Are You Smarter than a Fifth Grader?

(Bacterial Endotoxins Version)

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Various Bacterial Endotoxin Test (BET) assays, starting with the gel clot test and working through endpoint and kinetic assays, have been used in our industry for almost 40 years, and have performed remarkably well as tools for predicting pyrogenicity in parenteral products–all of which is thanks to the horseshoe crab. To think that this simple, ancient creature can make such a difference in the quality of the world's medical supply is astounding. Dr. Frederick Bang, one of the fathers of *Limulus* amoebocyte lysate (LAL) technology termed this, "serendipity." Isn't that the way most great discoveries are made?

The year was 1996, 18 years ago. I had the most exciting and humbling privilege of being a community advisor for a program that the local school system was hosting for fifth grade students called ExploraVision. ExploraVision was (and still is) sponsored by Toshiba, and it challenged students to pick a technology and research its past, describe its present, and predict its future. My job was to give the students an example for the expectations of the program, which I did using the whole LAL story, complete with discussions of Gram negative bacteria, endotoxin, rabbits and horseshoe crabs.

As part of the program, students were required to make "storyboards" of the past, present, and future of their chosen technologies. A storyboard is a picture without dialog, and a written caption that describes the scene. Students made storyboards of my presentation as practice for their own. I'm fortunate enough to have kept some of them, and I want to share them with you to provide some context and remarkable insight through the eyes of 10- and 11-year-olds into the history and future of the Bacterial Endotoxins Test. For brevity, I've supplied the pictures, but transcribe the captions that sit below them on the original boards. Spelling is not corrected.

We started out talking about pyrogens and about Gram negative bacteria, and about how humans and rabbits reacted similarly to the injection of endotoxin. I told the students about the 1996 ***past*** method for screening of medicine for fever causing agents, which was the rabbit pyrogen test. I explained that scientists would inject medicine into a rabbit's ear vein and take the animal's temperature after the injection. If the rabbit got a fever, it was assumed that people would also get a fever, so if the rabbit indeed got a fever, the medicine could not be sold. Below is the interpretation of this process by one particularly imaginative student:

In this scene, the laboratory technician, complete with full syringe and a rabbit in a cage is ready to inject the rabbit for a USP <151> rabbit pyrogen test. He encounters Arnold ShwarziaBunny who says, "Stand back, or I will Fire!!!" The technician responds, "Oh no, it's Arnold SwartziaBunny."

The caption to this story board reads:

Arnold: *Stop killing and testing on my family or I will fire*

Scientist: *But there isn't another way to test the medicine*

Arnold: *Well you better find another way or I will be back evil scientist!*

The issues of the rabbit pyrogen test were not lost on the fifth graders, and their concerns about animal testing are still valid. In spite of periodic and even some current concerns about the robustness of the LAL test, we continually prove that the BET is a much more sensitive and predictive methodology than the rabbit test with respect to the safety/pyrogenicity of the supply of parenteral

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products. Unfortunately, in spite of this history, we regress to using the rabbit pyrogen test all too often.

Then I told the fifth graders about the 1996 ***present*** state of testing and that, in fact, we did find another way to test medicine for endotoxin. I launched into the story of the LAL test, starting with the fact that horseshoe crabs were around at the time of the dinosaurs and that the Delaware Bay had the highest concentration of *Limulus* in the world (Jersey pride). I told them that the same kinds of bacteria that make horseshoe crabs sick also give people fevers (Gram negatives). I explained and showed them pictures about how the crabs are harvested, how they are bled (the blue blood was very cool), and how they're released back to the environment. We did a gel clot test in the classroom, and the students correctly read the test results.

The fifth graders "got it", which was great. One young man summed it up beautifully and concisely. I wonder where he is now.

Scientists now take blood from a horseshoe crab. They put the medicine and the blood in a test tube. The test tube goes into a machine that works as a warm water bath. After a period of time scientists take out the tube. If the substance inside clots when the tube is turned over, the medicine has endotoxin in it and it can't be sold.

Being very concerned about any animal's welfare, the fifth graders had significant reservations about the cruelty of the bleeding process, because horseshoe crabs can't talk or scream. Even with the "release back to the environment" part of the story, the students were concerned about animal injury, mortality and conservation.

