The Red Cell and Anemia

Part One: Introduction to Hematopoiesis and Its Routine Clinical Evaluation

I. Introduction

Hematopathology is not only the study of disease of the blood and bone marrow, but also of the organs and tissues which employ blood cells as principal effectors of their physiologic functions. Such would include the lymph nodes, spleen, thymus, and the many foci of lymphoid tissue found along the aerodigestive tract. Generally two types of medical subspecialists intensively practice in this area, the hematologist and the hematopathologist. The **hematologist** usually is a Board-certified internist who has completed additional years of training in hematology, usually as part of a combined fellowship in hematology and oncology. The thrust of this individual's work is toward the diagnosis and medical management of patients with hematologic disease, especially neoplasms, and medical management of other nonhematologic cancer. The **hematopathologist**, on the other hand, is usually Board-certified in anatomic and clinical pathology and has taken additional years of training in hematopathology. His or her principal activity is the morphologic diagnosis of conditions of the hematopoietic and lymphocyte-rich tissues and in the performance of laboratory testing that assists such diagnosis.[1]

Hematopathology is somewhat unique in its approach to the patient and the disease, in that 1) many diseases are understood at the molecular level, 2) the patient's tissue is easily obtainable in large quantities (in the case of peripheral blood, at least) and easily kept viable for special studies, and 3) the function of the blood (or at least the erythroid component) is relatively simple when compared to that of other organ systems. Because it is a scientifically integrated discipline hematology/hematopathology is an area which is intellectually gratifying to the eclectic individual who is well-rounded in various biomedical endeavors, including biochemistry, physiology, pharmacology, microanatomy, morphologic diagnosis, and patient care.

II. The Blood

A few nights working in a trauma center would tend to convince one that the body is just a huge bag of blood. In fact, an "average" 70 liter human body contains only about 5 liters of blood, or 7% by volume. In the normal state, blood has no business anywhere except in the confines of the heart and blood vessels and in the sinusoids of the marrow, liver, and spleen. Of the average 5 L of blood, only 2.25 L, or 45%,

---

[1]If you are destined to become a dean or departmental chairman, you are probably sensing a turf war here somewhere. Actually, if this *is* war, it is one of great chivalry compared to some of the other jurisdictional altercations in medicine. For instance, the war between plastic surgeons and cosmetic surgeons (yes, there *is* a difference) makes any tiff between internal medicine and pathology look like the War of Jenkins' Ear (no pun intended).

consists of cells. The rest is plasma, which itself consists of 93% water (by weight) and 7% solids (mostly proteins, the greatest proportion of which is albumin). Of the 2.25 L of cells, only 0.037 L (1.6%) are leukocytes. The entire circulating leukocyte population, if purified, would fit in a bartender's jigger. The total circulating platelet volume is even less — about 0.0065 L — or a little over one teaspoonful.

III. Erythrocytes

Structurally the simplest cell in the body, volumes have been written about the lowly red blood cell. The basic function of the rbc is the creation and maintenance of an environment salutary to the physical integrity and functionality of hemoglobin. In the normal state, erythrocytes are produced only in the skeleton (in adults only in the axial skeleton), but in pathologic states (especially myelofibrosis, which will be covered subsequently) almost any organ can become the site of erythropoiesis. Numerous substances are necessary for creation of erythrocytes, including metals (iron, cobalt, manganese), vitamins (B_{12}, B_6, C, E, folate, riboflavin, pantothenic acid, thiamin), and amino acids. Regulatory substances necessary for normal erythropoiesis include erythropoietin, thyroid hormones, and androgens. Erythrocytes progress from blast precursors in the marrow over a period of five days. Then they are released into the blood as reticulocytes, distinguishable from regular erythrocytes only with special supravital stains. The reticulocyte changes to an erythrocyte in one day and circulates for 120 days before being destroyed in the reticuloendothelial system.

Clinical laboratories measure several important parameters that reflect rbc structure and function. These measurements are used to 1) evaluate the adequacy of oxygen delivery to the tissues, at least as is related to hematologic (as opposed to cardiopulmonary) factors, and 2) detect abnormalities in rbc size and shape that may provide clues to the diagnosis of a variety of hematologic conditions. Most of these tests are performed using automated equipment to analyze a simple venipuncture sample collected in a universal lavender- (or purple-) top tube containing EDTA as an anticoagulant. Let us consider each of these tests.

A. Hemoglobin concentration in whole blood

Referred to simply as "hemoglobin," this test involves lysing the erythrocytes, thus producing an evenly distributed solution of hemoglobin in the sample. The hemoglobin is chemically converted mole-for-mole to the more stable and easily measured **cyanmethemoglobin**, which is a colored compound that can be measured colorimetrically, its concentration being calculated from its amount of light absorption using Beer's Law [2]. The normal range for hemoglobin is highly age and sex dependent, with men having higher values than women,

---

[2]Beer's law expresses the mathematical relationship between the molar concentration of a colored substance in solution and the amount of monochromatic light it absorbs. This relationship is the basis of the great majority of automated chemical tests run in the clinical laboratory.

and adults having higher values than children (except neonates, which have the highest values of all). At Hermann Hospital the young adult female normal range is 12 – 16 g/dL; for adult males it is 14 – 18 g/dL.

This is an easy test to perform, as hemoglobin is present in the blood in higher concentration than that of any other measured substance in laboratory medicine[3]. The result is traditionally expressed as unit mass per volume, specifically grams per deciliter (g/dL). Ideologues in lab medicine have been maintaining for years that this unit will be replaced by Système Internationale (SI) units of moles per liter, but this has not gained any significant acceptance in clinical medicine except in the most nerdly circles.

B. Erythrocyte count

Also referred to as just "rbc," this simply involves counting the number of rbcs per unit volume of whole blood. Manual methods using the hated hemocytometer[4] have been universally replaced by automated counting. The major source of error in the rbc count is an artificially reduced result that occurs in some conditions where rbcs stick together in the sample tube, with two or more cells being counted as one. The result of the test is expressed as number of cells per unit volume, specifically cells/µL. At Hermann Hospital, the normal range is $4.2 - 5.4 \times 10^6$/µL for females; for adult males it is $4.7 - 6.1 \times 10^6$/µL.

C. Hematocrit

This is also called the packed cell volume or PCV [5]. It is a measure of the total volume of the erythrocytes relative to the total volume of whole blood in a sample. The result is expressed as a proportion, either unitless (*e.g.*, 0.42) or with volume units (*e.g.*, 0.42 L/L, or 42 cL/L [centiliters/liter]). An archaic way of expressing hematocrit is "volumes per cent" or just "percent" (42%, in the above illustration). Small office labs and stat labs measure hematocrit simply by spinning down a whole blood sample in a capillary tube and measuring the length of the column of rbcs relative to the length of the column of the whole specimen. Larger labs use automated methods that actually measure the volume individually of each of thousands of red cells in a measured volume of whole blood and add them up. The volume of individual erythrocytes can be

----

[3]For instance, the concentration of hemoglobin in blood is approximately 300,000,000,000 times that of estradiol, the principal female sex hormone, which is also routinely measured in clinical laboratories.

[4]Doctors who demand that the lab perform a manual count for anything except platelets are usually found at the bottom of an elevator shaft the next morning.

[5]Not to be permutatively confused with "PVC" (premature ventricular contraction), or "CVP" (central venous pressure)

electronically determined by measurement of their electrical impedance or their light-scattering properties. The normal range is 0.37 – 0.47 L/L for females, and 0.42 – 0.52 L/L for males.

D. Erythrocyte indices

The three cardinal rbc measurements described above (hemoglobin, hematocrit, and rbc count) are used to arithmetically derive the erythrocyte indices – **mean corpuscular volume**, **mean corpuscular hemoglobin**, and **mean corpuscular hemoglobin concentration**. As much as we all hate memorization, it is important to know how to calculate these indices and have some idea of the normal ranges. We will consider these individually.

1. Mean corpuscular volume (MCV)

This is the mean volume of all the erythrocytes counted in the sample. The value is expressed in volume units, in this case very small ones – femtoliters (fL, 10^{-15} liter). The normal range is 80 – 94 fL. The formula for the calculation in general terms is

$$MCV = \frac{\text{hematocrit}}{\text{rbc count}}$$

When using specific units, decimal fudge factors are required; for example,

$$MCV \text{ (in fL)} = \frac{\text{hematocrit (in L/L)} \times 1000}{\text{rbc count (in millions/}\mu\text{L)}}$$

I think that it is easier to forget the fudge factors, use the first formula, multiply out the values while ignoring the bothersome decimal, and reposition the decimal in the final result so as to approximate the order of magnitude of the normal range. This is safe, since you will not see an MCV of 8 fL, or one of 800 fL.[6]

When the MCV is low, the blood is said to be **microcytic**[7], when high, **macrocytic**. **Normocytic** refers to blood with a normal MCV. Keep in mind that the MCV measures only *average* cell volume. The MCV can be normal while the individual red cells of the population vary wildly in volume from

---

[6]Except maybe in Lilliput or Brobdingnag, respectively.

