EXAMINING EFFECTS OF SPACE FLIGHT ON THE SKELETAL SYSTEM

BONE, CALCIUM, AND SPACE FLIGHT

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INTRODUCTION

Why do we **physically** stop growing when we have reached less than a third of our life expectancy? Aquatic species such as mollusks, crustaceans, and some fish grow **indefinitely**. Can you imagine? Some of these species never stop growing! One giant clam weighed 600 pounds and may have been 100 years old; a giant squid can grow to 50 feet; a 2800 pound turtle has been reported. One reason that this is possible may be that the water in which these creatures live helps support their great weight. But landwellers, such as ourselves, have to support their body weight without this buoyant help (buoy = float), so we have evolved ways of limiting our size. Thank goodness, or otherwise, we might call our world the "land of the giants !" It is interesting, however, that studies have shown that astronauts **actually become taller in space** since their spinal column is not constantly being compacted by the downward force of gravity. We'll discuss this later in the chapter.

Certainly, it is not just the skeletal system that is challenged on a daily basis by the gravitational forces we encounter here on Earth. We've already discussed how gravity influences the muscles in our body. And we will discuss in this chapter some of t he connective tissues that contribute to the structure of our bodies. So, we must keep in mind that it is the components of the **musculoskeletal** system - bone, muscles, and connective tissue - that perform **together** with pe rfect teamwork. Their mission is threefold:

- to **support** the body,
- to **shield** our delicate internal organs, and
- to make the body **mobile**.

No team has ever been more carefully equipped and organized. In this chapter, we will discuss three primary aspects of the **skeletal system**: the skeleton's structure and function; the formation and breakdown of bone; and the important chem icals (minerals, salts, and hormones) that participate in bone metabolism. In addition, we will examine a particular space flight investigation that was carried out to look at how bone formation and calcium metabolism change in microgravity. Let's begin w ith a look at how the Earth's gravity has influenced the development of our skeletal structure.

EARTH PHYSIOLOGY

The very detailed, intricate, and harmonious workings of our senses, the nervous system, and the muscles would all be impossible without the approximately 206 bones of the human skeleton (not counting the tiny **sesamoid bones** like sesame seeds - embedded in the tendons of the thumb, big toe, and other pressure points).

Support for the body here on Earth is supplied by the bones

Figure 1. The different types of joints in the body.

The bones provide the movable framework that **gives shape to motion.** Bones are **rigid** (but slightly flexible) structures, that, when combined with the 68 **joints** of the body, permit **fluid motion** of various types. **Pivot joints, hinge joints, gliding joints,** and **ball-and-socket joints** allow the widest range of motion; the ball-and-socket joint at the shoulder is among the most maneuverable in the body (Figure 1). Normally the ends of bones at joints are coated with a tough but flexible tissue called **cartilage**, which provides a wear-resistant surface for a lubricating fluid that surrounds the joint. This fluid is known as **synovial fluid**, which is a secretion of egg-white consistency that acts as a " joint oil." Infection, in jury, disease, or wear and tear can cause cartilage to become damaged and deteriorate, resulting in **arthritis**.

Supported by bone and activated by muscle, the body enjoys an incredibly wide range of movement, as forceful as sledge-hammering, as gentle as blinking. The same hand that pats a puppy, pounds a desk. The same foot that teeters on tiptoe, kicks a field goal. The limits of mobility are broad and the limitations relatively few. We cannot, for example, touch left forefinger to left elbow, or turn the head to look directly behind us. Even so, by stretching ligaments and muscles to make the joints unusually limber, some of us are able to wrap our feet around our neck, or do backbend flips across a padded floor or lawn. Some people can also hyperextend their elbows or even bend their thumb backwards to touch their forearm, causing everyone around them to gasp!

The thumb alone would convince anyone that the architect of our body (whoever that may be to each one of us) **had to be a genius!** Of the thousand or so different functions we perform daily with the 19 bones in each hand, 2 are demonstrated in Figure 2. Neither of these functions would be possible without the thumb. **In a precision grip**, a flexed finger and opposing thumb grasp an object in a posture that assures accuracy and fine control; **in a power grip**, the object is held between the flexed fingers and palm while the thumb exerts counterpressure. Each hand can perform both grips at once - with a small object grasped in your palm, you can pick up another with thumb and forefinger. Let's discuss the development, general arrangement, and function of the bones in our bodies.

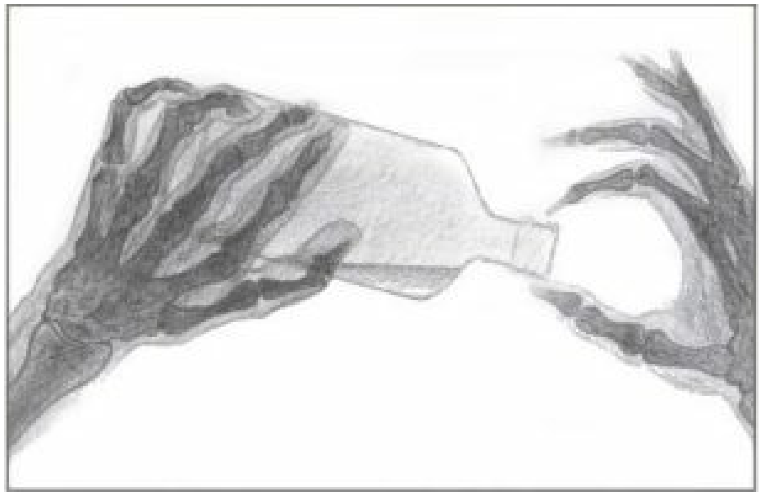


Figure 2. The thumb is capable of precision grips that allow for accuracy and fine control of certain movements as well as power grips that allow for firm handling of objects.

Bone Development and Structure

Because bone is made up of minerals and is hard, many people think that it is not living material. But a bone in a living animal consists of both **living tissue** and **non-living substances**. Within the "alive bone" are blood vessels, nerves, collagen, and living cells including:

- **osteoblasts** (cells that help **form** bone), and
- **osteoclasts** (cells that help **eat away** old bone).

In addition, bone contains cells called **osteocytes**, which are mature osteoblasts that have ended their bone-forming careers. These cells engage in metabolic exchange with the blood that flows through the bones. The **nonliving**, but very important, substances in bone are the minerals and salts.

Besides the metabolically active cellular portion of bone tissue, bone is also made up of a **matrix** (a bonding of multiple fibers and chemicals) of different materials, including primarily **collagen fibers** and **crystalline salts**. The crystalline salts deposited in the matrix of bone are composed principally of **calcium** and **phosphate**, which are combined to form **hydroxyapatite crystals**. As you can see, the **chemical formula** for hydroxyapatite crystals includes molecules of calcium (Ca), phosphate (PO_4), and hydroxide (OH):

$$Ca_{10}[PO_4]_6[0H]_2$$

In particular, it is the collagen fibers and the calcium salts that help to strengthen bone. In fact, the collagen fibers of bone have great **tensile strength** (the strength to endure stretching forces), while the calcium salts, which are similar in physical properties to marble, have great **compressional strength** (the strength to endure squeezing forces). These combined properties, plus the degree of bondage between the collagen fibers and the crystals, provide a bony structure that has both extreme tensile and compressional strength (Figure 3).

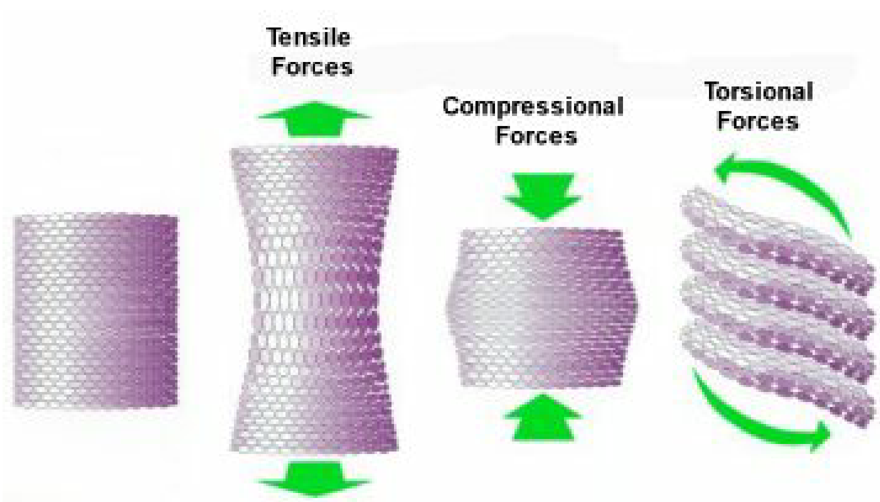


Figure 3. Examples of compressional forces (squeezing), tensile forces (stretching), and torsional forces(twisting).

Thus, bones are constructed in exactly the same way that **reinforced concrete** is constructed. The steel of reinforced concrete provides the tensile strength, while the cement, sand, and rock provide the compressional strength. However, the **compressional strength of bone is greater than that of even the best reinforced concrete, and the tensile strength approaches that of reinforced concrete**. But, even with their great compressional and tensile strengths, neither bone nor concrete has a very high level of **torsional strength** (the strength to endure twisting). In fact, bone fractures often occur as a result of torsional forces that are exerted on an arm or a leg. First, let's examine the major structural components of our bones and then let's briefly discuss how bone develops.

Bones can be classified according to their shapes as: **long** (including the arm and leg bones), **short** (including the bones of the wrists and ankles), **flat** (including the ribs and the bones of the skull), and **irregular** (including the vertebrae along our spine). In describing the general structure of bone, a long bone will be used as an example (Figure 4). At each end of such a bone there is an expanded portion called an **epiphysis**, which forms a joint with another bone. The shaft of the bone, which is located between the epiphyses, is called the **diaphysis**. Except for the **articular** cartilage that covers the very ends of each epiphysis, the bone is completely enclosed by a tough covering called the **periosteum**. Within the periosteum lies a bony layer called **compact bone**, which is solid, strong, and resistant to bending. The epiphyses are composed largely of **spongy (cancellous) bone**, which **provides the greatest amount of elastic strength** since the epiphyses are subjected to the greatest forces of compression.

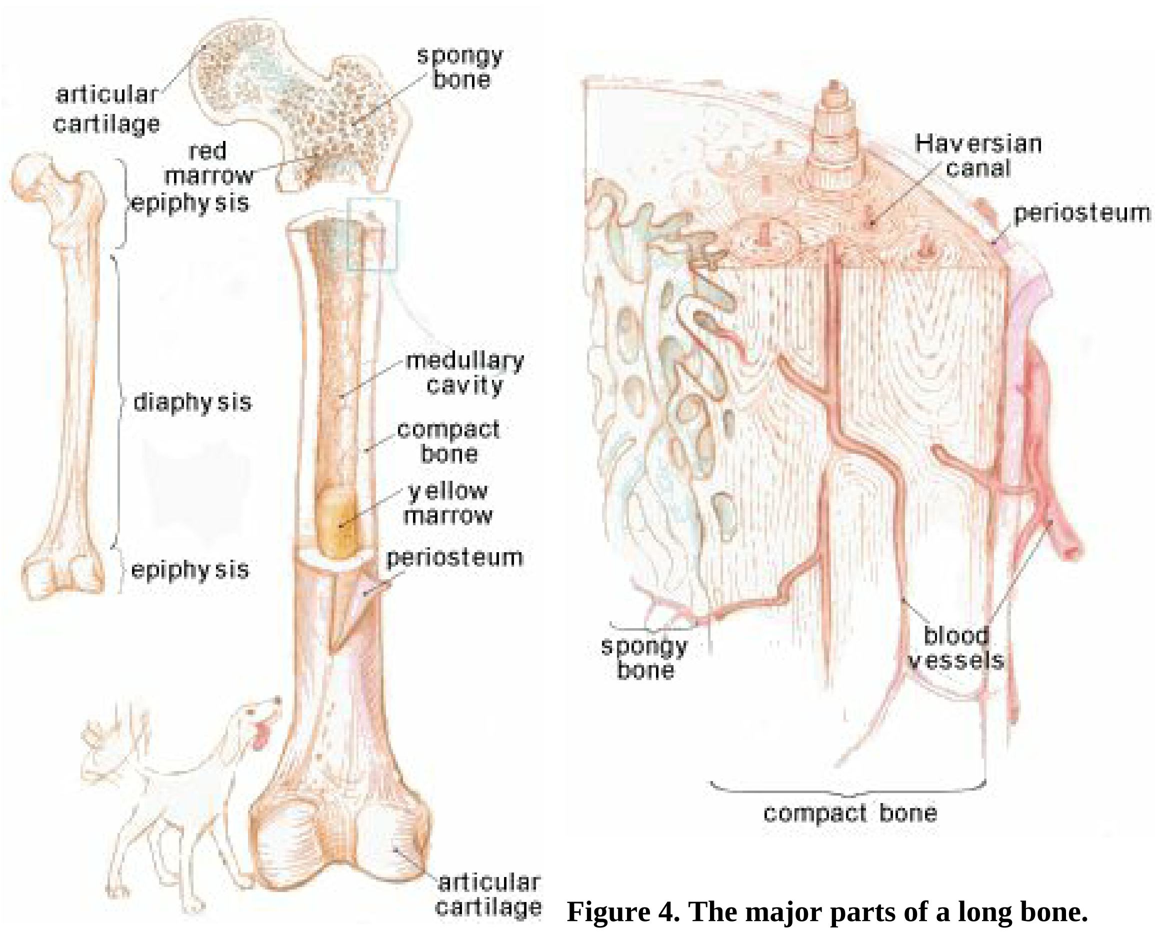


Figure 4. The major parts of a long bone.

The compact bone in the diaphysis of a long bone forms a rigid tube with a hollow chamber called the **medullary cavity**. This cavity is continuous with the spaces of the spongy bone and is filled with a specialized type of connective tissue called **marrow**. The marrow in the medullary cavity of an adult bone is usually of a type called **yellow marrow**, which functions as fat storage tissue. The marrow in the spaces of spongy bone is likely to be **red marrow**, which functions to produce various types of blood cells. In an earlier chapter, we discussed how the red bone marrow participates in the formation of red blood cells (RBCs) through the process of erythropoiesis. But since we are focusing on the bones in our bodies, let's examine the process of bone formation, or **ossification**.

In the fetus, most of the skeleton is made up of **cartilage**, a tough, flexible **connective tissue** that has **no minerals or salts**. As the fetus grows, osteoblasts and osteoclasts slowly replace cartilage cells and ossification begins.

Ossification is the formation of bone by the activity of osteoblasts and osteoclasts and the addition of minerals and salts. Calcium compounds must be present for ossification to take place. Osteoblasts do not make these minerals, but must take them from the blood and deposit them in the bone. By the time we are born, many of the bones have been at least partly ossified.

