MEASURING SARCOMERE LENGTH USING LASER BEAM DIFFRACTION PATTERNS

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INTRODUCTION

Contraction in striated muscle results from interaction at cross linkages between the thick myosin filaments and the thinner filaments of actin. Shortening occurs as the thin filaments are pulled inward and interdigitate more with the thick filaments. Since the thin filaments are joined to the Z-band, the inward movement pulls the Z bands together and the sarcomere shortens. The shortening of the sarcomere can be demonstrated in living muscle simply by observing the change in the width of the light (I-Band) between the darker A-bands.

Because the I-bands of the sarcomere behave as light emitting slits similar to those in Young's Two-Slit Experiment (Beesley, 1971), his theory and experiment, can be used to analyze the spacing of the bands. When coherent light passes through two such closely spaced slits, a set of light bands called fringes can be projected onto a screen (Figure 1). The distance between the fringe bands is geometrically related to the distance between the slits and can be calculated.

One thing that makes this possible is the remarkable uniformity in length of each A-band thick filament, the uniformity in individual sarcomere length (Z to Z line), and the cross sectional alignment of I and A bands within the cell and muscle. As a result, frog sartorius muscle which is about 3 mm wide by 0.5 mm thick and 30 mm long will act as an excellent diffraction grating.

Laser light passing through the muscle will then project a diffraction pattern onto a screen that can be geometrically analyzed. This experiment takes advantage of these principles.

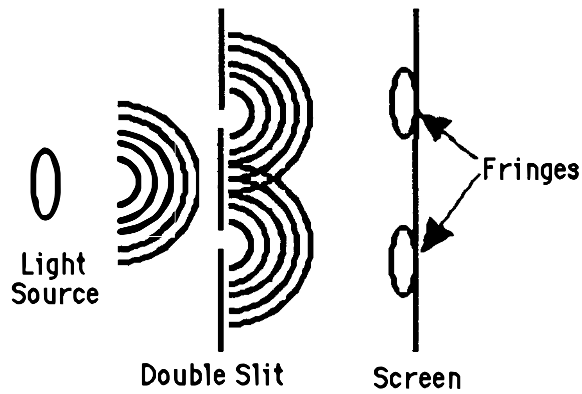


Figure 1

MATERIALS - SPECIAL APPARATUS

The requirements are minimal and many variations are possible. Before the development of laser technology scientists used a source of intense monocromatic light to illuminate the muscle preparation. This is still possible. Should you need to develop such a setup refer to Appendix A.

Laser Setup—The simplest arrangement uses an adjustable optical bench, a simply constructed muscle chamber, an adjustable manipulator for stretching the muscle, and a laser as diagrammed in Figure 2. An inexpensive He/Ne Laser, which works well as the light source, and the optical bench, can often be borrowed from a physics department. A clear lucite metric ruler is used to measure the fringe bands.

Muscle Support Chamber—The only requirements for the muscle support chamber are: (1) the belly of the muscle must be supported or compressed into a flat position; (2) one must be able to vary the length of the muscle as needed; (3) the aperture which allows the passage of the light beam must be restricted so that only about 0.5 mm of the length and 3 mm of the width of the muscle is illuminated (with a laser this in not nearly so critical); and (4) provision must be made for stimulating the muscle.

