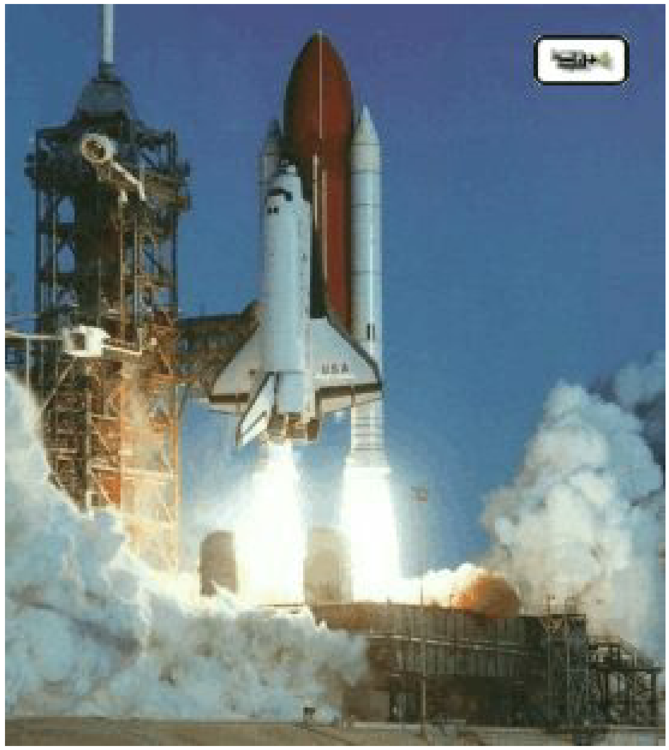
EXAMINING THE EFFECTS OF SPACE FLIGHT ON BLOOD AND ITS COMPONENTS



THE INFLUENCE OF SPACE FLIGHT ON ERYTHROKINETICS IN HUMANS

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The Major Components of Blood

Among all the body's systems, the blood is unique: it is the only tissue in the body that flows. This flowing tissue, endlessly making its course from the heart to the remotest parts of the body and returning, is a sea in which the body is bathed. Blood has two distinct parts (Figure 2). **Plasma**, the liquid part of the blood, makes up about 55% of the blood volume. Since there is a total of 5 liters of blood in the body of an average adult, the **plasma volume (PV)** in the body is about 2.75 liters. Plasma is a yellowish solution consisting of about 91% water, and the other 9% is a host of substances indispensable to life. Among them are: **nutrients** such as glucose, fats, and amino acids; **chemicals** important to the body, such as sodium, potassium, and calcium; special **proteins**, such as fibrinogen, albumin, and various globulins that produce antibodies, which fight off viruses and other unwelcome intruders in the body; and **hormones**, which are regulatory substances such as insulin, and epinephrine, more familiarly known as adrenaline, which speeds up the heart rate whenever some emergency requires a greater blood flow to the muscles.

The role of plasma in the body is to help transport food and oxygen to the cells of the body and to carry wastes away from the cells. In addition, with its potent arsenal to draw upon, plasma plays a crucial role in maintaining the body's chemical balance, water content, and temperature at a safe level. That is, the plasma serves the body by helping to maintain **homeostasis**, or a stable internal environment in the body. In fact, essentially all the organs, tissues, and fluids of the body perform functions that help to maintain the body as a stable system. By analyzing plasma, medical doctors can find out what types of nutrients are circulating throughout the body, and they can measure the levels of hormones and other constituents that plasma helps to transport.

The cellular portion of blood normally makes up about 45% of the blood volume and it consists primarily of three cellular components (Table 1): white blood cells (**WBCs**, also known as **leukocytes**), platelets, and red blood cells (**RBCs**, also known as **erythrocytes**). The WBCs constitute the blood's mobile security system. Some WBCs are endowed with the curious ability to wiggle out of the bloodstream and back in again. The WBCs can move like an amoeba, slipping through thin walls of capillaries and wandering among cells and tissues. They converge together in great numbers wherever invading bacteria, viruses, fungi, or parasites gain entry into the body, destroying them by swallowing them or by synthesizing **antibodies**, which are complex proteins that react with and destroy these foreign substances. Whenever white cells mobilize for action, the body compensates by manufacturing more. Double the usual number may appear in the blood within hours. Often this rising white cell count, as physicians describe it, serves as an early tip-off that a dangerous infection has entered the body.

Element	Diameter (in um)	Number (per mm^3)	Scientific notation (per mm^3)	Main function
red blood cells	7 - 8	4,500,000 - 52500,000	4.5×10^6 5.5×10^6	oxygen transport
white blood cells	9 - 12	7,000 - 10,000	7×10^3 1×10^4	defense against microorganisms
platelets	2 - 4	300,000	3×10^5	blood-clotting

Table I. Comparison of some characteristics of the cellular components of blood.

The smallest of blood's three cellular components are the platelets, named for their resemblance to tiny plates. Their main function was discovered when doctors observed that people with low platelet counts were especially vulnerable to bleeding. It is now well known that platelets are vital to blood clotting. When they touch the roughened surface of a torn blood vessel, they burst apart, releasing chemicals that set off a reaction in the blood leaking out. The result is that they convert one of the plasma's proteins, fibrinogen, into a network of fibers that trap RBCs - thereby forming a clot which seals the leak.

The main focus of this chapter will be the examination of the most abundant of all the cells of the body, the red blood cells, or RBCs. These RBCs outnumber the WBCs about 700 to 1. Theirs is the exclusive and all- important job of picking up oxygen in the lungs, carrying it to the rest of the body, and carrying waste carbon dioxide back the other way. Their life is hectic and brief: after about three or four months they grow old, are eaten, and then replaced by new recruits sent into the bloodstream from the bone marrow. As mentioned previously, the study of the activity of the RBCs is called **erythrokinetics**. Erythrokinetics involves looking at the entire lifetime of a RBC from its "birth" (it is born in the bone marrow), through its passage around the body (each RBC travels around the body in about a minute), all the way through its destruction. A normal lifetime for each RBC is 90 to 120 days.

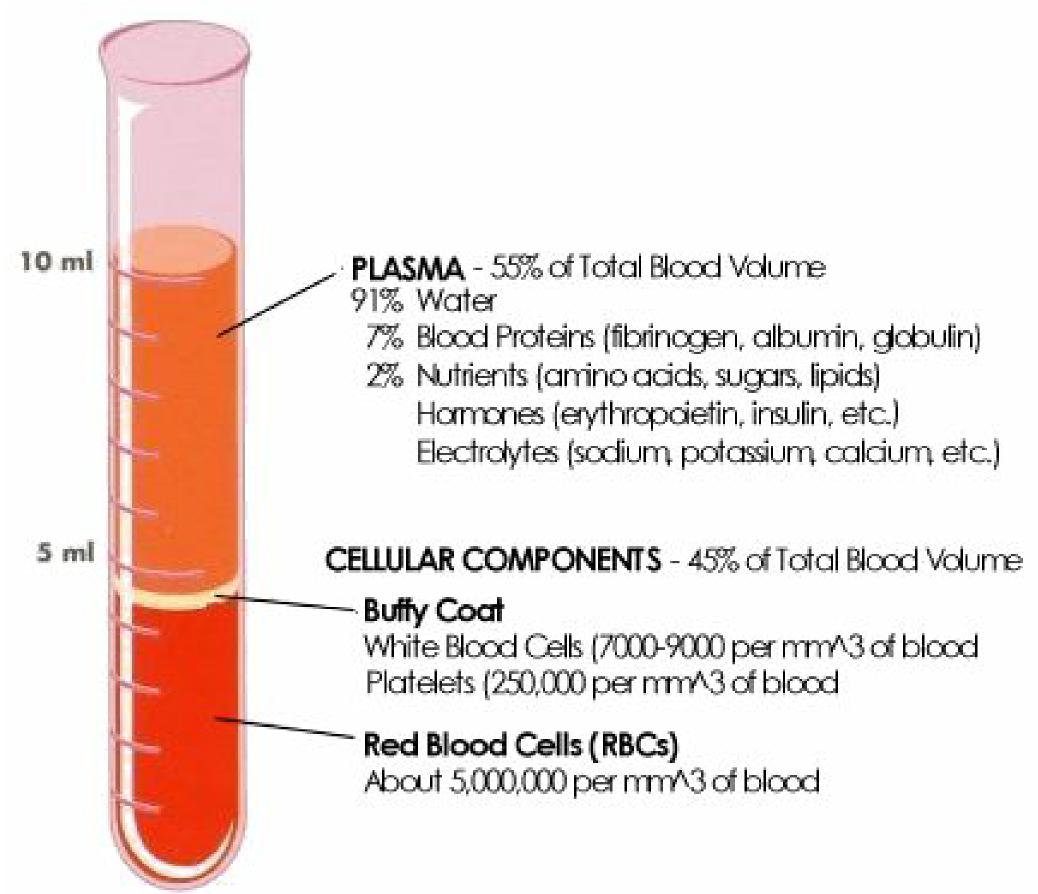
The RBC's effectiveness as an oxygen-carrier is due to its content of **hemoglobin**, a compound of protein and iron which gives blood its red color. Each RBC contains nearly 300 million hemoglobin molecules! And, when a person lives in an area at sea level where atmospheric pressure equals 760 torr each hemoglobin molecule can carry four oxygen molecules, which means that each RBC can carry 1.2 billion oxygen molecules! Hemoglobin has a chemical way of latching onto oxygen as the blood passes through the lungs and holding it in its grip until the destination is reached. As the blood passes through the tissue capillaries, the hemoglobin will not release oxygen into the tissues if too much oxygen is already there, but, if the oxygen concentration is too low, sufficient oxygen will be released by the hemoglobin to re-establish an adequate tissue oxygen concentration. (This is another example of a **homeostatic control mechanism**.) When, for any reason, the hemoglobin content in the bloodstream dips below the minimum for body needs, the result is **anemia**, meaning (although not literally) "no blood." Anemia is a reduction in the number or volume of RBCs. This reduction in RBCs results, obviously, in a reduction in the amount of hemoglobin and, therefore, in a reduction in the body's oxygen-carrying capacity. Another cause of anemia could be a diet deficient in iron-rich foods.

Normal RBCs are biconcave disks that are capable of changing their shape as they- pass through capillaries. Actually, the RBC is a "bag" that can be deformed into almost any shape without rupturing the cell. They are remarkably flexible and remarkably small. In normal men (if there are any!!), the average number of RBCs ***per cubic millimeter*** is 5,200,000 (300,000) and in normal women 4,700,000 (300,000). The number of RBCs varies in the two sexes and at different ages. Also, if a person moves to a higher altitude, for instance, to the mountains), the number of RBCs present in that person may not be enough to supply the body with oxygen. This is because as you go into higher altitudes, the atmospheric pressure becomes lower. For each liter of air you breathe at lower atmospheric pressures, there are fewer molecules of air, including fewer molecules of oxygen. With fewer molecules of oxygen available, your lungs are not able to supply each RBC with the amount of oxygen to which it is accustomed. In this condition, the RBCs cannot deliver enough oxygen to the body (including the brain) and you will become dizzy. Therefore, the body responds by increasing the production of RBCs so that the oxygen needs of the body can be met.

HEMATOCRIT

A common laboratory test can tell a physician a great deal about the **volume** of red cells in a blood sample. The volume of RBCs refers to the amount of space that the RBCs occupy within the blood. If whole blood (the cellular portion together with the plasma) is placed in a special **hematocrit** tube (a small test tube) and then spun very rapidly in a centrifuge, the heavier components will quickly settle to the bottom of the tube (Figure 2). When the centrifuge spins, the RBCs are forced to the bottom of the tube because they are the heaviest element in the blood. The WBCs and platelets are lighter so, as the hematocrit tube spins, they come to rest on top of the heavier RBCs in a layer called the **buffy coat**. Above the buffy coat rests the plasma. From the hematocrit tube, one can approximate the percentage of space that the RBCs occupy in the total sample. At sea level, the hematocrit of a normal adult male averages about 47, which means that 47% of the blood volume is RBCs, while that of a normal adult female is 42.

Figure 2. Blood can be separated into its components by putting it into a centrifuge and "spinning it down." The parts separate according to their relative "weights." This test tube shows the components of blood in their relative ratios. It shows a he matocrit of 45 because the RBC layer together with the "buffy coat" layer make up 45% of the total volume of centrifuged blood (4.5 m. out of 10 ml).