[7]For some reason the "mic" in "microcytic" is pronounced like the "mic" in "Mickey Mouse," not like the "mic" in "Michael Jackson." The problem with "micro-" and "macro-" is similar to that with such medical antonymic pairs as "adduct" and "abduct," and "hypo-" and "hyper-." These roots sound similar (especially when pronounced by a fatigued Texan) but have exactly opposite meanings. I would imagine that this produced analogous problems in the Classical languages from which these terms sprang. For instance: "Yes, Leonidas. The messenger was a Macedonian with a brutish accent, but I distinctly understood him to say that the Persians are approaching Thermopylae with a *micro* army which is *hypo* equipped. I say take 300 hoplites up there to polish them off, and the rest of us can stay here in Sparta and, uh, initiate the new recruits."

one to the next. Such an abnormal variation in cell volume is called **anisocytosis**. Some machines can measure the degree of anisocytosis by use of a parameter called the **red cell distribution width (RDW)**. This is simply a standardized parameter (similar to the standard deviation) for mathematically expressing magnitude of dispersion of a population about a mean. The normal range for RDW is 11.5 – 14.5 %.

2. Mean corpuscular hemoglobin (MCH)

The MCH represents the mean mass of hemoglobin in the RBC and is expressed in the mass unit, picograms (pg, 10^{-12} gram). The value is determined by the formula,

$$\text{MCH (in pg)} = \frac{\text{hemoglobin (in g/dL)} \times 10}{\text{rbc count (in millions/μL)}}$$

Again, a fudge factor is required in this equation, so it helps to get some feel for the normal range (27 – 31 pg) and gestalt the decimal point, as described for MCV, above. Since small cells have less hemoglobin than large cells, variation in the MCH tends to track along with that of the MCV. The MCH is something of a minor leaguer among the indices in that it adds little information independent of the MCV.

3. Mean corpuscular hemoglobin concentration (MCHC)

This is the mean concentration of hemoglobin in the red cell. Since whole blood is about one-half cells by volume, and all of the hemoglobin is confined to the cells, you would correctly expect the MCHC to be roughly twice the value for hemoglobin in whole blood and to be expressed in the same units; the normal range is 32 – 36 g/dL. The value is calculated using the formula,

$$\text{MCHC (in g/dL)} = \frac{\text{hemoglobin (in g/dL)}}{\text{hematocrit (in L/L)}}$$

Cells with normal, high, and low MCHC are referred to as **normochromic**, **hyperchromic**, and **hypochromic**, respectively. Again, these terms will have importance in anemia classification.

IV. Leukocytes and the leukocyte differential count

To consider the leukocytes together as a group is something of a granfalloon[8], because each type of leukocyte has its own function and ontogeny semi-independent of the others. To measure the total leukocyte count and allow this term to mean any-

_____

[8]**Granfalloon**, noun, a false or misleading grouping together of objects or concepts based on irrelevant commonalities; from Kurt Vonnegut's *Cat's Cradle* (1963), in which it is offered:

"If you wish to study a granfalloon
Just remove the skin of a toy balloon."

An essential goal of medical nosology is the elimination of granfalloons from our body of knowledge.

thing to the doctor is a travesty, yet the "wbc" count has traditionally been considered a cardinal measurement in a routine laboratory workup for just about any condition. I cannot emphasize too much that to evaluate critically the hematologic status of a patient, one must consider the individual absolute counts of each of the leukocyte types rather than the total wbc count. For such a critical evaluation, the first step is to order a **wbc count with differential**. In many labs, the result will be reported as a **relative differential**, something like this:

WBC	6000	/µL
segmented neutrophils	60	%
band neutrophils	2	%
lymphocytes	25	%
monocytes	8	%
eosinophils	3	%
basophils	2	%

Your first task is to multiply the wbc count by each of the percentages given for the cell types; this gives you an **absolute differential**. Now you're in business to get some idea as to the pathophysiologic status of the patient's blood and marrow. Thus, the illustration above becomes:

WBC	6000	/µL
segmented neutrophils	3600	/µL
band neutrophils	120	/µL
lymphocytes	1500	/µL
monocytes	480	/µL
eosinophils	180	/µL
basophils	120	/µL

The total wbc count is invariably done using an automated method. Routinely, the differential count is done "by hand" (*i.e.,* through the microscope) in smaller labs, and by automated methods in larger facilities. The automated methods are amazingly accurate, considering the fine distinctions that must often be made in discerning one type of leukocyte from the other. One manufacturer's machine can quite reliably pick out one leukemic blast cell in eight hundred or more leukocytes. Now we shall consider each of the leukocyte types individually.

A. Neutrophils

The most populous of the circulating white cells, they are also the most short lived in circulation. After production and release by the marrow, they only circulate for about eight hours before proceeding to the tissues (via diapedesis), where they live for about a week, if all goes well. They are produced as a response to acute body stress, whether from infection, infarction, trauma, emotional distress, or other noxious stimuli. When called to a site of injury, they phagocytose invaders and other undesirable substances and usually kill themselves in the act of doing in the bad guys.

Normally, the circulating neutrophil series consists only of band neutrophils and segmented neutrophils, the latter being the most mature type. In stress situations (*i.e.*, the "acute phase reaction"), earlier forms (usually no earlier than myelocytes) can be seen in the blood. This picture is called a "left shift." The band count has been used as an indicator of acute stress. In practice, band counts tend to be less than reliable due to tremendous interobserver variability, even among seasoned medical technologists, in discriminating bands from segs by microscopy. Other morphologic clues to acute stress may be more helpful: In the acute phase reaction, any of the neutrophil forms may develop deep blue cytoplasmic granules, vacuoles, and vague blue cytoplasmic inclusions called **Döhle bodies**, which consist of aggregates of ribosomes and endoplasmic reticulum. All of these features are easily seen (except possibly the Döhle bodies), even by neophytes.

The normal range for neutrophil (band + seg) count is 1160 – 8300 /μL for blacks, and 1700 – 8100 /μL for other groups. Keeping in mind the lower expected low-end value for blacks will save you much time (and patients much expense and pain) over the course of your career. Obesity and cigarette smoking are associated an increased neutrophil count. It is said that for each pack per day of cigarettes smoked, the granulocyte count may be expected to rise by 1000 /μL.

B. Monocytes

These large cells are actually more closely related to neutrophils than are the other "granulocytes," the basophil and eosinophil. Monocytes and neutrophils share the same stem cell. Monocytes are to histiocytes (or macrophages) what Bruce Wayne is to Batman. They are produced by the marrow, circulate for five to eight days, and then enter the tissues where they are mysteriously transformed into histiocytes. Here they serve as the welcome wagon for any outside invaders and are capable of "processing" foreign antigens and "presenting"[9] them to the immunocompetent lymphocytes. They are also capable of the more brutal activity of phagocytosis. Unlike neutrophils, histiocytes can usually survive the phagocytosis of microbes. What they trade off is killing power. For instance, mycobacteria can live in histiocytes (following phagocytosis) for years.

The normal range for the monocyte count is 200 – 950 /μL.

---

[9]This sounds very high-tech and diplomatic to describe activities that must be very gloopy and unsympathetic. I think more descriptive phraseology would be "…capable of 'busting' foreign antigens and 'taking them downtown.'"

C. Eosinophils

These comely cells are traditionally grouped with the neutrophils and basophils as "granulocytes," another granfalloon. Current thinking is that eosinophils and neutrophils are derived from different stem cells, which are not distinguishable from each other by currently available techniques of examination. Although the hallmark of the eosinophil is the presence of bright orange, large, refractile granules, another feature helpful in identifying them (especially on H&E-stained routine histologic sections) is that they rarely have more than two nuclear lobes (unlike the neutrophil, which usually has three or four). The normal range of the absolute eosinophil count is 0–450 /µL.

Eosinophils are capable of ameboid motion (in response to chemotactic sub-stances released by bacteria and components of the complement system) and phagocytosis. They are often seen at the site of invasive parasitic infestations and allergic (immediate hypersensitivity) responses. Individuals with chronic allergic conditions (such as atopic rhinitis or extrinsic asthma) typically have elevated circulating eosinophil counts. The eos may serve a critical function in mitigating allergic responses, since they can 1) inactivate slow reacting sub-stance of anaphylaxis (SRS-A), 2) neutralize histamine, and 3) inhibit mast cell degranulation. The life span of eos in the peripheral blood is about the same as that of neutrophils. Following a classic acute phase reaction, as the granulocyte count in the peripheral blood drops, the eosinophil count temporarily rises.

D. Basophils

The most esthetically pleasing of all the leukocytes, the basophils are also the least numerous, the normal range of their count in peripheral blood being 0 – 200/µL. They are easily recognized by their very large, deep purple cytoplasmic granules which overlie, as well as flank, the nucleus (eosinophil granules, by contrast, only flank the nucleus but do not overlie it). It is tempting to assume that the basophil and the mast cell are the blood and tissue versions, respectively, of the same cell type. Actually it is controversial as to whether this concept is true or whether these are two different cell types. The following table presents some of the contrasts between mast cells and basophils:

|	Basophils	Mast cells
Nuclear morphology	segmented	round or ovoid
Mitotic potential	no	yes
Peroxidase content[10]	+	-
Acid phosphatase[11]	-	+
Alkaline phosphatase	-	+
PAS reaction[12]	++++	+

In active allergic reactions, blood basophils decrease in number, while tissue mast cells increase. This reciprocal relationship suggests that they represent the same cell type (*i.e.*, an allergen stimulates the passage of the cells from the blood to the site of the allergen in the tissues). Some experiments with animals have also shown that mast cells are marrow-derived and are capable of differentiating into cells that resemble basophils. Conversely, some recent evidence suggests that basophils (as well as eosinophils) can differentiate from metachromatic precursor cells that reside among epithelial cells in the nasal mucosa

Without invoking religion or Alexander Pope ("Whatever *is*, is right," *An Essay on Man*, 1732-34) it is hard to see any useful role of the basophil/mast cell in human physiology. The mast cell is the essential effector of immediate (Type 1) hypersensitivity reactions, which produce only misery, dysfunction, and occasionally death for the hapless host.