In long bones, the growth and elongation (lengthening) continue from birth through adolescence. **Elongation** is achieved by the activity of two cartilage plates, called **epiphyseal plates**, located between the **shaft** (the diaphysis) and the **heads** (epiphyses) of the bones (Figure 5). These plates expand, forming new cells, and increasing the **length** of the shaft. In this manner, the length of the shaft increases **at both ends**, and each head of the bone moves progressively apart. As growth proceeds, the thickness of the epiphyseal plates gradually decreases and this bone lengthening process ends. In humans, different bones stop lengthening at different ages, but ossification is fully complete by about age 25. During this lengthening period, **the stresses of physical activity result in the strengthening of bone tissue.**

In contrast to the lengthening of bone, the **thickness and strength** of bone must continually be maintained by the body. That is, old bone must be replaced by new bone all the time. This is accomplished as bone is continually deposited by **osteoblasts**, while at the same time, it is *continually being reabsorbed (broken down and digested by the body)* by **osteoclasts** (Figure 5). Osteoblasts are found on the outer surfaces of the bones and in the bone cavities. A small amount of osteoblastic activity occurs continually in all living bones (on about 4% of all surfaces at any given time) so that at least some new bone is being formed constantly. Normally, in fact, except in growing bones, **the rates of bone deposition and absorption are equal to each other so that the total mass of bone remains constant.**

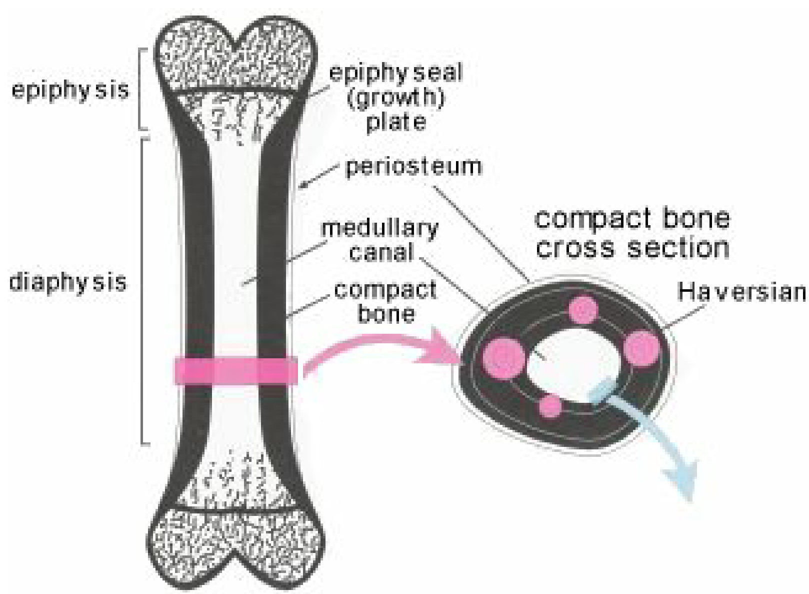
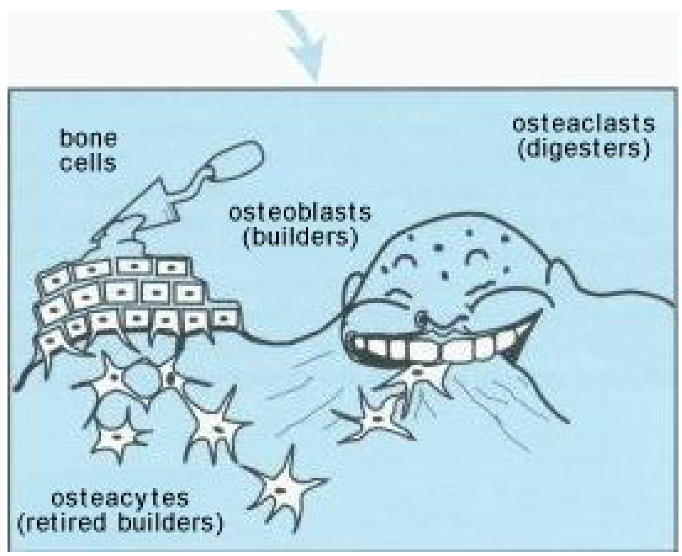


Figure 5. Long bones continue to grow and elongate (lengthen) though adolescence. This process is called ossification.

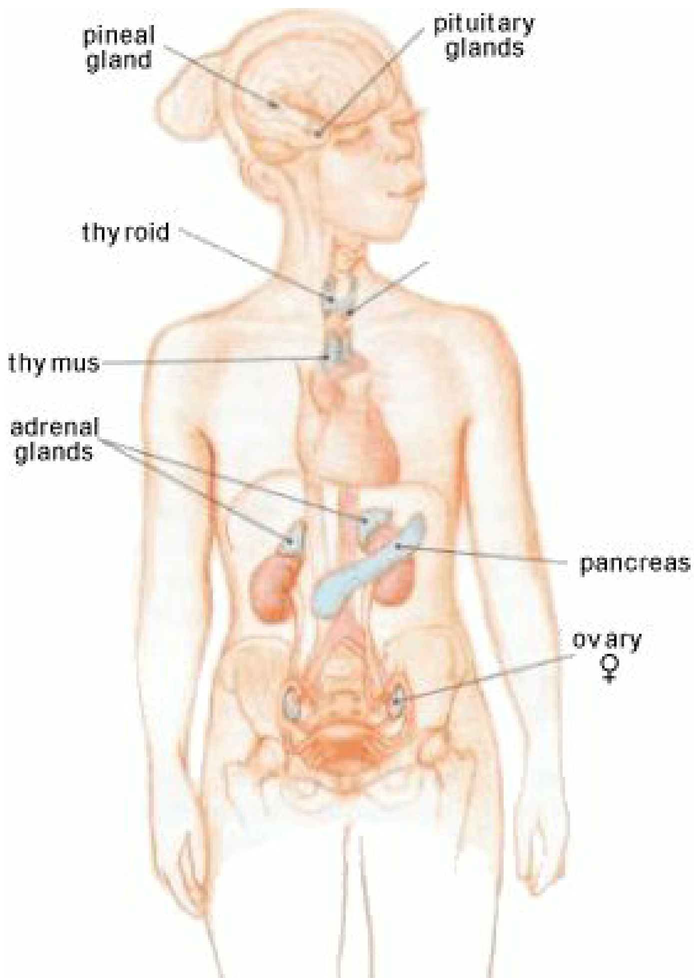


Usually, osteoclasts exist in small but concentrated masses, and once a mass of osteoclasts begins to develop, it usually eats away at the bone for about three weeks, eating out a tunnel that may be as large as 1 millimeter in diameter and several millimeters in length. At the end of this time the osteoclasts disappear and the tunnel is invaded by osteoblasts instead; then new bone begins to develop, Bone deposition then continues for several months, the new bone being laid down in successive layers of concentric circles on the inner surfaces of the cavity until the tunnel is filled. Deposition of new bone ceases when the bone reaches the surface of the blood vessels supplying the area. The canal through which these blood vessels run, called the **haversion canal** (Figure 5), therefore, is all that remains of the original cavity. This process continues until about age 40, when the activity of osteoblasts slows and bones become more brittle. Let's look at some important factors that are necessary to produce healthy bone.

Bone development is influenced by a number of factors, including **nutrition, exposure to sunlight, hormonal secretions, and physical exercise**. For example, exposure of skin to the ultraviolet portion of sunlight is favorable to bone development, because the skin can produce **vitamin D** when it is exposed to such radiation. Vitamin D is necessary for the proper absorption of calcium in the small intestine. In the absence of this vitamin, calcium is poorly absorbed, the bone matrix is deficient in calcium, and the bones are likely to be deformed or very weak. **Vitamins A** and **C** also are needed for normal bone growth and development.

Hormones that affect bone growth and development include those secreted by the pituitary gland, thyroid gland, parathyroid glands, and the ovaries and testes (Figure 6). The pituitary gland, for instance, secretes **growth hormone (GH)**, also called **somatotropin**, which stimulates activity in the epiphyseal plates. This hormone is the main regulator of height. Somatotropin plays many roles in the body: it stimulates bone and muscle growth, maintains the normal rate of protein synthesis in all body cells, and speeds the release of fats as an energy source for growth. Other hormones play a part in maintaining the strength and health of the bone matrix by functioning to **control the level of blood calcium**. In fact, calcium is needed for a number of metabolic processes other than for bone formation, including blood clot formation, nerve impulse conduction, and muscle cell contraction. When a low blood calcium condition exists, the **parathyroid glands** respond by releasing **parathyroid hormone (PTH)**. This hormone stimulates **osteoclasts to break down bone *tissue*** , and as a result, calcium salts are released into the blood. On the other hand, if the blood calcium level is excessively high, the **thyroid gland** responds by releasing a hormone called **calcitonin**. Its effect is opposite that of parathyroid hormone; **it inhibits osteoclast activity allowing osteoblasts to form bone tissue**. As a result, the excessive calcium is stored in bone matrix. The actions of these hormones are both excellent examples of some important **negative feedback loops** present in our bodies (Figure 7). Without adequate supplies of these important chemicals, the bones will not develop or grow normally (figure 22).

Figure 6. Certain endocrine glands secrete hormones that are important.



Women, especially if slim, are more prone to bone loss after **menopause** (when women stop menstruation, age 45-60). Race is another factor that affects ones tendency to lose bone. White and Asian women are especially vunerable to a bone-thinning disease called **osteoporosis** (which means "porous bone"), which strikes one in four American women over 60. Usually, in osteoporosis, the **osteoblastic activity in the bone is less than normal**, and consequent the rate of bone deposition is reduced. This results in brittle bones in the elderly, and therefore, their bones are more susceptible to fracture.

Older people may also develop a hump in the upper back, often referred to as a **dowager's hump**. Some women can lose as much as **one-third of their skeletal structure by age 75**. This is twice the rate of bone loss in older men, who have about 30% more bone mass to start with. **Osteoporosis afflicts more people than any other bone disease.** There are many causes of osteoporosis:

- the lack of physical stress on the bones because of less activity;
- malnutrition, including not enough calcium in the diet, to the extent that sufficient bone matrix cannot be formed;
- lack of vitamin C, which is necessary for osteoblasts to create healthy bone matrix;
- the lack of **estrogen** secretion, which occurs in women after menopause (estrogen is also important to stimulate osteoblast activity); end,
- old age, when the body simply cannot form sufficient bone matrix.

In order to prevent the development of osteoporosis, **extra calcium** is needed in the diet, **exercise** can help by stimulating the formation of new bone, and **estrogen therapy** (estrogen is an important ovarian sex hormone in females) can curb bone loss in women after menopause. Let's briefly discuss exercise and how "loading" the bones helps them to stay strong.

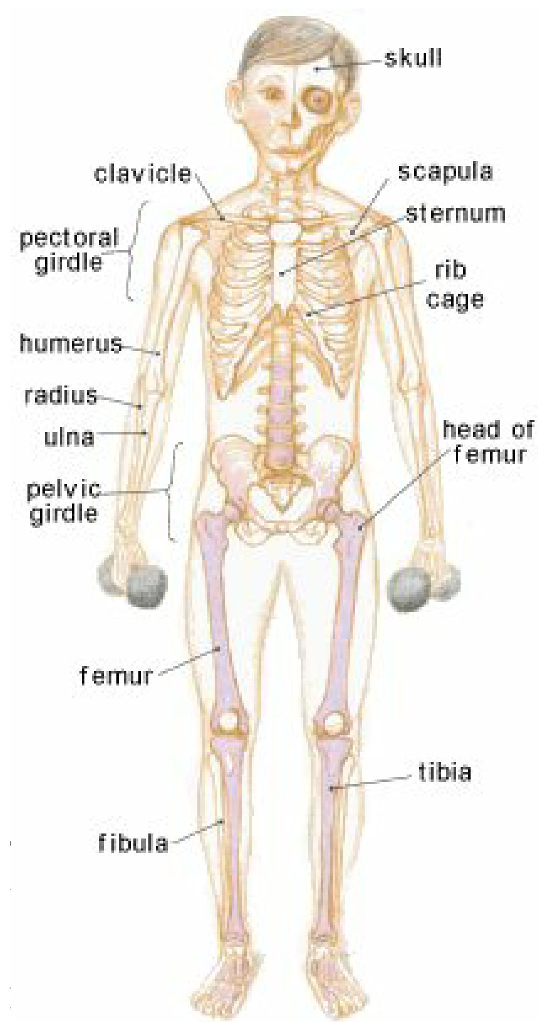
Bone is deposited **in proportion to the compressional load** that the bone must carry. For instance, the bones of athletes become considerably heavier than those of nonathletes. Also, if a person has one leg in a cast but continues to walk on the opposite leg, the bone of the leg in the cast becomes thin and as much as 30% decalcified within a few weeks, while the opposite bone remains thick and normally calcified. Therefore, continual physical stress stimulates calcification and osteoblastic deposition of bone, producing stronger bones. Now that we are familiar with what bone is and how it is formed, let's examine how bones serve to support, protect, and move our bodies.

Bone Function

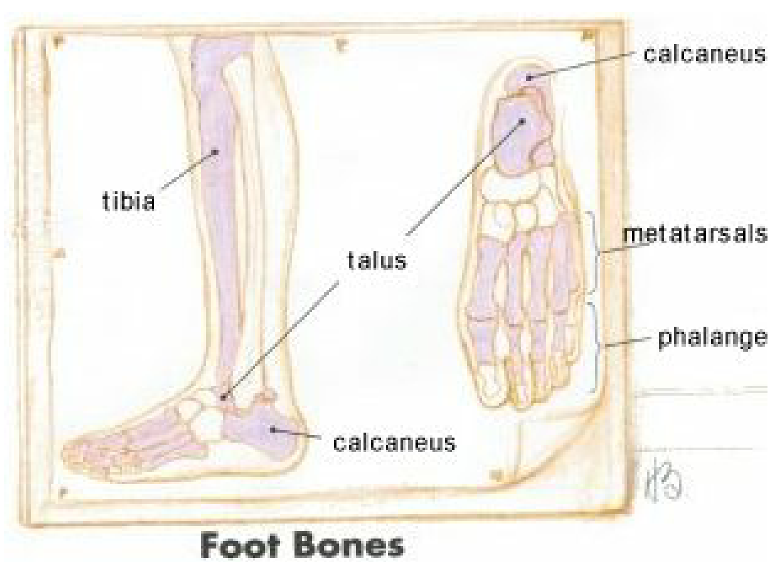
As mentioned previously, bones are generally classified into four types according to shape: **long, short, flat,** or **irregular**. They range in size from the all-powerful leg bone the **femur-** about 20 inches long, more than an inch across at midshaft to the **pisiform**, the smallest of the wrist bones, shaped like a split pea; this bone lies at the base of the little finger, familiarly known as the pinkie. But whatever their size or shape, almost every bone in the body is designed to fit a particular need. The most notable exception is the coccyx, our tailbone.

The **arrangement of the individual bones** is as precise, orderly and purposeful as the full skeletal system itself, and their distribution from top to bottom is extremely **balanced**. Most of the bones in our body are structured in a **symmetrical** fashion. That is, many of our bones are **matched** on each side of the body. This matched design allows us to balance and stabilize ourselves in the face of the various forces that act on our bodies. Although we will be discussing only the skeletal system, keep in mind that the sensory and balance organs of the nervous system, the muscles, **and the bones work** together to help achieve this stability. Let's start at the top and work our way down the major parts of the skeleton!

Figure 8. The anti-griavity bones.

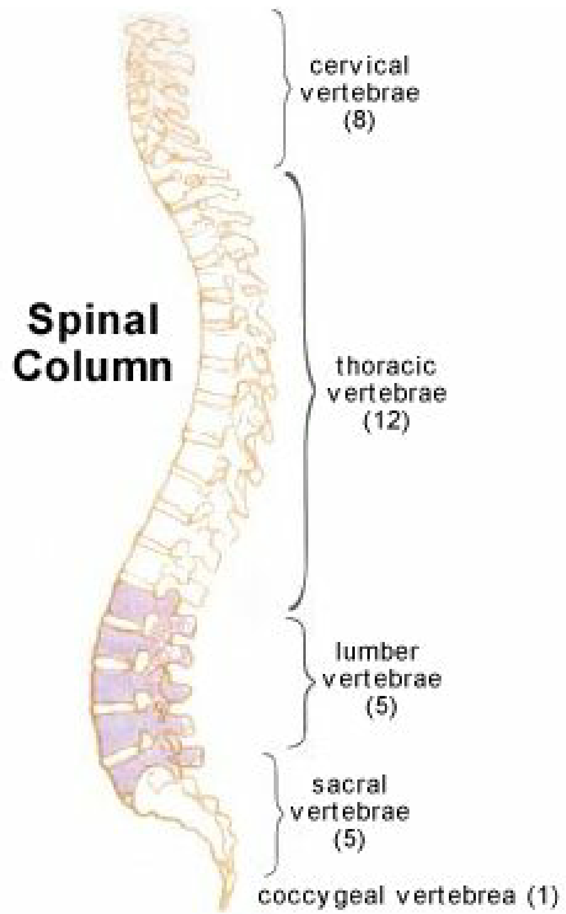


The **skull**, the "top" of the skeletal system, has 29 bones that are fused together to form the **cranium**, or brain case, the face, and the ear bones. The only part of the skull that can move freely is the **jawbone**. The spine, to which are attached the **pectoral (shoulder) girdle, rib cage** and the **pelvic (hip) girdle**, has 26 **vertebrae**. The ribs number 24, 12 on each side. The two girdles, so named because of their shape, mark the upper and lower limits of the body's trunk, or central area. From them, respectively, stem the bones of the upper and lower limbs, the arms and the legs, respectively. **Each limb** has 30 bones apiece. Of the 60 bones in the two upper limbs, all but 6 are concentrated in the hands and wrists; of the 60 bones in the 2 lower limbs, all but 8 are concentrated in the ankles and feet. Thus, appropriately, more than half of all the bones in the body support those parts of our bodies that maintain the busiest daily work schedule - our extremities.



the **kinds** of daily work that the upper extremities perform compared with the lower extremities. The skeletal components of the lower extremities are primarily involved in opposing gravity. They are considered our **anti-gravity bones** (Figure 8). The **femur**, for example, must withstand great weight and pressure. Its shaft is shaped like a hollow cylinder - an excellent design, as any engineer knows, for **maximum strength with a minimum of material.** Because of this special construction, the thigh bone can take **enormous pressures**, depending upon the weight of the person and the activity at the moment. In a 125-pound woman who is simply taking a walk, for example, some points of the femur withstand a pressure of 1200 pounds per square inch (psi). If, by some whim, she were suddenly to start jumping, this bone would be equally capable of resisting the far greater stresses involved.

