, such as aerobic respiration, TCA cycle, and mitochondrial electron transport, are abstract and intangible to most students. Students' content learning can be improved in these areas if they get an opportunity to experiment with any of the reactions involved in these processes. Also, such experiments and related questionnaires, if designed appropriately, can help test students' hands-on skills as well as conceptual and procedural understanding.

There have been some reports describing experiments to test and analyse these skills in students' conceptual understanding in the laboratory (Vartak *et al.*, 2013; Latourelle *et al.*, 2019) as well as in the field (Dresner *et al.*, 2014). As part of a project carried out at our Centre, our major objective is to develop innovative experimental tasks in various areas of biology so as to nurture as well as test the HOC skills in high school students.

Here we present the results of an experimental test along with the subsequent questionnaire that was designed to probe students' above-mentioned skills.

METHODOLOGY

Subjects

The subjects in this study were a group of 35 students from different high schools all across India. They were all high achievers (top 1% meritorious students in biology) in their respective classes in the routinely conducted tests. They participated in a biochemical experiment which had been developed by the authors.

Materials

Laboratory Manual for Students (Box 1, shown on p. 4 – p. 5)

Multiple–choice Questionnaire (Box 2, shown on p. 6)

All materials mentioned in the Laboratory Manual for Students (Box 1) were prepared by the instructor beforehand. All reagents used were of analytical grade.

Methods

The participating students were asked to carry out the biochemical experiment independently by following the instructions of the laboratory manual (Box 1). After performing the biochemical experiment, they were asked to answer the questionnaire (Box 2).

INTERPRETATION OF THE BIOCHEMICAL EXPERIMENT

The experiment described in Box 1 involves electron transport chain observed *in vivo* in aerobic respiration. Using mitochondrial membrane systems from liver cells, the flow of electrons is studied. However, the path of electrons is blocked in the complex IV by the addition of NaN_3 in the reaction mixture and providing an external electron acceptor, dichlorophenolindophenol (DCPIP). This results in the change in the colour of reaction mixture as a result of the reduction of DCPIP. Thus Set 5 indicates the specific enzyme reaction involving succinic acid as a substrate, succinate dehydrogenase as the enzyme, and DCPIP as the terminal electron acceptor molecule. Sets 1 – 4 serve as controls. Set 1 shows a small reduction in the optical density (OD) value in spite of the absence of the specific substrate which is succinic acid.

Box 1: Laboratory Manual for Students

Biochemical Experiment on Succinate Dehydrogenase

Introduction to the Experiment:

Among the various enzymes involved in aerobic respiration, succinate dehydrogenase is unique as it is linked to both TCA cycle and electron transport chain (White, 2004). This enzyme catalyses both the oxidation of succinate to fumarate and the reduction of FAD to $FADH_2$. The electrons then travel through the components of the electron transport chain to the terminal electron acceptor, which is oxygen in case of aerobic respiration (Figure below).

The reaction that takes place in intact mitochondria can also be studied *in vitro* using different compounds as electron acceptor molecules. To study the activity of succinate dehydrogenase *in vitro,* dichlorophenolindophenol (DCPIP) has been used as an electron acceptor in this experiment. This compound is blue when oxidized and colourless when reduced. To divert the flow of electrons from Complex III & Complex IV to DCPIP, sodium azide (NaN_3) which acts as an inhibitor of cytochrome a_3 in Complex IV (See Figure below) needs to be added.

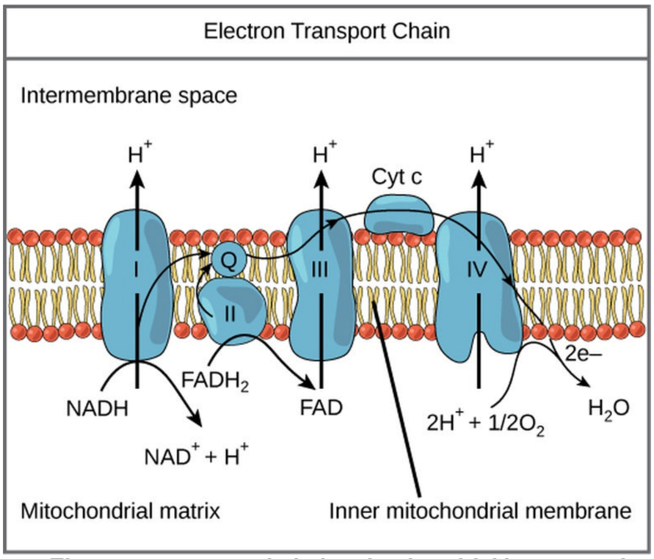

Figure: Electron transport chain in mitochondrial inner membrane.
(Source: https://commons.wikimedia.org/wiki/File:Figure_07_04_01.jpg)

Materials and Methods:

Freshly obtained chicken liver extract, Potassium phosphate buffer pH 7 (0.5 M), Succinic acid solution (0.15 M), DCPIP solution (1.5 mM), NaN_3 solution (0.2 M), Cuvettes, Micropipettes, Colorimeter, Test tube stand

Preparation of Chicken Liver Extract: Cold conditions (4°C) need to be maintained throughout the preparation of extract until use in the experiment. 2.5 g of freshly obtained clean frozen chicken liver is cut into small pieces and transferred into a mortar. 10 ml of cold phosphate buffer is added and the tissue is macerated well using a pestle to prepare a homogenous crude extract. The extract is transferred to a tube and centrifugation is carried out at 10,000 rpm for 10 minutes. The supernatant obtained is stored at 4°C till use.