E. Lymphocytes

In the immune/inflammatory response, if the neutrophils and monocytes are the brutes, the lymphocytes are the brains. It is possible to observe the horror of life without lymphocyte function by studying the unfortunate few with hereditary, X-linked, severe combined immune deficiency. Such individuals uniformly die of systemic infections at an early age (except for the "bubble boys" of yesteryear, who lived out their short lives in antiseptic prisons). The

---

[10]**Peroxidase** catalyzes the following reaction:

$$2\ R-CH_2-OH\ +\ H_2O_2\ \rightarrow\ 2\ R-CHO\ +\ 2\ H_2O$$
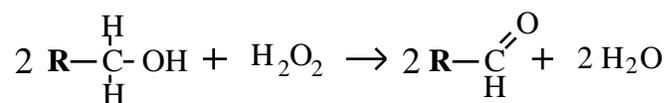
The "purpose" of the enzyme is to generate bactericidal aldehydes from peroxide generated naturally by phagocytes. Whether peroxidase is important to basophil function is not known.

[11]Acid and alkaline phosphatases are among the most commonly measured enzymes in the laboratory. Nevertheless, their functions in physiology are unknown.

[12]Periodic acid-Schiff reaction (PAS) is an oft-used stain to detect the presence of large quantities of complex polysaccharides in anatomic structures. The reaction is positive for any molecular species that has a large number of diglycol (…-CHOH-CHOH-…) linkages.

functions of lymphocytes are so diverse and complex that they are beyond the scope of this text (and the scope of the author, it must be admitted). What follows are a few general remarks concerning examination of lymphocytes in peripheral blood.

After neutrophils, lymphocytes are the most numerous of the circulating leukocytes. The normal range of the lymphocyte count is $1000 - 4800/\mu L$. Their life span may vary from several days to a lifetime (as for memory lymphocytes). Unlike neutrophils, monocytes, and eosinophils, the lymphocytes 1) can move back and forth between the vessels and the extravascular tissues, 2) are capable of reverting to blast-like cells, and 3) when so transformed, can multiply as the immunologic need arises.

In normal people, most of lymphocytes are small, innocent-looking round cells with heavily "painted-on" nuclear chromatin, scant watery cytoplasm, and no granules. A small proportion of normal lymphs are larger and have more opaque, "busy-looking" cytoplasm and slightly irregular nuclei. Some of these have a few large, dark blue granules, the so called "azurophilic granules." It has been maintained that these granulated cells are T cells (*i.e.*, T-cells that have a surface receptor for the IgG Fc region) or natural killer (NK) null-cells. Other phenotypes of lymphocytes are not recognizable as such on the routine, Wright-stained smear and require special techniques for identification.

When activated by whatever means, lymphocytes can become very large (approaching or exceeding the diameter of monocytes) and basophilic (reflecting the increased amount of synthesized cytoplasmic RNA and protein). The cytoplasm becomes finely granular (reflecting increased numbers of organelles), and the nuclear chromatin becomes less clumped (the better to transcribe you with, my dear!). Such cells are called "transformed lymphocytes," "atypical lymphocytes," or "viral lymphocytes" by various votaries of blood smears. Although such cells are classically associated with viral infection (particularly infectious mononucleosis), they may also be seen in bacterial and other infections and in allergic conditions. A morphologic pitfall is mistaking them for monocytes (a harmless mistake) or leukemic blasts (not so harmless).

V. Platelets

The main thing to remember about platelets is to *look for them first.*. A typical tyro maneuver is to study a blood smear for an hour looking for some profound hematological abnormality, never to realize there is nary a platelet in sight. It is therefore necessary to discipline yourself to first check for a normal number of platelets when sitting down with a slide, before being seduced by the midnight beauty of the basophil's alluring granules or the monocyte's monolithic sovereignty. The normal platelet count is $133 - 333 \times 10^3/\mu L$.

Platelets are counted by machine in most hospital labs and by direct phase microscopy in smaller facilities. Since platelets are easily mistaken for garbage (and vice

versa) by both techniques, the platelet count is probably the most inaccurate of all the routinely measured hematologic parameters. Actually, you can estimate the platelet count fairly accurately (up to an absolute value of about $500 \times 10^3/\mu L$) by multiplying the average number of platelets per oil immersion field by a factor of 20,000. For instance, an average of ten platelets per oil immersion field (derived from the counting of ten fields) would translate to 200,000/μL (10 × 20,000). Abnormal bleeding generally does not occur unless the platelet count is less than 30,000/μL, if the platelets are functioning properly. Screening for proper platelet function is accomplished by use of the **bleeding time** test (covered later in the block).

VI. Other cells in peripheral blood

Plasma cells sometimes appear in the peripheral blood in states characterized by reactivity of lymphocytes. Old time hematologists often maintain that the cells that look exactly like plasma cells on the smear are really "plasmacytoid lymphs," and it is usually nonproductive to argue this point with them. **Endothelial cells** occasionally get scooped up into the phlebotomy needle during blood collection and show up on the slide. They are huge and tend to be present in groups. **Histiocytes**, complete with pseudopodia and phagocytic vacuoles, may appear in states of extreme reactivity, especially in septic neonates. **Nucleated red cells** may also be seen in small numbers in the peripheral blood of newborns; however, *in adults, even a single nucleated rbc on the slide is abnormal*, indicating some sort of serious marrow stress, from hemolytic anemia to metastatic cancer. **Myeloblasts** are always abnormal and usually indicate leukemia or an allied neoplastic disease. Rarely they may be seen in non-neoplastic conditions, such as recovery from marrow shutdown (aplasia). Later stages of myeloid development (promyelocyte, myelocyte, metamyelocyte) may be represented in the peripheral blood in both reactive states and leukemias.

VII. Bone marrow examination

This is one of the most common biopsy procedures performed on both outpatients and the hospitalized. Two types of specimens are generally obtained, the **aspirate** and the **core biopsy**. The site of biopsy is usually the posterior iliac crest (via the posterior superior iliac spine) in adults and the anterior tibia in children, although other sites are available. After local anesthesia is applied to the periosteum and overlying skin, a small needle (usually the "University of Illinois needle") is introduced (or crunched actually) into the medullary space through a small skin incision. About 0.5 mL of marrow material is aspirated and smeared onto several glass slides and stained with a stain identical or similar to the Wright stain used on peripheral blood. Some material usually remains in the syringe where it is allowed to clot. It is then fished out of the syringe, processed like all other biopsy tissue, embedded in paraffin, sectioned, and stained with hematoxylin/eosin and other selected stains. The core biopsy, generally performed after the aspirate is done, is taken with a larger, tapered needle, typically the "Jamshidi needle." This yields a core of bone (similar to a geologic core sample) which is fixed, decalcified, processed, and

sectioned. The H&E-stained core biopsy and aspirate clot sections are best for assessment of marrow cellularity and the presence of metastatic neoplasms or granulomas. The Wright-stained aspirate smears are best for studying the detailed cytology of hematopoietic cells.
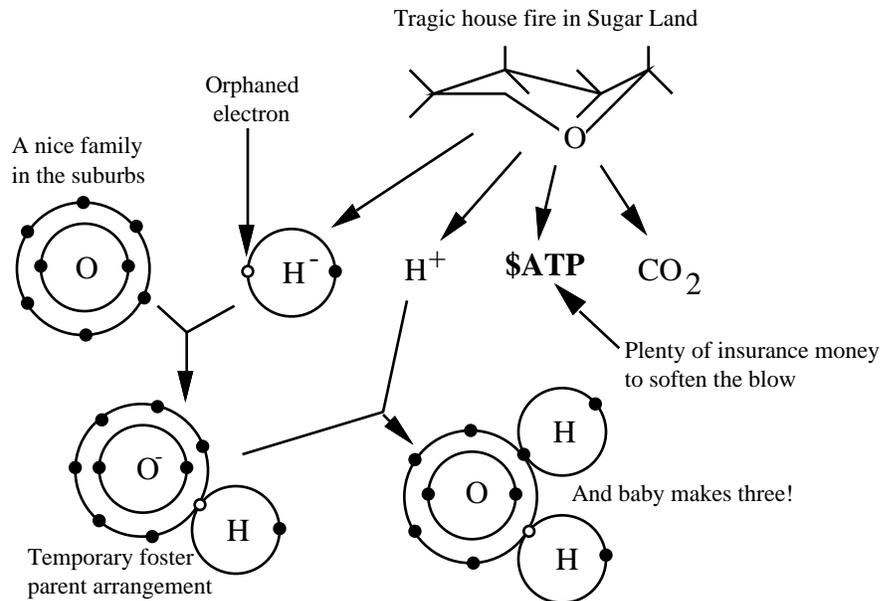
The bone marrow biopsy procedure produces some pain for the patient, since it is impossible to anaesthetize the inside of bone. The level of pain ranges from mild discomfort to agony, depending on the individual's pain threshold and level of apprehension. Some physicians elect to precede the biopsy with a benzodiazepine or other minor tranquilizer. Generally the aspiration action produces much more pain than the core biopsy.

