

Update #2 from the Lab Team

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Greetings!

It's Jesse, here with the second update from the Hawke Lab Team at York University. Peter did an excellent job of describing his project in the first update, and I plan to shed some light on my project in this update.

We have been hard at work in the lab at York University perfecting our technique using the cryostat machine. As Peter mentioned, the cryostat is the device we use to take slices of muscle tissue. We operate it using a crank or handle, much like playing with a Jack-In-The-Box. Although clearly we are not just playing around! We need to perfect our work with the mouse tissue now, because once we begin using the seal tissue we can't afford to waste any of our samples as they are very precious. When we use the cryostat, the

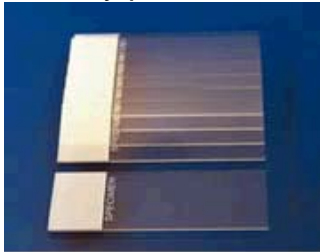


Figure 2: Microscope Slides (2)

tissue sample is mounted on a stage. As we turn the crank, the sample is brought down over a very sharp blade which slices a thin section (8 microns thick) for us to mount onto a microscope slide. Once we have cut serial sections (two or three sections in a row), we can use histological techniques to see our muscle tissue in more detail as seen in figures 3, 4, and 5.

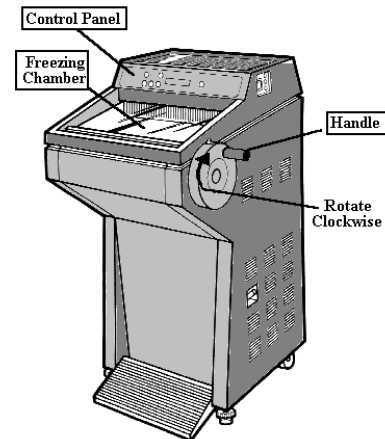


Figure 1: Cryostat (1)

It can be very difficult to get good cross-sections using the cryostat. Ice crystals can form when the muscle tissue is frozen and these crystals can distort the image of the cell by disrupting the inside of the muscle fibre (cytoplasm) and bursting organelles within the muscle fibres. This is called *freeze fracture*. In order to prevent freeze fractures from occurring, we must make sure that once the muscle sample is taken from the animal it is immediately fixed in the OCT compound and frozen in a proper way. Most tissue is snap-frozen, which means it is immersed in liquid nitrogen which is around -200°C (-328°F). However, that would not work with muscle tissue. Muscle tissue must be frozen at a slightly warmer temperature to ensure that the muscle does not become too brittle. Thus, muscle is frozen using iso-pentane (also called 2-methylbutane) that has been cooled in liquid nitrogen. Freezing the muscle immediately will prevent water droplets that are present in the muscle from forming large crystals and causing freeze fractures. We must also make sure that the tissue is not frozen too quickly, which will cause the sample to become brittle and break very easily. As well, if the muscle that has been embedded in OCT compound is left in iso-

pentane for too long, the muscle and embedding may crack. Once the tissue has been frozen, it must be kept in a -80°C freezer until we are ready to cut the tissue in the cryostat.

Figure 3 shows a muscle cross-section that I stained with haematoxylin. Haematoxylin is one of the most widely used staining dyes, as it can stain a number of different components in tissue, such as muscle glycogen and muscle protein. We use haematoxylin to check the orientation of the muscle sections we have cut. Our goal is to make transverse sections that cut the muscle perpendicular to the length of the fibre. This gives a clear view of all the muscle fibres and other muscle constituents. If the blade on the cryostat is angled incorrectly, the cross-section would show fibres that are cut length-wise. This view would be useful if we wanted to look at things that make up an individual muscle fibre.

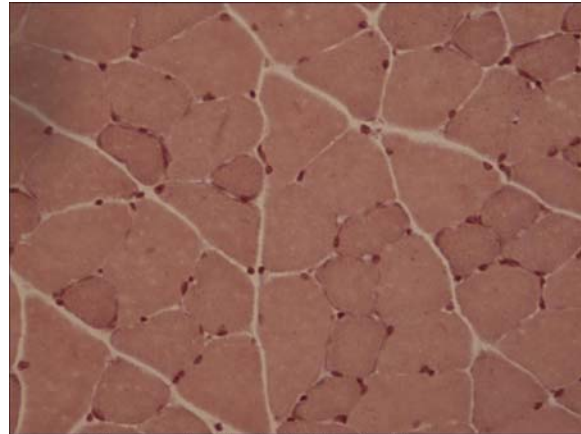


Figure 3: Mouse muscle stained with Haematoxylin

However, in my study I am interested in the distribution of intramuscular fat and the fibre type throughout all of the fibres that compose a given muscle section, rather than just one individual fibre. Once we have adjusted the blade in the cryostat and taken our first section, we stain the section with haematoxylin and look at the slide under a microscope. If the muscle is orientated properly, we will see an image similar to Figure 3 and can continue to cut more sections. However, if the muscle is not angled properly in the cryostat then the tissue will be cut longitudinally and the fibers would have an oval appearance.