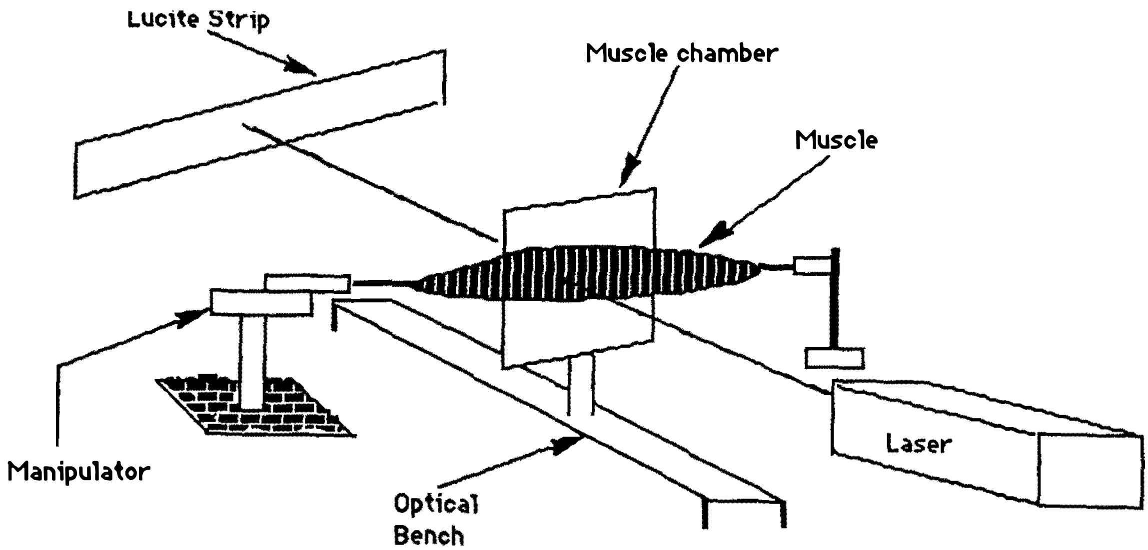


Figure 2

Figure 3 shows the design of a simple vertical support which will allow passage of the beam horizontally. The support is constructed out of two plastic or glass microscope slides. To one of the slides is affixed two flattened silver wires approximately 1 cm apart. Superglue is a suitable affixative. Note: be sure the wires are bare after gluing to the slide. To the lower half of one slide, plastic spacer similar to those used in electrophoresis, are used to separate the two slides and provide a reservoir for Ringer's solution needed to moisten the muscle. (Note: an alternative approach might be to use a glue gun or Mortite strip caulking to lay down a ridge around the glass).