For a procedure that involves invasion of bone, the marrow biopsy is remarkably free of complications. Bleeding and infection may occur but are rare, even in severely thrombocytopenic and immunosuppressed patients. It is highly recommended that med students learn how to perform this useful procedure during the clinical years of their training.

Part Two: Anemia – Pathophysiologic Consequences and Clinical Investigation

I. Introduction

Free oxygen, the plant kingdom's unique gift to this planet, is a highly reactive, dangerous substance capable of laying waste the delicate molecules that form the basis of life. How peculiar that we, as aerobes, have traded the security of a languid existence in a reducing milieu for the high-stakes, fast-lane life of free-flowing ATP, the dear currency that gives us the strength, speed, and mental facility to profoundly alter our world. Aerobic respiration, for all the complexity of the chemical reactions of intermediary metabolism, simply boils down to the body's need to find something to do with the spare electron left over from the destruction of the glucose molecule. This orphaned lepton, bereft of binding energy by its repeated violation at the hands of the cytochrome gantlet, finds no comfort in the carbon dioxide rubble of its former hexose home. Should it not find the succor of oxygen, it would escape to a feral existence of unsavory chemical reactions, where it would find itself in the company of the opprobrious Free Radicals, miscreants whose only purpose is the steric vandalism of the macromolecular cathedrals of life.

Tragic house fire in Sugar Land

Orphaned electron

A nice family in the suburbs

O

H⁻

H⁺

$ATP

CO_2

Plenty of insurance money to soften the blow

O⁻

H

Temporary foster parent arrangement

O

H

H

And baby makes three!

It has been said that all damage to the body from any pathologic state in the end is caused by hypoxia at some level. If this is true, the story of pathology is the story of hypoxia. Preventing or correcting hypoxia is then the ultimate goal of all medical specialties. Pulmonologists and cardiologists deal with hypoxia at the gross mechanical level, but hematologists do so at the finer cellular and molecular levels. The physicochemical properties of hemoglobin and biochemical housekeeping in the erythrocyte are both in their purview, but what hematologists contend with at the grossest level is anemia.

II. Definition of anemia

Anemia may be defined as **any condition resulting from a significant decrease in the total body erythrocyte mass**.[13] Measurement of total body rbc mass requires special radiolabeling techniques that are not amenable to general medical diagnostic work. Measurements typically substituted for rbc mass determination take advantage of the body's tendency to maintain normal total blood volume by dilution of the depleted rbc component with plasma. This adjustment results in decrease of the total blood hemoglobin concentration, the rbc count, and the hematocrit. Therefore, a pragmatic definition of anemia is a state which exists when the hemoglobin is less than 12 g/dL or the hematocrit is less than 37 cL/L.[14] Anemia may ex-

---

[13]Literally, anemia means "without blood." A better term to reflect the true pathologic state would be "oligoerythremia," which is not in the lexicon. If you commit neologism in medicine, you will be criticized for "confusing everyone with nonstandard terminology," unless you are on the faculty at Harvard or Stanford, when the same behavior will be considered "a bold, iconoclastic stroke of intellectual honesty to ensure the accuracy and precision of scientific communication."

[14]Note that the hematocrit tracks along at about three times the value of the hemoglobin, and nine times the value of the rbc count (expressed in millions/µL). This arithmetic relationship breaks down when microcytosis or macrocytosis exist.
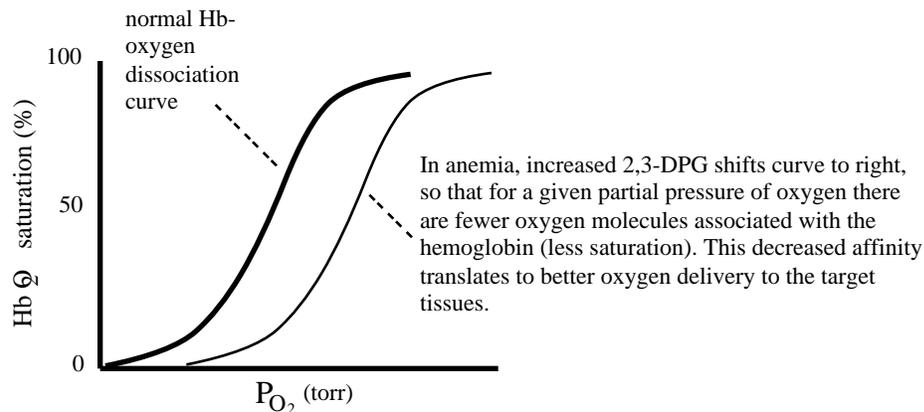
ist as a laboratory finding in a subjectively healthy individual, because the body can, within limits, compensate for the decreased red cell mass.

III. Physiologic compensation for decreased rbc mass

Each physiologic mechanism will be discussed below. It should be noted that, although there are many adjustments that can be made, one that *cannot* is decrease in the tissue requirement for oxygen. Actually, overall body oxidative metabolism *increases* in anemia because of the energy requirement of the compensatory activities.

A. Decreased hemoglobin oxygen affinity

Increased oxygen extraction of anemic blood by the tissues produces increased concentration of deoxyhemoglobin in the rbc, which stimulates the production of 2,3-diphosphoglycerate (2,3-DPG). 2,3-DPG shifts the hemoglobin-oxygen dissociation curve to the right, thus allowing the tissues to more easily strip the hemoglobin of its precious electron-accepting cargo:

normal Hb-oxygen dissociation curve

In anemia, increased 2,3-DPG shifts curve to right, so that for a given partial pressure of oxygen there are fewer oxygen molecules associated with the hemoglobin (less saturation). This decreased affinity translates to better oxygen delivery to the target tissues.

B. Redistribution of blood flow

In anemia selective vasoconstriction of blood vessels subserving certain nonvital areas allows more blood to flow into critical areas. The main donor sites who sacrifice their aerobic lifestyle are the skin and kidneys. Shunting of blood away from cutaneous sites is the mechanism behind the clinical finding of **pallor**, a cardinal sign of anemia. Although the kidney can hardly be thought of as a nonvital area, it receives (in the normal state) much more blood flow than is needed to meet its metabolic requirements.

C. Increased cardiac output

The heart can respond to tissue hypoxia by increased cardiac output. The increased output is matched by decreased peripheral vascular resistance and decreased blood viscosity (thinner blood flows more freely than thick blood), so that cardiac output can rise without an increase in blood pressure. Generally, anemia must be fairly severe (hemoglobin < 7 g/dL) before cardiac output rises.

IV. Clinical signs and symptoms of anemia

When the above mechanisms are overwhelmed by the increasing magnitude of the anemia, or when the demands of physical activity or intercurrent illness overwhelm them, a clinical disease state becomes apparent to the physician and to the patient. The severity of clinical symptoms bears less relationship to the severity of the anemia than to the length of time over which the condition develops. An acute hemorrhagic condition may produce symptoms with loss of as little as 20% of the total blood volume (or 20% of the total red cell mass).[15] Conversely, anemias developing over periods long enough to allow compensatory mechanisms to operate will allow much greater loss of rbc mass before producing symptoms. It is not terribly uncommon to see a patient with a hemoglobin of 4 g/dL (hematocrit 12 cL/L), representing a loss of 70% of the rbc mass, being reluctantly dragged into a clinic by relatives concerned that he or she is looking a bit washed out.[16]

When symptoms do develop, they are pretty much what you would expect given the precarious state of oxygen delivery to the tissues: dyspnea on exertion, easy fatigability, fainting, lightheadedness, tinnitus, and headache. In addition, the hyperdynamic state of the circulatory system can produce palpitations and roaring in the ears. Pre-existing cardiovascular pathologic conditions are, as you would expect, exacerbated by the anemia. Angina pectoris, intermittent claudication, and night muscle cramps speak to the effect of anemia on already compromised perfusion.

Clinical signs of a slowly developed anemia are pallor, tachycardia, and a systolic ejection murmur. In rapidly developing anemia (as from hemorrhage and certain catastrophic hemolytic anemias), additional symptoms and signs are noted: syncope on rising from bed, orthostatic hypotension (*i.e.*, the blood pressure falls when the patient is raised from the supine to the sitting or standing positions) and orthostatic tachycardia. Keep in mind that if anemia develops through rapid enough bleeding, the hematocrit and hemoglobin will be normal (since in hemorrhage the rbc's and plasma are lost in proportion). Because of this, your appreciation of these clinical signs will serve you better in diagnosing this type of anemia than will the laboratory.

V. Classification of anemias

Anemias can be classified by cytometric schemes (*i.e.*, those that depend on cell size and hemoglobin-content parameters, such as MCV and MCHC), erythrokinetic

---

[15]By contrast, it is common and inconsequential to acutely lose 10% of the total blood volume, the amount lost when donating a "unit" of blood. On the other side, acute loss of 40% produces arterial hypotension, and 50%, shock and death.

[16]Don't think this kind of thing happens only in such places as Chad (1 physician per 47,640 persons). In 1988, I saw this exact scenario happen here (1 physician per 404 persons). Moreover, the patient was the young son of a Houston physician.

schemes (those that take into account the rates of rbc production and destruction), and biochemical/molecular schemes (those that consider the etiology of the anemia at the molecular level.