Figure 4 is an example of a freeze fractured muscle tissue that has been stained with haematoxylin. Notice the large ice crystals that have formed and disrupted our image of the muscle fibres.

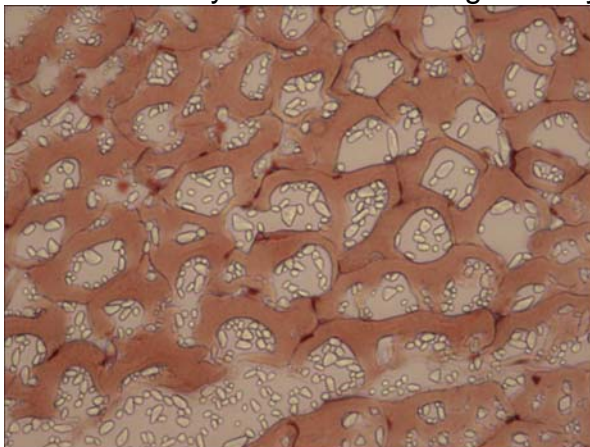


Figure 4: Freeze fractured mouse muscle stained with Haematoxylin

The large, grey coloured circles are ice crystals that have expanded from small water droplets. The smaller yellow shapes are the glue (called permount) that we use to hold the cover slip in place over the slide. When the tissue is taken out of the freezer, the muscle sample can warm up from -80°C (-112°F) to above zero or even room temperature. If the tissue becomes too warm, the ice crystals can melt. When the tissue is

placed back in the freezer, the water droplets in the muscle re-freeze and expand, leading to more severe freeze fracturing.

Figure 5 is an image of a muscle section stained with Toluidine blue. Toluidine blue is another common dye that is used as a quick orientation guide when looking at tissue cross-sections under a light microscope. Notice that the muscle fibres are transverse sections.

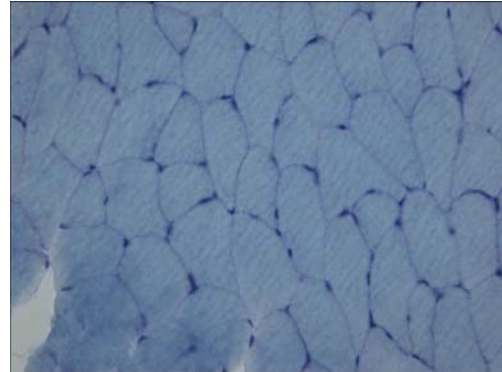


Figure 5: Mouse muscle stained with Toluidine blue

My work with the Weddell Seal muscle will involve analyzing how the distribution of fat within the muscle fibres relates to muscle fibre type as the seal develops from a pup to a juvenile seal and finally to an adult.

Fat is one of the main sources of energy that is used by the body to fuel all of our daily activities. Fat stores, called adipose tissue, store the majority of the energy that is used by the body. The bonds between atoms in the adipose tissue are broken down, releasing energy that can be used to power your muscles. All of the fat stores in the human body account for 60% of the energy contribution when we are resting, and can provide an even larger portion once we start doing physical activity.

Fat plays a particularly important role when it is located throughout muscle, hence the term *intramuscular* fat. Muscle requires a lot of energy in order to meet its metabolic demands, and fat is the primary supplier of that energy to muscles during work. The energy molecule that is most often used in the body is ATP, which can be derived from molecules stored in fat.

One of the staining procedures that I will be performing is called *Oil-Red-O*. *Oil-Red-O* is used to identify fat or lipids located within tissue sections. By using this type of staining procedure, I will be able to locate the areas of intramuscular fat within the muscle tissue.

In addition to investigating the intramuscular fat in seal muscle, I will also be studying the different muscle fibre types and how the distribution of muscle fibres change as the Weddell Seal develops from a pup to a juvenile to an adult. There are three types of muscle fibres that exist in the body: Type I, IIa, and IIx fibres.

