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Assessing Undergraduate Expressions of Biochemical Mechanisms

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Assessing Undergraduate Expressions of Biochemical Mechanisms

by

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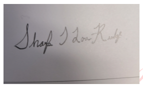

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Abstract:

Approaches toward teaching metabolism often result in students perceiving biochemical pathways as isolated from one another. This leads to students' compartmentalization of the knowledge acquired within biochemistry courses rather than the extension of that knowledge beyond specified chapters. As a result, students face challenges demonstrating a thorough understanding of the subject matter. Thus, we sought to investigate this phenomenon by exploring how well students delineated mechanistic relationships between biochemical substances and processes. Students were presented with worksheets seeking details regarding the substances present within a collection of metabolic pathways and were asked to model those pathways within a "map." The students were then presented with short answer questions relating to those metabolic pathways. Responses to these questions were analyzed to characterize their understanding of the metabolic concepts they learned.

To analyze the student data collected, we utilized van Mil et al.'s model for molecular mechanisms. Within this model, biochemical substances (e.g., molecules, enzymes, organs) are categorized as "entities," while actions between them are categorized as "activities." To characterize how well students articulated mechanistic relationships within the metabolic pathways they described, all entities and activities identified within student responses were categorized into three levels of specificity. All molecules and enzymes were grouped into the highest, or "L", level of entity specificity, all pathways were grouped into the intermediate, or "L-1" level of entity specificity, and all organs were categorized within the lowest, or "L-2" level of entity specificity. Activities were categorized in the same way, with their level depending on whether the actions between entities were described as occurring at a molecular, pathway, or

bodily level. A match between specificity levels, such as an “L” entity used with an “L” activity, communicated an ability to describe mechanistic relationships with appropriate specificity.

In analyzing our data, we found a mismatch between the entities and activities within student responses. For example, students, on average, used L level entities 13 times more than L level activities. These findings suggest that students may have trouble describing molecular-level mechanisms with appropriate specificity. This could indicate a lack of understanding of those mechanisms.

Introduction

Within this research, our aim was to observe how biochemistry students demonstrate their understanding of biochemical concepts, specifically in expressing mechanisms of mammalian metabolic pathways. Many researchers in biochemistry education have targeted issues involving student understanding of academic material, and many have attempted to enhance student understanding through the development of novel instructional strategies. Specifically, many sources have identified that students exhibit difficulty demonstrating an adequate understanding of mechanistic relationships within metabolic pathways. Long, et al. observed that students struggled to demonstrate understanding of chemical mechanisms within a pathway (2021). Fardilha, et al. observed that students faced difficulties integrating different pathways, or conceptualizing mechanistic relationships between pathways (2009). Both sources hypothesized different origins of this lack of conceptual understanding: mechanistic relationships on a chemical level and mechanistic relationships between pathways, respectively. Based on these hypotheses of where the difficulties originate, they each developed teaching and activity-based strategies to try and improve student performance within academic courses. Following Long, et al.'s observation that students face difficulties connecting mechanistic relationships between molecules within pathways, they hypothesized that these difficulties originate from a lack of understanding of how substrates and enzymes interact within metabolic pathways to produce products, as well as how these processes are regulated (2021). To address this, Long, et al. developed a 3-dimensional animation that visually represented mechanistic relationships between molecules that students struggled to mentally connect (2021). Long's study, then, established that difficulties in understanding how molecular entities relate to one another within a pathway, as well as how metabolic pathways relate to those molecular interactions, presents the major obstacle that biochemistry students face when learning metabolism in biochemistry. Fardilha, et al. attempted to improve students' understanding of fatty acid metabolism through the development and utilization of a learning exercise that established real-life context to fatty-acid metabolism prior to students' pursuit of information about the biochemical specificities of the metabolic pathway. The development of the instructional method was based on the idea that if given a context for which a metabolic pathway fits, students will struggle less to synthesize information about the pathway. This study, therefore, establishes both the lack of synthesis between functions of the pathway and between the molecular pathway and effects at a bodily level to be the root cause of the conceptual difficulties that students face in learning metabolism. Each of these studies established that the lack of connection between different biochemical levels, such as the molecular, pathway, or bodily levels, serves as the major obstacles that students face while trying to learn the mechanisms of metabolic pathways. Each of these studies also developed an instructional method that improved student performance. After utilization of the 3-D animation created by Long et al., students were better able to connect mechanistic relationships between molecules in metabolic pathways (2021). After utilization of the learning exercise developed by Fardilha et al., students demonstrated improved ability to connect mechanistic relationships between metabolic pathways (2009). The improved student success rate after the employment of each of these instructional methods not only implies that the hypotheses regarding the difficulties students face in understanding material may be evidentially supported, but also that if a more fundamental/comprehensive root-cause of the conceptual difficulty that students face when learning metabolism was discovered, more, targeted, educational strategies could be developed that further enhance student understanding of the material.