An example: sickle cell anemia

Cytometric classification: normochromic, normocytic
Erythrokinetic classification: hemolytic
Biochemical/molecular classification: DNA point mutation producing amino acid substitution in hemoglobin beta chain

A. Cytometric classification

Because cytometric parameters are more easily and less expensively measured than are erythrokinetic and biochemical ones, it is most practical to work from the cytometric classification, to the erythrokinetic, and then (hopefully) to the biochemical. Your first job in working up a patient with anemia is to place the case in one of three major cytometric categories:

1. Normochromic, normocytic anemia (normal MCHC, normal MCV).

These include:

a) anemias of chronic disease

b) hemolytic anemias (those characterized by accelerated destruction of rbc's)

c) anemia of acute hemorrhage

d) aplastic anemias (those characterized by disappearance of rbc precursors from the marrow)

2. Hypochromic, microcytic anemia (low MCHC, low MCV).

These include:

a) iron deficiency anemia

b) thalassemias

c) anemia of chronic disease (rare cases)

3. Normochromic, macrocytic anemia (normal MCHC, high MCV).

These include:

   a) vitamin B$_{12}$ deficiency

   b) folate deficiency

B. Erythrokinetic classification

You would now want to proceed with classifying your case based on the rate of rbc turnover. If this is high, a **normoregenerative** anemia exists. Such anemias are seen in hemolysis (excess destruction of rbc's) or hemorrhage (loss of rbc's from the vascular compartment. In these cases, the marrow responds appropriately to anemia by briskly stepping up the production of rbc's and releasing them into the bloodstream prematurely. There are several lab tests that allow you to determine if increased rbc turnover exists:

1. Reticulocyte count

A sample of blood is stained with a supravital dye that marks reticulocytes. An increased number of reticulocytes is seen when the marrow is churning out rbc's at excessive speed (presumably to make up for those lost to hemolysis or hemorrhage). Most labs will report the result of the reticulocyte count in percent of all rbc's counted. At Hermann the normal range is 0.5-1.5 %. Making clinical decisions based on this raw count is somewhat fallacious.

> *For instance*: A normal person with an rbc count of 5,000,000 /µL and an absolute reticulocyte count of 50,000 /µL would have a relative retic count of 1.0%. An anemic person with 2,000,000 rbc's/µL and the *same* 50,000 retics/µL would have an apparently "abnormal" relative retic count of 2.5 % and could be misdiagnosed as having high turnover.

Clearly, one needs to find some way to correct the raw retic count so as to avoid this problem. One can easily calculate the **absolute retic count** (in cells/µL) by multiplying the rbc count by the relative retic count. The normal range for the absolute retic count is 50,000-90,000 /µL. Another parameter that has found popularity is the **reticulocyte production index** (RPI), calculated as follows:

$$\text{RPI(\%)} = \text{relative retic count (\%)} \times \frac{\text{hematocrit (cL/L)} \div 45}{\text{fudge factor}}$$

The fudge factor is extracted from the following table:

Patient's Hematocrit Value (cL/L)	Fudge Factor
40-45	1.0
35-40	1.5
25-34	2.0
15-24	2.5
<15	3.0

The RPI has the advantage of being reported in percent, just like the raw retic count, so the same normal range (0.5-1.5 %) can be used. In addition,

the fudge factor in the denominator lends an air of conservatism by allowing for the premature release of reticulocytes in high-turnover states. As can be appreciated from the table, the lower the hematocrit, the earlier the release of the retics into the blood. For a severe normoregenerative anemia, the retics are kicked out two days early. They then circulate for three days instead of the normal one day before maturing into regular rbc's.

2. Serum unconjugated bilirubin and urine urobilinogen concentration

When red cells, at the end of their 120-day life-span, go to the great spleen in the sky, they are systematically dismantled. Through a series of biochemical steps too boring to go into even here, the heme is changed into bilirubin.[17] The bilirubin is greedily scarfed up by the liver, conjugated with glucuronide, squirted into the alimentary tract in the bile, and converted to urobilinogen by evangelical colonic bacteria. The urobilinogen is excreted in the stool (most of it) or reabsorbed and excreted in the urine (very little of it).[18] This is summarized in the next diagram.
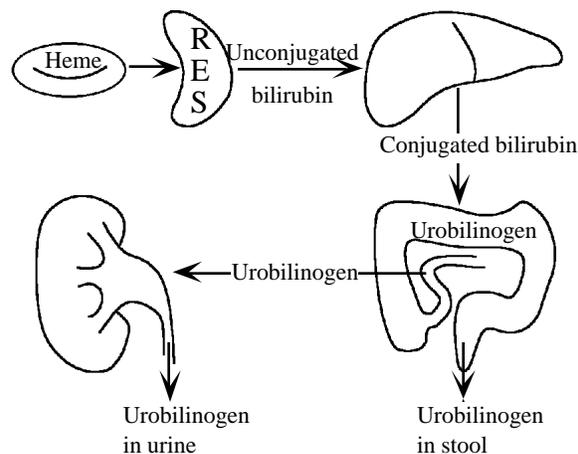
In cases of accelerated rbc destruction, the capacity of the liver to capture bilirubin is saturated (analogous to the situation in the Texas penal system), and the concentration of unconjugated bilirubin in serum increases, occasionally to the point of producing clinical jaundice. Moreover, the increased production of urobilinogen that results is reflected by increased urobilinogen concentration in the urine.[19] Unconjugated bilirubin is not water soluble and

---

[17]The phase of the reaction where the porphyrin ring is broken yields carbon monoxide as a by product. This is the only known human metabolic reaction where CO is generated

[18]Unlike bilirubin, urobilinogen is colorless and is not the reason that the urine is yellow. The normal urine color is due to substances called "urochromes," the chemical composition of which is a subject of much evasion in biochemistry texts.

[19]Also by increased *stool* urobilinogen, which is actually a more sensitive indicator of hemolysis. This is not often measured in clinical medicine, since it is so hard for many to accept on an emotional level that something as funky as stool could possibly yield such exquisite biochemical information.

therefore will not be excreted in the urine[20], despite its elevation in the serum.

3. Serum haptoglobin concentration

When an rbc is destroyed, the liberated hemoglobin binds mole-for-mole with a serum protein, haptoglobin. The "purpose" of this reaction is to keep the kidneys from squandering iron (free hemoglobin is freely filtered by the glomerulus, but hemoglobin-haptoglobin complexes are too big to muscle their way through, so that they are safe to bumble their way back to the reticuloendothelial system where they can be properly disassembled). The serum haptoglobin concentration then decreases. Laboratory measurement of haptoglobin is fairly easy and yields useful information to assist in documenting decreased rbc life span.

In the case of hemolysis which takes place in the bloodstream (rather than in the RES), so-called **intravascular hemolysis**, additional biochemical phenomena are observed (see diagram, below). Free hemoglobin in excess of that which binds haptoglobin is rapidly filtered into the urine. What remains in the plasma spontaneously degrades into metheme and globin. A portion of metheme binds albumin to produce a measurable compound, **methemalbumin**, while the remainder binds to a measurable serum protein, **hemopexin**, which then decreases in serum concentration. All of the substances whose names are boxed in the diagram are those whose laboratory measurement is feasible and helpful in documenting hemolysis.
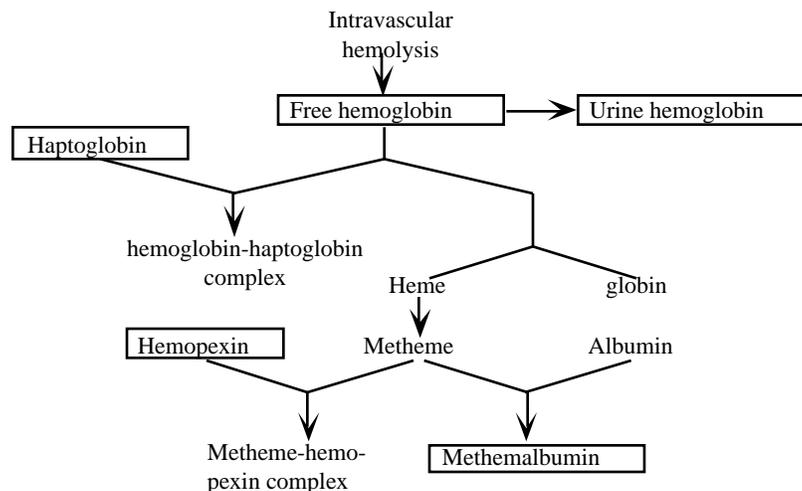
Also, overordering 24-hour stool specimens for *anything* wears thin on the nursing staff and is probably a leading cause of "accidental" death among physicians.

[20]When bilirubin does appear in the urine, it is of the water-soluble *conjugated* variety. Unlike urobilinogen, conjugated bilirubin is pigmented and turns the urine a dark amber. This is *not* seen in hemolysis, but in hepatocellular diseases, where enterohepatically recirculated conjugated bilirubin resorbed from the gut cannot be handled with sufficient alacrity by the diseased liver.

4. Bone marrow biopsy

This can be used to directly observe any accelerated production of rbc's. The ratio of the number of myeloid to erythroid precursors (the M:E ratio) tends to decrease in high-production states, and the marrow becomes hypercellular. Marrow biopsy is not usually performed *just* to measure the M:E ratio, but to answer other hematologic questions that have been raised.

