Student Difficulties with the Interpretation of a Textbook Diagram of Immunoglobulin G (IgG)*

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Konrad J. Schönborn‡, Trevor R. Anderson‡§, and Diane J. Grayson¶

From the ‡School of Molecular and Cellular BioSciences, University of Natal (Pietermaritzburg), P/Bag X01, Scottsville 3209 and ¶Faculty of Science, University of South Africa, P. O. Box 392, Unisa 0003, South Africa

Diagrams are considered to be invaluable teaching and learning tools in biochemistry, because they help learners build mental models of phenomena, which allows for comprehension and integration of scientific concepts. Sometimes, however, students experience difficulties with the interpretation of diagrams, which may have a negative effect on their learning of science. This paper reports on three categories of difficulties encountered by students with the interpretation of a stylized textbook diagram of the structure of immunoglobulin G (IgG). The difficulties were identified and classified using the four-level framework of Grayson et al. [1]. Possible factors affecting the ability of students to interpret the diagram, and various teaching and learning strategies that might remediate the difficulties are also discussed.

Keywords: Student’s conceptual and reasoning difficulties, textbook diagrams, teaching and learning.

Over the past three decades, a major focus of science education research has been the identification of the reasoning and conceptual difficulties of students. Furthermore, it has been shown that if such difficulties are not addressed they can hinder students’ learning and understanding of science [2]. A large number of student difficulties have been reported in physics (e.g. see Ref. 3), chemistry (e.g. see Ref. 4), and biology (e.g. see Ref. 5). In biochemistry, however, only a few such difficulties have been identified by formal research. Fisher [6], for example, has published research on student difficulties with protein synthesis, whereas Anderson and Grayson [7] and Anderson et al. [8] have identified a range of conceptual and reasoning difficulties in the area of metabolism. Recently, a methodological framework has been developed for the identification and classification of such difficulties [1].

Research in various disciplines has shown that diagrams can be extremely useful for clarifying and integrating concepts, for the mental representation of text, and for the construction of useful mental models of abstract phenomena such as chemical structures and biochemical reactions and processes [9, 10]. What has not, however, always been acknowledged is that the interpretation of diagrams is a highly cognitively demanding task [11] that can lead to numerous misconceptions and incorrect ways of reasoning that are very difficult to correct through conventional teaching methods [12, 13]. Although extensive literature exists on the general use of, and difficulties with, diagrams in other scientific fields (e.g. see Refs. 14–17), very few research reports have been published on the effectiveness of diagrams in the field of biochemistry. Núñez de Castro and Alonso [18] have shown that textbook diagrams of enzyme-catalyzed reactions are often too simplified and exclude essential chemical steps, whereas Menger et al. [19] have reported that the presentation of micelle structure in texts can be misleading, especially when they are presented as “spokes of a wheel.” Crossley et al. [20] have presented preliminary results that suggest that some students misinterpret diagrams of the electron transport chain of mitochondria.

The results presented in this paper are part of a much broader study aimed at identifying and classifying students’ conceptual and reasoning difficulties with the interpretation of various types of diagrams representing the structure of immunoglobulin G (IgG)* and its interaction with antigens. Types of diagrams being investigated range from realistic types (e.g. an electron micrograph of antibody-antigen complexes) to more abstract types (e.g. an ELISA graph). In this paper we present evidence for difficulties shown by students when interpreting a stylized-type diagram of IgG (see Fig. 1) and suggest possible sources of each difficulty and potential ways of remediating them during teaching.

STUDY DESIGN AND METHOD

The study was done in 2000 with 130 second-year biochemistry students who had studied a module on immunology, as well as 21 third-year students who had studied the same course the previous year. The textbook diagram

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‡ To whom correspondence should be addressed. E-mail: anderson@nu.ac.za.

§ The abbreviations used are: IgG, immunoglobulin G; V, variable; C, constant; I, interviewer; S, student.
chains, connected by interchain disulfide bonds. These chemical heavy (H) and light (L) polypeptide chains, connected by interchain disulfide bonds, represent the two identical light (k) and constant (C) domains shown in light red and gray, respectively. The solid black lines are meant to represent the two identical heavy (γ) and two identical light (κ) polypeptide chains, connected by interchain disulfide bonds. These lines also depict the characteristic “Y” shape of the IgG molecule in a k₂γ₂ structural designation. The bivalency of the IgG molecule is represented by two antigen molecules (shown in dark red) attached to the variable regions of the antigen-binding domains of the antibody.