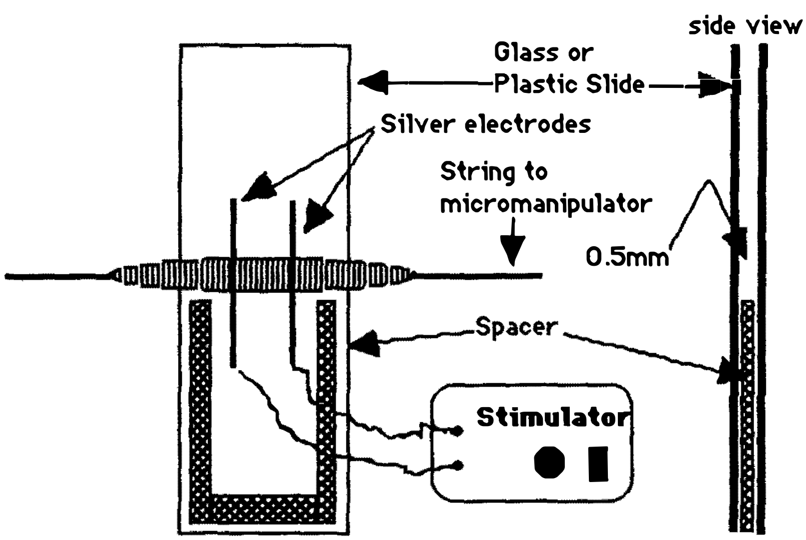
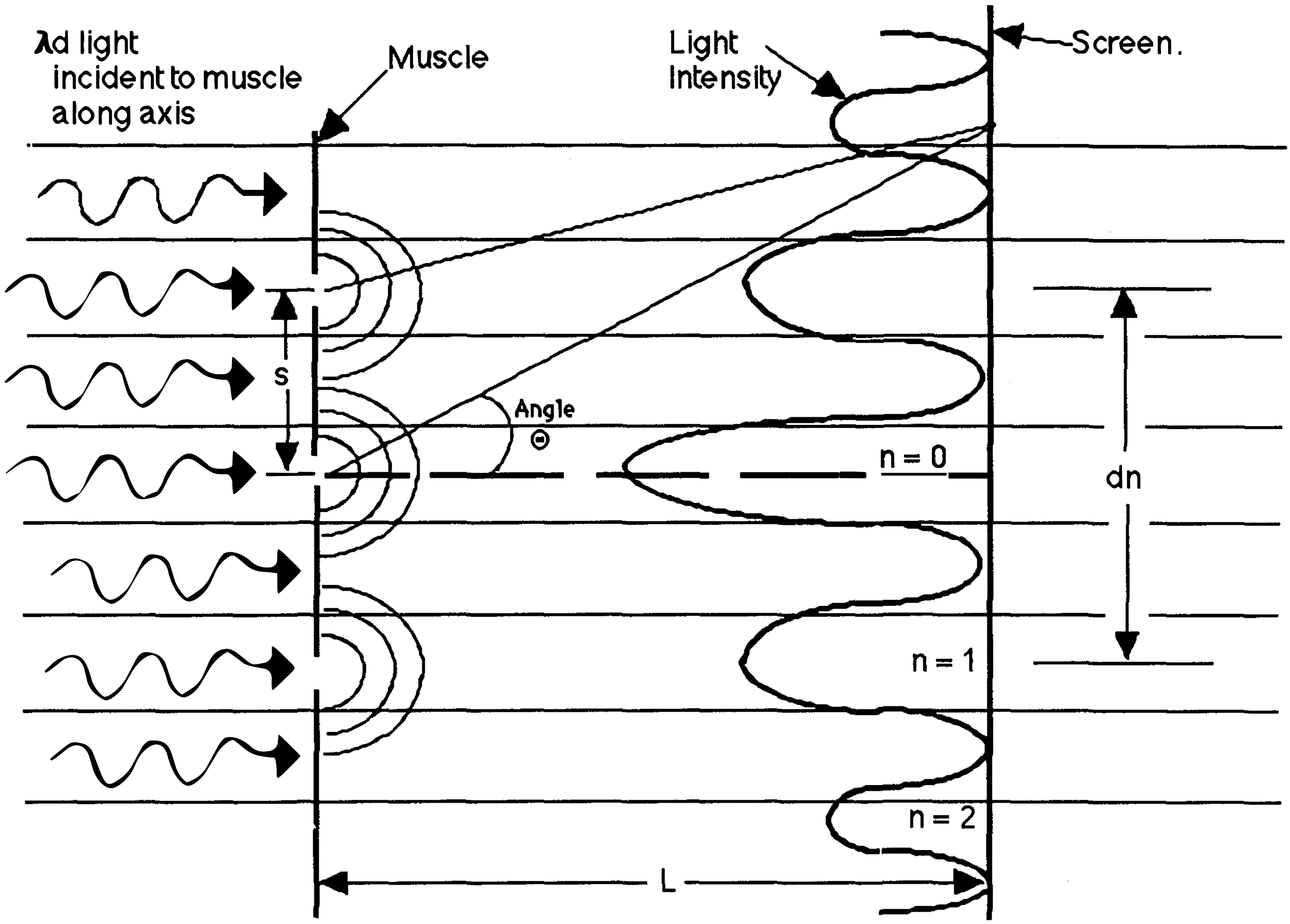


Figure 3

EXPERIMENTAL PROCEDURE

1. It is necessary to dissect and remove a sartorius muscle (not gastrocnemius) complete with the pelvic bone. Identify the sartorius muscle running on the middle ventral surface of the thigh from the tibial insertion to the pelvic origin. Carefully cut a slit lateral to the tibial tendon and pass the thread under the tendon and tie it. Be careful not to damage the muscle. Now lift the thread and cut the tendon. Beginning at the tibial end, dissect the sartorius free from the other muscles. Remove the legs including the symphysis. Leave the epiphysial sockets. Cut the urostyle bones near the pelvic end. Leave the muscle attached to the circular pelvic bone. Keep the preparation cool and moist with Ringer's Solution.
2. Lay the muscle flat over the silver electrodes on slide 1. Add Ringer's solution to the enclosed area. Place the other slide lightly on the muscle to ensure flatness. Clamp the slides together with a rubber band or paper clip.