The normoregenerative anemias are in contrast to those characterized by inadequate marrow response to the degree of anemia. These are the **hyporegenerative** anemias. In such cases, the reticulocyte production index is decreased. The classic example is **aplastic anemia**, in which there is primary marrow failure to produce enough erythrocyte mass. As you have probably come to expect, the distinction of these categories is not always absolute. For instance, in thalassemia major there is a degree of hemolysis (generally associated with the normoregenerative states) and inadequate marrow response to the degree of anemia.

C. Biochemical classification

Finally, one should attempt to determine the etiology of the anemia as specifically as possible. In some cases (*e.g.*, iron deficiency), etiologic classification is easily attained; in others (*e.g.*, aplastic anemia) the biochemical mechanism of disease may be hopelessly elusive. Generally, biochemical tests are aimed at identifying a depleted cofactor necessary for normal hematopoiesis (iron, ferritin, folate, B_{12}), an abnormally functioning enzyme (glucose-6-phosphate dehydrogenase, pyruvate kinase), or abnormal function of the immune system (the direct antiglobulin [Coombs'] test). We will consider each of these tests in greater depth when we consider the specific anemias in the next sections.

# Part Three: Nutritional Anemias and Anemia of Chronic Disease

I. Iron metabolism and iron deficiency anemia

A. Iron and its metabolism

The fourth most abundant element in the earth's crust, iron is only a trace element in biologic systems, making up only 0.004% of the body's mass. Yet it is an essential component or cofactor of numerous metabolic reactions. By weight, the great proportion of the body's iron is dedicated to its essential role as a structural component of hemoglobin. Hemoglobin without iron is totally useless (in fact, hemoglobin with Fe^{+++} instead of the normal Fe^{++} is the ugly brown **methemoglobin** and is also worthless as an oxygen carrier). Without sufficient iron available to the rbc precursors, normal erythropoiesis cannot take place, and anemia develops. On the other hand, iron is a toxic substance.

Too much iron accumulating in vital structures (especially the heart, pancreas, and liver) produces a potentially fatal condition, **hemochromatosis**. Clearly, iron, like oxygen, is another of the deleterious substances that evolution has led biologic systems into flirtation with.

Most of the iron not circulating in the rbc's is stored in the Fe^{+++} (ferric) oxidation state. This iron is stored in marrow histiocytes in the form of **hemosiderin**. When iron is needed by the erythron, the hemosiderin gives up its iron to nearby rbc precursors who line up around the histiocyte like pigs around a trough. Hemosiderin is easy to see microscopically in smear or section preparations of marrow, due to the ferric iron's ability to produce an intense blue color in the Prussian blue stain. This reaction is the basis of the routine "iron stain" done on bone marrow specimensto assess adequacy of depot iron. Erythrocytes would not be expected to stain positively, since they contain ferrous iron. Because the body is dealing with such an essential but dangerous and biologically rare substance (and because you have become resigned to endless memorization in your hazing as medical students), you would expect that there would be some kind of complicated mechanism for the absorption and transport of iron.

Storage (27%)

Fe(III)

Transferrin (0.08%)

Other (6%)

Fe(II)

Hemoglobin (67%)
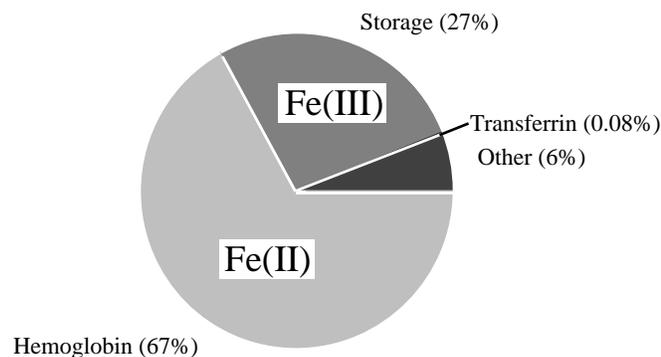
Iron is present in greatest concentration in meat and dark green vegetables. The U.S. R.D.A. for adults is 10 mg for males, 18 mg for menstruating females. The average daily American diet contains about 10 mg iron, of which only about 1 mg is absorbed. What goes in must come out, and in the adult male, the 1 mg/day iron loss occurs almost exclusively in the stool. For reproductive--aged females, an additional route is the menstrual flux, which accounts for a wildly variable incremental loss. While the average monthly menstrual blood loss is 40 mL (equivalent to 16 mg iron), some women who consider themselves healthy may lose up to 495 mL blood (200 mg iron) per menstrual period, or an average of about 7 mg iron per day (200 mg iron ÷ 28 days/cycle). It is not surprising that iron deficiency anemia is relatively common in women of this age group.

Following ingestion, iron is absorbed primarily in the duodenum, although any portion of the small bowel is efficient at iron absorption (in contrast to the situ-

ation with B_{12}, as noted below). Only ferrous iron can be absorbed. The normal gastric acidity provides an optimal environment for the reduction of any ferric iron to the ferrous version. In states of iron depletion, a greater proportion of iron is absorbed than in states of normal iron depots. After uptake, the ferrous iron is transported to the subepithelial capillaries (possibly by intracellular **transferrin**), and released into the bloodstream. There it is oxidized[21] to Fe^{+++} and again taken up by plasma transferrin. It is then conveyed to the erythron (and reduced again to the ferrous version) or to marrow histiocytes for eventual incorporation into hemoglobin. Storage iron exists as part of a ferric iron-apo-protein complex called **ferritin**. Ferritin molecules are soluble and are present in plasma in concentration equilibrium with ferritin molecules in histiocytes. Therefore, decreased iron stores (as is seen in impending iron deficiency anemia) are reflected by decreased serum concentration of ferritin, a substance easily measured in clinical laboratories.[22] In marrow histiocytes, most of the ferritin molecules glom up into visible (through the microscope, that is) blobs of cytoplasmic inclusions rich in iron and poor in apoprotein; this substance is called **hemosiderin**. Hemosiderin is easily seen with the Prussian blue stain but can even be observed in unstained preparations of marrow, if present in sufficient quantities, due to the natural golden brown color of iron itself. Since hemosiderin is not soluble, it does not float around in the plasma with ferritin.

B. Iron deficiency anemia

When there is insufficient iron available for the normal production of hemoglobin, anemia results. The cells which are produced are small and pale, and indices from such specimens show low values for MCHC and MCV. Therefore, the classic anemia that occurs in iron deficiency is *hypochromic, microcytic*. Early or mild cases of iron deficiency anemia (IDA) show microcytosis without hypochromia. Since this is a hyporegenerative anemia, the retic count or RPI would be expected to be low; however, because so many cases of IDA are due to chronic bleeding, it is not uncommon to see patients with episodes of hemorrhage that have produced an *elevated* RPI on clinical presentation. It would appear that the marrow is able to produce a transient response to bleeding, but over the long haul it is a day late and a dollar short. Another finding commonly seen on clinical presentation is **thrombocytosis**, again probably reflecting marrow response to bleeding. The *sine qua non* of

---

[21] The enzyme that performs the ferrous-to-ferric oxidation is **ceruloplasmin**, probably better known for its role in copper transport.

[22] Unfortunately, the serum ferritin is not always decreased in iron deficiency; many systemic diseases, especially cancer, produce marked elevation of serum ferritin, even in the face of iron deficiency. Therefore, a low serum ferritin indicates iron deficiency, but a normal or increased serum ferritin indicates nothing. This is a good example of a laboratory test which has excellent *specificity* but poor *sensitivity* in detecting a specific condition.

IDA is the observation that there is essentailly *no* iron in the marrow (that's zero, zilch, nada), since erythropoiesis can occur normally as long as at least some storage iron is present. In iron-deficient states, one of the body's clever reactive phenomena is the increase in production of transferrin. This is sometimes measured as **total iron binding capacity** of serum (TIBC). Without the availability if iron, a heme precursor, **protoporphyrin**, and a porphyrin side-reactant, **zinc protoporphyrin**, accumulate in the red cell. These may also be measured. In summary, the laboratory features of IDA are:

Hypochromic, microcytic anemia	Variable retic count, variable platelet count
Absent marrow storage iron	free erythrocyte protoporphyrin
serum iron, TIBC, serum ferritin	erythrocyte zinc protoporphyrin

C. Causes of IDA

Iron stores can be depleted either through insufficient intake or excessive loss. In America, the combination of our meat-rich diet and fortification of our staples (such as Wonder Bread, Quaker Instant Grits, and Kellogg's 40% Bran Flakes) with added iron makes dietary insufficiency a very rare condition. The one exception to this is the case with milk-fed infants. Bovine milk has almost no iron. An iron-deficient state in such babies often is sown in the fertile soil of an antenatal life in a mother who was also overtly or borderline iron-deficient (iron requirements are markedly increased in pregnancy due to the demands of developing the fetal tissues). Fortunately most infant formulas are fortified with iron now. Moreover, today's parents are so paranoid about iron deficiency that it is surprising that the typical child of the 1990's can get through an airport without setting off any alarms. Still, nutritional cases of IDA do occur.

Although dietary deficiency of iron is rare, individuals with gastrointestinal lesions producing malabsorption syndromes may fail to assimilate sufficient iron to maintain the erythron, even in the face of adequate iron intake.