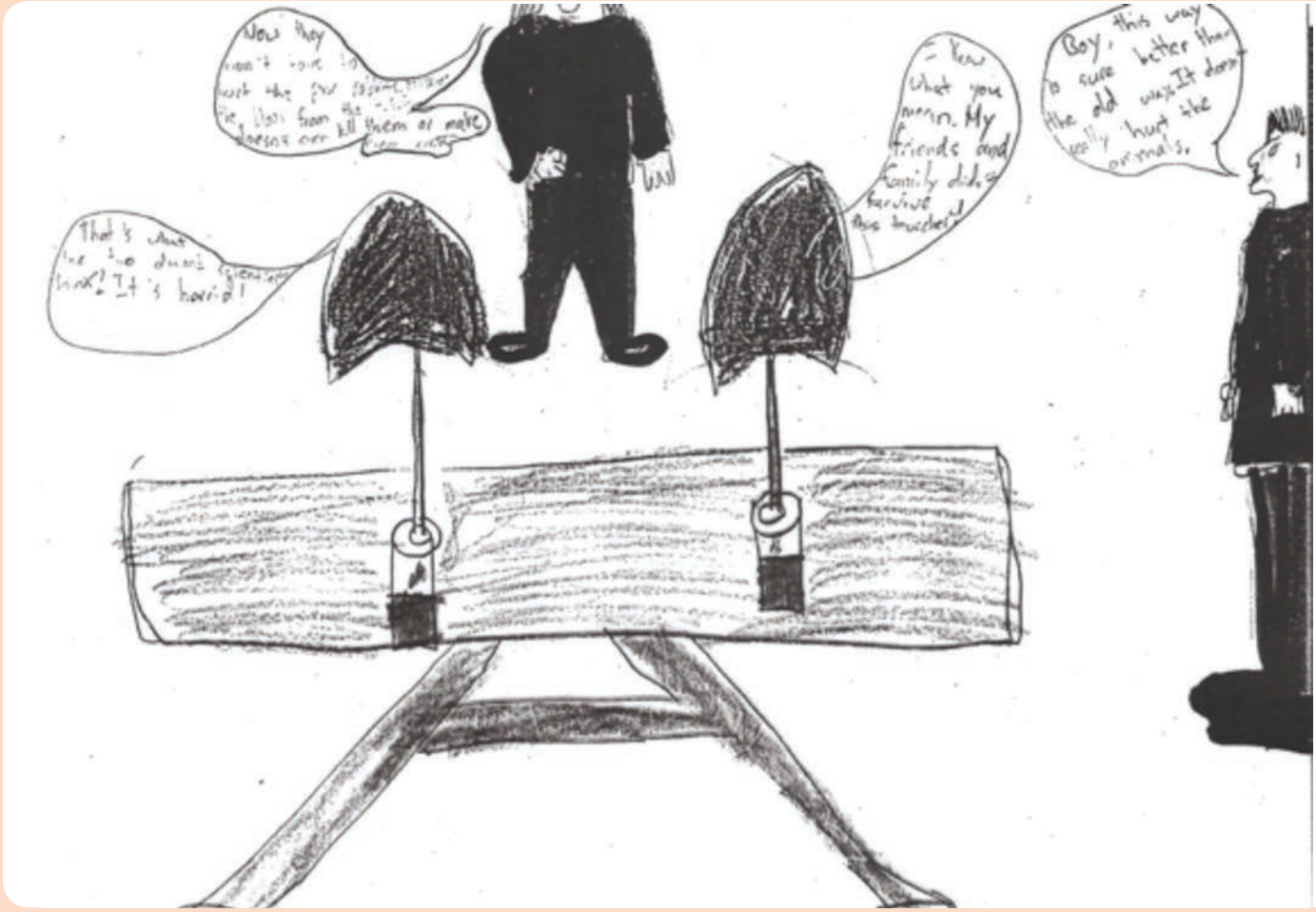
In this picture of crabs being bled, the crabs and scientists are talking:

Scientist: Now they don't have to hurt the poor rabbits. Milking the blood from the HSC doesn't even kill them or make them sick.

Animal Rights Activist: Boy, this way is sure better than the old way. It doesn't really hurt the animals

Horseshoe crab 1: That's what the two dumb scientists think! It's horrid!

