**Illuminations**

As educators, we are continually designing new methods and procedures to enhance learning. During this process, good ideas are frequently generated and tested, but the extent of such activities may not be adequate for a full manuscript. Nonetheless, the ideas may be quite beneficial in improving the teaching and learning of physiology. *Illuminations* is a column designed to facilitate the sharing of these ideas (illuminations). The format of the submissions is quite simple: a succinct description of about one or two double-spaced pages (less title and authorship) of something you have used for the classroom, teaching, laboratory, conference room, etc. You may include one or two simple figures or references. Submit ideas for inclusion in *Illuminations* directly to the Associate Editor in charge, Stephen DiCarlo (sdicarlo@med.wayne.edu).

**How Does Conjugated Bilirubin Appear in the Bloodstream?**

In our university, undergraduate medical students (in the Bachelor of Medicine and Bachelor of Surgery Programme) are required to prepare “special study modules” as part of course requirements. This exercise provides students with an opportunity for an in-depth study of areas not covered in the core curriculum. They are assigned a facilitator who guides the student in selecting the topic, selecting learning resources, and in preparing a written document (~3,000 words) that is submitted for evaluation. This manuscript, which is presented as a dialogue, summarizes what happened between the facilitator, E. S. Prakash (ESP), and his student, Yugaseelan Tarmalingam (YT), in one of the special study modules. In this instance, YT, a second-year undergraduate medical student, had a specific question: How does conjugated bilirubin leave the hepatocytes in Dubin-Johnson syndrome? This question

![Diagram depicting the relationship between bile canaliculi, hepatocytes, and perisinusoidal space of Disse. The bile canaliculi are oriented perpendicular to a plate of hepatocytes. MRP2, multidrug resistance protein 2 [also called multispecific organic anion transporter (MOAT)]. Yugaseelan’s literature review indicated that multidrug resistance protein 3 (MRP3) is located in the basolateral membrane of the hepatocyte and enables secretion of conjugated bilirubin into the bloodstream. For details, please see the text.](image-url)
first occurred to him during a problem based learning tutorial on jaundice. After answering this question, we were motivated to write this short article to Advances in Physiology Education once we verified that information about the actual mechanism of secretion of conjugated bilirubin into the bloodstream has still not been incorporated into commonly used textbooks of physiology.

YT: How does conjugated bilirubin leave the hepatocytes in Dubin-Johnson syndrome?

ESP: Bilirubin diglucuronide, a water-soluble molecule, is secreted by hepatocytes into bile canaliculi (2). However, even normally, a small fraction of bilirubin in blood is conjugated, but it does not normally exceed 0.4 mg/dl (2). Resorption of conjugated bilirubin from the intestine is negligible (3). How does conjugated bilirubin ever appear in the bloodstream in normal individuals, let alone those with Dubin-Johnson syndrome? Is turnover of liver cells responsible for causing some conjugated bilirubin to leak into blood? Obstruction to flow of bile is characterized by a predominance of conjugated bilirubin in blood (2). My understanding of this is shown in Fig. 1, i.e., a rise in intrabiliary pressures as a result of intra-/extrahepatic obstruction to bile flow will eventually overcome the resistance of tight junctions between the apical ends of hepatocytes, which is the only barrier that normally prevents bile from mixing with blood. If this happens, conjugated bilirubin will appear in the bloodstream. Furthermore, the conjugated hyperbilirubinemia of obstructive jaundice is accompanied by raised blood levels of alkaline phosphatase (2), an enzyme from bile duct epithelial cells, indicating that the above explanation is correct.

YT: If passive reflux of bile via these tight junctions is adequate to explain the entry of conjugated bilirubin into the bloodstream, it cannot still explain why conjugated bilirubin levels in blood are raised in Dubin-Johnson’s syndrome, a genetic condition caused by mutations in the gene coding for the canalicular multispecific organic anion transporter (cMOAT) gene, a molecule responsible for transporting conjugated bilirubin into bile (5). This condition is characterized by conjugated hyperbilirubinemia in the absence of intra-/extrahepatic cholestasis and a concomitant rise in blood levels of hepatic transaminases or alkaline phosphatase (4). So, there must be a mechanism for secretion of conjugated bilirubin into the bloodstream on the basolateral (sinusoidal) membrane of hepatocytes.

ESP: Probably it makes homeostatic sense to have conjugated bilirubin and xenobiotics also transported via the hepatoctye basolateral membrane into the circulation so that they may be excreted in the urine.

YT: Review of a recent report by Belinsky et al. (1) indicates that multidrug resistance protein (MRP)3 located in the basolateral membrane of hepatocytes transports conjugated bilirubin into circulation. Normally, the secretion of conjugated bilirubin into bile is mediated by MRP2 located in the apical membrane of hepatocytes. Under conditions of hereditary (Dubin-Johnson syndrome) or acquired MRP2 deficiency, the isoform MRP3 is upregulated in the sinusoidal membrane of hepatocytes. MRP3-null mice have significantly reduced serum levels of bilirubin diglucuronide after bile duct ligation compared with wild-type mice. MRP3 may serve as an alternate route of elimination of bile acids and bilirubin glucuronides when MRP2 function is defective, such as under cholestatic conditions (1).

ESP: Well done Yugaseelan! This “discovery” has come about specifically because you insisted on reviewing bilirubin dynamics in the liver in Dubin-Johnson syndrome.

REFERENCES

Yugaseelan Tarmalinggam
E. S. Prakash
School of Medicine
Faculty of Medical and Health Sciences
Asian Institute of Medicine, Science and Technology
Bedong 08100, Kedah Darul Aman, Malaysia
E-mail: dresprakash@gmail.com
doi:10.1152/advan.00080.2007