Student understanding of the diagram was investigated at the end of the module by means of written tests and interviews questions. Both the diagram and its caption were supplied to students during the questioning processes. Whereas the written questions were given to both groups of students, only ten volunteers participated in interviews, whereas those that are known to researchers but have not been extensively explored are classified at Level 3 or partially established. Level 2 difficulties are those that are suspected on the basis of teaching or learning experience, whereas difficulties that emerge unexpectedly from analysis of the data are classified at Level 1. In each case the incidence of the difficulty was calculated and recorded.

RESULTS AND DISCUSSION

The results revealed the existence of three general categories and several subcategories of difficulties with the diagram. This paper will focus only on the three general categories, namely process-type difficulties, structural-type difficulties, and DNA-related difficulties.

Process-type Difficulties—Students demonstrating the process-type difficulty thought that the IgG diagram represented various complex processes, rather than a simple non-covalent binding interaction between antibody and antigen molecules. For instance, some students interpreted the diagram as showing an antigen either in the process of entering the antibody structure or attacking the antibody, analogous to the way a foreign agent might “attack” or “invade” a host, i.e. they may have incorrectly linked the everyday meaning of the body being prone to an attack to their interpretation of the single biomolecular event of antibody-antigen interaction. This is illustrated by the following examples of student quotations.

“Antigen entering the κ and γ. Shows the pathway on which the antigen goes through. The V region first, then the C region.” (Response to probe a).

Interviewer (I): Now . . . the next step . . . then you told me a bit . . . now tell me related to this (points to antigen).

Student (S): Related to this . . . mmmm . . . I think it (antigen) goes straight and breaks those two strands (S-S bonds).

“The diagram is trying to represent regions . . . regions where an antigen may attack.” (Response to probe a).

Other students showing the process-type difficulty thought that the antibody itself was capable of performing
the major immune function of destroying the antigen, either directly by some chemical process or by surrounding and engulfing the antigen as in the case of phagocytosis i.e. they were unable to distinguish between the concept of antibody-antigen binding and other secondary immune response processes. This is shown by the following three student quotations.

“After binding to the antibody, antigen will be destroyed due to a chemical rxn (reaction) that may take place between the binding sites.” (Response to probe g).

“Region(s) V and C show the different parts of the antibody which are meant to destroy the antigen. The composition of chemicals released in region V are different to the one(s) in region C. (Response to probe a).

I: Ok, once it is joined (Ag) what does it do? 
S: Umm . . . (pause) . . . then I would think that the antibody surrounds it (Ag) and kills it.

A student who thought that the diagram represented an antibody undergoing cell division showed another process-type difficulty. The following quote supports this interpretation.

“Cell (C), cell division takes place, two cells (V) are formed. Cell C old mature structure attaches 2 cells with black lines or bonds. Young immature cells (V) are attacked by Ag.” (Response to probe a).

Finally, related to the difficulty above, some students in interviews appeared to interpret the heavy and light polypeptide chains as being able to grow from an origin within the structure of the antibody. The following two quotes demonstrate this interpretation.

S: These strings (polypeptide chains) . . . they . . . I would say they originally came from this big black molecule (C-region) . . .
I: Ja (yes) . . .
S: They . . . and it . . . they come apart (indicates at hinge region), they bind into the antigens and they start . . . they know where to bind because they start at the C-region . . .
I: All right, so, if the antibody was by itself here, if the antigens weren’t here on this picture, how would it look?
S: These black lines (heavy/light chains) wouldn’t be out here (points), it (they) will (would) be compacted inside so there’s just one sphere (lower C-region) . . .
I: . . . Yes . . .
S: . . . And then umm, it will come into contact with the antigens, and then sense the contact, and then these lines will protrude in and change the . . . (pause).