RED BLOOD CELL MASS

The **total red blood cell mass (RBCM)**, where mass refers to the quantity of material, in the circulatory system is regulated very closely by the body. Not only does the body work to make sure that an adequate number of red cells is always available to provide sufficient tissue oxygenation, but the body must also work very hard to make sure that there are not ***too many*** RBCs, so that the cells do not become so concentrated that they impede or clog blood flow. Therefore, RBC production is controlled very tightly by the body in a process called **negative feedback** (Figure 3). Most control systems of the body respond to the body's needs through this kind of process. What it means is that if the body senses too much of something (for example, if the body senses too much oxygen availability), the body will respond by causing a reaction in the opposite direction by decreasing something (for example, a decrease in RBC production). Conversely, if the body senses too little oxygen, the negative feedback mechanisms will cause the body to increase RBC production. So you can really think of a negative feedback mechanism as more of an "opposite reaction" system. In other words, if there is MORE of something than needed, it makes LESS of something, which will cause the body to return to its homeostatic condition. If there is LESS of something than needed, it makes MORE of something else, which will cause the body to return to homeostasis. This concept should become clearer as we discuss the specifics about RBC production next.

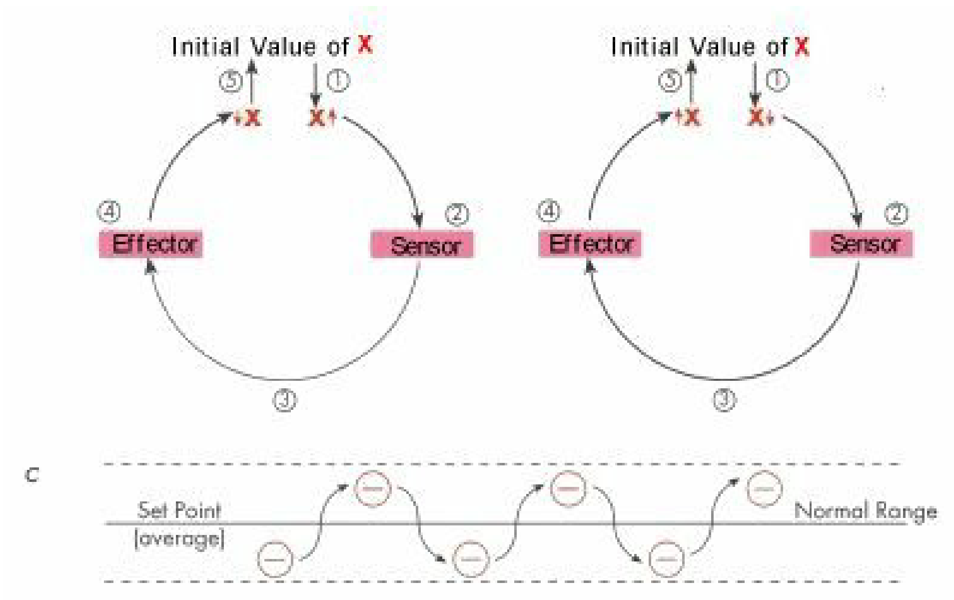


Figure 3.
a) A rise in some factor of the internal environment (X) is detected by a sensor. The sensor activates an effector (generally, nerves or hormones), which causes a decrease in this factor (iX). In this way the factor returns to its initial level and homeostasis is maintained. The circled numbers indicate the sequence of events.
b) A negative feedback hop in which a decrease in some factor of the internal environment (lX) is reversed by the actions of an effector.
c) Negative feedback loops (indicated by negative signs) work to maintain stability in our bodies within a normal range.

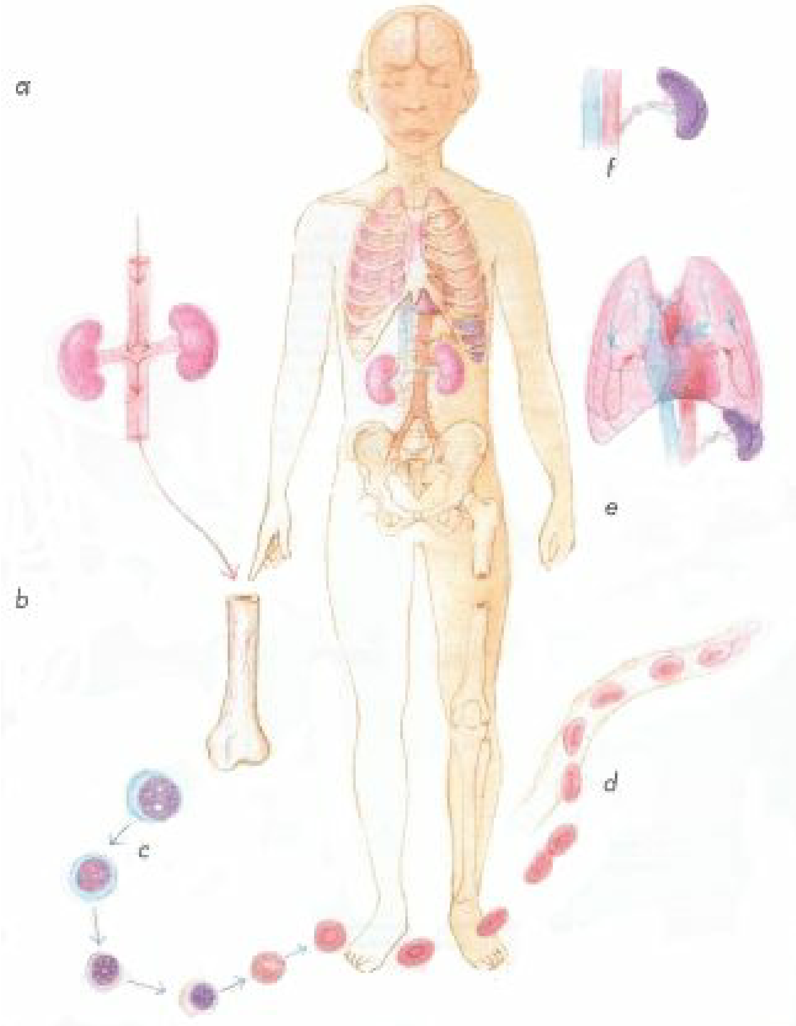
ERYTHROPOIESIS

The term **erythropoiesis** (erythro = RBC, and poiesis = to make) is used to describe the process of RBC formation or production. In humans, erythropoiesis occurs almost exclusively in the **red bone marrow**. (The yellow bone marrow is primarily composed of fat, but, in response to a greater need for RBC production, the yellow bone marrow can turn to red marrow.) The red bone marrow of essentially **all** bones produces RBCs from birth to about five years of age. Between the ages of 5 to 20, the long bones slowly lose their ability to produce RBCs. Above age 20, most RBCs are produced primarily in the marrow of the vertebrae, the sternum, the ribs, and the pelvis. Let's examine how RBCs are produced and, ultimately, how they are destroyed.

The organ responsible for "turning on the faucet" of RBC production is the **kidney** (Figure 4). The kidneys can detect low levels of oxygen in the blood. When this happens, the kidneys respond by releasing a hormone called **erythropoietin**, which then travels to the red bone marrow to stimulate the marrow to begin RBC production.

Now, once the erythropoietin stimulates the red bone marrow to begin manufacturing RBCs, a series of events occurs. In the bone marrow there are many special **stem cells** from which RBCs can be formed. As these cells mature, they extrude their nucleus as they slowly fill with hemoglobin until they are bright red **reticulocytes** ready to escape the bone marrow and squeeze into the blood capillaries to begin circulating around the body. In a blood sample, the reticulocytes can be distinguished from RBCs because they still contain some speckles or pieces of their nucleus. Within a few days, this reticulocyte completely loses all its nuclear material and becomes a full-fledged RBC that is ready to serve the oxygen needs of the body. After about three to four months, the RBC has worked so hard that it begins to weaken. The membranes of old RBCs become very fragile and the cells may rupture during passage through some tight spots in the circulation. These old and damaged RBCs are "eaten" primarily by the **spleen**, and most of the leftover components (especially the iron from the hemoglobin) are recycled to form new RBCs.

The production of new RBCs occurs as the need arises. A natural need always exists to produce new RBCs to replace the ones that have gotten old, or have been damaged, and have "died." Old RBCs die every day in our bodies and more new ones are also born every day. The body can also increase production of RBCs in response to special needs. As mentioned previously, new RBCs must be produced when a person enters a high altitude environment. At very high altitudes, where the quantity of oxygen in the air is greatly decreased, insufficient oxygen is transported to the tissues, and red cells are produced so rapidly that their number in the blood is considerably increased. Therefore, it is obvious that it is not the concentration of RBC's that controls the rate of red cell production, but instead, it is the functional ability of the RBCs to transport oxygen to the tissues **in response to the tissue demand for oxygen** that controls the rate of RBC production. In other words, it's just like the economic concept of "supply and demand." If the supply of oxygen is LESS than what the body demands, the MORE RBCs are produced. If the supply of oxygen is MORE than what the body demands, the FEWER RBCs are produced. This wonderful negative feedback mechanism works fine on Earth. How about in space?



The RBC lifetime is about 120 days.

Figure 4. The life cycle of a red blood cell.

a) **Kidneys** respond to a lower than normal oxygen concentration in the blood by releasing the hormone **erythropoietin**.
b) Erythropoietin travels to the **red bone marrow** and stimulates an increase in the production of **red blood cells (RBCs)**.
c) The red bone marrow manufactures RBCs from **stem cells** that live inside the marrow.
d) RBCs squeeze through blood vessel membranes to enter the circulation.
e) The **heart** and **lungs** work to supply continuous movement and oxygenation of RBCs.
f) Damaged or old RBCs are destroyed primarily by the **spleen**.

SPACE PHYSIOLOGY

We have already described in the previous chapter how space flight affects the organ systems of the body (the heart and the lungs), and we even discussed how some of the smaller structures of the body, the blood vessels, are affected in space. Now we are taking our discussion to even smaller, microscopic levels by examining flight affects our body at the **cellular level**. We know that space flight somehow affects the activity of the cells in our blood, including the RBCs. Let's examine how the **erythrokinetics** of space flight is different from that on Earth.

What has surprised scientists is the fact that, for space travelers, the **percentage** or **concentration** of RBCs in the bloodstream stays about the same even though plasma volume decreases in space. That is, the "space normal" hematocrit is about the same as the "Earth normal" hematocrit. What does this suggest? Remember that as humans are exposed to the microgravity of space, there is a loss of body fluid (including a loss of plasma) due to the headword fluid shift One would imagine that as the plasma level is decreased in the bloodstream, then the **concentration** of blood cells in the bloodstream would increase. If this were true, the hematocrit would rise. Data has indicated that the inflight hematocrit measurements for astronauts do not change to any appreciable extent from the preflight hematocrit, which means that the percentage of RBCs in the blood inflight is not different from the percentage of RBCs in the blood preflight, even though there is less plasma. Since there is a decrease in plasma volume, and since the hematocrit does not change, this suggests that the **number of RBCs must decrease**. We call this reduction in RBCs "space anemia."

There are different theories that exist to explain this decrease in the number of RBCs. One, called the "hemoconcentration" theory, suggests that, while in space, the body detects an overabundance of fluids in the upper part of the body. The astronauts find that they are not thirsty and they want to drink less while, at the same time, their kidneys are stimulated to remove this excess fluid, part of which is plasma. We have already learned a great deal about this in the previous chapters. The removal of plasma causes the blood to becomes "thicker," because as fluid is eliminated, the percentage of RBCs per volume of blood increases. This may cause an overabundance of oxygen- carrying ability. When the kidney detects this overabundance of oxygen, the kidney reduces the production of erythropoietin, which, in turn, suppresses RBC formation. This theory suggests that the production level of RBCs decrease. Data suggests that this is not the only theory to consider.

There have been alternative suggestions made to explain the space flight reduction of RBCs. These include the possibility that the "space anemia" is due to the loss of muscle mass which occurs in space flight. Because muscles are used less in microgravity (there isn't even any "walking around" to do in space) the muscles lose mass and require less oxygen. With a lower oxygen requirement, the blood can reduce its oxygen-carrying capacity. This theory suggests that the body responds to this lower oxygen requirement by reducing the number of RBCs produced.

If the last theory we discussed is true, that the muscles require less oxygen, another possible explanation of how the blood can reduce its oxygen-carrying capacity would be to increase the destruction rate of RBCs. If this theory is correct, then the proportion of circulating reticulocytes (immature RBCs) in the bloodstream would increase above normal. This is because the normal production line in the marrow would continue kicking out reticulocytes, while at the same time, many of the mature, healthy RBCs already in the bloodstream would be destroyed.