$$n\lambda = S(\sin\theta_n)$$

$$\text{But } \sin\theta_n = \frac{\frac{dn}{2}}{\sqrt{L^2 + \left(\frac{dn}{2}\right)^2}} \approx \frac{dn}{2L}$$

$$\text{Then } n\lambda = \frac{S\,dn}{2L}$$

$$\text{Or } S = \frac{2nL\lambda}{dn}$$

Figure 4

3. Mount the slide-support onto the holder of the optical bench. Attach the string to the adjustable manipulator and the pelvis to an immovable support. Attach the wires to the stimulator. Adjust the muscle to its approximate maximal length in the body. Mount a slit diaphragm in front of the mounted muscle (Note: this is an optional control of the laser beam width). Mount the laser source to one end of the optical bench. Mount a white projection screen at the other end of the optical bench. Switch on the light, adjust the height and position of all parts of the setup so that a good view of the projected beam can be seen. If set up correctly you should be able to see a diffraction pattern similar to Figure 4 on the screen.

   After viewing the diffraction bands, two measurements must be made: (a) the distance between the first order bands (see figure 5) and (b) the distance from muscle to viewing screen. It is most accurate to measure between bands on either side rather than to estimate the distance from a band to the midline. Measure similarly for second order and higher bands. (Note: if you use the projection screen, you have the advantage of using the measuring scale on the bench, but the disadvantage of having to measure accurately the distance between the bands. Should this be your preference you might try using a caliper to measure the band distance. Optionally, you could project the beam across the room to a screen with the advantage of being able to measure longer distances with less error.)

4. Repeat the measurements at lengths above and below the starting length. In order to use shorter lengths the muscle must be preshortened by stimulation. If a muscle is stimulated and allowed to shorten, then it elongates again only if pulled out by a load. This necessary load is very small, less than the muscle weight. But if a muscle is resting on a flat surface it will tend to remain short and the filaments remain interdigitated. Loosen the tension on the manipulator a little. Stimulate the muscle with several single shocks of 1mSec duration and 1 to 10 volts. (Note : a 1.5 volt battery could be used with a simple key switch for stimulating.) This will shorten the muscle to a new length which can be recorded. Measure the diffraction band separations, slacken threads, and shorten even further. The muscle will not shorten by much more than 30% in this way.

   Next extend the muscle to various lengths beyond the starting value, up to 30% extension, and measure the band separations.

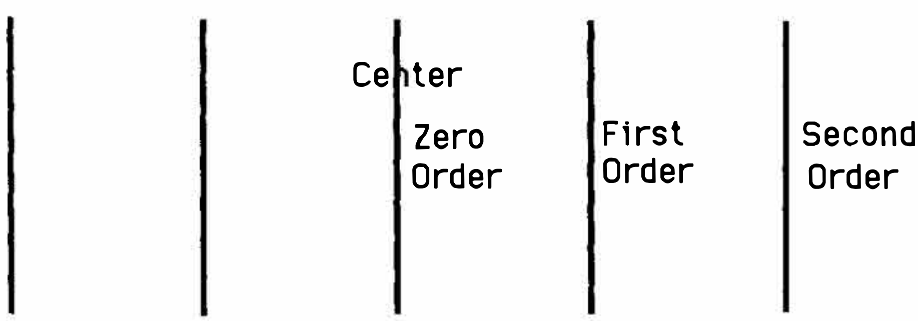


Figure 5

CALCULATIONS

1. From the properties of a diffraction grating, the sarcomere length is given by:

$$S = \frac{2 n l \lambda}{dn}$$

where:
1 = distance from muscle to screen
dn = distance between the nth order bands of brightness
n = order of the bands (1, 2, and 3)
0.5896 = wave length in m (Na line is 0.6328)

Calculate the sarcomere length at each muscle length for the various values of n.