The student quoted above also thought that the antigens were represented in the diagram by the entire top two spheres, probably because the student interpreted the arrow-shaped feature, used for the antigen, simply as a diagram label.

The difficulties described above suggest that, in many cases, the students are focusing on surface-level features of the diagram when extracting meaning from it, a type of reasoning that has been shown to be common among novices. In a seminal study conducted by Chi et al. [26], novices (beginning physics students) and experts (experienced physicists) were asked to sort a number of mechanics problems into categories. The novices generally grouped problems together that involved similar surface features, such as inclined planes, whereas the experts grouped problems according to the physics principles needed to solve them, such as conservation of energy. In other words, novices tended to focus on the surface structure of the problems, whereas experts focused on the deep structure of the problems. In this paper, we shall use the term “surface-level reasoning” to mean the cognitive process employed by students who focus on the surface-level features of the diagram and “deep-level reasoning” to describe a process in which students focus on the deeper structure of the diagram. Deep-level reasoning may be used, for example, to understand a structure or cellular process or to solve a problem.

The interpretation given above of difficulties shown by students may originate from the way the features of the diagram are artistically presented. For example, in the case of the cell division-type difficulty, students may have inappropriately transferred what they had previously learned about biology diagrams and concepts of mitosis or binary fission to what was being presented graphically in the diagram. Such inappropriate transfer of information may well be a consequence of surface-level reasoning. Furthermore, students probably interpreted the diagram literally instead of recognizing the stylized nature of the diagram when suggesting that the arrow-shaped antigen (Fig. 1) was in the process of penetrating the antibody (another example of surface-level reasoning). This interpretation might have been enhanced further by the fact that, in the diagram, the antigen is both pointing at the space between the light and heavy chains and is of the same width as the space, suggesting a possible pathway of entry. Thus it is possible that the arrow shape of the antigen and channel-like features of the diagram led students to consider incorrectly the processes of phagocytosis and endocytosis when attempting to interpret the diagram.

The process-type difficulty category initially emerged unexpectedly (level 1) from student answers to free response questions. Thereafter, more specific written probes and interview questions helped us gain greater insight into the nature of each difficulty, allowing the difficulties to be classified higher up the framework at Level 3. At this level the incidence of the difficulty was found to range from 15 to 54% depending on whether second or third-year students responded to the probes and which probes they were given.

**Structural-type Difficulties**—Students who showed the structural-type difficulties interpreted incorrectly the way in which various structural features of IgG were represented in the diagram. These included the way in which disulfide bonds, variable and constant amino acid regions, and light and heavy chains were represented. The following student quotes illustrate these difficulties.

“Heavy and light chains and (with) H-bonds between them.” (Response to probe d).

“Show(s) how three atoms are bonded to form a molecule. The antigen binds to the V region of the molecule. It shows that all three atoms are bonded by the C region . . .” (Response to probe a).

“Black lines (are) some form of bond or attachment holding the three cells together, blood cells, biconcave-type shape.” (Response to probe a).

“The colored areas represent different areas . . . of red
blood cells." (Response to probe e).

"The colored (gray) region represents different amino acid residues attached to the backbone (black line) of the antibody." (Response to probe f).

From the quotes above it is clear that the spherical structures, used in the diagram to represent the three-dimensional structure of the constant and variable domains, were confusing for those students who thought that the spheres were red blood cells, whole atoms, or even amino acids. In addition, the black lines in the diagram were also confusing to the students who thought that the shorter ones represented hydrogen bonds (rather than disulfide bonds) and the longer ones single bonds (rather than amino acid chains) holding the spheres together.

A possible source of the structural-type difficulties is the fact that biochemistry textbooks often use more than one convention to represent a single structural feature of a molecule and that many of the conventions that textbook authors use are not conventions at all but are idiosyncratic representations. For instance, whereas the disulfide bond is represented as a short straight black line in the present diagram (Fig. 1), it is often represented in other diagrams either as “-S-S-” or as a yellow colored bar, presumably to denote the presence of sulfur (this in itself could cause a misconception, because not all chemical compounds containing sulfur are yellow in appearance). Student confusion might have been compounded further by the fact that in the diagram both the disulfide bond and the polypeptide chain are represented as straight black lines, whereas the rigid, frame-like appearance of the black lines implies a mechanical support capability. The way in which the variable and constant regions are depicted as large spheres may also be a source of confusion. They look like separate, ball-like structures, possibly leading some students to believe that they are not part of the actual antibody structure.