A fourth theory for the reduction in RBC mass has to do with the well-documented fact that astronauts lose calcium from their bones under conditions of microgravity. (We'll be talking more about this in a later chapter.) The loss of body calcium could disrupt the bone architecture, which would result in the loss of bone strength, a condition that is similar to a disease called **osteoporosis**. This alteration of bone metabolism may also affect the bone marrow, and, therefore, affect normal RBC production in the marrow. To summarize the theories, a decrease in the number of RBCs can occur in space under the following conditions:

(a) after the body eliminates "excess" fluid and the kidneys detect that the blood has become too "thick," the kidneys may suppress the production of erythropoietin resulting in a **decrease in RBC production**;
(b) as muscle mass is lost and their oxygen requirement is reduced, the kidneys detect an overabundance of oxygen-carrying capacity in the blood, which may cause them to suppress the production of erythropoietin, resulting in **a decrease in RBC production**;
(c) for the same reason shown in (b), the body may respond to the overabundance of oxygen-carrying capacity in the blood by **increasing the destruction rate of RBCs**; and
(d) as astronauts lose calcium in their bones, the structure and function of the bone and its marrow may change and may result in a decrease in RBC production.

Out of all four of these theories, there are two main points that emerge. A decrease in RBC number can occur by **a decrease in RBC production** or **an increase in RBC destruction** (or a combination of both).

A detailed investigation developed by Dr. Clarence Alfrey and his research team was designed to examine the effect of microgravity and the interaction of changes in body weight and plasma volume, which both decrease inflight, on the rate of RBC production. His experiment was also designed to examine the roles of the hormone erythropoietin in the production or reduction of circulating RBCs. The investigation measured:

- changes in plasma volume (PV) and red blood cell mass (RBCM),
- hematocrit levels (RBC volume per volume of blood),
- reticulocyte counts, and
- erythropoietin levels in the blood. The investigators also examined:
- erythrocyte production and survival, and
- the rate at which the bone marrow uses iron to produce RBCs.

Before we begin our examination of the results from Dr. Alfrey's study, let's review the original hypotheses that served as the foundation for the development of Dr. Alfrey's space flight study. In addition, just as in the previous chapter, we will participate in some Student Investigations that are designed to clarify certain important concepts related to the measurement of RBC activity. Dr. Alfrey's experiment was designed to support one of the following two simple hypotheses (and refute the other), or, alternatively, to support or refute them both:

Hypothesis 1
Red blood cell mass is reduced during space flight as a result of a decrease in red blood cell production, which in turn is due to a decrease or inhibition of erythropoietin production.

Hypothesis 2
Red blood cell mass is reduced during space flight as a result of increased destruction of red blood cells.

YOUR PERSPECTIVE

Before describing some of the actual experimental procedures that Dr. Alfrey and his team carried out, it is important for you to understand more clearly some of the scientific techniques and concepts related to his study. The first exercise has been designed to provide you with background and basic information about hematocrit. You will be given some specific information about how certain conditions affect a person's hematocrit. Then you will be asked to use that information to match certain hematocrits with certain described individuals.

The second exercise will familiarize you with the concept of **concentration**. You will see how measuring the concentration of a dye in a liquid can help determine the **volume** of that liquid. The third exercise will use what you learned in the second exercise to explain how to determine how quickly RBCs are produced in the bone marrow. **RBC production** rates are determined by a **radioactive marker** (or **tracer**) method that utilizes the concept of concentration. Low levels of radioactivity, when used in controlled situations, can be a valuable research tool for the determination of various rates and volumes within the body. Dr. Alfrey's investigation utilized three different radioactive elements to accomplish various measurements for his study. In the third Student Investigation, a specific technique will be described that uses a **radioactive marker** to actually "mark" or "target" certain cells in order to determine the efficiency of RBC production in the bone marrow. The changes in concentration of these markers over time will give important information about the rate at which the marker is being utilized in the body. You will carry out a graphical analysis of the results from this technique.

None of the Student Investigations are difficult. Keep in mind that all three of the activities relate to actual situations that occur every day in many laboratories around the country.

Enjoy your journey!

STUDENT INVESTIGATION 1

How Do Hematocrit Values Vary Among Populations?

Background

In research, it is often desirable to draw conclusions about the scientific results obtained from different **populations**. A population is a group of individuals related by some factor that sets them apart from another group. The population could be related by the fact that they are all humans (as opposed to, for instance, dogs), or that they are all men, or that they are all humans between the ages of 30 and 40 years old. Researchers carry out studies to determine the average behavior of a given population in the area of interest. Here, we will look at hematocrit values and try to guess what population those values were obtained from.

In the Introduction section to this (Dr. Alfrey's) experiment, we learned that the hematocrit is a determination of the percentage of RBCs per unit of blood volume; that is, a hematocrit value of 47 (which is an average value for men) suggests that 47% of the entire blood volume consists of RBCs. The hematocrit value varies between different populations, and hematocrit values can change according to the state of health of an individual, the oxygen availability, or because of heavy bleeding, when many blood cells can be lost. Review the Introduction section along with Figure 2 before proceeding with this exercise.

Procedure

1. Each student should complete this exercise independently.
2. Observe the test tubes illustrated in Figure 5. Imagine that these represent blood samples of five different subjects. Imagine further that these test tubes have been centrifuged and the components of blood have separated to enable us to make a deter mination of the hematocrit level.
3. Use the scale on the left side of the test tubes and write down the hematocrit values on a separate sheet of paper.
4. When all of the hematocrit values have been determined, answer the questions at the end of this exercise. Review your results with the whole class.

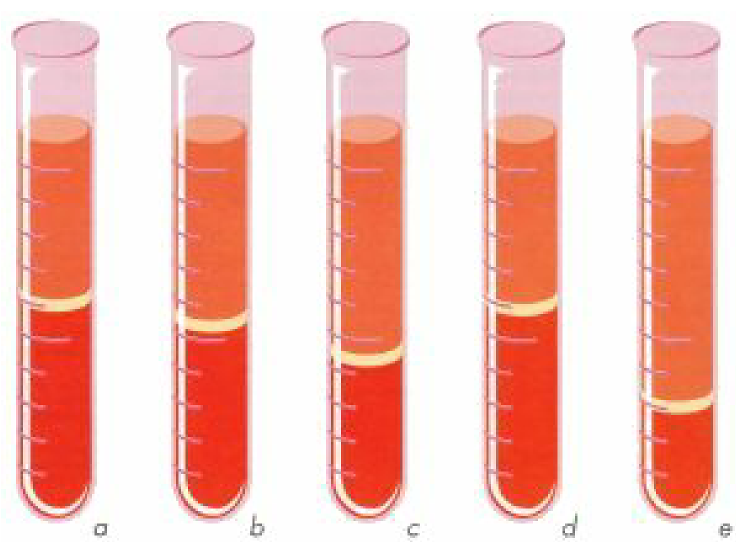


Figure 5. The test tubes contain blood samples collected from five different individuals. Each test tube has been spun in a centrifuge to separate the blood components.

Questions

1. Which blood sample was probably taken from a female? On what information do you base your decision?
2. Which blood sample was probably taken from an anemic person? On what information did your base your decision?
3. Which blood sample was probably taken from a person who smokes? (Hint: Smoking produces carbon monoxide in the body and it enters the bloodstream. Carbon monoxide has 200 times the affinity (attraction) for hemoglobin than does oxygen and won't readily "let go" of the hemoglobin available to

carry oxygen. How would this affect the body's RBC production?)

4. Which blood sample was probably taken from a person who lives at high altitude? On what information did you base your decision?
5. Which blood sample was probably taken from a male astronaut in space? On what information did you base your decision?

STUDENT INVESTIGATION 2

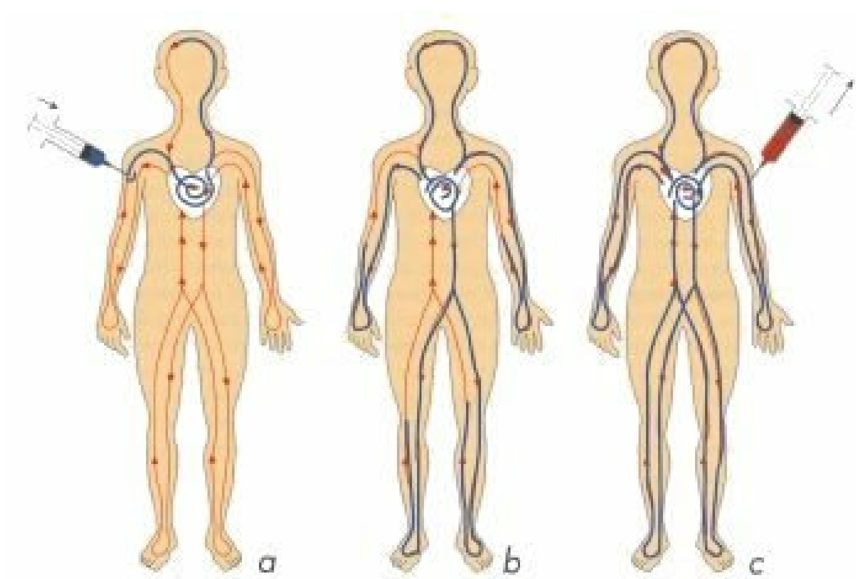
The Dilution Method for Determining Fluid Volumes

Background

How would you measure the volume of plasma in a person's body? If it were possible, the most accurate way to know how much plasma is in the body would be to take all of the blood out of a person's body, spin it in a large centrifuge (to separate the blood cells and the buffy coat) and then pour the plasma off the top of the cells into a large graduated cylinder to measure the total volume of plasma. Of course, this technique would be fatal to the poor individual who became involved in such a crazy effort! Therefore, a technique was developed to obtain a very accurate measurement of plasma volume in the body. The process for measuring plasma volume is called the "dilution method." Dr. Alfrey employed this method to determine the plasma volume for the astronauts who flew in space.

The dilution method involves injecting the astronaut **intravenously** (also known as **IV**, meaning in the veins) with a known quantity and concentration of a **dye** (also known as a **marker** or **tracer** because the dye is used to "mark" the plasma). This dye enters the blood stream and actually mixes with the blood (Figure 6a). After a short period of time, the dye has travelled and mixed with the blood throughout the entire circulation (Figure 6b). During this time, the blood dilutes the dye and then a sample of the blood is collected from the astronaut (Figure 6c). By comparing the **concentration of the dye that was initially injected (X in units of mg)** with the **concentration of the dye in the blood sample (C in units of mg/liter of blood)**, the investigator can determine how much the dye has been diluted. Using this information in a mathematical equation will yield information about the volume of plasma in the body.

Figure 6: (a) To determine blood volume, a person is injected with a known quantity of dye or marker substance. (b) The dye circulates within the blood vessels and becomes evenly distributed throughout the blood. (c) A blood sample is taken to determine how much the dye has been diluted (yielding a new level of concentration of dye in the sample.) The blood volume can be determined based on how much blood must have been present to dilute the dye to its new concentration.



$$\text{Volume of plasma (liter)} = \frac{\text{Amount of dye injected (mg)}}{\text{Concentration of dye in blood sample (mg/liter)}}$$

$$\text{V (liter)} = \frac{\text{X (mg)}}{\text{C (mg/liter)}}$$

The dye that is used in this technique must be chosen very carefully so that none of the dye is lost in other parts of the body and so that an accurate plasma volume determination can be made. What would happen if the dye escaped from the bloodstream and seeped into the rest of the body? Remember that the blood serves as a vehicle to carry various substances to the cells and tissues of the body. Constant exchanges are taking place through the blood vessel membrane where oxygen, vitamins, minerals, and many other things pass in and out of the bloodstream. In order to make sure that the dye does not pass through the wall of the blood vessel and enter the rest of the body, a dye is chosen that binds directly to proteins in the blood that are too large to pass through the blood vessel wall. This insures that the concentration of the dye is not affected by the possibility of its disappearance into other parts of the body.

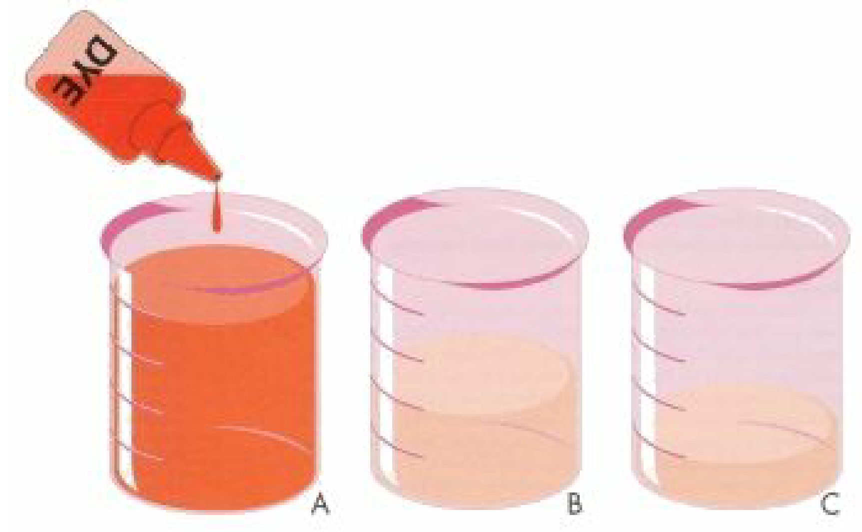


Figure 7: The three containers have different fluid volumes. A known amount of dye has been added to container A. It is your job to determine how much dye to add to containers B and C in order for the concentration of dye in each container to match the concentration in container A.