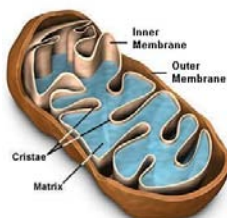


Figure 6: Mitochondria (3)

Type I fibres (also called slow twitch fibres) use mainly cellular respiration to provide their energy needs. Cellular respiration is the process that humans, seals, and many other animals use to make ATP. ATP is made by an organelle called the mitochondria, which is found inside the cells of all plants and

animals. The mitochondria have a specialized structure which allows them to make energy in the form of ATP using glucose and oxygen. A molecule called myoglobin helps mitochondria during cellular respiration by carrying oxygen from the blood vessels into the cell. Slow twitch fibres have a large number of mitochondria. For this reason, slow twitch fibres are also called *slow-oxidative* fibres (from the word oxygen) and have a red colour. As you breathe in air, your blood carries the oxygen to all the cells of your body so that the mitochondria can produce ATP. Thus, slow twitch fibres are surrounded by a lot of blood vessels, which supply oxygenated blood. Slow twitch fibres can't contract as quickly or often as Type II fibres, but they are far more resistant to fatigue.

Type IIX fibres (also called fast twitch fibres) use mainly anaerobic metabolism, which means they don't use oxygen to make ATP. These fibres rely only on the first step of cellular respiration, called glycolysis, which does not require oxygen. Since fast twitch fibres don't require the use of oxygen, they don't have as much mitochondria or myoglobin as the slow twitch fibres. For this reason, fast twitch fibres are not as efficient at using energy as the other fibre types. At the same time, this process is extremely useful for small bursts of intense physical activity. Thus, these fibres are also called *fast-glycolytic* fibres (FG). The low mitochondrial and myoglobin content gives the muscle a white appearance, such as the muscle typical found in a chicken breast. Have you ever heard your family argue at the dinner table over who is going to get the dark meat and who is going to get the white meat? Well, this is what they are arguing about: who gets what type of muscle fibre.

Type IIA fibres (also called intermediate fibres) have muscle characteristics that fall in-between fast twitch and slow twitch fibres. They are highly adaptable fibres, and can improve their oxidative capacity through endurance training. Conversely, these fibres also have the ability to produce quick contractions like fast-glycolytic fibres and can undergo anaerobic metabolism. Thus, these fibres are also called *fast-oxidative glycolytic fibres (FOG)*.

Figure 7 shows a picture of a Weddell Seal from last year's expedition. Below it, you can see images of three different muscle groups that were studied. The swimming muscles (the hindlimb and the longissimus dorsi) have a larger percentage of slow twitch fibres, which causes the solution to stain as a dark blue colour. The non-swimming (pectoralis) muscles have a larger proportion of fast twitch fibres, which appear as a lighter blue colour. My work will involve comparing the intramuscular fat distribution of the Weddell Seal with the types of muscle fibre.



Figure 7: Weddell Seal Muscle

As mentioned earlier, I will be utilizing the Oil-Red-O staining procedure to look for intramuscular fat. In addition, I will be using another staining procedure called *Metachromatic* staining that I will use to determine muscle fibre type. By taking serial sections of the Weddell Seal tissue, I will be able to overlay one muscle section stained with Oil-Red-O with a serial section stained using Metachromatic staining. This will allow me to study four major aspects of the seal muscle as it develops from a pup, to a juvenile and then to an adult:

- 1) Determine the fibre areas
- 2) Calculate the percentage of intramuscular fat per fibre
- 3) Find the number of fat droplets and the average droplet size
- 4) Determine the relationship between fat content and fibre type

Over the next few weeks I will finish practicing my cutting technique with the mouse muscle and will graduate to using the Weddell Seal tissue. I will also be learning how to perform the Oil-Red-O and Metachromatic staining procedures, and will have some beautiful pictures of my tissue for you by my next update. I hope you are all enjoying working with the Polar Science team and I look forward to answering any questions you may have!

Challenge Question #1: Why does water expand as it freezes?

Challenge Question #2: Why do slow twitch muscle fibres have a red colour? If you are eating chicken for dinner, and you ask for a piece of dark meat, what kind of muscle fibre are you eating: fast or slow twitch fibres?

Challenge Question #3: In humans, what type of athlete would have a larger proportion of slow twitch fibres and which would have a greater proportion of fast twitch fibres? What type of athlete would have intermediate fibres?

Figure References

Figure 1: University of Hawaii. *Cryotome*. Retrieved Friday October 13, 2006 from

<http://www.botany.hawaii.edu/bot606/606Photo/BasicAnatomy/Cryotome/Image188.gif>

Figure 2: Electron Microscopy Sciences. *Histology and Light Microscopy*. Retrieved Friday

October 13, 2006 from <http://www.emsdiasum.com/microscopy/products/histology/slides.aspx>

Figure 6: Carlton, R. *Mitochondria*. Retrieved Friday October 13, 2006 from

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