The femur is not, by any means, the only anti-gravity bone in our bodies but it is the largest. The **spine, pelvic girdle, tibia (lower leg),** and the **bones of the foot** (particularly, the **talus** and **calcaneus** bones) are all important in our day-to-day "struggle" to stand and move against gravity.

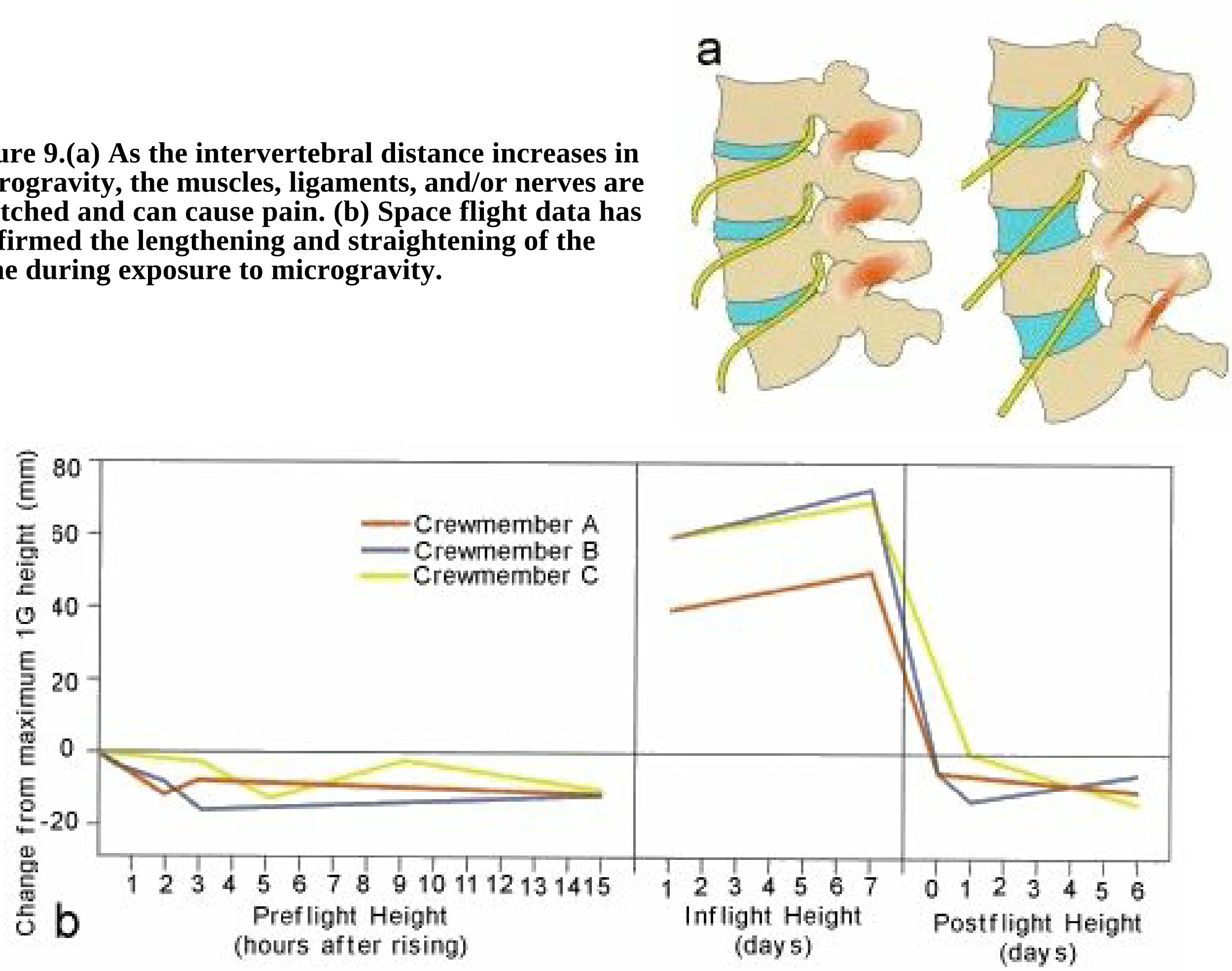


Another superb example of how each bone was designed with a purpose is the **vertebrae** of the spinal column. To help bear the weight of the body, it is formed like a solid cylinder, but it actually consists of alternating layers of bone and cartilage. These compressible cartilage disks between the vertebrae absorb shock and keep the vertebrae from grinding together when the spine bends. At the back of the bony cylinder, a ring permits passage of the **spinal nerve cord**, and also serves to protect it. At the back of the ring are three sharp projections, or **spurs**, which join with the ribs and anchor the muscles of the back. The flexibility of the spine and its ability to stretch and compress contribute to the actual height changes that occur to all of us during the day. In fact, when you have reached the exciting time in your life when you have begun to drive a car, notice how you must adjust your rear-view mirror upwards in the morning and then read just it downwards in the evening. This happens because, after a full day of fighting off gravity, you actually shrink in size! Let's discuss how the absence of gravity might affect your height as well.

After about age 25, a person's height can go only one way - and that is **down**. A man or woman might lose an eighth of an inch between ages 25 and 40 as the spongy disks between the vertebrae in the spine shrink, causing the bones to move closer together. The back begins to bend forward after age 40. From age 20 to age 70, a woman may shrink about 2 inches, while a man might lose about an inch. In space, however, **there is a height increase** as the human vertebral column lengthens and straightens, probably because gravity does not compress the body. In fact, on past U.S. space flights, more than two-thirds of astronauts reported back pain. This back pain may be associated with the stretching of the spine. On previous space missions, spinal measurements were performed and the astronauts were found to **increase their height from 6 to 8 cm above their greatest early morning height here on Earth** (Figure 9), and there was a flattening of the normal spinal curve.

Space flight also causes bone loss presumably because astronauts are not required to stand and support themselves to create "loading forces" on the bones. This bone loss could be a limiting factor for long-duration missions, such as a Mars expedition or extended stays on a space station. Before effective **countermeasures** can be devised, a thorough knowledge of **how much** bone loss occurs, **which bones** are affected, and **how fast** the bone loss appears during weightlessness is needed from actual space flight data and from Earth-based models that simulate the **disuse** (lack of use) of bones. We are going to examine a space flight investigation designed by Dr. Emily Morey-Holton and her team from across the United States that uses laboratory rats to study the effects of space flight on bone formation and calcium metabolism. This results of this and other related investigations can offer some important clues and strategies about how to cope with some of the debilitating bone problems that exist here on Earth, such as osteoporosis. Let's get started!

Figure 9.(a) As the intervertebral distance increases in microgravity, the muscles, ligaments, and/or nerves are stretched and can cause pain. (b) Space flight data has confirmed the lengthening and straightening of the spine during exposure to microgravity.



SPACE PHYSIOLOGY

Earth, Mars, and outer space all differ in their gravitational characteristics. Of course, we are all familiar with the force of gravity here on Earth, and our bodies are well adapted to moving around in this kind of environment. Even though humans have never set foot on Mars (although we hear periodic stories about Martians who live there), we know from remote experiments that the gravitational force there is equivalent to about 38% of Earth's gravity. The gravitational force on Mars **would** require that we support our bodies to stand, walk, and run. But, of course, our weight would be lower on Mars and this means that our musculoskeletal system would not experience the same loading characteristics that it does on Earth (Figure 10). Presumably, then, our bones and muscles would become weaker on Mars if we did the same kind of things there everday that we do here on Earth.

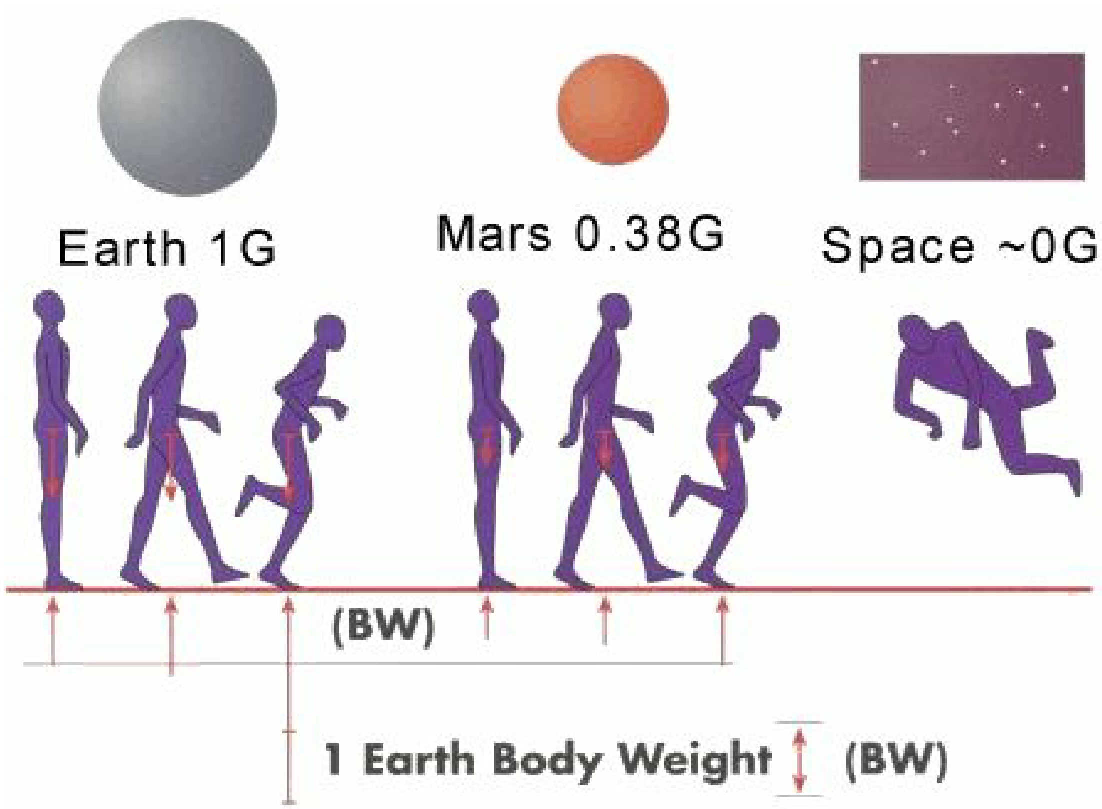


Figure 10. Earth, Mars, and outer space differ in the external forces that each exerts on the body.

In the microgravity of **outer space**, the musculoskeletal system is used even less intensively and in a different way than it is on either Mars or Earth. The absence of gravitational force results in changes to load-bearing tissues, causing a reduction of bone and muscle. We know this from previous studies that have been done on long-duration missions such as Skylab (28-, 59-, and 84-day missions) and from research carried out on very long missions aboard the various Soviet/Russian space stations (missions lasting up to a year). Astronauts in space "float" from place to place instead of using their legs to walk around. In addition, the metabolic state of the musculoskeletal system may be altered by changes in dietary intake and exercise levels, and also by the space motion sickness that many people who go into space experience at the beginning of their trip. The physiological changes that occur within the astronauts while in space, however, are **appropriate** for space flight. Remember, the body responds to the environment that it finds itself in, so in space, the body establishes a **space-normal** condition. **Any problems that occur only appear when the astronauts return to Earth.**

The muscle and bone losses, primarily from weight-bearing tissues, contribute to a reduced fitness of astronauts when they return to Earth. And as you know by now, changes in other body systems have occurred as well. In previous chapters, we have examined how the cardiovascular system, blood, kidney and endocrine, and muscle systems have been affected by space flight. In this chapter, we will focus on the effects of space flight on the skeletal system.

Once the astronaut returns home, the body will respond again to the "new" environment of Earth. The body's goal is to reach an **Earth-normal** condition as soon as possible. Therefore, in order to study how the body has changed while in space, it is essential that experiments be performed **as early as possible** after the shuttle has landed. In the case of bone, the **physical changes** that take place begin to recover slowly. However, the **chemical** changes that have taken place probably begin to reverse themselves immediately upon return to Earth.

There are two main reasons, then, that laboratory rats offer researchers a valuable opportunity to study bone changes that have occurred either here on Earth or in space. First, the bones of the animals are **accessible**. That is, the bones can be removed from the rats and studied directly. Secondly, the rats are small enough that their bones react much more quickly to environmental changes. Their bones grow much more rapidly than human bones and, therefore, the bones also **change** more rapidly. This occurs because rats have a much shorter lifespan than humans and all of thier life processes, including bone formation, are accelerated. In particular, this becomes very important if we want to study bone changes that have occurred on short space missions. In contrast, the bones of astronauts are not accessible directly nor will they change as dramatically as the rat bones will. The use of laboratory rats, then, magnifies the view that researchers can obtain about how the bones are affected by changes in external forces.

Another advantage to using laboratory rats, instead of larger animals like monkeys, for the study of bone changes is that they can be **housed in different kinds of cages on the same space mission**, thus allowing one to see how cage configuration (rather than space flight itself) might affect their bone growth. For instance, Dr. Holton was interested in determining how bone changes in space would be influenced by **group housing** (five rats to a cage) as compared with **individual housing** (one rat per cage). The results of such a study could be important in understanding how to provide the best and most natural conditions possible for rats used for research purposes, and in determining that the **pure effect** of space flight on the animal's bones was being measured.

Dr. Holton carried out her bone study using laboratory rats on two different space missions. Her primary hypotheses that served as the basis for her experimental design included:

Hypothesis 1

> Gravity is necessary for normal development of bone structure and decreased gravity or skeletal unloading causes defective skeletal growth.

Hypothesis 2

> The response of bone to space flight will be localized and will differ not only from bone to bone, but also at different sites within the same bone.

Hypothesis 3

> The type of housing (group vs. individual) will influence the bone response to space flight and the recovery from space flight.

Before we begin our examination of Dr. Holton's space flight results, let's participate in two "Student Investigations" designed to clarify certain important concepts from this chapter. These activities will prepare you to understand more about bone structure and strength, and besides, they should be fun!

Your Perspective

For this section, you are going to participate in some activities that were designed to clarify certain important concepts related to bone structure and strength. Student Investigation 1 involves an activity that will demonstrate how bone growth characteristics in our limbs are related to our overall skeletal structure, particularly our height. You will be asked to measure the upper arm bone and the heights of a number of different students. From this data you will participate in a graphing exercise that will demonstrate for you how the length of the bone in the arm is related to a person's height.

Student Investigation 2 involves an activity that will help you understand how different materials compare in their strength characteristics. We will examine how the **size** (or cross-sectional areas) and the **kind** of materials influence how well they can stand up against:

- compressional (squeezing) forces,
- tensile (stretching) forces,
- torsional (twisting) forces.