In this exercise, you will be asked to add a certain number of "drops" of dye to a certain volume of water. Then you will have to determine how many drops you must add to different volumes of water in order to obtain the same concentration of dye in the liquid (Figure 7). By referring to the equations above, Dr. Alfrey's investigation was designed to **calculate V** (the volume of blood) by **measuring X** (the amount of dye added to the blood) and **measuring C** (the concentration of dye per liter of blood). You will be **calculating X** (the amount or number of drops of dye) by **measuring V** (the volume of water) and **measuring C** (the concentration of dye per volume of water). It takes some very sophisticated analysis equipment to determine the concentration of dye in a blood sample. You probably do not have access to this equipment, so your determination of the concentration of dye in the water will have to be made by your eyes! This exercise is simply to point out how different volumes of liquid can affect the concentration of a dye. In the final part of this exercise, you will use what you have learned to calculate the actual plasma volume for some of the astronauts in Dr. Alfrey's study before, during, and after a space flight mission.

Materials

Three large glass beakers, each capable of holding at least one liter and all three the same size.

- A bottle of dye
- A stir stick
- Water

Procedure

1. Students should work in groups. All students must read the entire lab together before beginning their activities.
2. Fill beaker #1 with one liter of water. Fill beaker #2 with a 1/2 liter of water. Fill beaker #3 with 1/4 liter of water. Be as accurate as possible with your volume measurements.
3. Put 24 drops of dye into beaker #1 and stir it until the dye is completely mixed. Count the drops carefully and then record the number on your data sheet.
   At this point, each group should select two individuals to step aside and calculate how many drops **should be** added to beakers #2 and #3 so that the concentration of dye in those beakers will be the same as beaker #1. They will use the equation shown in the background section. For this equation, V is known and C = 24 drops/liter. These two individuals should keep their results to themselves until the rest of their group can finish adding dye to each of the remaining two beakers. At the end, the calculated results will be compared with the actual number of measured drops of dye that were added to the beakers. The rest of the group will continue with the next step.
4. Set beaker #2 next to beaker #1 so that you can compare the two. Begin adding dye **slowly**, drop by drop, to beaker #2. Stir as each drop is added. Add enough drops until you have determined that the concentration of

dye in beaker #2 matches the concentration of dye in beaker # 1. **Don't forget to count the drops as you add them to the beaker.** Record the number of drops you added to beaker #2.

5. Set beaker #3 next to both beaker #1 and #2. Again, begin adding dye slowly, drop by drop, to beaker #3. Follow the same procedure for beaker #3, counting and stirring after each drop, until you have determined that the concentration of dye in all three beakers is the same. Record the number of drops you added to beaker #3.

Now, compare the calculated results with the results obtained by adding drops of dye. Hopefully, the two results come very close to matching. Any difference in the two results can be attributed to the lack of an accurate way to "measure" the concentration of dye in the liquid.

Now, we will look at some actual **raw data** and results that Dr. Alfrey obtained during his space flight investigation. Table 2 includes various data including the amount of radioactive iodine (dye) that was originally injected into the bloodstream of each astronaut (x), and the concentration of that dye in the blood sample that was removed from the astronaut at different times during the mission (c). From these data, you are to calculate the plasma volume (v) using the equation v= x/c. You will be able to see how the plasma volume changed over time during the mission. Your teacher will review with you: the various parts of the table; how to carry out your calculations; and, later, what the correct answers are. You know, people are actually paid to do this sort of analysis!

The Influence of Space Flight on Erythrokinetics in Humans Plasma Volume Data

	GCPM	**NCPM/mL**	**Net Count Injected**	**Net Counts In Post-injected Plasma**	**Plasma Volume in mL**	**% Change from Preflight Value**
Preflight Air Background Injection Solution Pre-injection Plasma Post injection Plasma Volume Injected Volume of Samples	32 342591 32 273 2 ml 1 ml	34255 0 241	685118			Not Defined
FD-2 Air Background Injection Solution Pre-injection Plasma Post injection Plasma Volume Injected Volume of Samples	32 342591 82 382 2 ml 1 ml	342559 50 350	685118			%
FD-8 Air Background Injection Solution Pre-injection Plasma Post injection Plasma Volume Injected Volume of Samples	32 685132 182 737 2 ml 1 ml	685100 150 705	1370200			%
R+O (Landing Day) Air Background Injection Solution Pre-injection Plasma Post injection Plasma Volume Injected Volume of Samples	32 1370264 552 1625 2 ml 1 ml	1370232 520 1593	2742464			%

GCPM = **Gross** Counts Per Minute
NCPM = **Net** Counts Per Minute = GCPM (sample) - GCPM (air background)
NCPM/ml = NCPM divided by the volume of the **sample** or 1 ml
Volume of **samples** = volume of injection solution and plasma that was counted = 1 ml
Net counts Injected = NCPM/ml (Injection solution) time **volume injected** (which was 2 ml)
***** **Net** counts in Post-injected Plasma = NCPM/ml (post-injection Plasma) - NCXPM/ml (Pre-injection Plasma)
***** Plasma Volume (PV) = Net Counts Injected divided by Net Counts in Post-injection Plasma
***** % Change = (FD PV - Preflight PV) divided by Preflight PV
The air background is the same because all of the above count rates (counts per minute) were determined on the same day.

THE SPACE FLIGHT INVESTIGATION

Recall that the space flight investigation developed by Dr. Alfrey was designed to examine the erythrokinetics of space flight. The fluid shift that we've already discussed in the previous chapter has a major impact on the activities of the RBC. This, of course, makes sense, because RBCs are part of most of the fluid (blood) that shifts toward the upper part of the body. Therefore, not only is there a fluid shift but there is also an RBC shift. In response to these shifts, not only is there an elimination of fluids from the body soon after an astronaut arrives in space, but there is also an elimination of RBCs. We know this because the hematocrit stays about the same in space. That is, the ratio of plasma to RBCs in the blood on Earth is the same as the ratio of plasma to RBCs in space. The question is: Are RBCs eliminated because of a decrease in their production **or** because of an increase in their destruction **or** because both occur simultaneously?

Why is this question important? Well, a reduction in production is definitely different than an increase in destruction. Here on Earth, we understand well how the body's oxygen demand can influence the production of RBCs. However, in a healthy person, the destruction of mature RBCs should only occur when RBCs have gotten old or have somehow been damaged. On Earth in a healthy average sized adult, this only happens to about 0.8% of the total RBCs per day. Of course, in almost all cases, these are also replaced everyday. An increased destruction rate would mean that healthy, young RBCs are being destroyed and this is something we have never seen in healthy human beings. Therefore, the question is important because answering it could provide important information about how the RBC system works.

In order to fully characterize RBC activity in space, Dr. Alfrey's team designed a full set of measurements that look at different aspects of RBC production and survival. The preflight, inflight, and postflight portions of this experiment were done on two space missions and the results are very interesting. We will review each major measurement set to see the changes in preflight, inflight, and postflight conditions of the astronauts. For each measurement set, we will include information about why the measurements are important, the equipment and techniques used to make the measurements, the expected results, and the actual results. These measurement sets were designed to study changes in:

(1) plasma volume, RBC mass, total blood volume, and hematocrit;
(2) erythropoietin levels and reticulocyte counts; and
(3) RBC production and survival.

Ultimately, however, the measurement sets were designed to support or refute Dr. Alfrey's hypotheses:

Hypothesis 1

Red blood cell mass is reduced during space flight as a result of a decrease in red blood cell production, which in turn is due to a decrease or inhibition of erythropoietin production.

Hypothesis 2

Red blood cell mass is reduced during space flight as a result of increased destruction of red blood cells.

Just as in the previous chapter, all of the results that you will see involve **real experimental data**. They were obtained during the course of preparing for, during, and after one or more space flight missions. After you review the scientific result s that are presented in each of the next three sections, you will be asked to break into small groups to work on the development of a presentation that members of your group will deliver to your classmates. Each small group will take one of the three sect ions and develop a plan for how to presenting the information in a clear and concise way.

Let's move on to examine Dr. Alfrey's investigation.

STUDENT INVESTIGATION 3

Determining the Efficiency of RBC Production in the Bone Marrow

Background

Each RBC contains about 200 - 300 million hemoglobin molecules, and each hemoglobin molecule has four atoms of iron, which attract four oxygen molecules. (The oxygen is obtained from the lungs and it attaches to the iron so that it can be delivered to the cells and tissues of the body.) RBCs being manufactured in the marrow obtain iron from the plasma. This iron is then incorporated into the hemoglobin. In fact, most of the iron in the body is in the hemoglobin of the RBCs. When RBCs are destroyed, iron is returned to the plasma where it will be used again to form new RBCs. Using a **radioactive marker** (or **tracer**) method (using radioactive iron - Fe59), we can determine the rate at which the marrow removes iron from the plasma, and, therefore, determine the rate at which the marrow is producing the RBCs.

The method to measure the amount of radioactive iron in the blood involves using a **scintillation counter**. The radioactive iron is continually decaying and it gives off gamma rays. The scintillation counter will count the number of gamma rays being given off by the radioactive iron. The number of counts that is registered by the scintillation counter is proportional to the amount of radioactive iron present in a blood sample.

The protocol used in determining the production rate of RBCs is the following:
(You will not be carrying out the actual protocols, but you will carry out a graphing exercise to illustrate the concept.)

1. Three astronauts on a mission are serving as the subjects for this study.
2. Each astronaut is given an injection of a radioactive iron marker (called Fe59). (At this point the iron races to the bone marrow to be absorbed and to be used in the production of the RBCs.)
3. Every half hour, a blood sample is taken from each astronaut to continue over a period of two hours. The samples are collected and analyzed to determine how quickly the Fe59 is taken up by the marrow. (The rate at which the Fe59 disappears from the blood is an indication of how efficiently the bone marrow is working to absorb the Fe59, and, therefore, how efficient the bone marrow is in producing RBCs.)
4. The data is shown in Table 3. Plot this data on a graph that your teacher will provide to you like Figure 8 and then answer the questions that follow.

Table 3. Data reflects the percentage of Fe59 remaining in the plasma for each astronaut. The measurements were made at regular time intervals after injection so the disappearance rate of Fe59 could be determined The Fe59 disappearance rate reflects the rate of absorption of Fe59 by the bone marrow.

% Fe^{59} Remaining in Plasma

Hours After Injection of Fe^{59}

Hours after injection	Astronaut 1	Astronaut 2	Astronaut 3
0.5	95	95	93
1.0	90	95	85
1.5	86	93	78
2.0	81	92	70

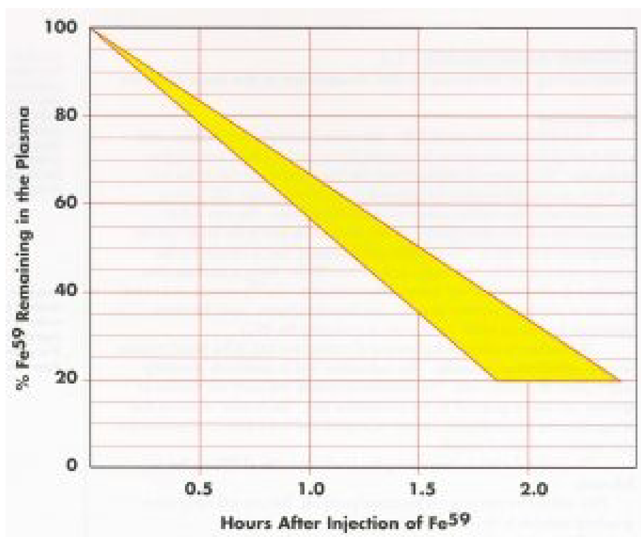


Figure 8. Graph of the efficiency of bone marrow uptake of iron from the blood. The area in the triangle represents the normal (Earth value) range.

Questions

1. Plot the results of your bone marrow Fe59 absorption study from all three astronauts on a copy of the graph in Figure 8. Do the results suggest a normal, decreased, or increased ability by the marrow to absorb Fe59 in space compared with Earth? What does this suggest about the ability of the bone marrow to produce RBCs?
2. How many molecules of oxygen are carried by an RBC? (Hint: Read the Background section for this Student Investigation).

The experiments that you have just completed have prepared you to understand some of the analytical methods that Dr. Alfrey and his team were used for the completion of their study. Appropriate analysis is the key to obtaining results that are correct and understandable. Let's now move on to discuss the actual methods and results that Dr. Alfrey obtained in his examination of how erythrokinetics change when a human enters the microgravity of space.