Other studies in molecular genetics, chemistry, and molecular and cellular biology, as opposed to biochemistry, uncovered a lack of synthesized knowledge across different ontological levels of reasoning or across different levels of categorical organization (information or physical levels). For instance, within molecular genetics, Duncan et al. explored student difficulties by analyzing how students demonstrated where different biophysical entities fit in within a larger ontological scheme of molecular genetics (2007). They discovered that students struggle to provide mechanistic explanations that connect entities to the process at different levels and to connect how genetic information relates to expression at a physical level. Talanquer, et al. uncovered that within chemistry, students struggle to synthesize how a chemical reaction relates to functions at other levels because of the simultaneous consideration of multiple factors (Talanquer, et al., 2016). Within cell biology also, it was found that when performance was observed, students failed to assign appropriate context to certain mechanisms, unable to synthesize the mechanisms with a physiological effect (Southard et al., 2017). Within all of these disciplines, then, difficulty integrating and synthesizing knowledge across levels of reasoning prevailed as an obstacle that students faced when attempting to understand and critically connect concepts within the material. This concept that ontological connections between and within different levels of reasoning generally presents itself as an obstacle within many related academic disciplines, in conjunction with that of students' failing to connect certain concepts within metabolism, as established by Long et. al. and Fardilha et. al., suggests ontological reasoning between and within all ontological levels of metabolism, not just between molecules/between molecules and pathways (Long et al.) or between pathways (Fardilha et. al.), could be where students fundamentally struggle when learning metabolism in biochemistry. Based on this hypothesis, then, which was also buoyed by evidence that educational strategies targeting connections between a given pair of ontological levels were deemed effective in improving student performance (Fardilha, et al., 2010; Long, et al., 2021), our study sought to analyze students' demonstration of mechanistic understanding between and within ontological levels of reasoning to determine how well students organize and synthesize mechanistic information within and between levels of reasoning in metabolic pathways. We sought to investigate the way that students explained mechanistic relationships within the three main levels of ontological reasoning in metabolism to determine if deficiency in mechanistic reasoning at any level may potentially contribute to the difficulty students face in synthesizing information between ontological levels or in understanding mechanistic relationships within metabolism as a whole. We intended that the knowledge gleaned would ultimately later lead to more targeted and effective instructional strategies. Our strategic framework for analyzing student data was developed through adapting concepts from van Mil's theoretical framework for modeling molecular mechanisms (van Mil, et al., 2011). Van Mil proposes that all biochemical entities and activities (e.g., biochemical substances and actions) can be distinctly categorized. Additionally, he proposes that entities and activities can be further categorized into levels of specificity, utilizing the terminology "L", "L-1", "L-2"... etc. to describe the level of specificity that entities/activities reside within, with "L" being the highest level of specificity (van Mil, et al., 2011). He goes further to address how this framework can be used to model and explain biochemical processes (van Mil, et al., 2011). Within our adaptation, however, we use van Mil's approach to label information within student responses to identify the ontological levels at which students reference, explain, and connect concepts within metabolic mechanisms. Using this strategic framework, we analyzed student data with the purpose of observing the specificity with which students describe metabolic mechanisms to both characterize the ontological level the entity/activity that the student used occupied and to identify potential areas at which the development of novel educational strategies could definitively target areas of lacking comprehension, specifically as it applies to how students connect mechanistic information across the molecular, pathway, and bodily levels.