For this activity, you will be responsible for **predicting** how various materials will respond to these external forces and then you will **design** and **perform** the actual experiment following certain guidelines. Finally, from the results, you will develop an understanding about how bones from people of different ages and sizes (height and overall body size) compare in strength. Let's get started!

Student Investigation 1

Predicting Height from the Length of Limb Bones

Background

The formation of bone by the activity of **osteoblasts** and the addition of **minerals** and **salts** is known as the process of **ossification**. This process begins in the fetus before birth and continues to some extent until about the age of 25. Although **every person's bone development schedule is unique to that individual** (just as the schedule at which they begin to walk or when they begin to talk is very individual and unique), a general timetable, Table 1, has been developed that indicates the various ages at which certain bones for the "average" person will complete ossification.

As you can see, during your high school years, the bones of the upper limbs and shoulder area are completing ossification. Also, by the ages of about 18 to 23, the bones of the lower limbs have been fully formed. In the average person, once ossification of the upper and lower limbs is complete, the size of the two major arm bones (the **humerus** and the **radius**) as well as the size of the two major leg bones (the **femur** and the **tibia**) have grown to a length that is **proportional to the person's height**. In fact, so precise is the relationship between these various bones and height that anthropologists and forensic scientists, with one dried bone as a clue, can closely estimate its owner's former living height.

Table I. Ossification timetable in humans.

Ossification Timetable

Third month of embryonic development	Ossification in long bones beginning
Fourth month	Most primary ossification centers have appeared in thediaphyses of bone.
Birth to 5 years	Secondary ossification centers appear in the epiphyses
5 years to 12 years in females, or 5 to 14 years in males	Ossification is spreading rapidly from the ossifcatio centers and various bones are becoming ossified
17 to 20 years	Bone of upper limbs and scapulae becoming completely ossified
18 to 23 years	Bone of the lower limbs and os coxae become completely ossified
23 to 25 years	Bone of the sternum, clavicles, and bertebrae become completely ossified
By 25 years	Nearly all bones are completely ossified

For your information, an **anthropologist** is a person involved in studying humans in relation to their physical character, distribution, origin, racial background, social structure, and culture. Anthropologists are often involved with digging for and finding the remains of people who lived years and years ago. Certain kinds of remains, such as bones, can serve to provide information about the physical characteristics of these ancient people. A **forensic scientist** is a person who was trained to take evidence from crime scenes and determine from that evidence who may have been involved in the crime. Both of these groups know that certain bones serve as excellent sources of information to determine a person's height. Such a determination is accomplished using a set of standard equations that contain the scientific information gathered from hundreds of studies. For instance, if a 17.9 inch femur is found, this value is inserted into the following equation:

Height in inches = (1.880 x femur length) + 32.010

and the result is that the femur belonged to a person who had a height of five and a half feet, assuming this person was a male. If the person were a female, a different equation must be used:

Height in inches = (1.945 x femur length) + 28.679

and the result is that the femur belonged to a woman whose height was about five feet, four inches. Now, let's look at the two equations above.

Both follow a very important form that is equivalent to the **equation of a line**:

y = mx + b where: y = the **height** of the person,
m = the **slope** of a graph of the line,
x = the **length of the person's limb bone**, and
b = the **y-intercept** on a graph of the line.

Any equation of the form **y = mx + b** has a graph that is a straight line. The **y-intercept, b**, is the y value when x = 0. Thus, the point (O,**b**) lies on the graph of the straight line. The number **m** is called the slope, and it is an indication of how the line slants. The greater the slope, the **steeper** the line slants upward, from left to right. If the slope is negative, the line slants downward from left to right (Figure 11).

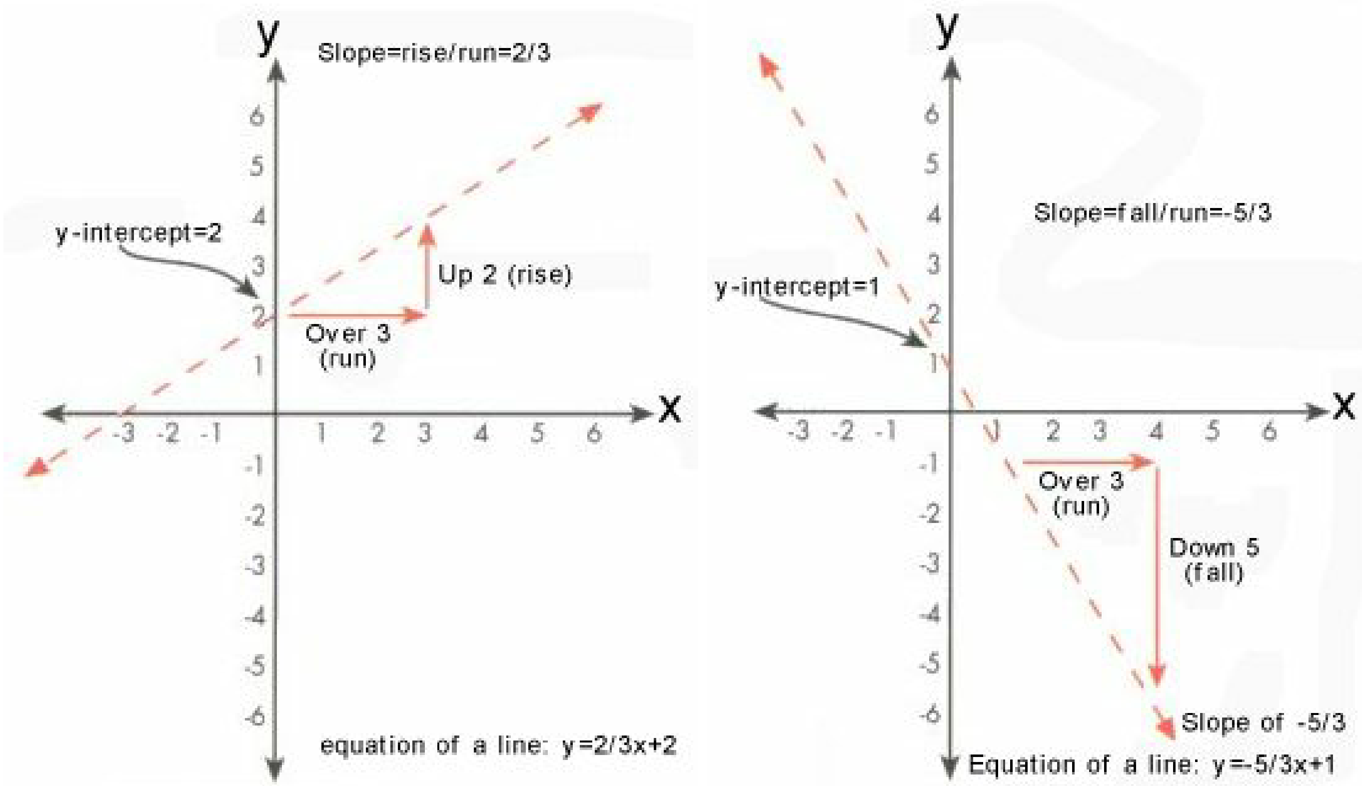


Figure 11. To determine the slope of a line on a graph, divide the number of vertical units by the number of horizontal units, or remember the relationship rive/run (for positive slopes) and fall/run (for negative slopes). The y-intercept is indicated on the graphs.

The fact that the length of the limb bones and its associated height is described by a linear equation such as this means that if you were tograph, for a group of different height individuals, the length of a particular limb bone along the x-axis and their height along the y-axis, **the points on the graph should fall generally along a straight line.** However, the slope of each line will always be positive.

You must be wondering why we have gone into detail about determining the equation of a line. Well, for this exercise, you will be doing just that. But that's not all. A small competition between groups is involved here! Let's begin.

Materials

Tape measures
Graph paper
Rulers

Procedure

Everyone should read all of the steps before beginning.

Step 1

Break into groups based on your teacher's instruction. For each person in the group, **two measurements will be taken**:

- the length of the upper arm limb (the humerus), and
- the height of each person.

For the measurement of the humerus, bend the subject's arm at the elbow and feel the **tuberosity**, or "knot," on the side of the elbow. This is essentially one of the ends of the humerus. Also, feel at the shoulder a similar "knot;" this should be the top of the humerus. Carefully measure the entire length of this bone. Then measure the subject's height very carefully. Record this data for each person before you begin the next step.

Step 2

The data for each person should include the two measurements. Graph the data with the length of the humerus along the x-axis and the subject's height along the y-axis. Once all of the data is graphed, draw a straight line along the data points and extend it so that your line intersects with the y-axis. This point will be the **y-intercept.** Next determine the **slope** of the line based on the model in Figure 11. Finally, determine the equation of your line.

Step 3

After each group has completed their data collection and has determined the equation for their lines, it will be time to determine which group came up with the **most accurate equation**. Select two or three "new" people from another group and switch them with two or three members of your own group. Select people of different heights. Each group will then measure the length of the humerus bone for each of the "new" persons and determine their height using two methods:

- first, **using your graphs**, your group will **estimate** height, and
- second, **using your line equations**, each group will **calculate** each new person's height.

Record this data. Then **measure** the person's height to see how closely your **graph and your line equation** were able to predict the height of these students. Which group came up with the most accurate graph? Which group came up with the most accurate line equation? They (there may be two groups) are the "winners!" The other groups will have to perform an adaptation of the very famous song "Dry Bones" to the rest of the class! The words to this song are shown at the end of this exercise.

Step 4

Based on your experiences, each group must answer the following questions.

Questions

1. What are the sources of error that might have been responsible for:
    - data points not lining up,
    - the graph not being accurate enough to estimate the "new" person's height, and
    - the equation not being accurate enough to calculate the "new" person's height.
2. What kind of sample population would yield the best set of data points and, therefore, the best line equation?
3. How will the age of the sample population affect your results? How old must your subjects be to determine height from the radius, the tibia, and the femur?

Dry Bones

`Adapted from the original African American Spiritual`

The foot bone connected to the leg bone,
The leg bone connected to the knee bone,
The knee bone connected to the hip bone,
The hip bone connected to the back bone,
The back bone connected to the shoulder bone,
The shoulder bone connected to the neck bone,
The neck bone connected to the jaw bone,

The jaw bone connected to the head bone,
Now hear us sing our song.
Them bones, them bones gonna walk around,
Them bones, them bones gonna walk around,
Them bones, them bones gonna walk around,
Now hear us sing our song.

Student Investigation 2

The Influence of Applied Forces on Different Materials

Background

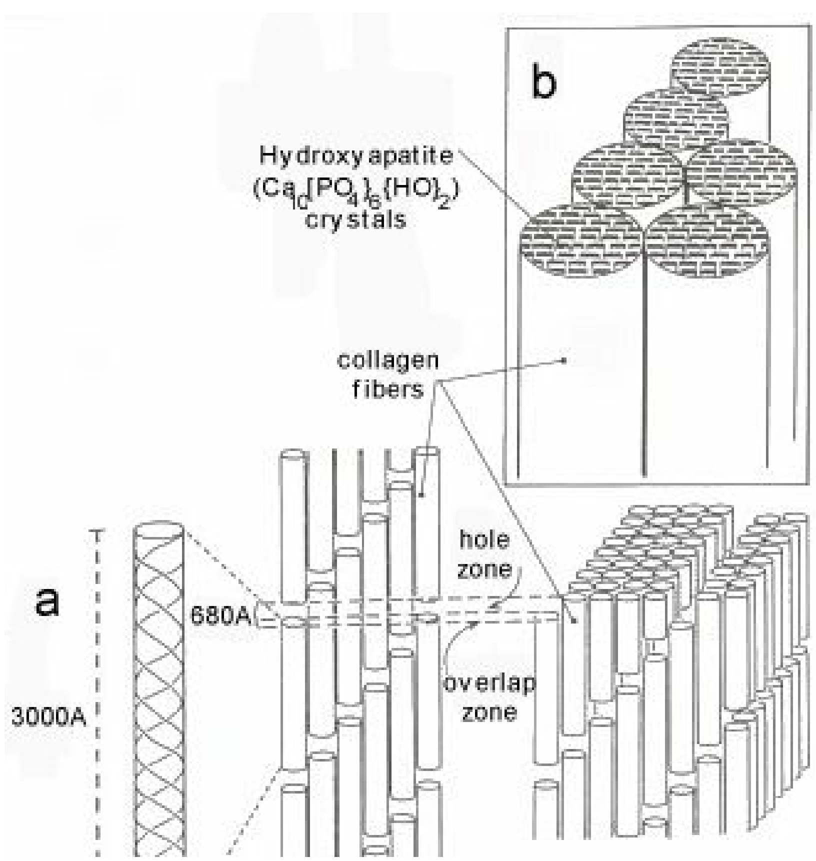
External forces applied to a solid object may compress, stretch, twist, or bend it out of shape. The ability of an object to return to its original form when the external forces are removed is called the **elasticity** of the solid. If too much deformation occurs, the object will not return to its original shape - its elastic limit has been exceeded. The study of the elastic properties of materials is an important area of **physics. This shouldn't scare you** because all of us have experience with bending, twisting, smashing, and stretching different materials almost every day of our lives. In fact, every time we walk or run, our bones are being "smashed" down to some extent constantly. And if any of you have ever broken a bone, you might have done so because the bone was twisted. In this exercise we will first learn a little about how different forces affect different materials and then we will actually **apply** different forces to things and **observe** how they are affected. That's right, **you will actually be designing an experiment** where you will be able to smash, bend, twist, stretch, and break different things, in a way that you will define! But let's begin with some background information to understand how different forces affect different materials.

Essentially all materials yield to some extent under the influence of applied forces, including bone. Ultimately, the change in shape or volume of a body when outside forces act on it is determined by the **strength** of the material. The strength of a material depends largely on three main factors:

- the **kind of material**, or what it is made of;
- the **physical characteristics** of the material receiving the force, including **cross sectional area, geometry (shape)**, and **density (how close together the molecules are)**; and,
- the **molecular forces holding the material together** (either electrochemical forces, or physical binding forces).Of course, the ability of the material to withstand forces also depends on the **kinds of forces** that are being applied.

Let's examine the electrochemical and binding nature of the molecular forces in bone. **Compact bone** is composed of repeating segments of **collagen fibers** that appear every 680 Angstroms (the angstrom (A) is a unit of length and is equal to 10 cm) along its length; **hydroxyapatite crystals** lie **within** to each segment of the fiber, bound tightly to it (Figure 12). This intimate bonding prevents **shear** in the bone; that is, it prevents the crystals and collagen fibers from slipping out of place. Such stability is essential in providing strength to the bone. In addition, the segments of adjacent collagen fibers **overlap each other**, also causing hydroxyapatite crystals to be overlapped like bricks keyed to each other in a brick wall. This produces a very orderly 3-dimensional collagen/crystal composite. The hydroxyapatite crystals ($Ca_{10}[PO_4]_6[OH]_2$) themselves contain electrochemical forces that keep the **calcium, phosphate,** and **hydroxide** molecules together in the right combination.

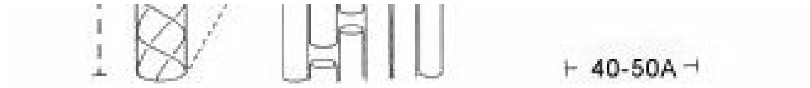
Figure 12.



As mentioned earlier in the chapter:

- collagen fibers of bone have great **tensile strength** (the strength to

endure being pulled apart);
- calcium salts have great **compressional strength** (the strength to endure being squeezed).



These combined properties, plus the degree of bonding between the collagen fibers and the crystals, provide a bony structure that has both extreme tensile and compressional strength. In fact, bones are constructed in exactly the same way that reinforced concrete is constructed. The steel of reinforced concrete provides the tensile strength, while the cement, sand, and rock provide the compressional strength. Bone, on a weight basis, is stronger than concrete.