2. Plot sarcomere length vs. muscle length over the range of lengths used. Note the linearity and the value at the initial length. Is it about 2.5 μ?

3. If a muscle chamber for recording tension is available, measure tetanic tension over the same range of lengths. From the diffraction experiment results, plot the curve relating sarcomere length to tetanic tension. The tension should be maximal at about 2.5 μ, zero at 3.5 μ and at about 1.3 μ.

Literature Cited

Sandow, A. 1936. Diffraction patterns of the frog sartorius and sarcomere behavior under stretch and during contraction. *J.C.C.P. 9*:55-75.

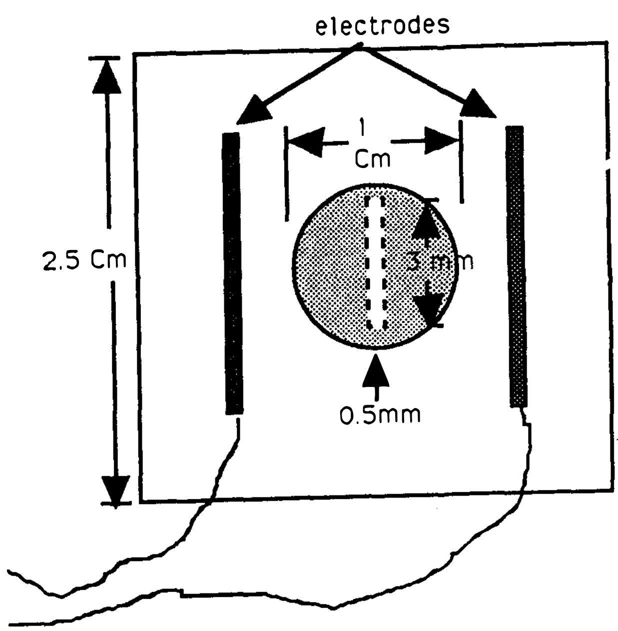
Beesley, M.J. 1971. *Coherence. Lasers and their Applications.* Barnes & Noble, New York. p. 26-29.

APPENDIX A

The illustration, at the right, shows a lucite box about 2.5 cm square with 1 cm sides. A 1 cm hole (round or square) is cut in the center of the bottom. The platform for supporting the belly of the muscle is centered over the hole about 5 mm above the bottom of the box. Holding pins are stuck in corks glued to the bottom of the box.

The top of the supporting platform can be made from a cover glass or polished lucite. All but the 0.5 x 3 mm area in the center should be made opaque by painting or placing black paper on the bottom. For the electrodes, flatten one end of a piece of silver wire (about 22-25 gauge) and stick one electrode on each side of the transparent center.

Attachments must be provided to restrain the muscle. One simple way is to create a transparent window. Mount a microscope cover glass over a hole raised about 1/4 inch above the rest of the container base and stick a cork on both sides for securing the pelvic bone with a pin. Secure the tibial end with thread around two pins.



Genetics Computer Group
University Research Park
575 Science Drive, Suite B
Madison, WI 53711-1060

June 7, 1995

Dr. Buzz Hoagland
Westfield State College
Department of Biology
Westfield, MA 01086

Dear Dr. Hoagland:

I have tried to reach you about your kind invitation to be the keynote speaker at the Association of Midwest College Biology Teachers (AMCBT) meeting on September 29. I do have that date open, and I would be happy to speak, but I think I should decline for two simple reasons.

The first reason is that I am not really a distinguished teacher of biology. Although I loved being a graduate teaching assistant in cell biology, most of my experience with education is from the point of view of a student—perhaps not a very good one at that. I did not like biology; the birds and the bees put me to sleep. Ecology and evolution were the only macrobiologies in which I could stay awake. My subjects were physics, chemistry, and biochemistry, and begrudgingly in later life, genetics.