Methods

Context

Undergraduate biochemistry students at both the University of Alabama in Huntsville and the University of North Alabama learned information organized within modules that taught mammalian metabolism, which is the material that the examination question within this study is based upon. Lehninger's Principles of Biochemistry 7th Edition was supplementarily supplied as a source of information to students, beyond course material. After receiving lectures on the material, students worked together to complete a worksheet modeled after Yung, et al.'s research study, regarding information that students had been taught within the learning modules. The students worked on the worksheet for multiple days before they then independently responded to the following exam question: "The ketogenic-diet ("keto-diet") is a high-fat, very low-carbohydrate diet that shares many similarities with the Atkins low-carbohydrate diet. Use your cellular metabolic map and worksheets to explain in complete sentences the metabolic impacts the ketogenic-diet would have on metabolism. Be sure to answer the question by referencing the entities (by bolding) and activities (by underlining) you provided in Part II of Worksheets A and B. Which pathways are upregulated? Which pathways are downregulated? Why?".

Analysis

Student responses to exam questions were analyzed using the program Dedoose. To assess how specifically students described biochemical relationships, we used the program to sort descriptions of biochemical mechanisms within exam questions. Our method of categorization heavily resembled Van mil et al.'s proposed framework (2011) for modifying educational strategies relating to the mechanistic explanations of biological functions. Key words and phrases relating to biochemical nouns, or entities, were categorized as "Entities". Key words or phrases relating to the physical or actionable relationship between "Entities" were categorized as "Activities". Using the Dedoose coding function, words and phrases deemed as "Entities" or "Activities" we individually tagged and tallied within each exam response.

To gain insight into the specificity with which students had described the biochemical mechanisms, we then stratified the previously coded "Entities" and "Activities" into three levels of specificity, adapting Van mil's heuristic approach to hierarchically representing biological mechanisms. Information about each level of specificity is summarized in *Table 1.* We then categorized each of the "Entities" and "Activities" as being an L Level, L-1 Level, or an L-2 Level, which corresponded to the specificity the "Entity" or "Activity" possessed, as related to molecular mechanisms within metabolic pathways. The L Level represented "Entities" or "Activities" at the molecular level, relating to molecules and molecular functions. The L-1 Level represented "Entities" or "Activities" at the pathway level, relating to metabolic pathways. The L-2 Level represented "Entities" or "Activities" at the bodily level, relating to the general function of metabolism for the body. The L-Level communicated highest specificity while the L-2 level communicated lowest specificity. Molecules, primarily substrates and products of the pathways, were categorized as L Level "Entities". Pathways were categorized as L-1 Level

"Entities". Organs and general mentions of "the body" were categorized as L-2 Level "Entities". *Table 2* provides examples of common "Activities" found within student responses at each level of specificity.

We used the Dedoose coding function to tag and tally "Entities" and "Activities" as belonging to the L Level, the L-1 Level, or the L-2 Level. From this tally, we then found a frequency count of "Entities" and "Activities" at each level of specificity. To gain insight into how specifically students were describing biochemical mechanisms within the metabolic pathways, we compared the average frequency of "Entities" at each level of specificity to the average frequency of "Activities" at each level of specificity. A high correlation between "Entities" and "Activities" at the same specificity level would represent a satisfactory description of the given mechanism at that level of specificity, and a high correlation between "Entities" and "Activities" at the L Level of specificity would represent a satisfactory description of the mechanism on a molecular level. A low correlation between "Entities" and "Activities" at a given level of specificity would represent a less satisfactory description of the mechanism at that level, and a less satisfactory description of the mechanism on a molecular level at the L Level of specificity. *Table 3* is representative of the two scenarios that a high and low correlation between "Entity" and "Activity" specificity levels represents as illustrated within real student responses.

From the frequency count of "Entities" and "Activities" at each level of specificity, we generated percentages of each as a function of total "Entities" or total "Activities" within student exams (see *Equation 1*). We calculated the average ratios of "Entities" to "Activities" at each level of specificity within each exam (see *Equation 2*), and these results were then summarized, graphically represented, and analyzed.

Results

We found that for all "Entities" identified within the student exams, 53% were categorized as L Level (the molecular level), 31% were categorized as L-1 Level (the pathway level), and 16% were categorized as L-2 Level (the bodily level) (*Figure 1a*). We found that for all "Activities" identified within the student exams, 8% were categorized as L Level, 49% were categorized as L-1 Level, and 43% were categorized as L-2 Level (*Figure 1b*).