The much more important cause of iron depletion is **chronic blood loss**. In females, this is usually due to menses. Other more sinister causes include chronically bleeding lesions of the gastrointestinal tract, from reflux esophagitis, to peptic ulcers, to gastric or colorectal adenocarcinomas. Because these bad guys may be lurking asymptomatically, spilling erythrocytes here and there for months, all cases of iron deficiency anemia must be thoroughly investigated for the presence of bleeding sites. This is especially true *in cases involving females who are not of reproductive age and in all males. In these demographic groups, to simply treat IDA with iron and not investigate for bleeding lesions is unequivocal gross negligence.*

D. Treatment of IDA

Oral iron preparations are available for treatment of theses cases. The cheapest and probably best absorbed is non-coated **ferrous sulfate** ($FeSO_4$), al-

though many other products are satisfactory. It is important to specify on the prescription that non-enteric coated preparations be used, so as to maximize iron availability for absorption. Since acute iron overdose is a potentially fatal toxicosis, iron tablets should be kept out of reach of children.

In certain cases, such as in gastrointestinal malabsorption syndromes, it may be necessary to give **parenteral iron**. This preparation, **iron dextran** (Imferon™), may be given IM or IV. Since it may produce anaphylactic shock, it needs to be given under direct physician supervision. **Transfusion**, which immediately restores all iron stores to surfeit, is very dangerous in chronically anemic patients because of the demand the increment in blood volume puts upon the already taxed heart. It is almost never indicated in iron deficiency anemia.

II. Anemia of Chronic Disease (ACD)

This is a condition seen in individuals suffering from chronic infections, noninfectious inflammatory diseases (such as rheumatoid arthritis), and neoplasms. The molecular basis for ACD is not known. The following pathogenetic observations have been made to help characterize the anemia:

Decreased rbc life span. This appears to be due to a factor or factors extrinsic to the red cell. The chemical nature of such factor(s) is completely unknown.

Impaired iron metabolism. Intestinal absorption of iron is compromised. Despite this, iron accumulates in the marrow histiocytes, but its uptake into rbc precursors is impaired. Therefore the marrow shows decreased sideroblastic iron in the face of increased histiocytic iron. The concentration of transferrin in the plasma is decreased, as is that of iron.

Refractoriness to erythropoietin. The cause is unknown but appears to be an effect independent of the impairment of iron transfer.

The anemia, is usually said to be normochromic/normocytic, but most patients actually have a slightly decreased MCHC (thus hypochromia). A minority of patients will be microcytic as well. The serum iron is decreased, as is the transferrin (or TIBC) in contrast to iron deficiency anemia, where transferrin is elevated. The absolute retic count is normal or slightly elevated. Bone marrow biopsy shows increased histiocytic iron and decreased sideroblastic iron, but no other morphologic findings are characteristic of this condition.

III. Megaloblastic anemias

These are a number of conditions which have in common the failure to synthesize adequate amounts of normal DNA. The anemias are macrocytic, since hemoglobinization is allowed, but cells mature more slowly in the marrow; therefore, the cells vegetate in the marrow, slowly maturing but stuffing their greedy little figurative mouths with iron, making hemoglobin, and getting larger as a result. Although some of these obese cells make it out of the marrow, many more never mature prop-

erly and eventually are destroyed before they have tasted the thrill of the extramedullary hunt. This phenomenon is referred to as **ineffective erythropoiesis**. Such marrows are packed with erythroid precursors, even in the face of severe anemia. The rbc precursors are notable morphologically for their immature, sometimes even blast-like chromatin in large nuclei. Such cells are called megaloblasts. Megaloblastic changes are not limited to the erythroid precursors, but are also seen in myeloid precursors. In some cases of megaloblastic anemia, there is concomitant leucopenia and thrombocytopenia, reflecting the troubled development of granulocytes and platelets as well.
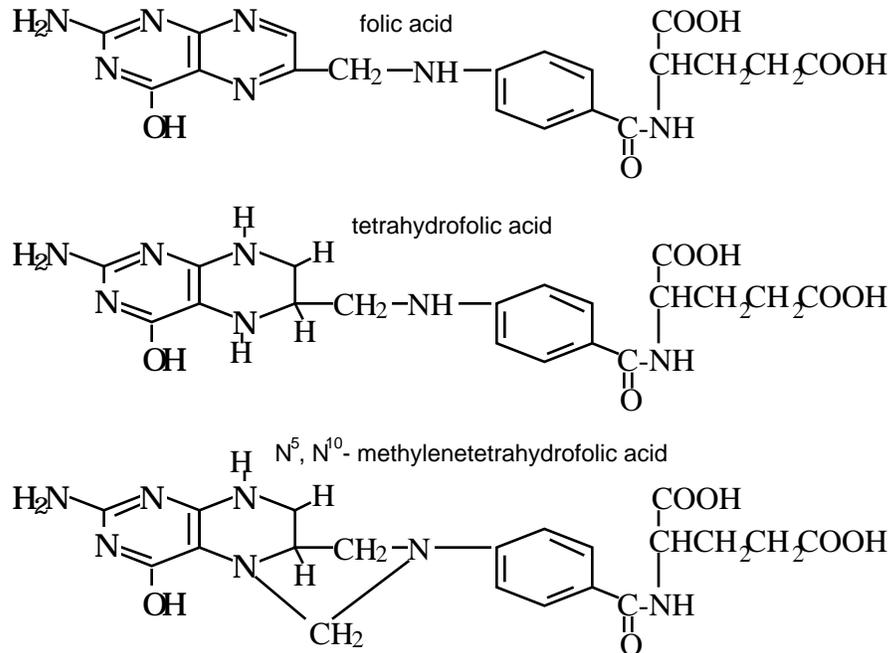
A. Pathogenesis of megaloblastic anemias

The megaloblastic state results from an imbalance between supply of co-factors necessary for DNA synthesis and demand for DNA production. The two co-factors which are the most important are **folate** and **vitamin B_{12}**. When these are deficient, megaloblastic change results. On the other hand, increased demand for DNA in physiologically hyperproliferative states, such as cancer and hemolytic anemia, can cause megaloblastic change even in the face of freely available folate and B_{12}. To understand why folate and B_{12} are so important to DNA synthesis, it is necessary to gird one's loins for a trip back to Biochem. DNA differs from RNA in that 1) deoxyribose is used instead of ribose, and **thymine** is used instead of **uracil**. The structural formulas below show that uracil and thymine differ only by a silly little methyl group.
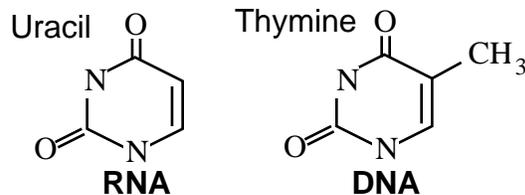
Unfortunately, the methyl is necessary for the magic DNA enzymes to recognize the molecule as DNA and work their wonders on the double helix. It seems that the body can make just about any organic molecule out of yesterday's BK Broiler and a few trace metals, but in this case a special set of reactions is necessary for the finishing touches on thymine. To stick the methyl group on the ring, folate is required, and B_{12} helps out.

B. Folate

Just to make casual observers think we're studying real science here, let us look at the chemical structure of folate and its metabolites.

H_2N ... N ... N — folic acid — COOH
OH — CH_2-NH — $CHCH_2CH_2COOH$ — C-NH ... O

H_2N ... N ... N — tetrahydrofolic acid — COOH
OH — H — CH_2-NH — $CHCH_2CH_2COOH$ — C-NH ... O

H_2N ... N ... N — N^5, N^{10}- methylenetetrahydrofolic acid — COOH
OH — H — CH_2-N — $CHCH_2CH_2COOH$ — C-NH ... O — CH_2

Note that "folate" and "folic acid" are basically the same thing and differ only in whether the carboxylic acid groups are dissociated, this in turn dependent only on ambient pH. **Folic acid** is a vitamin found in abundance in many foods, especially asparagus, broccoli, endive, spinach, and lima beans. The daily requirement is only 50 µg (Cf. 10,000 µg for iron). The body's folate reserves last about four months. The vitamin is rapidly absorbed by the proximal jejunum. Folic acid is metabolically inactive until it is converted into **tetrahydrofolic acid** (THF). A key enzyme in this conversion is **dihydrofolate reductase**, which is the target enzyme inhibited by the anticancer drug **methotrexate**. THF is capable of methyl group transfer by picking up the one-carbon group from the amino acid serine and sticking it on uridylate, thus producing thymidylate, which in turn goes off to seek its fortune in DNA as a courier of genetic messages. The methyl-carrying version of THF is called N^5,N^{10}**-methylene**-**THF** and is shown above.

As complicated as all this seems, it only scratches the surface. Folate has been shown to play a role in no fewer than six biochemical reactions, including synthesis of methionine, synthesis of purines (thymine is a pyrimidine), and catabolism of histidine. Failure of folate to break down histidine results in accumulation of an intermediary metabolite, **formiminoglutamic acid** (FIGlu), which can be measured in the clinical laboratory as a marker for folate deficiency.

Deficiency of folate is seen among poorly nourished individuals, especially alcoholics, infants fed solely on milk, and pregnant women. Malabsorption syndromes often produce folate deficiency, and certain drugs (*e.g.*, phenytoin,

phenobarbital, primidone, isoniazid, and cycloserine) are associated with compromise of folate absorption and metabolism.