The second reason I should decline is that I am turning into a curmudgeon, believing now that technology is education's enemy, and that students should learn the hard-core disciplines in science like mathematics, thermodynamics, logic, and mechanics and, in social science, economics and history. I see ignorance everywhere floating in a sea of information, most of it so poorly presented that it robs the listener of his power to reason and reflect. Water water everywhere but not a drop to drink!

Knowledge, as opposed to information, is assembled not accumulated. Like the stones of a great building, everything learned must be shaped by reflection and then laid upon the edifice in sequence, while the whole assembly rests on a bedrock of understanding. Politics, technology, information, ideology, religion, enthusiasms of every kind—these are the enemies of knowledge.

Science students should bring some knowledge of mathematics and language to college. On arrival they should turn off the TV, shut down the computer, and develop a sense of scale from physics and chemistry. What are the fundamental units? What is mass, velocity, energy, inertia, momentum, and work? What forces are known? What is the relationship between internal energy, pressure, volume and temperature? What is the periodicity of the elements and where do the isotopes come from? What is the nature of the particles? How big is the universe and what is it made of? What does it weigh? How long has it been here? Where is it going? Which quantity is greater, the mass-energy of the stuff in the universe or the gravitational potential of its distribution? What reactions power the sun? Lay this knowledge in the foundation if you would be a scientist.

Students should read some biology. What is metabolism? How did life get started on this planet? What are the species and why are they so diverse? What is the basis of inheritance? Are all living things related? What are the materials of life and how do they pool and cycle through the biosphere? What conditions are necessary to life? Is the biosphere stable?

Civilization cannot survive without a knowledge of history. What is feudalism and what changed to allow the rise of industrial capitalism? What are nations and why are they so persistent and compelling? What is religion and what is its role in history? How did this nation come to separate its civil and religious affairs? What are the causes of war and peace? What is totalitarianism and why was it so important in this century? What is property?

Students should also have to learn some economics. What is money? What are savings and what is capital? How is wealth generated? What is trade? What is the relationship between unemployment, interest rates, inflation, wages, and the value of a currency? How are savings, investment, and the current account related? How does government spending affect investment, savings and aggregate demand? What is the relationship between risk and return in a well-functioning market? What is known about the distribution of wealth?

If a student must learn about information, let him learn some information theory. How is the rate of information transmission limited by bandwidth? How are waves represented with numbers and numbers with waves? What does it mean to compress information? How do you represent a real number? How is prior knowledge of a data structure related to the amount of information that must be stored in it?

All these things are known and should be taught.

But the serious student will spend his whole life struggling with questions of ultimate meaning. Who are we? How did we get here? Why does it appear that the particular is orderly while the general is chaotic? What is the relationship between what we know and what we believe? Can we control our destiny? How can we know good from evil? Why do we love and hate? What will be left to posterity from this time? What responsibility do we have to the future? Will anything at all about human civilization survive on a geological time scale? What is God?

The answers to questions like these are not known; indeed the answers are not the stuff of knowledge. Educators cannot teach these things except to show respect for their importance. But students, as they walk through life, will find these deeper questions daunting without some knowledge and without some appreciation for the disciplines that have brought humanity to this junction. Instilling this knowledge and appreciation is the principal mission of education.

Now to the subject at hand. Computers are not a legitimate academic discipline any more than cars or bicycles. There is no such thing as computer science. There is an engineering discipline for people who would build or program computers and there are a few branches of mathematics that assist those interested in algorithms. But, how to use computers, how to store and retrieve data from them, how to find resources on Internet—these are best learned in the context of a pressing application.

Access to computers will not help students learn anything of importance. Important things are learned from teachers in the classroom and from books in the study. Armed with knowledge and the power to learn, students can stand against the wind and slowly make their way, and with God's grace, help others to follow.

I do appreciate the invitation, but as you can see, my views are out of fashion on the subject of technology in the classroom.