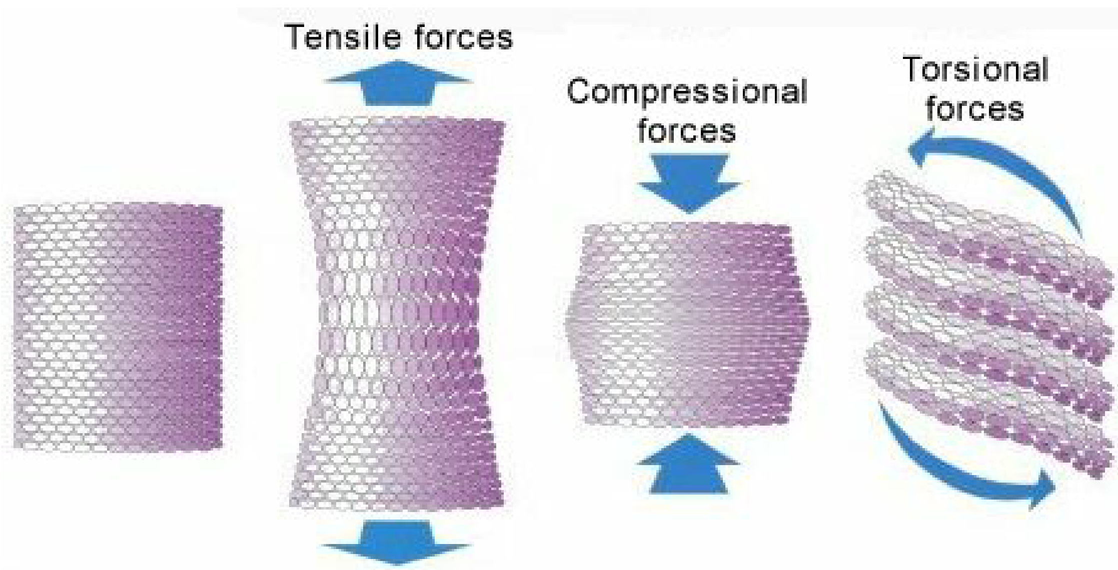


Figure 13. Uniform compressional and tensile forces applied to materials that we understand create predictable stresses. On the other hand, torsional forces are much more difficult to control and Often create more damage.

The **direction and magnitude of applied forces** will also influence how well a material can withstand those forces. **Uniform forces** are those that are applied evenly to a material, while **non-uniform** forces are those that are applied unevenly and can create the most damage. A uniform compressional force and a uniform tensile force are shown in Figure 13. These forces create **predictable stresses** on materials that are familiar to us. If we are familiar with a material, this means that the molecular forces within the material and the nature of the material is known. Normal human bone is fairly well understood, but a number of factors (e.g., age, gender, state of health including the presence of osteoporosis, etc.) can alter the molecular strength of a bone. Therefore, it is often difficult to determine how any particular bone that is not **normal** will behave under great stress.

The other force that is shown in Figure 13 is the torsional, or twisting force. It is much more difficult to predict how a material will respond to this kind of force because it is very difficult to produce a **uniform** twisting effect. Therefore, even with their great compressional and tensile strengths, neither bone nor concrete has a very high level of **torsional strength** (the strength to endure being twisted).

The term **strain** refers to the relative change in dimensions or shape of a material which is subjected to stress. That is, strain is the amount of deformation that occurs to a material under stress. Associated with each type of stress, or force, is a corresponding **strain**. **If the stress or applied forces are uniform, the strain on a material can be calculated**. In order to define tensile and compressional strains, let's consider an example where uniform stresses are being applied to a one-dimensional metal bar (Figure 14).

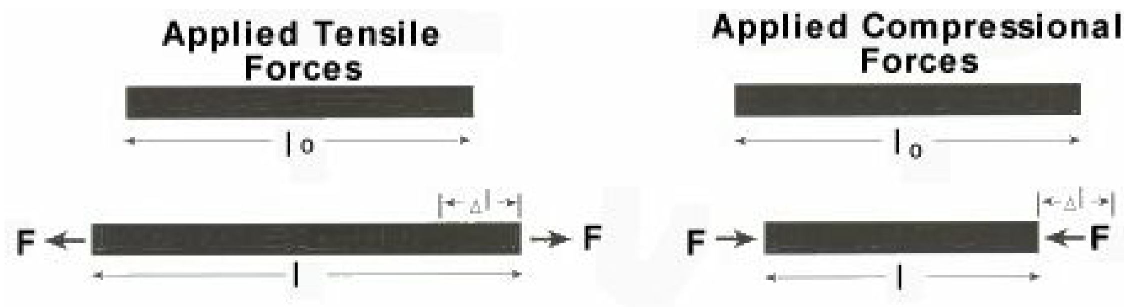


Figure 14. (a) A metal bar increases in length due to uniform tensile stress. (b) The bar decreases in length due to uniform compressional stress.

- The **tensile strain** on a body is defined as the ratio of the **increase in length** of the body to the **original length**.

$$\text{Tensile strain} = \frac{l - l_o}{l_o}$$

- The **compressional strain** of a body is defined as the ratio of the **decrease in length** of the body to the **original length**.

$$\text{Compressional strain} = \frac{l_o - l}{l_o}$$

We will not define **torsional strain** because twisting is more complicated to represent in a simple way, and our one-dimensional example of a metal bar is not an appropriate model to demonstrate torsional stress and strain. You have probably experimented with such forces without even knowing it by twisting an empty soda can, producing a torsional strain on the can.

Finally, when any stress is plotted on a graph against the resulting strain for a material, the resulting **stress-strain diagram** is found to have several different shapes, depending on the kind of material. As an example of a stress-strain diagram, Figure 15 illustrates the behavior of a particular metal when subjected to increasing tensile (stretching) stress. Let's examine the different sections of the graph.

(1) During the first portion of the curve (up to a strain of less than 1%), the stress and strain are **proportional**. This holds until the point a, the **proportional limit**, is reached. We know stress and strain are proportional because this segment of the line is straight. The fact that there is a region in which stress and strain are proportional is called **Hooke's Law,** named after a physicist named Robert Hooke (1635-1703). The ratio of stress to strain, or the stress per unit strain, is called an **elastic modulus** or **Young's modulus**. This relationship can be written as:

$$\text{Young's modulus (Y)} = \frac{\text{Stress}}{\text{Strain}}$$

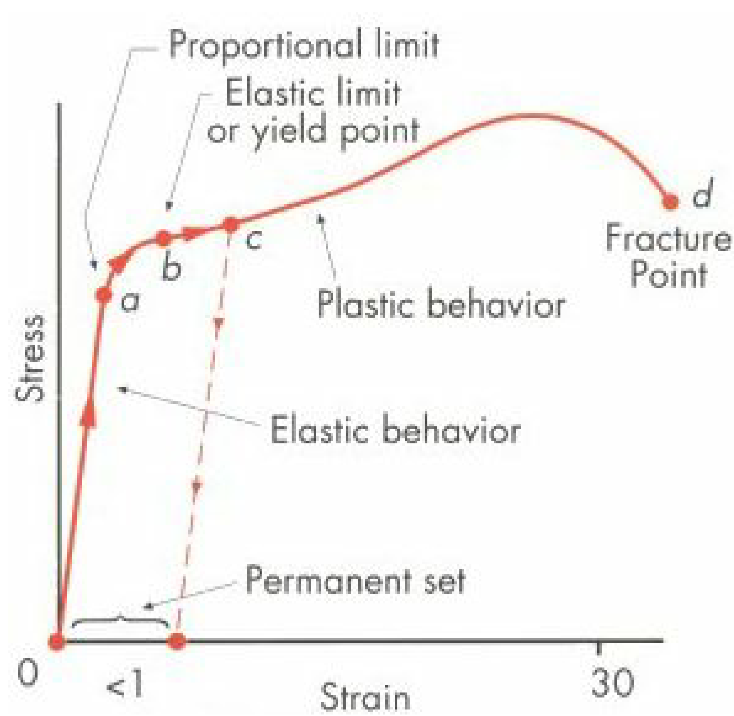


Figure 15. A typical stress-strain diagram for a ductile metal undergoing tension.

and is essentially the **slope** of the straight line on the stress-strain diagram. Every material has a unique Young's modulus value. That is, the stress required to produce a given strain depends on the nature of the material under stress. **The larger the Young's (electric) modulus for a material, the greater stress needed for a given strain**. That is, the greater the Young's modulus for a material, the better it can withstand greater forces.

(2) From a to b on the diagram, stress and strain are **not proportional,** but nevertheless, if the stress is removed at any point between O and b, the curve will be retraced in the opposite direction and the material will return to its **original shape and length**. In other words, the material will spring back into shape in a reverse order to the way it sprung out of shape to begin with. In the region Ob, then, the material is said to be elastic or to exhibit elastic behavior and the point b is called the **elastic limit**.

(3) If the material is stressed further, the strain increases rapidly, but when the stress is removed at some point beyond b, say c, the material does not come back to its original shape or length but returns along a different path to a different point, shown along the dashed line in Figure 15. The length of the material at zero stress is now greater than the original length and the material is said to have a permanent set.

(4) Further increase of stress beyond c produces a large increase in strain until point d is reached at which **fracture** takes place. From b to d, the metal is said to undergo **plastic deformation**. If large plastic deformation takes place between the elastic limit and the fracture point, the metal is said to be **ductile**. Such materials are capable of being drawn out like a wire or hammered thin like gold leaf. If, however, fracture occurs soon after the elastic limit is passed, the metal is said to be **brittle**.

In this section, you have been exposed to many new terms as we've reviewed different points related to the strength and elasticity of materials. Now it is time to **apply this knowledge** in a demonstration of how various kinds of stresses, or forces, will affect various kinds of materials. Your entire class will design the experiment in a way that will demonstrate many of the concepts described in this section. Then your class will be broken into small groups and, within each group, you will perform the experiment. Follow the guidelines for this activity that are provided by your teacher as well as the steps provided in the "Procedure" section below. And above all, **read everything before you begin**.

Materials

Various materials differing in:

- kinds of material,
- cross-sectional area,
- density, and
- geometry (shape).

Various instruments to produce forces such as:

- small household hammer,
- large rubber hammer,
- mortar and pestle,
- some kind of a stretching mechanism.

Procedure

Step 1

Before breaking into groups based on your teacher's direction, discuss as a class the different kinds of materials that you plan to use in your demonstration. The class should select items that will demonstrate three different levels of response (no response, partial response, and total fracture) to: (1) a compressional stress, (2) a tensile stress, and (3) a torsional stress. Think of things that would represent a variety of kinds of materials. And then think of things that vary in **cross-sectional area, shape, density, and elasticity**. Each student should make a list of these items, puffing them in categories similar to that shown in Table 2.

Your teacher will provide you with a separate copy of Table 2 for you to write on. Work with your teacher to help collect enough of each item for each group.

Step 2

Break into your groups and assign one or two students as the "stressors." These are the individuals who will be applying the forces to the various materials. **Before they begin to apply forces** to the first item, allow the other members of the group to predict, or rate, how the material will respond. Then the stressors can begin their demonstration, using either a compressional, tensile, or torsional stress. The stresses can be applied in a uniform or non- uniform manner to compare the responses of the various materials. Each student in the group, including the stressors, should make notes about each demonstration regarding the following points:

- the kind of material something is made of;
- the physical characteristics of the material (density, cross-sectional area, and geometry);
- the forces between the molecules in the material;
- the kind of forces that are applied; and
- the direction, relative magnitude, and uniformity of the applied forces.

Finally, it is important for your class to come up with a rating system so that you can rate each material in terms of its:

- elasticity;
- plasticity;
- fracture point, indicating how brittle the material is.

Step 3

Each student should write a report that includes his/her comments and observations about each material and each demonstration. Include at the end of this report the answers (in complete sentences) to the following questions:

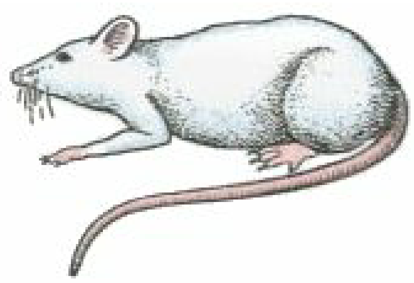
1. Which material that you tested was the **most elastic** and which was the **least elastic** of all the materials? What is the main difference between the two materials that makes them respond differently?
2. Which material that you tested was the **most brittle** and which was the **least brittle** of all the materials? What is the main difference between the two materials that makes them respond differently?
3. Do your answers to Questions 1 and 2 seem similar? Explain.
4. Draw a hypothetical stress/strain diagram comparing the two materials mentioned in Question 1.
5. Which material that you tested performed most similarly to how you understand a human bone would perform? What physical properties do the bone and this material have in common?

Well, we are finished with our "Student Investigations." Now that you more fully understand the concepts of bone growth and bone strength, let's move on to examine Dr. Holton's examination of how space flight affects these two bone properties.

Table 2. Example table of the physical characteristics of materials.

Demonstration	1	2	3	4	5	Test at least 10 materials
Kind of Material						
Cross-sectional area (large-med-small)						
Shape (regular-irregular)						
Density (high-med-low)						
Elasticity (high-med-low)						
Brittleness (high-med-low)						
Applied Force (compressional, tensile, torsional)						
Degree of Aplied force (strong-med-light)						
Comments						

THE SPACE FLIGHT INVESTIGATION



When bones develop and grow on Earth in the presence of gravity, they normally increase simultaneously in length, diameter, and mass. These three growth characteristics contribute to the strength of the bone. During space flight, in the absence of gravity, animal studies have shown that certain bones appear to grow in **length** at about the same rate as on Earth, but that the **diameter** of the bone is slightly smaller. In addition, the structure or "architecture" of the bone formed in space is different from that of animals left on Earth. Thus, for laboratory rats that have flown in space, **strength does not increase proportionally to the increase in bone size as it does on Earth**. In this section, we will discuss some of the actual results that Dr. Holton obtained with laboratory rats, primarily through **postflight** analyses that compared flight animals with control animals that remained on Earth.

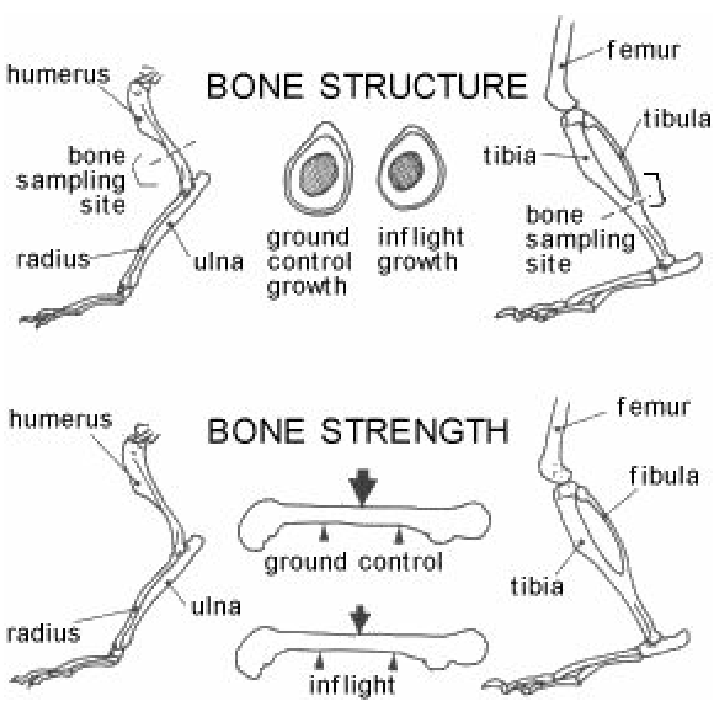
Ultimately, we would like to be able to apply the results of such animal studies to humans. after all, one of the main reasons to carry out such animal experiments is to be able to translate and adapt the results enable us to better understand the physical and chemical changes that have been observed in the astronauts.