I. Measurements to Understand Quantitative Changes in the Major Blood Components: Plasma Volume (PV), Red Blood Cell Moss (RBCM), Total Blood Volume (TBV), and Hematocrit

Before a scientist dives into an examination of **why** certain changes occur in the body, he or she must obtain a quantitative understanding of the symptoms of the changes. First of all, symptoms provide evidence that a change that has taken place. The quantitative measurements of those symptoms tell you the **magnitude**, or **how much**, of a change has taken place. Therefore, you must know the magnitude of the symptoms before you can begin to understand the source of the symptoms.

For instance, if you fall while skiing down a mountain and you feel extreme pain in your knee, the magnitude of the pain is a symptom that suggests you might have a serious injury. If you feel just a slight amount of pain only when you touch it, this lesser magnitude of pain could suggest that you might have just bruised your knee. And if you do not feel pain at all in your knee, then there is no reason to think that there is anything wrong with it and you can get up and continue skiing. The magnitude of the pain will determine what should be done next to diagnose the source of that pain.

For the investigation of the erythrokinetics of space flight, the first step for Dr. Alfrey's team was to determine the magnitude of the **volumetric changes** (volume is the amount of space that a substance occupies) of the major blood components, plasma and RBCs, as well as changes in the total volume of blood. Once the magnitude of the changes is understood, then Dr. Alfrey can begin to diagnose the source of the volumetric changes.

It has been known for some time that fluid volumes in the body decrease in microgravity. More specifically, scientists were aware that there are volumetric changes in some of the major blood components. Every astronaut studied after returning from space has been shown to have a decreased red blood cell mass (RBCM), a decreased plasma volume (PV), and, therefore, since

PV + RBCM = TBV,

there is also a decrease in the astronaut's total blood volume (TBV). The condition that results from the decreased RBCM has been termed "**space anemia**." A major part of Dr. Alfrey's investigation was to measure the magnitude of changes in PV, RBCM, and total blood volume (TBV) in astronauts. Hematocrit measurements were also performed. The unique feature of Dr. Alfrey's study was that, in conjunction with the determination of **how much** the fluid and RBC volumes decreased in space, he also carried out the most complete set of measurements to determine **why** the changes took place. In this section, we will only discuss the volumetric (how much) measurements that were carried out.

PV and RBCM were measured using the **dilution method** (described in Student Investigation 2). Remember, the dilution method involves injecting the astronaut with a known quantity and concentration of marker substance (a dye or a labeled molecule) that mixes with the liquid portion of the blood (plasma) and becomes evenly distributed throughout the bloodstream. Once the marker is evenly distributed, a blood sample is taken. By comparing the concentration of the marker that was initially injected with the concentration of the marker in the blood sample, the scientist can determine how much plasma must be in the system to dilute the marker to its new concentration. Using the formula

$$\text{Volume (liter)} = \frac{\text{X (mg)}}{\text{C (mg/liter)}}$$

or,

$$\text{V (liter)} = \frac{\text{Amount of marker substance injected (mg)}}{\text{Concentration of marker in blood sample (mg/liter)}}$$

one can determine the PV and the RBCM.

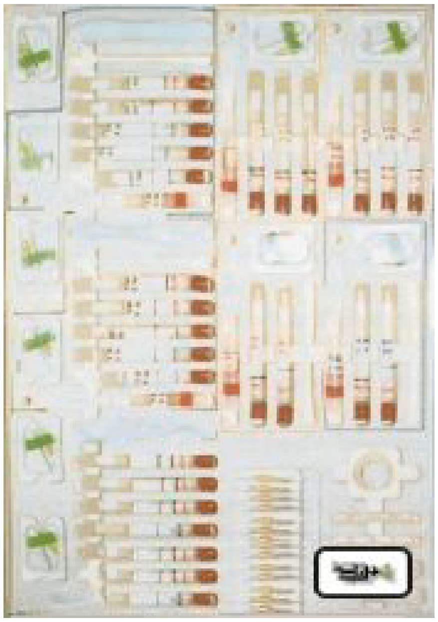


Figure 9. Blood samples collected from the astronauts are stored in a carefully packed Blood Sample Kit. The kit has Velcro attachments to secure the blood samples so that they do not float around the cabin of the spacecraft. Blood that will be returned to Earth for analysis is stored in the refrigerator or freezer to preserve it.

For Dr. Alfrey's determination of PV, astronauts were injected with specially prepared blood protein molecules called **albumin**. Before injection, the albumin molecules were labeled with a radioactive material, **125-iodine**, that attaches to the albumin and emits **gamma rays**. This radioactive property (the emission of gamma rays) served as the "marker" to distinguish the injected albumin molecules from those that the astronauts already have circulating in their bloodstream. The dose of radioactivity that the astronauts were exposed to was very low and safe.

At this point, you may be wondering what a gamma ray is. Well, in 1905, a thoughtful young man by the name of Albert Einstein came up with a new theory that simplified our understanding of light. Remember, light is a form of electromagnetic (EM) radiation. According to Einstein, light and other forms of EM radiation consist of individual bundles of energy that radiate from a source. These bundles of energy are called **photons**. The EM radiation (light) that you see shining from the Sun is composed of photons. The EM radiation (light) from a light bulb in your classroom or home is composed of photons. Not all EM radiation (Figure 10) is visible but all EM radiation is composed of photons, and all photons carry various amounts of energy. **Gamma rays are very high energy photons** that are not visible to the naked eye.

Note: All forms of electromagnetic (EM) radiation consist of photons. You have already been exposed (no pun intended!) to the EM spectrum before in the Introduction section of the book It is shown again to remind you that gamma rays fall in the very high energy range of the spectrum.

When this radioactive albumin is injected into the astronaut, it circulates around the bloodstream until it is mixed fully and distributed evenly with the rest of the blood. It takes about 30 minutes for the albumin to spread through the bloodstream. After this time, a blood sample is taken from the astronaut, and the amount of labeled albumin in that sample is measured by counting the number of gamma rays emitted from the blood sample. The number of gamma ray emissions is directly proportional to the number of radioactive labeled albumin molecules in the sample.

Now, as mentioned previously, it is impossible to see gamma ray emissions with the naked eye. Therefore, in order to be able to count the number of gamma rays that the sample emits, it is necessary to use a special gamma ray counter that can transform each gamma ray into a visible form of energy - light flashes. In order to accomplish this, the blood sample was placed inside a **sodium iodide crystal** (Figure 11), which is a widely used gamma ray **scintillation detector** (scintillate = to emit sparks or flashes). Every time a gamma ray is emitted from the blood sample, the crystal emits a flash of light. The number of light flashes reflects the number of gamma rays emitted from the blood sample. In Dr. Alfrey's experiment, the astronauts were injected with the labeled albumin preflight (to obtain a baseline measurement), twice inflight, and postflight. About 30 minutes after each injection, a blood sample was taken and PV was determined. The results are shown in Figure 12.

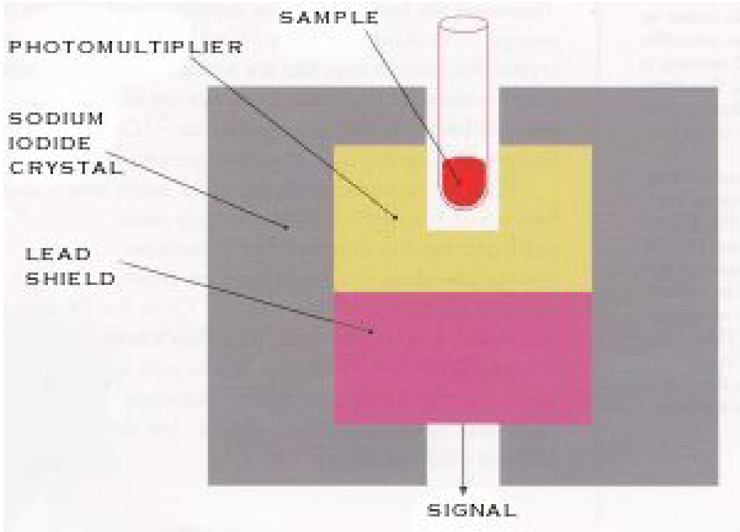


Figure11. A sodium iodide crystal is used to detect gamma rays. The energy from the gamma ray is transformed by the crystal into a visible form of energy- light flashes.

RBCM was also determined using the dilution method. However, the "markers" used in this case were radioactive labeled RBCs. Before the mission, a blood sample was taken from each astronaut and combined with a compound called sodium chromate (Na2CrO4). This compound specifically enters the RBCs in the blood sample where a radioactive isotope of chromium (51 Cr) sticks strongly to the hemoglobin. The blood sample is then spun in a centrifuge so that the plasma separates from the RBCs. The plasma is removed and all that is left are the radioactive 51 Cr labeled RBCs. The labeled RBCs are then injected back into the astronauts where they will circulate and distribute evenly in the bloodstream.

The same blood sample that is taken to determine PV can also be used to determine RBCM. Both the 1 25-iodine and the 51 Cr are gamma ray emitters. However, the two radioactive materials emit gamma rays that are different in energy. Therefore, when the blood sample is placed into the sodium iodide crystal, the gamma rays that are emitted from the 51 Cr are higher energy photons (and therefore brighter) than those that are emitted from the 125-iodine. Therefore, the very bright flashes are counted as 51 Cr gamma ray emissions and the dull flashes are counted as 125-iodine gamma ray emissions.

PV was measured preflight, at 22 hours and about 160 hours inflight, and then again postflight. RBCM was only measured preflight and within two hours postflight, but it is assumed that it decreased in a linear fashion throughout the mission (therefore, a straight line was drawn on the graph in Figure 12 between the preflight and postflight values). Once the PV measurement values and the RBCM values were obtained, Dr. Alfrey's team could easily determine total blood volume (TBV). Remember, the TBV in your system consists primarily of two main parts, the plasma and the RBCs. Therefore, TBV = PV + RBCM. The TBV was calculated by adding PV and RBCM. The data for PV, RBCM, and the calculated TBV are shown in Figure 12.

Now, what does the data indicate? Well, from the results shown in Figure 12, it is clear that early in the mission there was a drop in the PV followed by a decline in RBCM. As the flight continued, some of the early loss in PV was regained, thereby replac ing the TBV lost due to the decrease in RBCM. These changes suggest that the TBV is regulated precisely by changing the volumes of its two components, RBCM and PV.

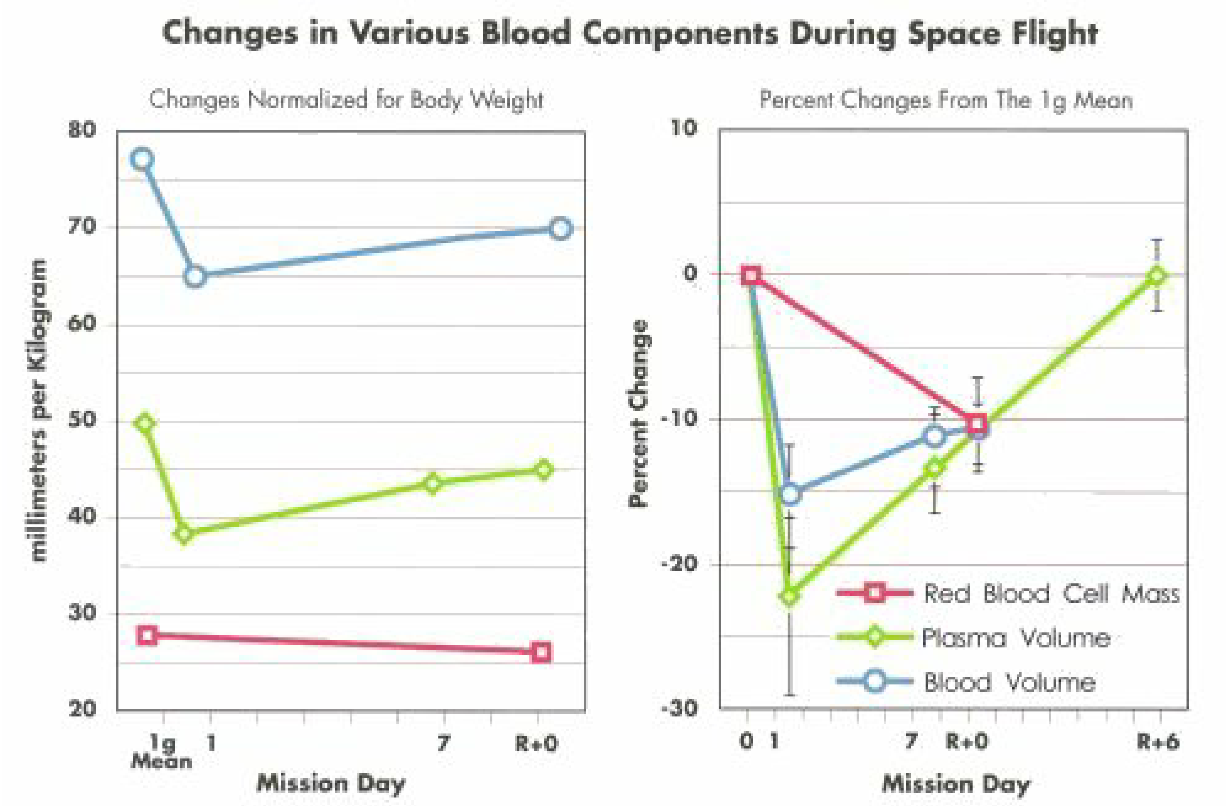


Figure 12. Plasma volume (PV), red blood cell mass (RBCM), and total blood volume (TBV) during space flight are all below preflight levels.