We found the average ratio of "Entity" to "Activity" at the L level of specificity per exam to be 10.5/0.8; We found the average ratio of "Entity" to "Activity" at the L-1 level of specificity per exam to be 6.1/5.3; We found the average ratio of "Entity" to "Activity" at the L-2 level of specificity per exam to be 3.1/4.6 (*Figure 2*).

We determined from the data that the majority of "Entities" identified within student responses were found to be L Level, while a minority of "Activities" identified within student responses were found to be L level. Additionally, we observed a weak match between L Level "Entities" and L Level "Activities". We observed a moderate match between L-1 Level "Entities"/"Activities" and between L-2 Level "Entities"/"Activities".

Discussion

The fact that the majority of the entities identified within student responses are at the molecular level suggests that students did not face difficulty identifying the molecular entities present within a given pathway. However, since a minority of activities are at the molecular level, it does suggest that students face difficulty using appropriate activities to express mechanistic relationships between L-level entities. A disproportion of students utilized language similar to that shown in the first student example within *Table 3* within their mechanistic explanations of relationships at the molecular level, or L-Level. Only a minority of students, as represented by the ratio exhibited in *Figure 2*, utilized matching language to describe mechanistic relationships at the L level, as represented by the second student example shown in *Table 3*. This identification of a mismatch between the chemical, pathway, and bodily levels is in line with some of the observations made by Long, et al, in that students struggled to understand mechanistic relationships between molecules. Similar to Long et. al.'s observations, also, students within this study were observed to struggle synthesizing detailed information at the molecular level to information at the pathway level, as students within this study used many pathway-level "Activities" to describe mechanistic relationships occurring at the molecular level. This, then, establishes that students do not face as much trouble connecting information between all ontological levels as they do specifically delineating mechanistic relationships at a molecular level, and connecting those detailed relationships to processes at other ontological levels. Therefore, regarding our initial hypotheses, we found that students non-specifically described mechanistic relationships between molecules, which suggests that reasoning at the molecular level, or L-level, may be the main obstacle that students face when learning and understanding metabolism. Since a moderate match, however, was found between L-1, or pathway level, entities and L-1 level activities, as well as between L-2, or bodily level, entities and L-2 level activities, it suggests that students struggle less to mechanistically explain pathway-level and body-level relationships with appropriate specificity, which further supports that students seem to only struggle describing metabolic mechanisms on a chemical level. This information deviates from the hypothesis formulated by Fardilha et al., in that the issue is not observed within relationships between pathways and between pathways and the body. The results of this study also differ slightly from what was discovered within research conducted by Duncan, Talanquer, and Southard, et al. Rather than observing a lack of mechanistic connection between entities and activities across ontological levels, as found within ontological levels of chemistry, molecular biology, and genetics, mechanistic explanations at the molecular level were found to be lacking in comparison to those at lower levels of specificity, which again implies that students struggled most connecting detailed mechanisms at the molecular level with mechanisms at other ontological levels. Once again, this observation is in line with our hypothesis that mechanistic reasoning within ontological levels, specifically at the molecular level, may pose itself as an obstacle that students face when understanding metabolic metabolism. Further investigation would be necessary to determine with certainty whether students are able to demonstrate nuanced knowledge of the mechanistic connections between all levels of mechanistic organization. This study established generally that in the explanation of mechanisms across levels, students are observed only to struggle connecting detailed mechanistic information at the chemical level to processes at other ontological levels of reasoning, due to the observed "mismatch" of L, L-1, and L-2 "Entities" and "Activities". One explanation for the lack of specificity students used within their explanations of mechanisms on a molecular level could be the fact that they were not specifically demanded to do so, however, which introduces another line of potential investigation to rule that out as the case. For instance, a similar study could be conducted, but this time with an exam question that specifically required that students explain metabolic pathways at the molecular level to observe whether students' demonstrations of mechanistic relationships at the L level would improve.