Bone mineral loss in astronauts has been documented in most early human space flights. Changes in calcium balance, decreased bone density, and inhibition of bone formation have also been reported. Data from Soviet/Russian flights suggests that:

- **dicephyseal bone** (bone in the shaft of the bone) formation may stop during weightlessness;
- the **rate of elongation** of long bones in the body is not affected by weightlessness, but
- the **rate of circumferential growth (diameter)** is decreased. In addition to the direct observations of the physical changes in bone growth, increased urinary calcium excretion has been observed in astronauts in Skylab and other flights.

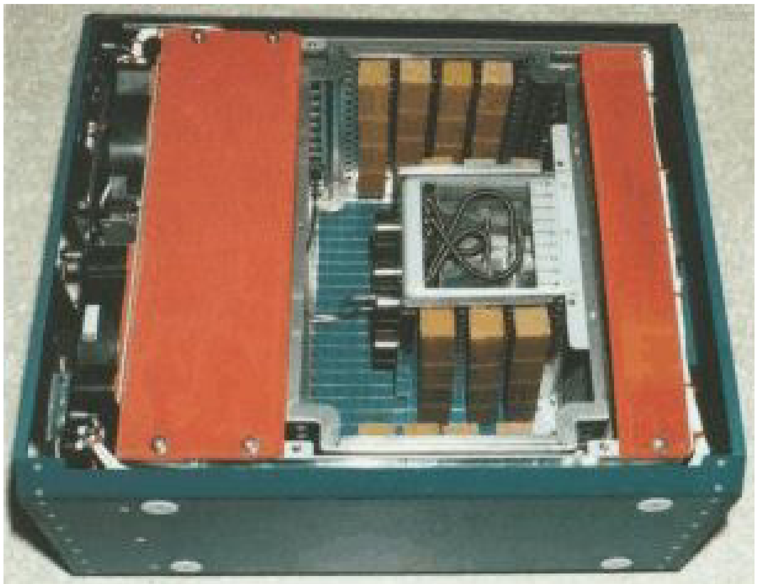
Dr. Holton's bone experiment was designed to characterizethe effects of microgravity on the structure and strength of certain **fore limb** and **hind limb bones** of the rat (Figure 16). For this experiment, **specific pathogen-free** (free of disease) male rats were used. These animals were raised and certified as disease-free so that they could be studied without any concern that the results were due to the presence of certain viruses or bacteria. Using such pathogen-free animals also safeguards the health of the humans who fly in space with the animals. All animals were given food and water ***ad libitum*** , or "anytime they wanted," throughout the experiment.

Figure 16. During space flight, changes in structure and strength have been noted in both the fore limbs and hind limbs of growing male rats.



The rats were subjected to a rhythm of normal day and night light cycles to mimic a "normal" environment and to match the cycles that the control animals would be exposed to on Earth. In fact, they were exposed to 12 hours of light, during times that generally matched the light cycle of Florida (9:00 a.m. to 9:00 p.m. Eastern Standard Time), their location while being prepared for launch at Kennedy Space Center. During the mission, the control animals were also kept on the same schedule. Keeping their "body rhythms" stable in this fashion was important to assure that the results of Dr. Holton's study would not reflect the stresses of changing body rhythms.

Figure 17. The Animal Enclosure Module.



The animals were either housed in a group fashion with five rats to a cage in a facility called the **Animal Enclosure Module (AEM)**, (Figure 17), or individually with one rat per cage in a facility called the **Research Animal Holding Facility (RAHF)**, which we have already discussed in the previous chapter. The rats were randomly divided into groups 13 days before launch (L-13). The various groups included:

- **A**nimal **E**nclosure **M**odule **c**ontrol animals (AEM-C);
- **A**nimal **E**nclosure **M**odule **f**light animals (AEM-F);
- singly housed (in a rat cage called a **VIV**arium) **c**ontrol animals (VIV-C);
- **R**esearch **A**nimal **h**olding **F**acility **F**light animals (RAHF-F);
- preflight control animals that were sacrificed at the beginning of the mission to establish baseline data for certain measurements.

After a nine-day mission, the AEM rats were removed from the shuttle within two hours of landing and the RAHF animals were removed within four hours. Half the animals in each group were sacrificed by decapitation as soon as they were received after landing (R+0). Their bones were processed within 20 minutes of decapitation and all Right rats were processed within six and a half hours after Right. Rapid processing of the Right animals (and their controls) was important to assure that the rats did not have much chance to begin readapting to the Earth's environment, thereby altering their **"space-normal"** condition. The remaining half of the animals were allowed to recover for nine days (R+ML, or Recovery + Mission Length) in order to understand how their bones readapt to an **"Earth-normal"** condition.

We will be examining the results of only a subset of Dr. Holton's various measurement sets that were performed on rats, including:

- bone length, circumference, mass, and strength measurements; and
- changes in calcium concentration and mineralization rates of bones.

So let's review some of the actual data from Dr. Holton's experiment to see how rat bones changed during space flight and to see if the type of housing arrangement for the rats might have influenced how their bones developed.

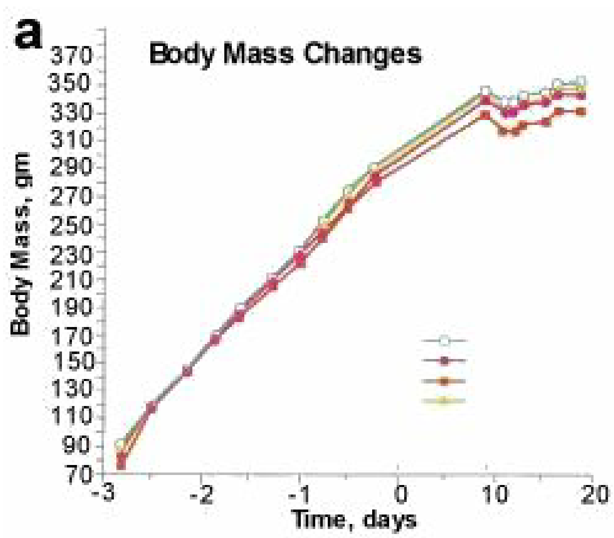
I. Measurement of Total Body Mass as well as Bone Length and Bone Mass in the Rat

There are certain kinds of rats that will continue to grow throughout their lives. In fact, unlike humans, **the epiphyseal plates (the plates responsible for the lengthening of bones) of rats never disappear**. It is possible for laboratory rats to live to be about three to four years old and grow to a size of 500-700 grams. The laboratory rats used in most scientific studies are younger and smaller; the rats that Dr. Holton used were about two months old and weighed just below 300 grams at the time of launch. Dr. Holton used the smaller, younger rats since they formed more bone during the short period of the mission, allowing her to observe and study more **bone growth** characteristics. The fact that they do continue to grow is one of the factors that makes rats such a good model for the study of bone formation. An interesting feature of Dr. Holton's study was an examination of how different housing arrangements might affect how the rat bones develop in space (and, of course, here on Earth for the control animals).

Before the rats were sacrificed, they were each weighed. Then, following decapitation, the different members of the dissection team worked quickly to process the various bones that will be used for the experiment. Once these bones had been appropriately p reserved, the general physical characteristics of certain individual rat bones were determined. These included **length, diameter**, and **weight**. We will review the results of bone length and mass measurements in this section. Changes in bone diameter will be examined in the next section.

The techniques for length and mass measurements are simple, the important factor being **accuracy** since the animals are very small and very small changes are of interest. In order to measure the length of the limb bones, a special **dial caliper** was used. This is a measuring instrument with two legs or " jaws" that can be adjusted. This instrument is accurate to 0.1 mm. In order to measure mass, the animals were placed on a very accurate electronic balance. Let's discuss the results of the rat's body mass and bone mass measurements.

Figure 18a.



a) Preflight and postflight rat growth during the space flight experiment.

The rats were 65 days old at launch and weighed an average of 285 ± 16 grams (mean value + standard deviation) two days before launch. The day of launch, the ground controls weighed an average of 295 ± 12 gm. Once the shuffle landed, the rats weighed an average of 331 ± 19 gm and by nine days later, the average weight was 342 ± 20 gm. **No significant difference in mean body weights was noted among any of the groups.**However, from Figure 18a, you can see that the **rate** of weight gain immediately began to change when the rats returned to Earth. Figure 18b is an expanded version of the postflight mass changes seen on Figure 18a. In fact, during the postflight period, the VIV-S (control rats housed singly in a rat cage called a vivarium) gained weight faster than the other groups. Also, both control groups gained significantly more weight than both flight groups in the postflight period. In particular, the flight animals exhibited a suppression of growth immediately after flight. However, after the second day back on Earth, all four groups continued their growth in a parallel fashion.

Dr. Holton also measured the **length** of certain antigravity bones, the **tibia**, the **femur**, and the **humerus**. These are considered the major **long bones** in the rat. Figure 19 is a graphical representation of the differences in postflight length measurements among the different bones. The tibia, femur, and humerus exhibited significant growth in length between launch and R+ML (Recovery or landing + mission length or 9 days). However, bone length was similar in all groups throughout this time.

Dr. Holton's team also measured the **bone mass** of the antigravity **femur** and two bones that are not used to oppose gravity, the first lumbar **vertebra** and one of the **ribs** of the animal. In reality, Dr. Holton processed the bones to dissolve the fat, so the measurements were not really of the bone mass, but instead, were of the fat free weight of the bone (in mg). It is referred to as bone mass, however.

Figure 20 indicates that bone mass in the vertebrea and rib did not appear to be affected by the flight. However, the femur in the individually housed flight rats (RAHF-F) was significantly smaller than its control (VIV-S) after R+ML. On the other hand, group-housed animals showed no differences in bone mass of the femur, whether on Earth or in space. In other words, the AEM-F rat femurs were very similar to the AEM-C rat femurs. What do these results suggest? Let's summarize the findings.

- The rats grew at a constant rate (linearly) throughout the flight period. This changed, however, during the postflight period when the rate of growth for the flight groups was significantly lower.
- The length of the rat's long bones continued to increase at a steady rate. The different housing arrangements did not seem to affect bone elongation.
- The bone mass for the vertebra and the rib was not affected by flight.

However, the RAHF-F femurs were significantly smaller in mass than similarly housed ground controls and were slightly smaller than the group-housed rats. The data suggests that singly-housed rats show a decrease in accumulation of bone mass during flight that becomes significant during the postflight period, compared with similarly housed controls. In terms of the femur results, the **length** remained the same but the bones **weighed less** for the animals that flew in space. This might suggest that the bone structure is different. We will see in the next section how the diameter of the bone was affected by space flight and we will examine if the bone continued to accumulate minerals at the same rate.

Finally, we will examine how any changes in the mineralization rate of long bones in the rat might affect the strength of those bones.

II. Changes in the Mineralization Rate and Strength of Rat Bones

Recall from an earlier section that new bone is continually being formed to replace old bone. That is, in contrast to the lengthening of bone, the thickness and strength of bone must continually be maintained by the body. This is accomplished as bone is continually deposited by osteoblasts, while at the same time, it is continually being reabsorbed (broken down and digested by the body) by osteoclasts (refer to Figure 5 earlier in the chapter). Osteoblasts are found on the outer surfaces of the bones and in the bone cavities. A small amount of osteoblastic activity occurs continually in all living bones (on about 4 % of all surfaces at any given time) so that at least some new bone is being formed constantly. Normally, in fact, except in growing bones, **the rates of bone deposition and absorption are equal to each other so that the total mass of bone remains constant**. Bone mineralization refers to the process of rebuilding bones, including the formation of the collagen/crystalline matrix, over time. Aging and certain disease states such as osteoporosis cause bones to mineralize at a reduced rate. This can also occur when bones are not "loaded" normally, which happens when a leg is in a cast or when ill patients are unable to get out of bed to walk around. When mineralization is reduced, the bones become weaker and more prone to fracture. Whole bone strength is largely dependent on the of the bone tissue, including:

- the **architecture** and **distribution** of bone mass, and
- the **geometry** of the bone.

During space flight, changes have been noted in both the fore limbs and hind limbs of growing male rats. Studies on the humerus and tibia have shown a decrease in the amount of bone formed during flight. In this section, we will examine Dr. Holton's results related to the growth of the diameter of bone and we will discuss how the strength of the bone may be affected by such a change. First, we will examine how the mineralization rate in rat bones is affected by space flight and how this mineralization rate can be determined by changes in bone growth diameter. Then, we will examine how any changes in the mineralization rate of bone can affect its strength, or stiffness. Let's begin with a discussion of how Dr. Holton measured the mineralization rates of the rat's tibia and humerus.

You already know that bone grows in length, but bone also grows around its diameter, similar to the way a tree trunk grows. If you have ever seen a cross section of a tree trunk, you can estimate the age of the tree by counting the rings. In fact, the wayto measure the rate of growth in a bone's diameter is by producing rings along the cross-section of the bone and timing how long it took to for the bone to grow from ring to ring. Fluorescent bone markers are used to produce colored rings along the diameter of the bone. The idea is simple.

First, the rat is injected with a bone marker. This bone marker will cover the outer boundary of the bone around the **periosteum**. Then, the bone continues to grow in diameter beyond the colored ring that was produced by the marker. After a certain amount of time, the rat is injected with a different marker that will, again, cover the outermost boundary of the bone with a different color. The bone will then continue to grow some more in diameter beyond the second colored ring. By measuring how much the bone grew and by knowing the amount of time it took to grow that much, one can determine the **rate of growth** of the bone. But, bone does not only grow "outward" along the edge of the periosteum, it also grows "inward" toward the **endosteum**, which is the inner boundary of the bone that lines the marrow cavity. Therefore, a marker must be used that can selectively mark both the inner and outer boundaries of the bone. In this way, one can determine if the bone grows faster "outwardly" or faster "inwardly" toward the marrow. The rate of growth of the diameter of a bone would reflect how quickly bone mineralization is taking place.

Dr. Holton performed her experiment in order to compare the mineralization rate of flight animals to that of the control animals. In addition, she was able to compare any effect that the housing arrangements had on bone growth and mineralization. In the cross sectional areas of bone depicted in Figure 21, the bone labels are marked. As you can see, the distance between the labeled rings shows the amount of bone growth between the various injections of fluorescent marker.

Tibia Cross Section: Control **Tibia Cross Section: Flight**

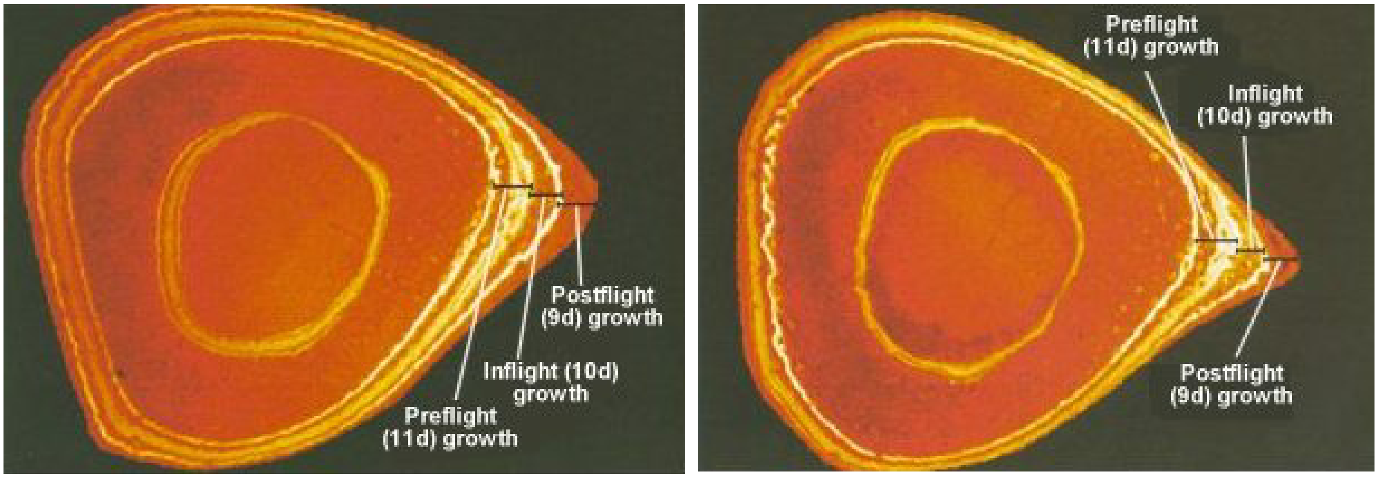


Figure 21. A pair of digitized fluorescent images of a cross section of bone from the tibia of a ground control and a flight rat. Preflight, inflight, and postflight bone growth distances are indicated.