The final measurement in this set involves the determination of hematocrit. You have already learned that hematocrit is a measure of the volume of RBCs in the body expressed as a percentage. What you may not have known is that there are **two different kinds of hematocrit values**. Most of the hematocrit determinations that are done routinely are called **peripheral venous hematocrit (PVH)**. This means that blood is taken from the peripheral (or outside surface) veins that are easily accessible. As you know, the peripheral venous hematocrit values are usually about 42 for women and 47 for men. However, the percentage of RBCs in the blood from the surface veins can differ somewhat from the percentage of RBCs in the blood of the veins and arteries th at are found deeper in the body. The deeper blood vessels usually have hematocrit values [called **total body hematocrit (TBH)**] that are lower than the hematocrit values found in the peripheral vessels (PVH). This is partly because there are a large number of capillaries in the deeper portions of the body that are smaller and have a lower percentage of RBCs and a higher percentage of plasma.

It is difficult and dangerous to obtain blood samples from deep within the body. This would require long needles to be penetrated deep into the core of the body. Therefore, an equation is used to calculate TBH:

FORMULA

The value in obtaining both TBH and PVH lies in their combination as a ratio (TBH/PVH). The value of that ratio can be compared with what would be considered a normal value. It is well known that, on Earth, the ratio of the TBH to the PVH (TBH/PVH) in a normal individual is .90. Dr. Alfrey's team determined the astronaut's TBH/PVH ratio for astronauts preflight, inflight, and postflight. The data for PVH, TBH, and for the ratio TBH/PVH is shown in Table 4.

From Table 4, you can see only small changes in the PVH. However, the TBH did increase early in the flight. These results illustrate the hazard in relying only upon the PVH as a true indicator of overall RBC volume in the body. The key determination for this set of hematocrit values is the ratio TBH/PVH. As mentioned previously, in healthy human beings here on Earth, a ratio value of .90 is considered normal. As you can see from Table 4, the ratio increases early inflight and then returns essentially to the preflight value.

In this section, we have merely been describing the magnitude of the symptoms that suggest that the erythrokinetics of space flight are different from that on Earth. Whether on Earth or in space, the body obviously works very hard to maintain a certain percentage of RBCs, and when that percentage changes, certain mechanisms are "turned on" or "turned off" to help maintain tight control of the number of RBCs in the body. In the next section, we will examine how the hormone erythropoietin acts to "turn on" or "turn off" the RBC faucet and how that mechanism may act differently in space than it does on Earth. Also, we will examine how the number of reticulocytes (immature RBCs) in the blood indicates the rate of production of RBCs.

Table 4. Peripheral venous and total body hematocrit data.

Peripheral Hematocrit

Crew Members	1 g		Inflight		Postflight	
	Mean	Standard Deviation	22 Hours	160 Hours	R + 0	R + 14
1	39	0.3	42	45	41	36
2	43	0.4	41	41	42	41
3	39	0.3	40	41	38	36

Total Body

Crew Members	1 g		Inflight		Postflight	
	Mean	Standard Deviation	22 Hours	160 Hours	R + 0	R + 14
1	34	0.008	42	34	36	-
2	38	0.008	41	40	38	-
3	34	0.010	40	37	34	-

Total Body Hematocrit/Peripheral Hematocrit

Crew Members	1 g		Inflight		Postflight	
	Mean	Standard Deviation	22 Hours	160 Hours	R + 0	R + 14
1	0.87	0.02	1.05	0.76	0.88	-
2	0.90	0.03	1.05	0.98	0.98	-
3	0.86	0.04	0.95	0.90	0.90	-

II. The RBC Faucet Measurement of Erythropoietin Levels and Circulating

The body (and in most cases, the brain) is always monitoring every physiological function. If staying alive is the body's goal, then **homeostasis** is one major strategy that the body uses to achieve that goal. In simple terms, if **any change** in **any body function** is detected by the brain, many mechanisms are activated to correct the change. As mentioned previously, this detection and correction process is almost always done using **negative feedback mechanisms**. If the kidney detects **too little** of something (oxygen, for instance, which is an indicator of too few RBCs), the kidney sends a message to the body to release **more** of something else (the hormone erythropoietin, for instance) to correct the situation. In our example, the erythropoietin travels to the bone marrow to stimulate increased production of RBCs. That is, the erythropoietin causes the "RBC faucet" to increase its flow. Conversely, too much oxygen (indicating too many RBCs) will cause less erythropoietin to be released, causing the "RBC faucet" to decrease its flow.

Now, it must be noted here that the homeostatic condition of the body on Earth is different from the homeostatic condition of the body in space. To state this in terms that you have heard before, the "Earth-normal" condition of the body is different from the "space-normal" condition of the body. The body goes through a natural process of **adaptation** when it senses the new environment of space. The brain develops a new set of criteria from which to judge the condition of the body, and the feedback mechanisms operate differently to reflect the new criteria. Therefore, the PV, RBCM, and TBV changes that we looked at in the previous section are normal changes for the body to undergo in space. And the space flight changes in erythropoietin levels and reticulocyte counts that you will look at in this section are also normal adaptive measures undertaken by the body in response to the new environment of space.

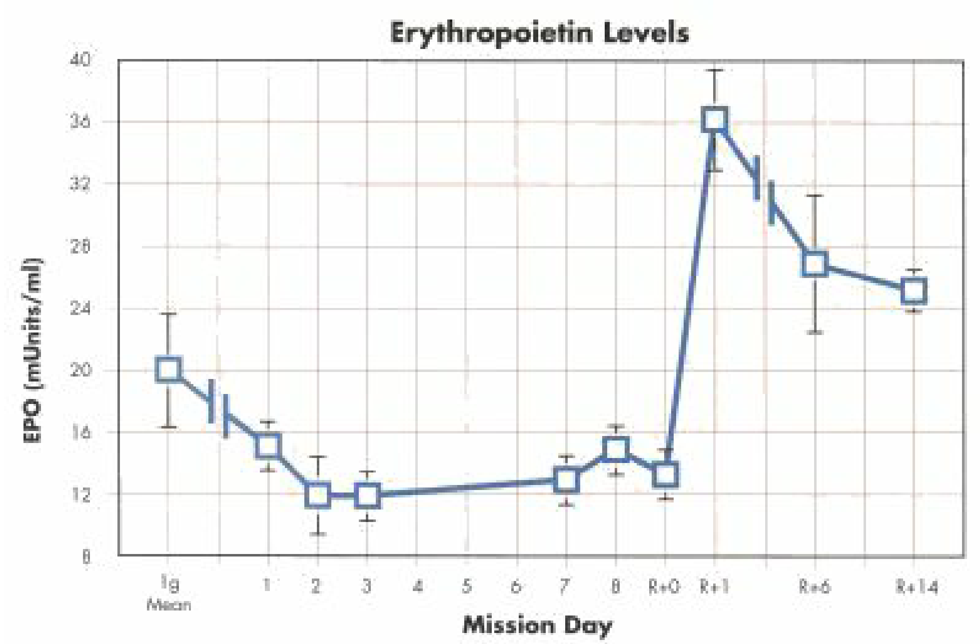


Figure 13. Preflight inflight and postflight erythropoietin (EPO) levels.

In Dr. Alfrey's study, erythropoietin levels were measured from blood samples taken preflight, six times inflight, and postflight. The inflight blood samples were frozen to assure their preservation for later analysis on Earth. As you can see from Figure 13, the inflight erythropoietin levels are lower compared to preflight levels. In fact, they are significantly lower (at times by up to 50%) until the end of the mission. The reduction in erythropoietin levels indicates where at least some of the responsibility rests for the reduction in RBCM seen in the last section. One day after landing, erythropoietin levels increased to twice preflight levels, presumably in response to the body's immediate need to replace the RBCM that decreased in space. At this point, the brain has had to reprogram itself to respond to the needs of the body under the conditions of Earth's gravity.

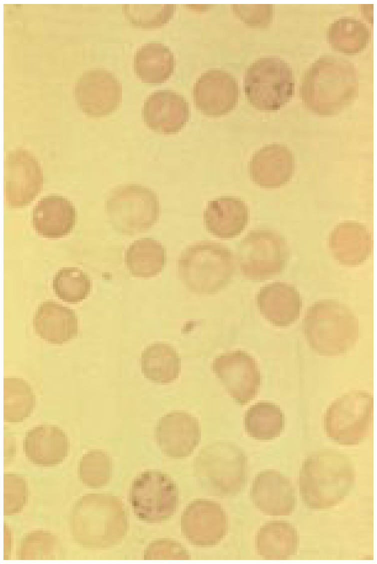


Figure 14. A smear slide showing selectively stained reticulocytes.

In the normal process of RBC production, there are certain **precursor cells** that must first develop in order to become full-grown RBCs. Precursor cells are the "babies" that eventually mature into full-grown RBCs. The bone marrow always has a supply of "RBC embryos," called **stem cells**, that wait for the signal from erythropoietin before they begin to develop. Once the erythropoietin signal arrives, these stem cells mature into the "RBC babies" known as **reticulocytes**. There are always a small number of reticulocytes that are released regularly by the bone marrow into the bloodstream, but if RBC production is increased for any reason, there are proportionally more reticulocytes circulating in the blood. If RBC production is decreased for any reason, there are then less reticulocytes released into the bloodstream. Therefore, the number of reticulocytes in the blood can indicate an increase or decrease in the production rate of RBCs that is occurring in the bone marrow.

The number of circulating reticulocytes in the bloodstream was determined before and after the mission in order to understand if the rate of production of RBCs is affected by space flight. This was done by taking a drop of blood from each of the four astronauts, adding a methylene blue dye that selectively marks the reticulocytes, and smearing the dyed sample on a glass slide. Under a microscope, the number of reticulocytes in the blood sample can be counted (Figure 14) and this number can be converted to a relative volume overall. Dr. Alfrey's results are shown in Table 5.

Table 5. Preflight and postflight reticulocyte counts.

What do these results indicate? That is, why do the erythropoietin levels and the number of reticulocytes decrease? Well, for one thing, the plasma volume decreases inflight. If the RBCM did not also decrease, the blood would be too thick with RBCs. Therefore, in order for the RBCM to decrease, the brain must signal the kidneys to slow down the release of erythropoietin. The consequence of decreased erythropoietin levels is a decrease in erythropoiesis. This means that there are less RBCs "born," which also means that the number of "baby RBCs," or reticulocytes, released by the bone marrow would decrease. It should be expected, then, that the decreased erythropoietin level was accompanied by a lowered reticulocyte count.

Another point to remember is that the body's oxygen requirement probably decreases inflight because astronauts do not utilize their muscles in the same way that they must on Earth, where we must always work against gravitational forces. Since RBCs carry the oxygen and there is less oxygen needed, then there are fewer RBCs needed by the body while in space.

We have talked about the negative feedback mechanism involving erythropoietin that is responsible for "turning on" or "turning off" the RBC production faucet. We have also looked at one of the factors (the number of reticulocytes) that indicate the rate of RBC production. In the next section we will look at the RBC production process more deeply and how other factors may become involved to either influence the rate of production or to affect the survival of RBCs.

III. RBC Production and Survival

Dr. Alfrey's entire experiment represents the first time that the basic mechanisms responsible for the observed reduction in RBCs has been so thoroughly studied preflight, inflight, and postflight. Up to this point, we have learned a great deal about his experiment. We first examined the quantitative changes in the major blood components resulting from space flight and found that, compared to Earth values,

- PV decreases,
- RBCM decreases, and
- TBV decreases.

We also learned about the two kinds of hematocrit values, peripheral venous hematocrit (PVH) and total body hematocrit (TBH) and found that, compared to Earth values,

- PVH remains about the same,
- TBH initially increases but then stabilizes, and
- the TBH/PVH ratio initially increases but also returns to an Earth-normal value.

Next we investigated the "first level" of indicators that verified that RBC production is decreased and found that:

- erythropoietin levels in the blood decrease, and
- reticulocyte counts decrease.

Both of these factors, when looked at together, indicate a reduced level of RBC production, which explains at least part of the reason for the RBCM decrease that occurs early in space flight.