Following the unexpected emergence of these difficulties in response to free response-type questions, more focused written and oral probes were designed to gain further insight into the nature of each difficulty. The results confirmed the existence of structural-type difficulties, with incidences ranging from 13 to 70%, depending on the specific subcategory of this difficulty. The difficulties were classified on the framework as established partially at Level 3.

**CONCLUSIONS**

The difficulties identified in this study suggest that there are at least three factors affecting the ability of students to interpret a diagram, the ability of students to reason with the diagram, students’ understanding (or lack thereof) of the concepts of relevance to the diagram, and the mode in which the desired phenomenon is represented diagrammatically. These three factors are interdependent, making it difficult to establish which factor is playing the major role. Nonetheless, it is useful to consider each factor independently to develop a clearer idea of where the difficulties lie and how they could be remediated.

With regard to reasoning difficulties, Hill [12] and Lowe [17] have shown that the ability of a learner to interpret abstract diagrams is related to the prior experience of the learner with such diagrams and requires the acquisition of good reasoning skills. Moreover, knowing how to read a diagram, in itself, is a skill that must be learned. Published teaching and learning strategies that might improve the diagrammatic reasoning skills of the learners and remediate their reasoning difficulties with diagrams include the following: explicitly explaining diagrams to learners and teaching them skills and strategies for reading, interpreting, and understanding diagrams [13, 29]; explicitly teach-
ing them diagram conventions [13]; cueing them to think more deeply about diagrams when interpreting them; getting them to consult with multiple representations of the same phenomenon; and getting them to draw their own diagrams of the same phenomenon depicted by the diagram [30].

Various strategies can also be used to try and remediate the conceptual difficulties relating to the diagram. If the conceptual difficulty has resulted from the exposure of the students to the diagram, it could be addressed in a preventative manner by either improving the way the diagram is represented or the ability of the students to reason with it by supplying them with the necessary domain-specific diagrammatic skills [30]. By contrast, if the conceptual difficulty already exists as part of the prior knowledge (before exposure to the diagram) of the students it would require special strategies to correct the difficulty as such difficulties tend to be resistant to change. Strategies that may be effective could include the following: making explicit to students what part of the immune reaction was being covered when teaching about IgG structure and function; clarifying to students what concepts the diagram is and is not representing; cueing them to make links to such concepts; and placing the role of IgG in the context of the overall immune response with, for example, the use of an overview flow diagram.

Concerning difficulties related to the mode of representation of the diagram, factors such as artistic embellishments, the particular visual devices and symbols used to represent the elements of the diagram, the confusing similarity of certain diagrams across different contexts, and attempts to portray the submicroscopic environment could all have contributed to student difficulties. The science education literature has a range of advice to textbook writers as to how these factors might be addressed to improve the usefulness of diagrams as teaching and learning tools (e.g. see Ref. 31). First, it is important to realize that a diagram that seems clear to an author may not be clear to a learner, and experts should not assume that novices would interpret diagrams and their conventions in the same way as they would. It follows that diagrams should contain sufficient visual support to help students conceptualize relationships and build mental models [11]. In particular, conventions should be standardized or specially designed for optimal clarity. In this regard, some biochemistry textbook authors (e.g. see Ref. 32) present the various conventions, as well as color codes, symbols, and icons used in the textbook, in a preface to help readers interpret their diagrams.

In conclusion, the findings presented in this paper confirm the results of other studies (e.g. see Refs. 12–14 and 30), which show that incorrect interpretation of, and reasoning with, diagrams in science can lead to misunderstandings and conceptual difficulties. Future research will focus on student difficulties with other diagrams in biochemistry with a view to devising criteria for evaluating the effectiveness of diagrams in achieving the intended understanding and learning outcomes in biochemistry. The results of such research will hopefully yield guidelines on how diagrams should best be used by teachers and learners and designed by textbook writers.

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REFERENCES