Given the information derived from this study, directions of future investigation could be targeted towards the improvement of instructional methods. As students were observed to have the most difficulty explaining mechanistic relationships between molecules, future research could aim towards developing instructional methods that place emphasis on relevant molecular relationships, as well as how those specific relationships relate to functions on a pathway level. This would serve the purpose of allowing students to better conceptualize how the "chemistry" connects to the "biology", which would allow for a more integrated knowledge base of concepts within metabolism. Rather than focusing upon how substrates and products drive pathway functions, for instance, entire molecular mechanisms could be elucidated and practiced to give a more comprehensive picture of how metabolic mechanisms occur within and across all ontological levels.

Conclusion

Within this study, responses to an exam question regarding mechanisms within mammalian metabolic pathways, were collected from undergraduate biochemistry students, and student expressions of mechanisms within and between different ontological levels of mammalian metabolism were observed within the responses. "Entities" and "Activities" were coded within student exams and categorized into three different levels of organization: the L-level, or chemical level, the L-1 level, or pathway level, and the L-2 level, or bodily level. Students' usage of "Entities" and "Activities" at each of these levels were tallied and compared, and it was determined that students, overall, used many L-level, or chemical-level "Entities" compared to L-level, or chemical level, "Activities". Additionally, it was found that "Entity"/ "Activity" usage at the L-1, pathway level, and L-2, bodily level, exhibited more of a "match" than that at the L level, chemical-level. Therefore, the conclusion was drawn that students had more difficulty explaining mechanistic relationships at the L level than at the L-1 level, L-2 level, or across levels, as many L-level Entities were used with L-1 and L-2 activities, as indicated by the lack of L-level Activities.

Future efforts could aim to develop instructional strategies that could improve student performance in the way of mechanistically explaining molecular relationships within metabolic pathways. Additionally, further research could focus upon determining with more certainty what exactly, causes students to struggle to mechanistically explain molecular relationships within pathways.

Figures

$$\frac{x\text{−}Level\ Entities}{Total\ Entities} \textbf{ or } \frac{x\text{−}Level\ Activities}{Total\ Activities}$$

Equation 1: *Equation used to create percentages of entities/activities at each level of specificity.*

$$\frac{\Sigma \frac{Entity\ at\ x - Level * Per\ Exam *}{Activity\ at\ x - Level * Per\ Exam *}}{n} = Average\ Ratio\ of\ \frac{E}{A}\ per\ exam$$

$$n = Number\ of\ exams\ analyzed$$

Equation 2: *Equation used to calculate ratio of entities to activities at each specificity level.*

Table 1: *Summary of each specificity level used to categorize each of the Entities and Activities identified within student exams.*

L-2	Lowest Specificity; “Body-Level” Entities and Activities
L-1	Intermediate Specificity; “Pathway-Level” Entities and Activities
L	Highest Specificity; “Molecular-Level” Entities and Activities

Table 2: *Examples of "Activities" found at each level of specificity. Representative of the way that "Activities" were categorized on the L, L-1, L-2 scale.*

L-2 Activity	L-1 Activity	L Activity
Produce	Upregulate	Phosphorylate
Break Down	Downregulate	Hydrolyze
Use	Increase	Decarboxylate
Utilize	Decrease	Oxidize
Make	Divert	Reduce
Burn	Overproduction	Convert
Cause	Overconsumption	Secrete
Allow	Inhibit	Transport

Table 3: *Example of student responses with and without "Entity" and "Activity" specificity level correlation.*

Student Example	**Level Correlation**
"**Ketones** are produced from **fatty acids**."	Entity/Activity **Mismatch**
"**Acetyl-CoA** that is formed during fatty acid oxidation would normally be oxidized to **CO2** and **H2O**…"	Entity/Activity **Match**