Wow! You have learned a great deal about Dr. Alfrey's experiment and his results up to this point. We now have one more level of information to absorb in order to obtain a clearer picture of those mechanisms that may be responsible for the decreased RBCM. This section deals with the issue of RBC production and survival.

Here on Earth,

production rate of RBCs = destruction rate of RBCs

so that a **steady state** always exists in the number of RBCs in our body. That is, there is always a constant number of RBCs in our bloodstream on Earth. As RBCs become old, they are destroyed by the body to make room for new RBCs that are born to replace the old ones. If destruction of RBCs occurs **before they are old**, however, then not only is the destruction rate increased but the survival rate of healthy RBCs is reduced. Let's state that idea one more time because you must understand the concept fully in order to appreciate this part of Dr. Alfrey's experiment.

If RBC destruction increases, then RBC survival decreases.

As an analogy, let's think about the consequences of war. It may seem strange to bring the subject of war into a scientific discussion, but it can serve as an example to clarify what we are talking about. During a war, there is an **increase** in destruction of human life. This means that there is also a **decrease** in the normal survival time (life expectancy) of those lives that were destroyed. Therefore, as the destruction of human life increases, human survival decreases. Now let's get back to the subject of RBCs.

We already know that, in space, the number of RBCs decreases early in flight. We have also learned that a reduced production level of RBCs is responsible for at least some of that early decrease in RBCs. The main point of this part of Dr. Alfrey's experiment is to document how much the **production rate** decreases in space and to determine whether the **survival rate** of RBCs might also be affected by space flight. Survival rate is determined by measuring **destruction rate** (remember the war analogy?).

In order to study both RBC production and survival, the astronauts were injected with radioactive 51 Cr labeled RBCs 21 days prior to launch and blood samples were taken from the astronauts at various points after the injection. With the blood samples, two types of information could be obtained. First, the rate of RBC destruction was determined in order to understand if RBC survival is affected by space flight. Second, the rate of RBC production was determined. Now, how can survival and production rates be determined using radioactive labeled

RBCs? Let's examine this question.

When the radioactive labeled RBCs were injected into the astronaut, then the astronaut essentially had two groups of RBCs circulating in the bloodstream. The two groups (radioactive and normal) of RBCs are identical in certain respects and completely different in other respects (Table 6). First of all, the two groups of RBCs **function identically**. That is, the radioactive RBCs (which remain radioactive their entire lifetime) carry oxygen to the cells of the body just as the normal RBCs do. Also, the radioactive RBCs circulate normally in the bloodstream and they also live as long as the normal RBCs.

Table 6. Similarities and differences between normal unlabeled RBCs and radioactive labeled RBCs on Earth.

Catergory	Description	Normal RBCs	Radioactive RBCs	Status
Function	To carry oxygen to the cells of the body.	Yes	Yes	Identical
Activities	Circulate all around the body for entire lifetime.	Yes	Yes	Identical
Destruction	After lifetime of 120 days they are destroyed.	Yes	Yes	Identical
Production	As RBCs grow old and are destroyed, new RBCs are produced to replace them.	Yes	No	Different
Identification	Emit gamma rays enabling accurate identification and quantification.	No	Yes	Different

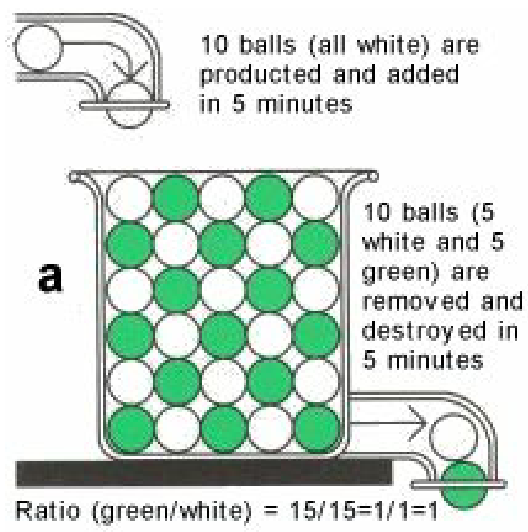
There are two main differences, however, between the two groups of RBCs. New radioactive RBCs **are not** produced by the body when the old ones die, whereas new normal RBCs **are** produced. Secondly, the radioactive 51 Cr that is attached to the labeled RBCs emits gamma rays, causing the labeled RBCs to be distinguishable from normal RBCs in a blood sample through the use of a **scintillation detector** (as discussed in a previous section). The number of gamma rays that are emitted is proportional to the amount of radioactive labeled RBCs in the sample. These differences are the key to why RBC survival and production can be measured.

As both groups of RBCs perform their function together in the body, a small percentage of them die every day. **Radioactive RBCs and normal RBCs die at the same rate.** Therefore, Dr. Alfrey's team can take blood samples at various intervals after the radioactive RBCs have been injected and watch how fast they disappear. This disappearance rate of the labeled RBCs will tell Dr. Alfrey how rapidly both the labeled and the nonlabeled RBCs are being destroyed. If they are being destroyed in space at the same rate that they are being destroyed on Earth, then normal RBC survival is not in question. If, however, they are being destroyed in space more rapidly than they are on Earth, then the RBC survival rate is decreased in space.

Once Dr. Alfrey has determined if the RBC destruction rate is affected by space flight, he can use that information to determine how much RBC production rate is affected by space flight. Let's illustrate this idea to make it a little bit easier to understand. Look at Figure 15. The green balls are radioactive RBCs and the white balls are normal RBCs. To begin with, there are equal numbers of green and white balls. As the balls are destroyed at the same rate, only the white balls continue to be replenished. Therefore, the **ratio** of green balls to white balls continually declines. That is, the green balls are becoming diluted by the continual addition of white balls. By determining how fast the green balls (radioactive RBCs) are being diluted by the white balls (normal RBCs), you can determine how fast the white balls (normal RBCs) are being produced.

Up to this point, we have discussed the basic principles behind the measurements of RBC survival and RBC production. Let's look at each measurement in a bit more detail, along with Dr. Alfrey's results.

Figure 15. An example of RBC destruction rate.



A. RBC Survival To measure the rate of survival of RBCs, the disappearance rate of **total RBC 51Cr radioactivit** was measured. This is shown as **TCrRBC** in (figure 16), which is a short way of writing "total RBC chromium." That is,

the disappearance rate of the total RBC chromium (TCrRBC) is proportional to the RBC survival rate.

A major point to understand about this test is that the disappearance of total RBC 51 Cr radioactivity is caused by either early death or removal. Early death would include **hemolysis** (premature bursting of a healthy RBC) and **phagocytosis** (the attacking and killing of healthy RBCs by cell-eating phagocytes). The removal of RBCs refers to **phlebotomy** (taking too many blood samples for tests like these).

This test depends on the measurement of radioactivity in the **total amount of labeled RBCs**. If RBCs die earlier than they normally do (from hemolysis or phagocytosis), or if too much blood is removed in the process of taking blood samples (phlebotomy), the total number of RBCs will decrease more quickly than normal. Dr. Alfrey can make corrections in his data to account for any loss of blood due to phlebotomy; therefore, the disappearance rate of TCrRBC will indicate if unexpected hemolysis or phagocytosis is taking place. If so, RBC survival will be decreased and if not, RBC survival will be normal.

To determine the survival rate of RBCs, Dr. Alfrey's team took blood samples at various intervals after the initial injection Of 51 Cr labeled RBCs. **The same labeled RBCs that were injected into the astronauts to determine RBCM (which we talked about in a previous section) are used to carry out measurements of both RBC survival and production**. For each blood sample that is taken, the amount of radioactivity in the sample is determined by using the scintillation detector that we discussed earlier. From doing the original RBCM measurement from a blood sample taken soon after the injection of radioactive RBCs, Dr. Alfrey knows how much radioactivity was in the astronaut's system to begin with. This initial measurement is considered the 100% reference point to compare each subsequent measurement. Therefore, the level of radioactivity in the next blood sample that is taken will have some percentage less than the first measurement. Then the next blood sample that is taken will have some greater percentage less than the first measurement, and so on. If these percentages are plotted on a graph relative to the time that the samples were taken, the slope of the curve connecting the points will indicate the **disappearance rate** of the labeled RBCs from the bloodstream. This disappearance rate indicates how fast the RBCs are being destroyed and whether RBC survival is normal or reduced.

The open square data points in Figure 16 indicate the rate of survival (TCrRBC) of RBCs for the astronauts in Dr. Alfrey's experiment. As you can see, measurements were taken five times preflight, and a final measurement was taken after the mission. No inflight measurements were taken for this determination; however, it is assumed that the rate of survival continued in a linear fashion from the preflight value to the postflight value. Therefore, a straight line was drawn between those data points.

B. RBC Production

To measure the rate of production of RBCs, the disappearance rate of 51 Cr **per gram of hemoglobin** in the blood sample is determined. This is shown as **Cr/gHb** in Figure 16,.which is a short way of writing "chromium per gram of hemoglobin." That is,

the disappearance rate of chromium per gram of hemoglobin (Cr/gHb) is proportional to the RBC production rate.

The main point to understand about this test is that as new unlabeled RBCs are produced in the bone marrow, they are released into the bloodstream. The release of new unlabeled cells dilutes the circulating labeled RBCs. Over time, the ratio of labeled RBCs to unlabeled RBCs decreases. The rate at which the labeled RBCs become diluted with unlabeled RBCs is proportional to the rate of new RBC production.

This measurement is not affected by the destruction of RBCs or by the removal of RBCs when blood samples are taken. That is because this measurement does not depend on the **actual amount of RBCs in the system** (as the RBC survival measurement does); it only depends on the **relative ratio** of unlabeled RBCs to labeled RBCs. RBC production can be determined, then, by looking at relative changes in the ratio of radioactive RBCs to normal RBCs. The change in this ratio can be determined by looking at how fast the radioactive RBCs become diluted by the production of normal RBCs.

The round data points in Figure 16 indicate the rate of production (Cr/gHb) of RBCs for the astronauts in Dr. Alfrey's experiment. As you can see, measurements were taken five times preflight, six times inflight, and a final measurement was taken after the mission.

Now, we have covered RBC survival and production separately up to this point and two main questions have emerged:

1. Does the survival rate of RBCs in space differ from the survival rate of RBCs on Earth?
2. Does the production rate of RBCs in space differ from the production rate of RBCs on Earth?

We can now answer these questions. The preflight portion of Figure 16 indicates both the production and survival rates on Earth. As you can see, both rates are identical until the astronauts enter the space environment. At that point, the rate of production of RBCs slows down (compared to the rate of production on Earth) while the survival rate continues normally. The divergence of the two lines indicates a reduced production of RBCs during the flight. From this data, Dr. Alfrey concludes that the **decrease in production together with normal age-related death**

accounts for the observed decrease in RBCM.

Congratulations!

We have come to the end of the scientific description of Dr. Alfrey's experiment. It is left up to you to return to Dr. Alfrey's original hypotheses to determine which of the two were supported by the data. You and Dr. Alfrey now have the answers to make those determinations. But these answers will only lead scientists to develop a deeper set of questions to explore. That is what science is all about. It is a continual search for explanations and connections.

SPEAKING OF SPACE

Now is once again the time to practice the development and delivery of a scientific presentation. The scientific results that you have just examined are broken into three sections. For this activity, your teacher will assign three small groups to take one of the three sections and develop a plan for presenting the information in a clear and concise way. This activity is identical to the "Speaking of Space" activity from the previous chapter. Therefore, the guidelines from the last chapter are repeated here to serve as a reminder for you. There is one slight difference, however. In the development of your presentation, you should attempt to point out to your audience how different areas of science were blended to carry out Dr. Alfrey's experiment. Let's examine this idea a little more closely.

Dr. Alfrey's study incorporates some very interesting and important concepts related to biology, physics, mathematics, and chemistry. You may not have even realized that you were entering such worlds. A new challenge to you involves understanding and appreciating the connections between the biological, physical, mathematical, and chemical principles that you have been exposed to in this chapter. For instance, the dilution method for determining volumes that we covered extensively in this chapter includes the use of chemistry, physics, mathematics, and biology. For your assigned section, look back at the methods and principles used to explain the science and point out in your presentation how basic math was used for certain calculations, or how basic physics or chemistry principles were used to carry out the experiment. Help your audience appreciate how all of the sciences play a part in the background, design, and accomplishment of scientific experiments. The following guidelines will help you design a valuable presentation.

1. **Imagine that your small group is the actual scientific team that conceived, planned, and carried out the experiment**. (Of course, the astronauts actually carried out the inflight portion of the experiment but your role during this time was to oversee all of the activities and make sure that they were trained appropriately to do the right job.)

2. **You should design your presentation as if your audience has never heard the information before.** This means that you must first provide enough background so that your audience can understand the significance of the study. That is, explain why this study is important. Keep the audience foremost in your mind as you design your presentation and always make it as easy as possible for your audience to understand.