Sincerely,
John Devereux, Ph.D., President, Genetics Computer Group

Editor's Note: Despite his initial refusal, Dr. Devereaux presented his opinions in the keynote address to the 1995 AMCBT annual Meeting.

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Abstract of Workshop Session:

P.1.4. INSPIRATION: CONCEPT-MAPPING SOFTWARE TO ASSIST STUDENTS TO COPE WITH INFORMATION OVERLOAD
Leona Truchan, Alverno College, Milwaukee, WI
Inspiration is a powerful graphic software package that is easily mastered and can be used to create flow-charts, multiple graphics, and convert outlines into graphics. The workshop will give you an introductory experience using Inspiration and creating a graphic to show how easily it can be done. Students find that one or two graphics highlighting a 20-30 page chapter enables them to "see" what is significant and what are the unifying themes.

Abstract of Poster Session:

THE AVIAN LINK TO THE DINOSAURS
Don Robertson, Park College, Parkville, MO
Based on overall skeletal similarities, relationships between theropod dinosaurs are fairly apparent. From the 225 million year old *Herrerasaurus* to the 100 million year old *Sinornis*, the similarities become more apparent. In this family of animals, the pelvis evolved to a reversed position. Some of the younger species have left impressions of feathers in the fossil record. Also the *Sinornis* has a pygostyle, which is not seen in the 145 million year old *Archaeopteryx*. The pygostyle is where the tail feather fan attaches. Finally, the *Sinoris* was able to fully fold its forelimbs. These traits are all found on modern day birds. Their presence supports a relationship between theropod dinosaurs and modern day birds.

WHITE-TAILED DEER POPULATION CONTROL
Jason Stapleton, Park College, Parkville, MO
Population control methods of the white-tailed deer, *Odocoieleus virginianus*, across North America include hunting and contraception/sterilization. Intramuscular injections of porcine zona pellucida (PZP) were determined the most effective method of contraception/sterilization. Synthetic progestin would be an effective form of contraception if it did not require that animals be captured. Hunting was determined the most effective means of population control in general.

BROMELIADS AND DIVERSITY IN TROPICAL RAIN FORESTS
Laurie Baker, Park College, Parkville, MO
Previous studies of tank and atmospheric bromeliads have focused on morphology and physiology. Leaf arrangement and trichome structure and function aid in water retention and the acquisition of nutrients from accumulated litter. Outer cellular structure of roots aids in water retention. The formation of epiphytic masses in roots provides nutrition and habitat for rain forest animals. Bromeliad fllowers are attractive to birds, bats and flying invertebrates. The interaction between epiphytic bromeliad morphology, physiology and gaps created in rain forest canopies by falling trees may be an impetus to ascension of bromeliads toward the upper tree canopy. Because of their morphology and physiology, epiphytic bromeliads can be viewed as "keystone species" and strong interactors and as such contribute to the diversity of the tropical rain forest.

EFFECTS OF THYROID HORMONE ON BONE MINERAL DENSITY
Kristi Flaherty, Park College, Parkville, MO
A review of case studies that test for bone mineral density loss at the spine, hip, and femoral neck areas in postmenopausal women using the thyroid hormone compared to matched controls showed a decrease in the bone mineral density (BMD) of the postmenopausal women. Patients tested had a variety of thyroid histories and the doses of thyroid hormone varied with each study. Three studies provided evidence for no effect of thyroid hormone on BMD. Nine studies provided evidence that thyroid hormone does affect BMD. Four major factors which cause lower BMD levels, include previous treated Graves' Disease, over replacement of thyroxine, suppressive doses of thyroxine, and low Thyroid-Stimulating Hormone (TSH) levels. Thyroxine alone was found to have no effect on BMD, but the factors listed above were found to have a definite effect.

From the case studies I have reviewed, I believe that physiological doses of thyroxine do not cause BMD loss. When suppression doses are given, or overreplacement occurs, BMD loss does occur. A previous history of thyroid disease, such as hyperthyroidism, is shown to cause BMD loss.

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- -

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