Experimental Procedure:

In this experiment, the activity of succinate dehydrogenase is studied over a period of 30 minutes. Prepare the five sets of tubes for the experiment as given in Table. The entire experiment is carried out at room temperature (28°C). Instead of test tubes, 5 ml capacity cuvettes (diameter 1 cm), which can be inserted directly into the colorimeter to read the optical density (OD) values at 620 nm, are used. Thus, for each set, the reaction can be carried out in the same respective tube throughout.

Table: Experimental Protocol

Set No.	Phosphate buffer (I)	Succinic acid (II)	Sodium azide (III)	DCPIP (IV)	OD 620 nm Baseline reading*	Liver extract	OD 620 nm 0 min	5 min	10 min	15 min	30 min
1	2.6 ml	-	0.1 ml	0.1 ml		50 µl					
2	2.5 ml	0.1 ml	0.1 ml	0.1 ml		-					
3	2.6 ml	0.1 ml	0.1 ml	-		50 µl					
4	2.6 ml	0.1 ml	0.1 ml	-		-					
5	2.5 ml	0.1 ml	0.1 ml	0.1 ml		50 µl					

**Baseline reading refers to the OD values for each tube after mixing reagents I to IV as given in the table.*

Readings:

At the time mentioned in Table (0 min to 30 min), read and record the OD value at 620 nm of each cuvette.

This indicates that the extract being crude is likely to have some reducing equivalents in a small quantity which reduce DCPIP slightly. Set 2 indicates the stability of the electron donor and also takes care of any reaction that takes place between the electron donor, the substrate, and NaN_3. Set 3 takes care of any OD due to turbidity of the liver extract which can then be subtracted from the actual reading obtained in Set 5. The mixture of solutions in Set 4 is colourless and transparent and the OD is expected to remain close to zero throughout the experiment. The OD values of this set indicate that succinic acid, NaN_3 and phosphate buffer do not react with each other to give any change in OD.

RESULTS AND INTERPRETATION OF STUDENTS' PERFORMANCE

Performing the experiment in the given format allows us to test students' hands-on skills. The skills can be broadly divided into handling of glassware, pipetting of small volumes of liquids, and handling of instrument. The OD values obtained by an expert (Table 1) can be used as a reference. Baseline readings of Sets 1, 2 and 5 are good indicators of students' pipetting skills. Consistent and comparable values (compared to the values in Table 1) obtained by the students indicate accuracy in pipetting. Also, OD values obtained for each set are indicative of handling of the instrument, namely, the colorimeter. OD values less than 50% of the expected values would be the result of incorrect selection of wavelength, improper blanking, and/or errors in pipetting. If the students have obtained large negative values (- 0.05 onwards), it indicates that no proper blanking has been done by the student. Figure 1 shows that only 41% and 27% of students exhibited accurate pipetting and instrument handling skills, respectively.

In the questionnaire, Question 1 deals with analysis of five possible sources of the enzyme with focus on their locations in the cell and roles in aerobic or anaerobic respiration. It thus tests students' abilities, such as comprehension of text, comparing and contrasting the facts as well as predicting outcomes, which are considered as LOC skills. Fifty-seven percent of the students could answer it correctly (Figure 2). Question 2 deals with students' understanding of the processes that are underway in the five experimental sets followed by extrapolating the results to a new experimental set-up. Having understood the path of

Table 1: Data obtained by an expert

Set No.	OD 620 nm Baseline reading	0 min	5 min	10 min	15 min	30 min
1	0.75	0.69	0.64	0.63	0.63	0.64
2	0.74	0.72	0.71	0.70	0.71	0.70
3	0.01	0.06	0.06	0.06	0.06	0.06
4	0.01	0.01	0.01	0.01	0.01	0.01
5	0.72	0.70	0.58	0.49	0.42	0.34

electrons in normal electron transport chain and the effect of NaN_3 on it, students are expected to speculate the effect of omitting it in the experiment. This requires HOC skills such as application of a concept to a new situation and only 23% of the students exhibited this skill (Figure 2).

Box 2: Multiple-choice questionnaire

Questionnaire

Q. 1. Which of the following preparations will be a good alternative source of succinate dehydrogenase?

a. Cell membrane fraction of any aerobically respiring tissue.
b. Nuclear fraction of cardiac tissue.
c. Microsomal fraction of a tissue from secretory gland.
d. Crude cell lysate of bacteria isolated from a deep wound.
e. Crude extract of macerated muscle tissue.