3. **You must present information about the hypothesis, your methods, and the results**. Remember that a hypothesis should be a simple, basic statement about what you expect the results to indicate. Develop your own hypothesis based on what was actually expected before the results were obtained. The description of your methods should include information about your protocol, the equipment that was used and how it was used, information about who the subjects were and how many there were, and anything else that is relevant about your study. In planning for your presentation, you must also determine the best way to display your results. You may want to graph the data or present a table of values. If you choose to produce a graph, include a title, the units of measurement on each axis, a legend, and make it as clear as possible. Also, remember to tie the results of your study back to the hypothesis.

4. **Explain what the results indicate about how the body responds to space flight.** Also try to determine how the results might affect our understanding of human physiology here on Earth. Which, if any, health problems that we encounter on Earth might be helped by the knowledge you have gained from your space flight results?

Keep in mind that there are literally dozens (and sometimes hundreds) of people involved in carrying out a space flight investigation, each of whom is responsible for his or her very own specific aspect of the study, and each of whom is absolutely necessa ry to the success of both the individual experiment and the overall mission. There should be plenty of different roles for the different members of your team. There should certainly be a principal investigator who is in charge of the whole study, just as Dr. Alfrey was in charge of the real study that we've been learning about in this chapter. Also, a member of your team might serve as the engineer involved with the equipment. Another member of your team might be a physician or a physiologist that is assi gned to make sure all of the experimental procedures are carried out safely. There might also be various technicians that are responsible for collecting the data or producing the graphics. There are plenty of jobs for everyone. You may want to use more th an one person to present the experiment to your audience. And don't be afraid to use plenty of visual aids. Be imaginative but also be faithful to the main objectives of your experiment.

After each presentation, there should be a short question and answer (Q&A) period so the audience has the chance to ask relevant, thoughtful questions. Rely on your team members to help you answer the questions. Don't let this

Q&A session scare you. It is always a part of any well planned presentation. And remember, you will be on the other side of the fence asking questions of all the other groups! Good luck!

REVIEW QUESTIONS

Earth Physiology

1.
    A. Name three main cellular components of blood.
    B. Briefly describe the main function of each.

2. Describe the role of plasma in the body.

3.
    A. What is the blood test that measures the amount of red blood cells in the blood.
    B. Describe how this test is done.

4. Identify the part of blood that gives it a red color and state its role in carrying oxygen to the cells.

5. Describe the process which controls red blood cell production.

6. Identify the term used to describe the production of red blood cells and identify the main site of

production?

7. Name the organ of the body which triggers the production of red blood cells and explain how it is done.

8. Describe the events that take place after the red bone marrow is triggered o produce red blood cells?

9. What controls the rate of red blood cell production?

10. An average male of 70kg has approximately 5.0L of blood. Based on a hematocrit of 45%, calculate the total body volume of 1) RBC's and 2) Plasma.

    Hematocrit Value: .45 or 45%
    Plasma Proportion: .55 or 55%

Space Physiology

1. Why is the question of whether red blood cell elimination in space is due to a decrease in production or an increase in destruction important?

2. It is known that the astronaut's total blood volume decreases in space. What is the term used to describe this occurrence and why does this happen?

3. Identify the various blood measurements taken to gather data on the effects of space travel on blood.

4. Identify the method used to measure the plasma volume and the red blood cell mass, name the isotope used in the method, and describe the method used.

5. Identify and distinguish between the two types of hematocrit values. Explain why one method is used more often than the other.

6. Did Dr. Alfrey's studies show that erythropoietin levels increased or decreased during flight and what was the significance of this finding?

7. What measurement was made to monitor the production rate of red blood cells and explain how it was interpreted?

8. Explain the two main differences between unlabelled RBCs and labelled RBCs that allow the labelled RBCs to be detected in a blood sample?

CRITICAL THINKING

1.
    A. Given: An average person has about 7,000 white blood cells per mm of blood and 5,000,000 red blood cells per mm. Calculate the Ratio of white blood cells to red blood cells.
    B. Based on what you have learned what does this tell you about the production rate of each cell type and its function?
2. Describe how the rate of production of red blood cells is tied to the disappearance rate of the isotope 51-chromium?

REFERENCES

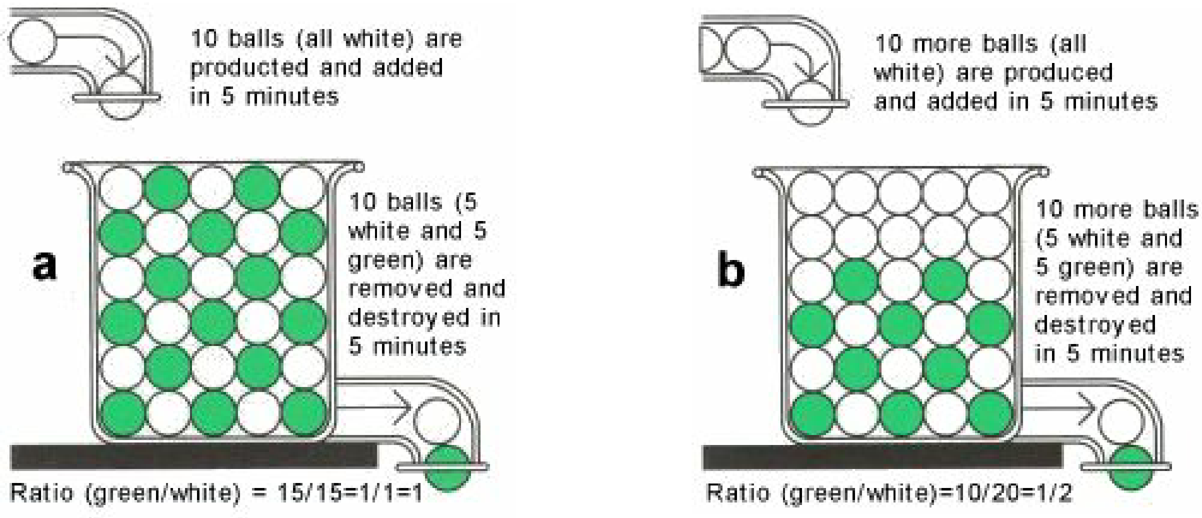
Certain parts of the text were excerpted and adapted from the following publications and other references:

For the section describing Earth Physiology:

1. Fox S1 (1987). **Human Physiology**, 2nd ed. William C Brown Publishers, Dubuque, Iowa.
2. Guyton AC (1986). **Textbook of Medical Physiology**, 7th ed. WB Saunders Company, Philadelphia, PA.
3. Nourse AE (1964). **The Body**. Life Science Laboratory, Time Incorporated, New York.
4. Oram RF (1989). **Biology Living Systems**. Merrill Publishing Company, Columbus, OH.

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

1. Udden MM, Driscoll TB, Leach-Huntoon CS, Alfrey CP (1994). Decreased production of red blood cells in man exposed to microgravity. **New England Journal of Medicine**, in press.
2. **Spacelab Life Sciences I: 180-dayPreliminoryResults**(1991). NASA Headquarters, Washington, DC.
3. Personal communications with Dr. CP Alfrey and Theda Driscoll, Baylor College of Medicine, Houston, TX, February 1994.



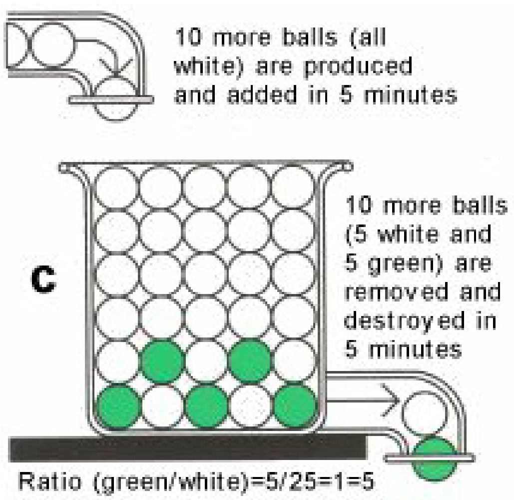


Figure 15. (a) A mixture of evenly distributed green and white balls is destroyed at the rate of 2 balls/minute. The production of only white balls continues at the same rate. (b) The ratio of green to white balls is reduced as the green balls become diluted by the white balls. (c) The ratio is further reduced each minute as the white balls continue to be produced.

Figure 16. Percent changes in the disappearance rate of Cr/gHb (round data points, indicating RBC production) and TCrRBC (open square data points, indicating RBC survival) following injection of ^{51}Cr labeled RBCs 21 days prior to launch until landing day. The divergence of the two lines indicates a reduced production of RBCs during flight.

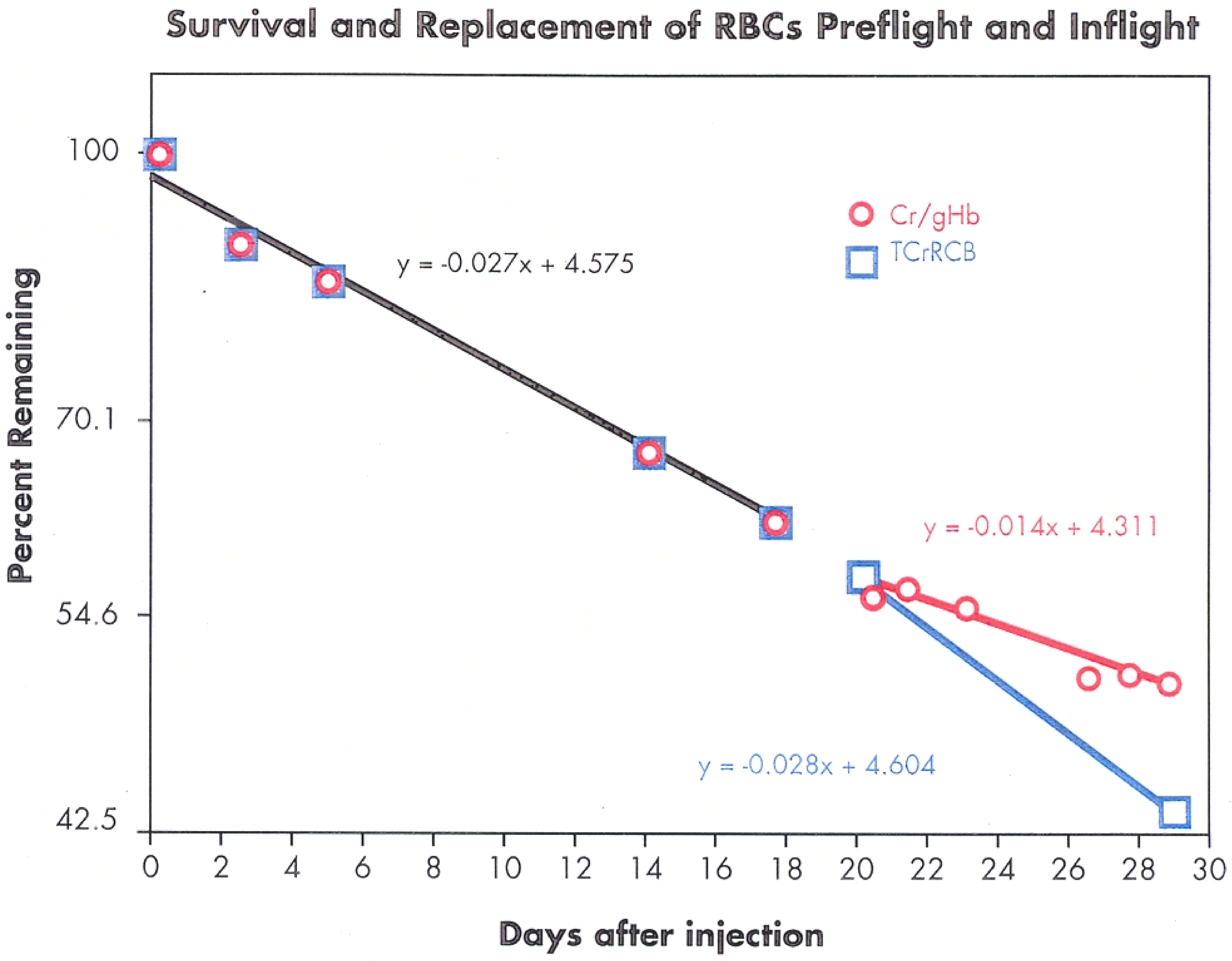


Table 5. Preflight and postflight reticulocyte counts.

Reticulocyte Count

	1g		Postflight	
	Mean	Standard Deviation	R + 0	R + 14
Crew Member 1	0.8	0.08	0.6	1.3
Crew Member 2	1.1	0.15	0.5	1.8
Crew Member 3	1.1	0.07	0.7	1.8