Key
Color = L Level Color = L-2 Level **Bold** = Entity Underline = Activity

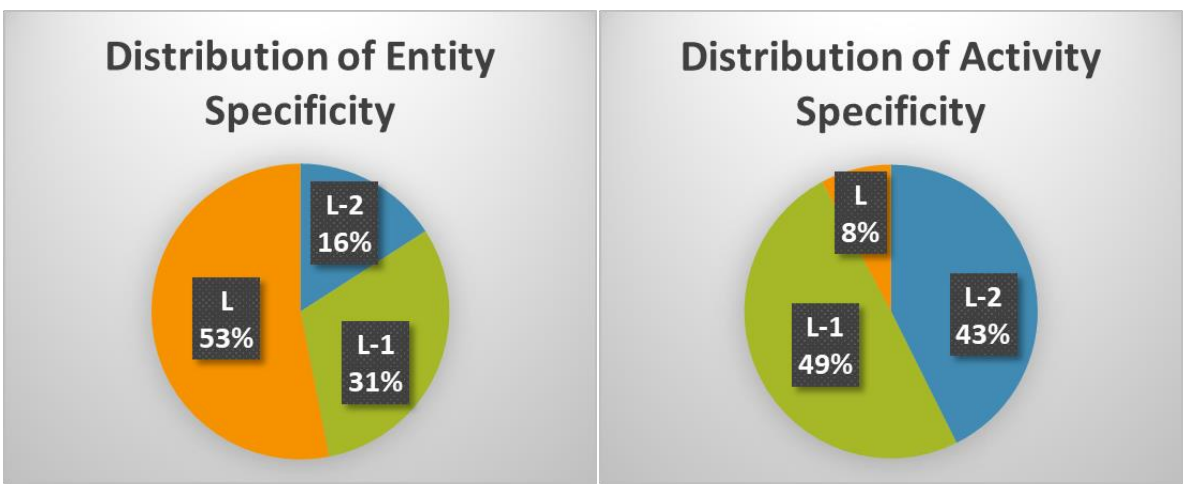

Figure 1a and 1b (respectively): *Graphical representations of "Entity" and "Activity" distributions within student exam responses. Percentages taken as percentage of all "Entity" or "Activity" found at* *each of the L, L-1, and L-2 Levels per total "Entities"/"Activities" identified within all student responses.*

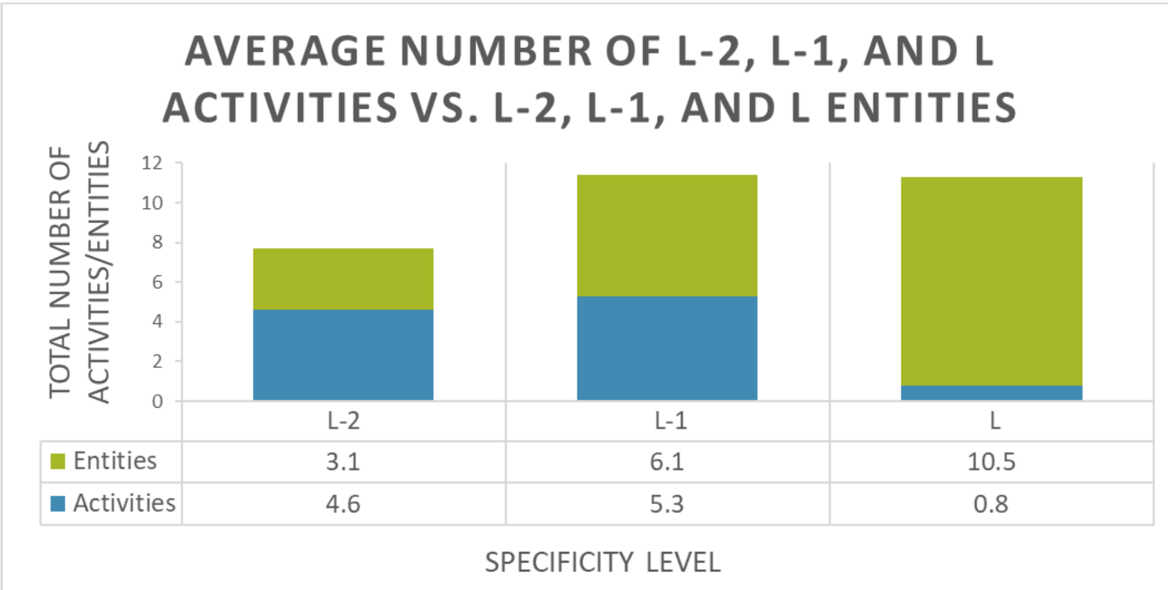

Figure 2: *Graphical and numerical representation of the average ratio of "Entities" to "Activities" at the L, L-1, and L-2 Levels. Ratios were found for each exam and averaged to yield the final average ratio depicted.*

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