Q.2. An experimenter added the 6th set with the following composition of solutions:

Set No.	Phosphate buffer	Succinic acid	Sodium azide	DCPIP	Liver extract
6	2.5 ml	0.1 ml	-	0.1 ml	50 µl

What result can be expected from this set?

a. OD lower than Set 1 but higher than Set 5
b. OD lower than Set 2 but higher than Set 1
c. OD lower than Set 5 but higher than Set 3
d. OD very close to zero reading
e. OD lower than Set 1 but higher than Set 3

Q. 3. Which set indicates the non-specific reduction of the electron acceptor?

a. Set 1
b. Set 2
c. Set 3
d. Set 4
e. Set 5

Q. 4. How can one obtain the value of non-specific reduction of the electron acceptor?

a. OD of Set 2 – OD of Set 1
b. OD of Set 5 – OD of Set 3
c. OD of Set 1 – OD of Set 5
d. OD of Set 2 – OD of Set 5
e. OD of Set 5 – OD of Set 4

Q. 5. Which set is indicative of the non-specific OD of the liver extract?

a. Set 1
b. Set 2
c. Set 3
d. Set 4
e. Set 5

Q. 6. Which of the sets indicates that the oxidized form of the electron acceptor is stable under the experimental conditions?

a. Set 1
b. Set 2
c. Set 3
d. Set 4
e. Set 5

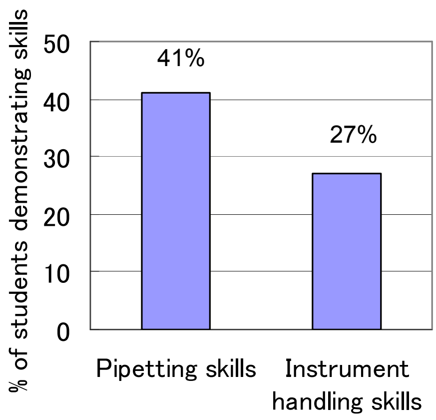

Figure 1: Students' accuracy in hands-on skills

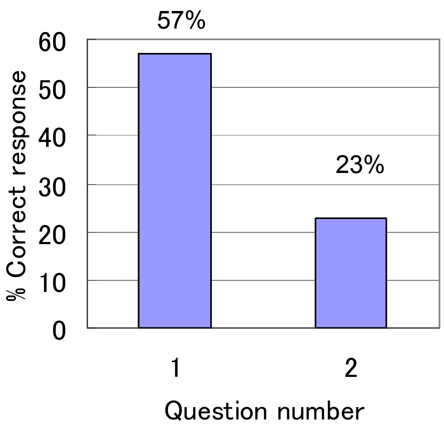

Figure 2: Students' responses to Questions 1 and 2 assessing LOC and HOC skills respectively

Procedural understanding is an important aspect of science process skills (Gott and Duggan, 1995; Roberts, 2001) and involves understanding about the rationale of the protocol being followed. In the present investigation, a reaction involving succinate dehydrogenase is underway in Set 5 while the other four sets represent different types of controls that are essential. Questions 3 – 6 probe students' understanding about the importance of appropriate controls in the experiment. This is a part of procedural understanding and as is evident from Figure 3, maximally up to 50% students exhibited this skill.

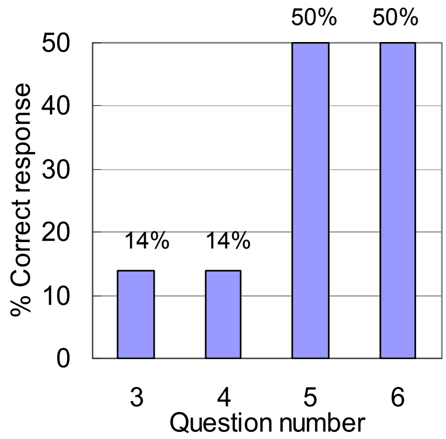

Figure 3: Students' responses to Questions 3 – 6 assessing procedural understanding

CONCLUDING REMARKS

It has long been realised that assessment reforms are greatly needed in higher education. Well–designed theory tests can help probe students' conceptual understanding, ability to interpret data and graphs as well as other cognitive skills. However, a well-designed laboratory exercise can additionally test students' hands-on skills and procedural understanding. Performing experiments in the laboratory takes students very close to a researcher's mind and allows them to actively participate in the process of collecting and analysing data. Also applying the acquired knowledge to novel situations can further strengthen students' HOC skills.

The experiment presented here is of a short duration and also does not require any sophisticated instrument or expensive chemical. The analysis shows that although the participating students were high achievers in the biology course, they were not very successful in getting practical (experimental) skills. This shows that there is a need to strengthen hands-on skills and procedural understanding in teaching regular biology courses at the high school level. Although this exercise was used as a testing module, the same module can be used as a learning tool for students if conducted as a guided inquiry session by a facilitator in the laboratory.

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