The preflight label is a **calcein dye** which was injected about 13 days before launch.

The inflight label is a marker called **declomycin**, which was injected 1.5 days before launch.

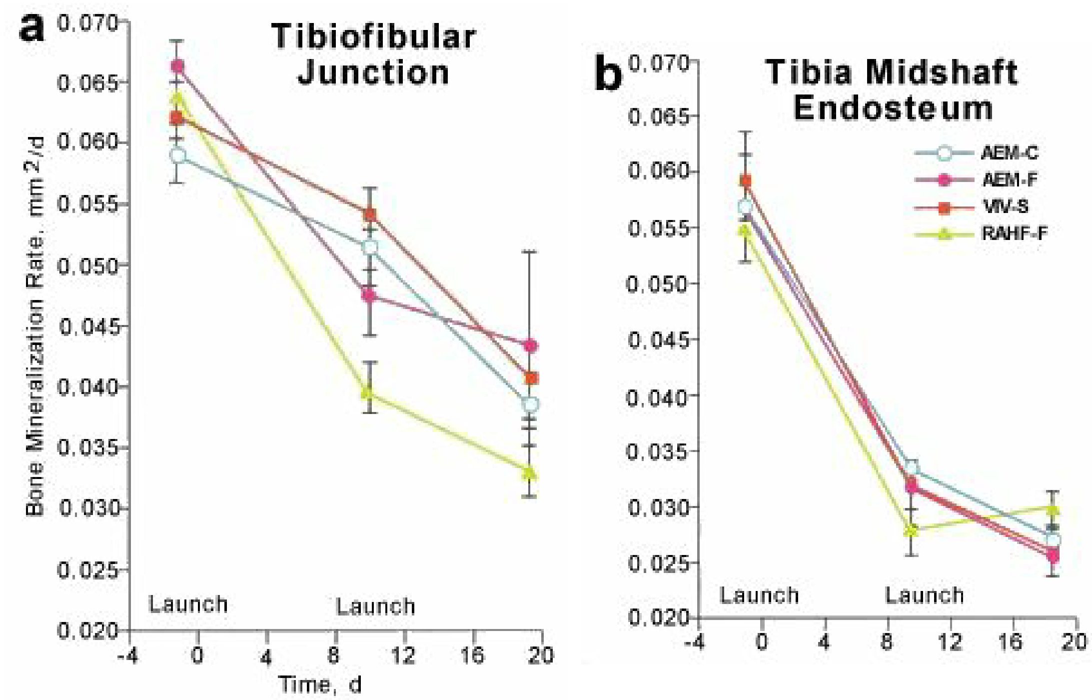
The postflight label was also **calcein** and was given at the end of the shuttle flight. The postilight distance leading to the bone surface represents nine days of growth. As mentioned, bone mineralizes both at the endosteal, or inner, surface of bone and at the periosteal, or outer, surface of bone. The most rapid bone growth usually occurs on the outer surface where muscle is attached.

Dr. Holton's results suggest that bone mineralization decreased over time. The results can be broken down into four parts:

(1) At the periosteal surface of the tibia (at a location on the bone known as the **tibiofibular junction**), the data from Figure 22a shows that the control groups experienced a 13% decrease in mineralization during the flight period. The periosteal mineralization for the flight rats, however, decreased significantly more during the flight period compared to the controls. Those that flew in a group housing arrangement, the AEM-F rats, experienced a 28.8% decrease and those that flew individually, the RAHF-F rats, experienced a 37.7% decrease in mineralization. Postflight, however, is another story. Although all rats exhibited reduced mineralization rates, the flight rats showed less of a decrease than the control rats.

(2) Like the periosteal surface of the tibia, the endosteal surface of the tibia showed decreased formation. Unlike the periosteal surface, however, the mineralization rate of the endosteal surface was very similar in all groups. Figure 22b shows this decrease averaged about 45%.

Figure 22 (a&b).



(3) In the humerus, no difference between flight and control animals occurred during the preflight period, but during the flight and postflight periods, the flight rats showed significantly reduced periosteal and endosteal mineralization. Figures 22c and 22d show the results of the periosteal and endosteal mineralization rate measurements, respectively.

Figure 22 (c&d).

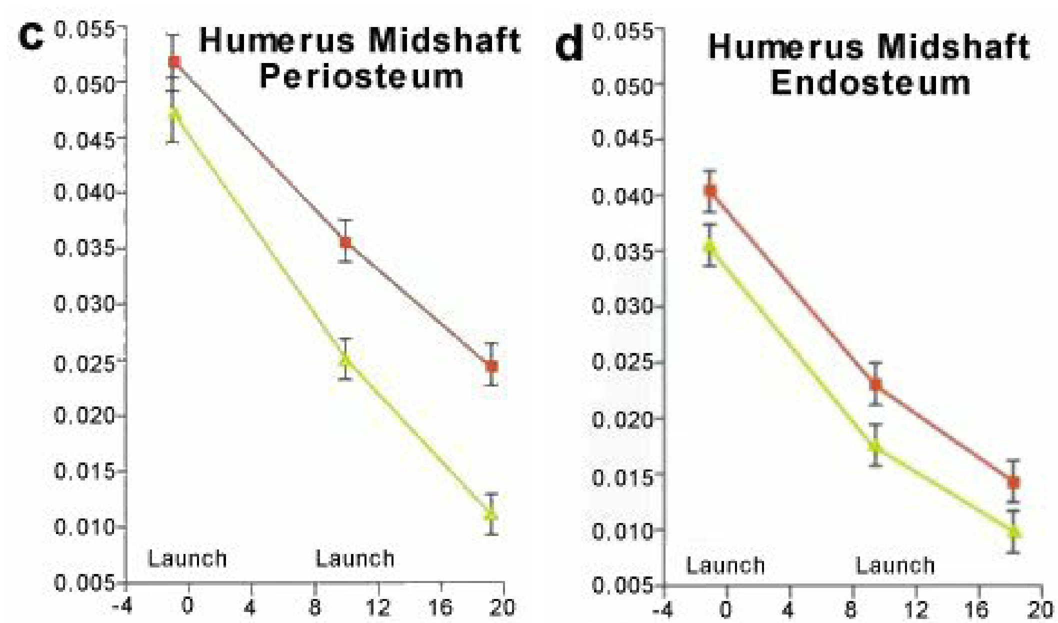
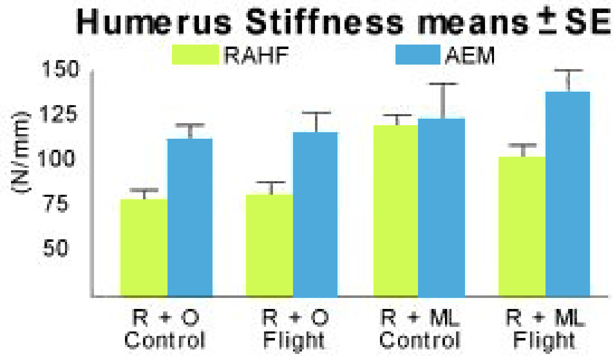


Figure 22 (a,b,c,d). Bone mineralization rates at the periosteal (outer) and endosteal (inner) surface of the tibia and the humerus of the rat.

Notice that the units of measurement for the bone mineralization rate is mm/day. That means that the measurement was based on how many square millimeters of bone were formed per day. What does all of this mean? Well, essentially, there was no real difference in mineralization rate due to flight at the endosteal surface of either the tibia or humerus. The main differences existed in the mineralization of the periosteal surface, with the largest difference being between the singly housed flight (RAHF-F) and control animals (VIV-C) for both the tibia and the humerus. This study was also designed to determine whether space flight affected the strength characteristics, or stiffness, of certain bones. In order to measure stiffness, the humerus and the tibia bones were placed onto a three-point bending chamber that was equipped to bend the bones while keeping them in a special "physiological fluid." This fluid was important to keep the bones in a state that resembles living bone. The bones were then bent until "failure," or until they fractured. All bone samples failed at a midpoint on the diaphysis. Figure 23 shows the results of the humerus stiffness test for animals that flew on both the RAHF (housed individually) and the AEM (group-housed).

In both cases, values for the flight and control animals are shown. From Figure 23, the data suggests the following:

Figure 23. The stiffness levels of the rat humerus for both the individually houses animals (RAHF) and the group-housed animals (AEM).



- The RAHF flight animals that were housed individually seemed to maintain the strength of their humerus bones compared to the control animals that stayed on Earth. However, their humerus bones were found to be significantly weaker than those of their matching controls after being home for nine days. That is, their bones did not seem to develop normally, even when they returned from space flight. From Figure 23, the data also suggests the following:
- The AEM flight animals that were housed in groups of five rats per cage seemed to have somewhat stronger humerus bones than their controls, both at landing and after nine days of recovery back on Earth. In addition, they seemed to have stronger bones overall than the animals that were housed individually in the RAHF. Does this mean that the animals that were housed in more crowded conditions get more exercise, thereby being able to "load" their bones more often You can imagine that they do have to constantly bounce off one another,but it is unclear if their stiffer bones can be fully attributed to their housing arrangement.

In space or during restricted activity or exercise, we've seen evidence of changes in the bone structure of rats, and in a previous chapter we learned of the decrease in the mass of the gravity-dependent muscles. These space flight effects certainly compound one another, rendering the animals weaker overall. Because of this weaker condition, the animals may be prone to bone fractures when exercise or structural loading is increased (i.e., return to Earth from space). The question that we are faced with is how all of this applies to the human system.We need to find out **what components** of the bone structure are changed, the **extent** to which they change, the **impact** of the changes on bone strength, and the **reversibility** of any changes. Only then can we develop countermeasures for astronauts to inhibit potentially damaging changes in bone structure during space flight This research can also help to design appropriate therapy for patients here on Earth who suffer from various forms of bone disease.

Now it is time to return to Dr. Holton's hypotheses:

Hypothesis 1

> Gravity is necessary for normal development of bone structure on Earth, and decreased gravity or skeletal unloading causes changes in skeletal growth patterns.

Hypothesis 2

> The response of bone to space flight will be localized and will differ not only from bone to bone, but also at different sites within the same bone.

Hypothesis 3

> The type of housing (group vs. individual) will influence the bones response to space flight and the recovery from space flight.

You have only been presented with a small portion of Dr. Holton's results. However, you should be able to provide general comments on how the results that you have seen either support or refute the hypotheses. It is left to your teacher to lead you in such a discussion.

CONGRATULATIONS!

We have just completed our examination of some of the important results of Dr. Holton's space flight investigation. These results, obtained using animal subjects, provide added information that should allow us to develop a better understanding of human bone function in space and on Earth.

Conclusion

We have one final activity left in this, a "Speaking of Space" activity that has been designed to provide a slightly different experience for you. Directions are provided in the next section.

SPEAKING OF SPACE

The following activity is important for a variety of reasons. This activity can be very helpful for you to understand all of the many little pieces that fit together in the world of science. Just like any major problem or issue that a person finds challenging, science can seem scary. However, it is nothing to be afraid of. The thing to do whenever you are faced with something challenging is to break it down into very small pieces, find a piece that you are comfortable with, and slowly add the pieces back together. All of a sudden, the fear is gone and you understand it in your own way. Let's do that with different aspects of this chapter.

For this activity, you are to return to the different parts of the chapter (including the Earth Physiology section, the Student Investigations, and the section that describes Dr. Holton's experiment) and just skim them. While skimming each part, keep an eye out for things that would fit into two different lists that you will be producing.

First, list all of the different science discipline areas that are involved in the examination and study of various aspects of bone. Give as many examples as possible of when and how these different science areas are used. The entire class will discuss and combine all of the different lists. Then each student will be asked to comment on his or her favorite science area as it applies to bone research.

Second, list all the different kinds of people that must have been "involved" with carrying out Dr. Holton's investigation including technicians, drivers, animal handlers, and so on, who were responsible for even the smallest part of her experiment. List as many people as you can and include a short comment on what they do. The entire class will discuss and combine all of the different lists of people and jobs. Then each student will be asked to select which job they would most like to do, **besides being an astronaut**, and explain why.

Remember this for the future: all you need to do is to break complicated things down into very small and manageable pieces and then they no longer look so complicated.

REVIEW QUESTIONS

Earth Physiology

1. Name four types of joints that allow a wide range of motion in our bodies and give an example of each.

2. Identify three types of cells that are found in bone and state their major function.

3. Describe the two components of bone that help to provide strength, and explain the contribution of each one to the strength of the bone.

4. Name the four classifications of bone that are based on their shape and give an example of each classification.

5. Identify the process of bone formation and how it occurs.

6. Describe how the lengthening of bones occurs during the growing years of a human.

7. How is the level of blood calcium regulated? Draw a diagram to indicate the negative feedback loop involved in this regulation.

8. Name the antigravity bones of our skeleton and explain why they are referred to as antigravity bones.

Space Physiology

1. Identify two reasons why a rat would make a better laboratory model for bone research in space than a horse.

2. Select one of Dr. Baldwin's hypotheses and, using any of the data sets in this chapter, provide an argument that supports or refutes the hypothesis.

3. Explain how an anthropologist or forensic scientist, with one dried bone as a clue, can closely estimate its owner's former living height. Which bones are proportional enough to a person's height to provide the most accurate estimation of height?

4. What three factors play a role in determining the strength of a material?

5. Describe the principles of Hooke's Law and Young's modulus and how they relate to one another.

6. Consider the various data sets that are presented in this chapter and select one that illustrates how the responses of the group-housed animals and the individually housed animals are different.

Critical Thinking

1. If you knew somebody that stands on their head all day, what bones in that person's body would be considered antigravity bones?

References

Certain parts of the text were excerpted and adapted from the following publications and other references:
For the section describing Earth Physiology:

1. Allison L (1976). **Blood and Guts: A Working Guide to Your Own Insides.** Little, Brown, and Company, Boston, Massachusetts.
2. Fox Sl 11987). **Human Physiology**, 2nd ed. William C Brown Publishers, Dubuque, Iowa.
3. Guyton AC (1986). **Textbook of Medical Physiology**, 7th ed. WB Saunders Company, Philadelphia, PA.
4. Nourse AE 11964). **The Body**. Life Science Laboratory, Time Incorporated, New York.
5. Oram RF (1989). **Biology: Living Systems**. Merrill Publishing Company, Columbus, OH.
6. **The Incredible Machine** (1992). Poole RM (ed.), National Geographic Society, Washington, DC.
7. Numerous personal communications with Dr. Emily Morey-Holton, NASA Ames Research Center, Moffett Field, CA.

For the section describing the space flight results and as influence for numerous figures and tables:

1. **Spacelab Life Sciences 1: 180-day Preliminary Results**(1991) NASA Headquarters, Washington, DC.
2. Morey ER (1979). Spaceflight and bone turnover: correlation with a new rat model of weightlessness. **Bioscience**. Vol 29, No 3, 168- 172.