Horseshoe crab 2: I know what you mean.



"The scientist is talking to the animal rights activist saying that it is a really good idea to do this because it doesn't harm the Horse Shoe Crab. The A.R.A (animal rights activist) is agreeing. The picture shows two Horse Shoe Crabs getting drained of blood. They hate it. It is pure horrer[sic], well that is what they know compared to the people that think that they know everything."

My friends and family didn't survive this tourcher[*sic*]!

The caption reads:

"The scientist is talking to the animal rights activist saying that it is a really good idea to do this because it doesn't harm the Horse Shoe Crab. The A.R.A (animal rights activist) is agreeing. The picture shows two Horse Shoe Crabs getting drained of blood. They hate it. It is pure horrer[sic], well that is what they know compared to the people that think that they know everything."

Not surprisingly there is continuing concern today about preservation of the horseshoe crab population due to erosion of spawning beaches, development on spawning beaches, and over-fishing of the horseshoe crab for the commercial purposes (horseshoe crabs are used for eel and conch bait). In fact there are businesses that are focused on hatching crabs in a controlled environment and releasing them back into the environment, as well as a number of groups concerned about conservation of this most passive but hugely important creature. Many mid-Atlantic states now have laws about over-fishing horseshoe crabs, not only for the medical relevance, but also because the eggs are food for the migrating Red Knot bird. For great information on horseshoe crab preservation, check out ERDG at www.horseshoecrab.org and the Atlantic States Marine Fisheries Commission at www.asmfc.org/species/horseshoe-crab.

I then challenged the fifth graders to predict the ***future*** of protecting the quality of the medicine supply. I was particularly proud to see one little girl (now 29 years old) who predicted that a woman scientist would invent a horseshoe crab replicating machine.

In this storyboard, the woman scientist says, "I've duplicated them!" and the horseshoe crab says, "Time to snap out of these straps and I'm home free!" The crab's buddies seem to be marching around the lab with protest signs that say, "Let us go!!"

The caption reads,

"When people find a way to duplicate the crabs, they won't have to take the crab's blood anymore.

Lady: *"I've finally duplicated them so now I can let them all go!"*

Crab: *"Time to take off these horrid straps, flick away that bottle of blood and go to my home."*

Lady: *"Hey, you helped us a lot. You should be glad."*

Crab: *"Would you be glad if you had lots of blood taken out of you?"*

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Lady: *"Hey, you helped us a lot. You should be glad."*

Crab: *"Would you be glad if you had lots of blood taken out of you?"*

Lady: *"No"*

Crab: *"Then don't you feel sorry for me"*

Lady: *"Well, yes but you can finally go home free. That's what I'm glad about."*

Lady: *"No"*

Crab: *"Then don't you feel sorry for me"*

Lady: *"Well, yes but you can finally go home free. That's what I'm glad about."*

What do we have today? We have cloned not the horseshoe crab, but a number of companies have cloned the essential proteins in the LAL cascade, eliminating the need for bleeding of live crabs. How prophetic.

After hearing the story of the Gram negative bacteria and how the endotoxin can cause fever, the story of the rabbits and their connection to people, and the story of the remarkable horseshoe crabs and their blue blood that detects very small quantities of endotoxin to keep our parenteral medicine safe, our fifth graders were free to choose technologies for their projects. Some projects were remarkably forward thinking and we see evidence of our students' thinking today. For example, one group designed a fingerprint-savvy trigger to unlock a gun only when the owner uses it– check out current Trigger Smart technology that uses RFID technology to keep guns out of the hands of children or intruders. Another group re-invented the toilet just a few years too early to engage in the Bill and Melinda Gates Foundation's "Reinvent the Toilet Challenge".

Another created a 360-degree television set so that multiple people can watch more than one show at a time. Samsung has come out with a TV where two people can watch different shows simultaneously, and of course we have DVR technology that can record 10 or more shows that air simultaneously.

So with my thanks to Chris our remarkable teacher, BettyAnn, the other community volunteer and about 60 fifth graders including Jonathan and Katie for their storyboards, and with my gratitude to horseshoe crabs everywhere, I must ask you—*Are you smarter than a fifth grader?*