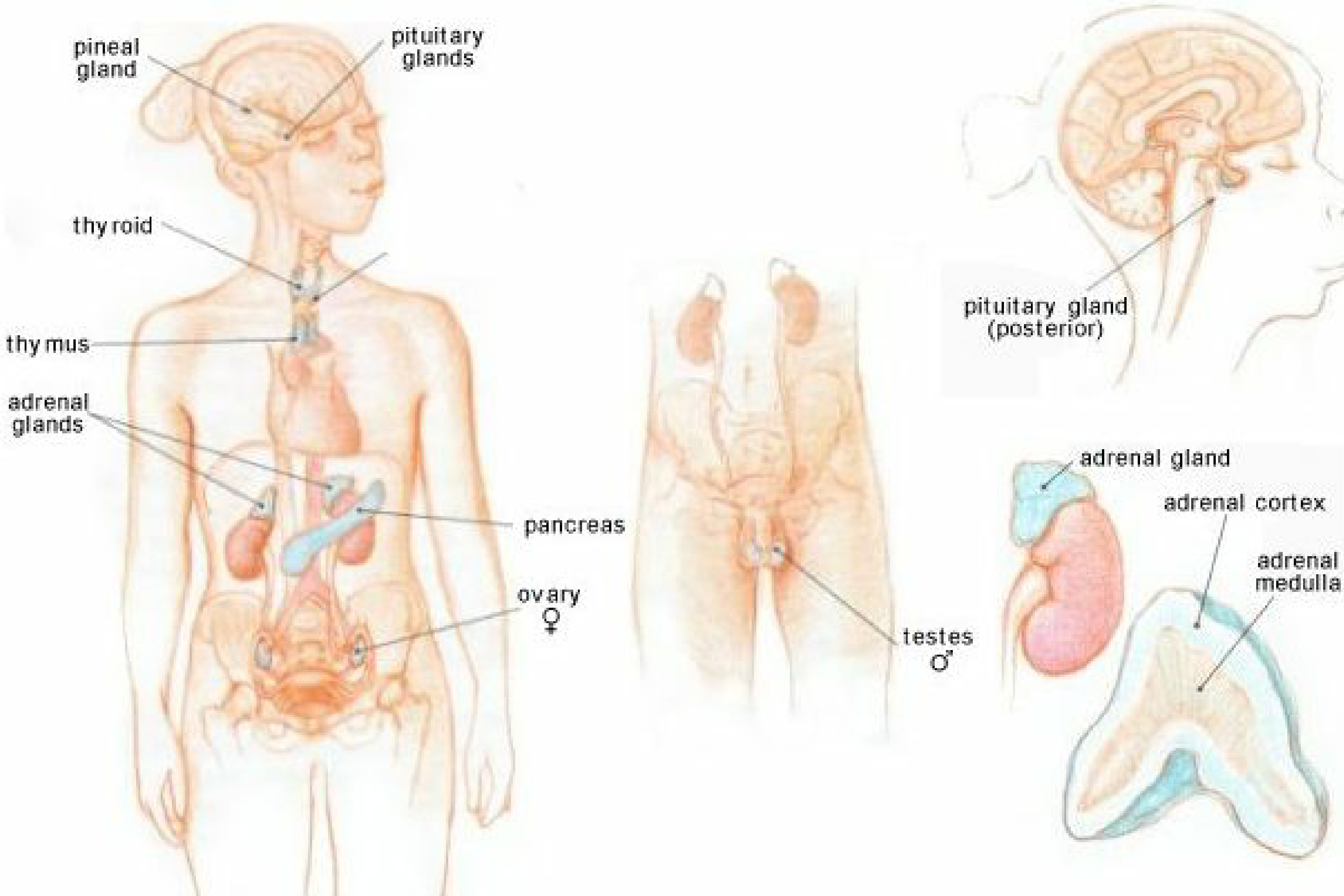


Figure 6. Certain endocrine glands secrete hormones that are important.

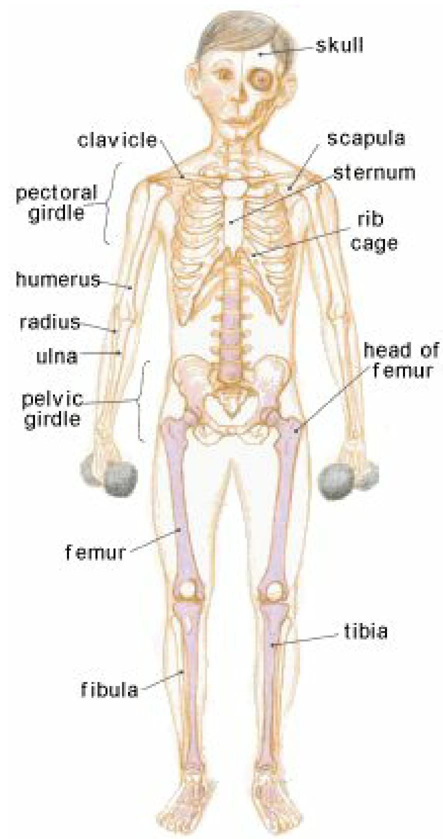


Figure 8. The arrangement of the skeletal system is orderly, symmetric, and balanced. The anti-gravity bones, indicated in the figure, are responsible for bearing the weight of the body in a gravitational environment.

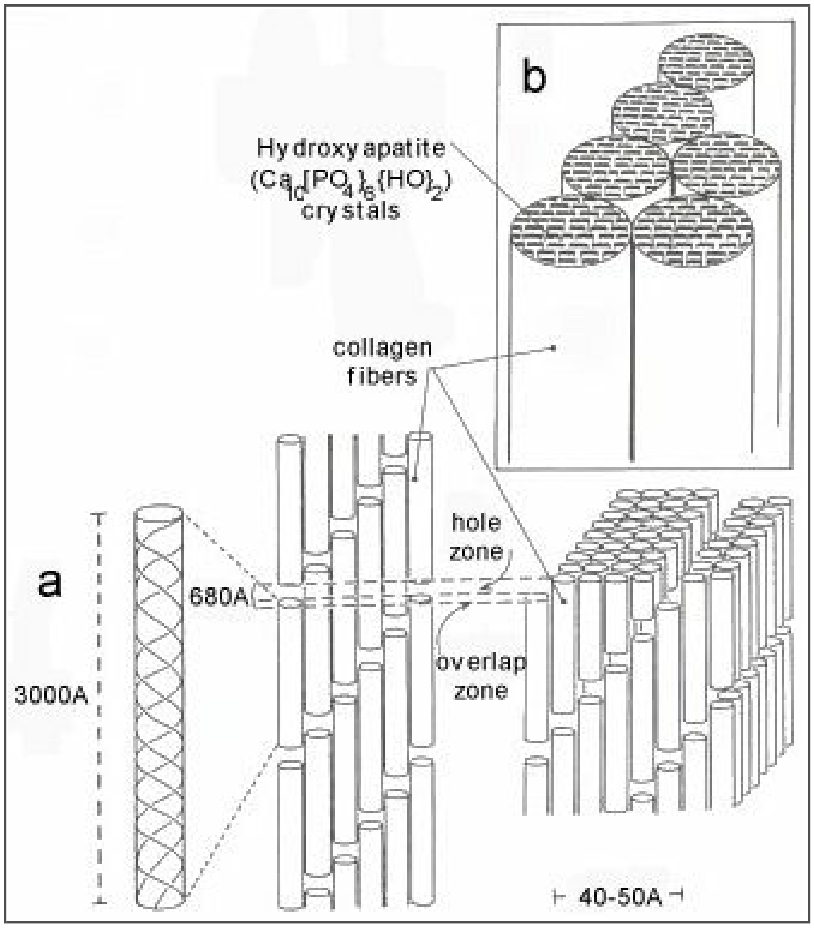


Figure 12: The molecular arrangement of collagen and hydroxyapatite crystals in compact bone. (a) Collagen fibers overlap adjacent fibers as they repeat every 680 Å. Hole zones are areas of low density bone collagen and overlap zones are areas of very high density bone collagen. (b) Hydroxyapatite crystals ($Ca_{10}[PO_4]_6[OH]_2$) are arranged in layers within each fiber, resenbling overlapping bricks (1 Å = 10^{-8} cm).

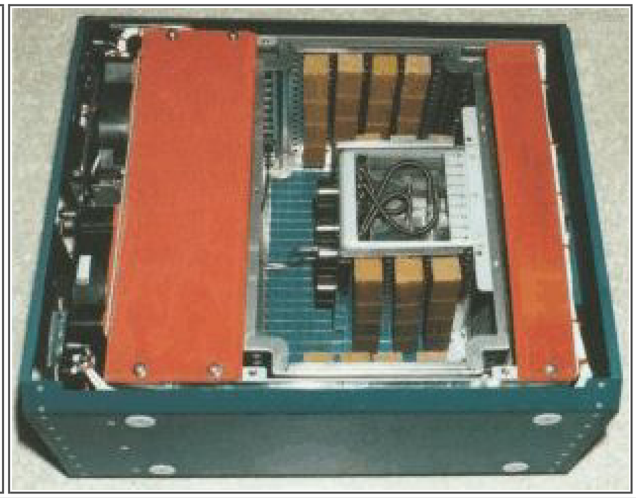


Figure 17. The Animal Enclosure Module (AEM) houses up to five rats in a single cage. This top-down view of the AEM reveals the animal's food bars that are lining the sides of the cage.

Figure 18b. b) The postflight weight gain is expanded in this view. The patterns of weight gain show an initial suppression of growth in the flight animals immediately after the flight. Data are plotted as mean ± standard error.

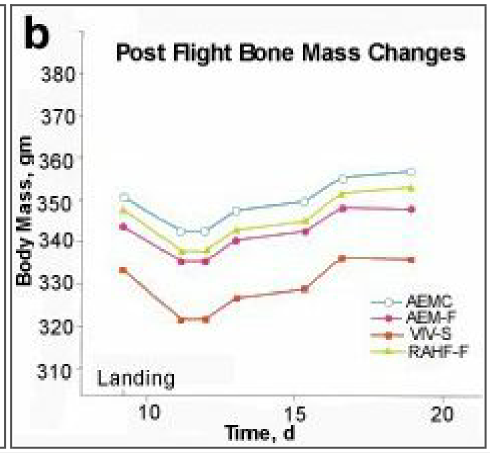


Figure 19. Bone length determinations during the experiment. No significant differences were noted in the length of any of the long bones during and after this mission between the flight and control animals.

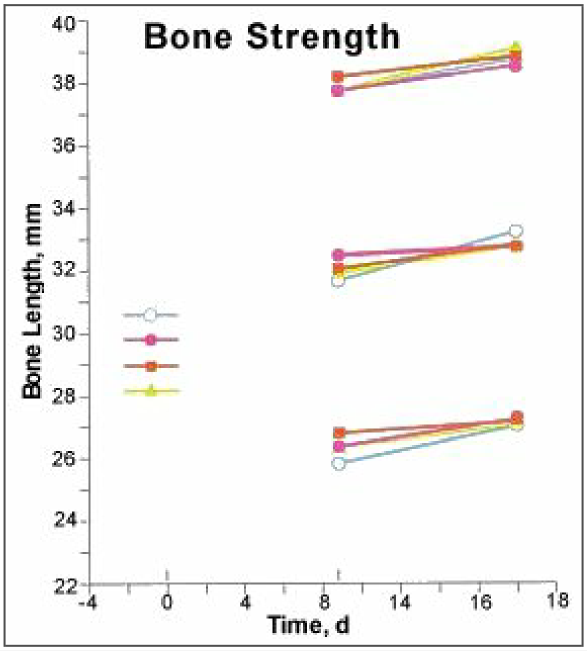
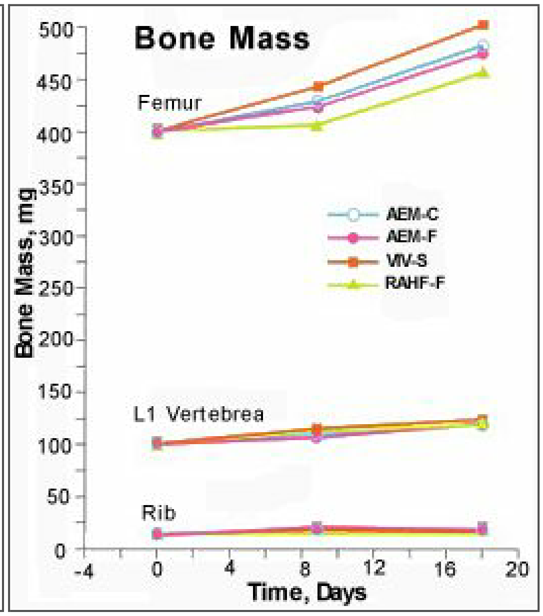


Figure 20. Bone mass determinations during the experiment. The mass of the anti-gravity femur from the singly housed flight rats (RAHF-F) is significantly different from that of its control (VIV-S). There are no mass differences between the AEM-F and AEM-C groups.



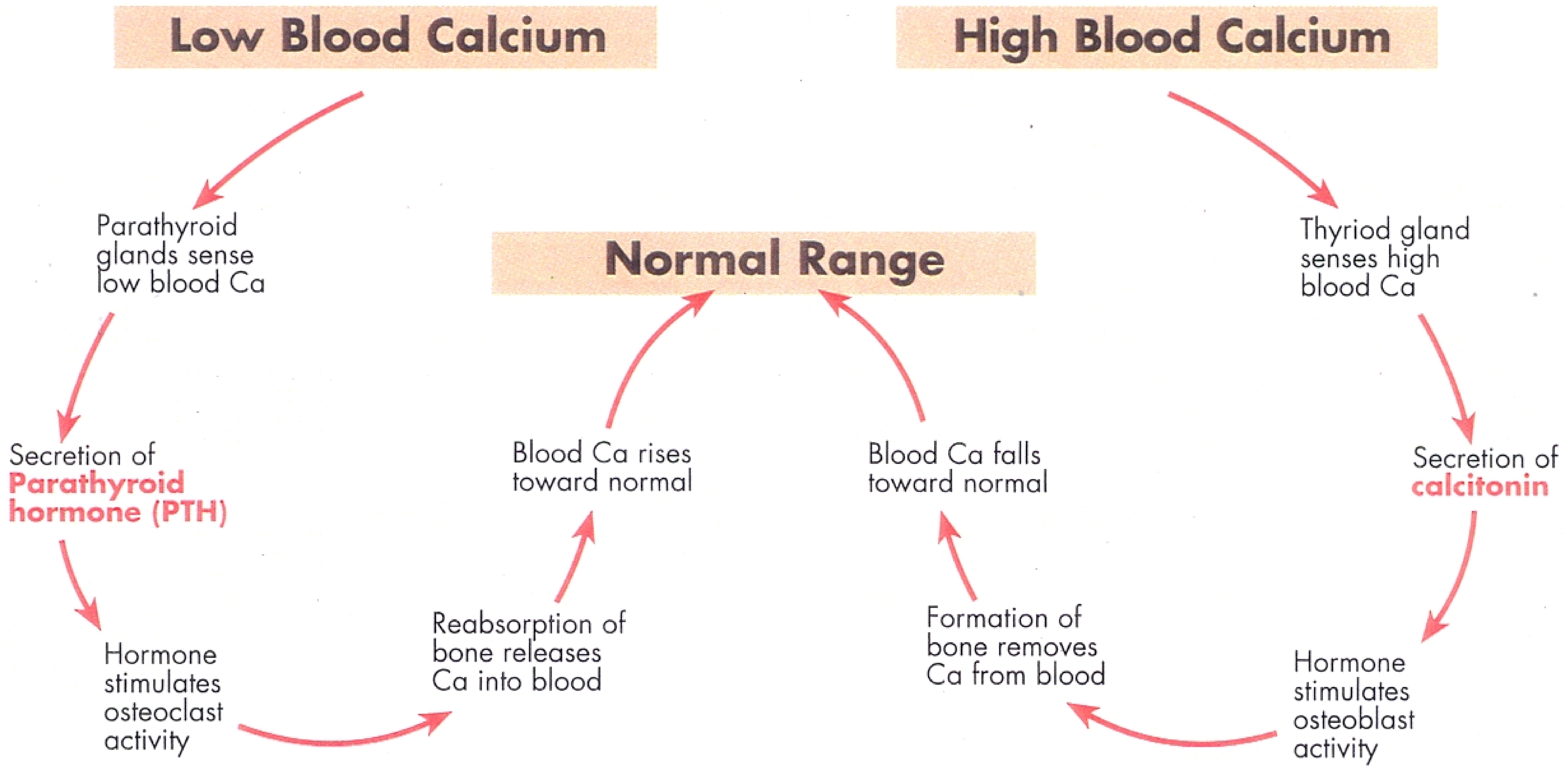


Figure 7. The parathyroid and thyroid glands function to control the level of blood calcium.