C. Vitamin B_{12}

Vitamin B_{12} is a substance whose biochemistry is complex. Basically it consists of a cobalt-containing porphyrin-like prosthetic group attached to a nucleotide.
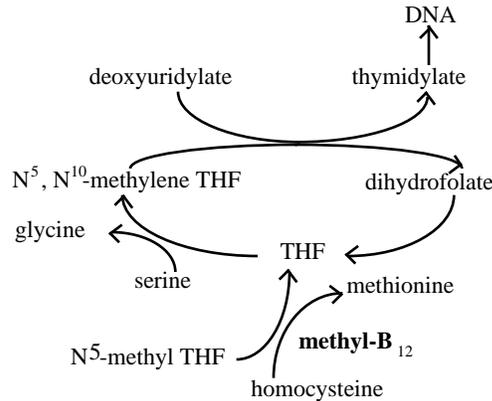
Its deficiency produces megaloblastic anemia due to its role in folate metabolism. During the many transformations of folate from one form to another, a proportion gets accidentally converted to **N^5-methyl-THF**, an inactive metabolite. This is called the "**folate trap**," since there is no way for active N^5,N^{10}methylene-THF to be regenerated except through a reaction for which a form of vitamin B_{12}, methyl-B_{12}, is a cofactor (see diagram, right). De-ficiency of B_{12} then produces a situation where more and more folate is trapped in an inactive form with no biochemical means of escape. The end result is failure to synthesize adequate DNA.

B_{12} deficiency also produces nervous system lesions not seen in folate deficiency. These lesions are manifest clinically as **combined systems disease**, a constellation of findings related to demyelination of axons in the spinal cord and cerebrum. These patients have decreased vibratory and proprioceptive senses in the extremities, spastic ataxia, disturbances of vision, taste, and smell, irritability, and somnolence. "Megaloblastic madness" is the term appended to the poorly documented cases of bizarre, sometimes psychotic behavior in B_{12}-deficient patients. Clearly, there must be something that B_{12} is supposed to do about which folate has little or no concern. The best guess is that it has something to do with B_{12}'s role as a cofactor for **methylmalonyl-CoA mutase**, which catalyzes the following reaction:
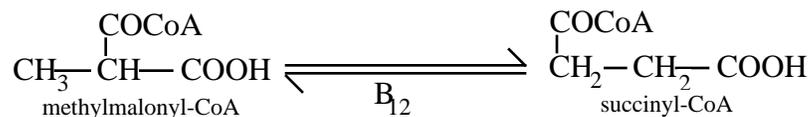
A few of you, the truly perverted, will recognize this reaction as the clever way the body has of dealing with odd-chain fatty acids, prestidigitating them into even-chain species that can be handily dispatched via beta-oxidation. Without this mechanism, no telling what sorts of havoc these three-carbon residues would raise floating around in the myelin-making factory. A nice spin-off is that **methylmalonic acid** accumulates and shows up in the urine, where it can be measured as a marker of B_{12} deficiency.

The **etiology of B_{12} deficiency** is more complicated than that of folate deficiency. One can develop deficiency through either of the following mechanisms:

1. Dietary deficiency.

B_{12} is only found in animal products,[23] but it is plentiful. Therefore, nutritional deficiency is seen almost exclusively in vegans.[24] Even so, this is extremely rare. Unlike the situation with folate, B_{12} body reserves can last for years.

2. Malabsorption states.

By far, this is the most common mechanism of disease development. The absorption of B_{12} is much more complicated than that of folate and iron. B_{12} is absorbed *only* in the terminal ileum. Absorption occurs *only* if the B_{12} is bound to a glycoprotein, unimaginatively named **intrinsic factor**, produced *only* by the parietal cells of the gastric mucosa.[25] Therefore, malabsorption can occur if 1) intrinsic factor is not produced, 2) intrinsic factor is neutralized, 3) there is a pathologic lesion of the terminal ileum, or 4) there are microorganisms present which compete successfully with the host for the B_{12}. Let us consider each mechanism in turn:

a. Intrinsic factor not produced

This is seen following total gastrectomy. One must always give maintenance parenteral B_{12} for life following gastrectomy. Intrinsic factor is also not produced in pernicious anemia (see below).

---

[23]So where did the animals get it? Carnivores get it from the flesh of herbivores. Herbivores have it made for them by commensal gut bacteria. Human gut flora does not make sufficient B_{12} to stave off the deficiency disease. If we made our own vitamin B_{12}, it wouldn't be a vitamin!

[24]Vegans are *pure* vegetarians who eschew even milk and eggs; they are not aliens from -Lyrae.

[25]Some theologians have maintained that the existence of humankind offers proof of the existence of God. If so, I would offer the corollary that the existence of the Byzantine mechanism of B_{12} absorption offers proof that God likes games with countless, arbitrary rules, like Dungeons & Dragons.

b. Intrinsic factor inhibited

Pernicious anemia (PA) is the classic term used to describe the megaloblastic anemia which develops as a result of autoimmune destruction of the gastric mucosa (atrophic gastritis) and autoantibodies directed against intrinsic factor. PA is said to occur most commonly in elderly Caucasians of northern European extraction and in younger African-Americans. There is a strong association with other autoimmune diseases in the same patient, especially Hashimoto's thyroiditis.

c. Terminal ileum lesions

Crohn's disease is a classic example. It typically affects the terminal ileum and can produce malabsorption because of massive tissue destruction in this area.

d. Competition for B_{12}

This occurs in various congenital or acquired anatomic abnormalities of the small intestine which foster overgrowth of our tiny little prokaryotic friends, all too happy to slurp up all that B_{12} at our expense. Another condition (a classic National Board question if there ever was one) is infestation with the fish tapeworm, *Diphyllobothrium latum*, also quite capable of quaffing a few cobalts.

Part Four: Hemolytic Anemias

I. Introduction and definitions

Previously we have looked at nutritional anemias and the anemia of chronic disease, in which the metabolic needs of erythrocyte development are not met. The result is failure to *produce* enough healthy red cells. Now we turn to conditions in which the erythrocyte construction industry is healthy, but where the red cells produced are incapable of surviving the normal 120-day life span. These *hemolytic* anemias may be due to either intrinsic defects in rbc structure/function or a hostile external environment in which the cells are forced to live. To start with a few definitions:

Hemolysis: Any condition characterized by a significantly decreased erythrocyte life span.

Compensated hemolytic state: A state of hemolysis in which the resulting increased erythrocyte production is able to keep up with accelerated rbc destruction, thus averting any anemia.

Hemolytic anemia: A state of hemolysis in which increased erythrocyte production is insufficient to keep up with accelerated rbc destruction, thus producing anemia. This anemia is characterized as normochromic/normocytic, except

when sufficient outpouring of the larger reticulocytes produces a resulting elevation of the MCV.

II. Diagnosis of hemolytic anemia

Diagnosis of hemolytic anemia is performed in four steps:

A. Establish that anemia exists.

The diagnosis of anemia has been previously covered.

B. Look for marrow response

The *sine qua non* for the diagnosis of hemolysis is demonstration of an attempted marrow response to erythrocyte destruction. The classic way to do this is with the **reticulocyte count**. Remember that you must correct the count for the degree of anemia to prevent overdiagnosis of hemolysis. The absolute retic count (in cells/µL) or, better, the **reticulocyte production index** (RPI) can be used to avoid this pitfall. Even so, one should never take a positive result out of context . A classic cause of reticulosis is recovery from a nutritional anemia (esp. iron and folate).[26] For this reason, you also need corroborating evidence of erythrocyte destruction, thus:

C. Look for erythrocyte detritus

We have previously discussed the fate of destroyed red cells and their component catabolites, such as free hemoglobin, methemoglobin, methemalbumin, bilirubin, and urobilinogen, as well as the specific binding proteins for these catabolites, such as haptoglobin and hemopexin (see *The Anemias: Classification and Clinical Investigation* earlier in this series). Laboratory measurement of some or all of these assists in the diagnosis of hemolysis.

D. Establish the pathophysiological mechanism of hemolysis

The first distinction to make is to determine whether the hemolysis is taking place in the sinusoids of the reticuloendothelial system[27] (**extravascular hemolysis**) or in the bloodstream proper (**intravascular hemolysis**). Both types produce indirect hyperbilirubinemia, urobilinogen in stool and urine, decreased serum haptoglobin, and reticulocytosis. In addition, assuming hemolysis is brisk enough to overwhelm the haptoglobin hemoglobin salvage mechanism, in-

---

[26]In particular, folate deficiency anemia in a neglected person, especially the alcoholic, will show dramatic reticulocyte response simply by ingestion of a routine hospital diet following admission. In such an instance, you may be prescribing specific treatment for the anemia without even knowing it.

[27]Now known as the "mononuclear phagocyte system." Pedants have pointed out that the RES was inapproporiately named, because the phagocytic lining cells of the sinusoids were not true endothelial cells but stationary macrophage-like cells. A sense of romance allows me a preference for the older term. After all, other fields have their traditionally inaccurate designations; *e.g.*, Voltaire's observation that the Holy Roman Empire was "neither Holy, nor Roman, nor an Empire."

travascular hemolysis produces hemosiderin in the urine sediment, free hemo-globin in the serum (which may be grossly visible), and free denatured hemo-globin in the urine. Some intravascular hemolytic conditions due to mechanical destruction of rbc's produce the helmet-shaped **schizocytes** (or "schistocytes" if you are a Græcophobic Latinophile), which can be seen on the routine peripher-al blood film. Extravascular hemolytic anemias may produce **spherocytes**, which are the result of an rbc having a narrow escape from the clutches of the RES.

The next determination to make is the mechanism of rbc destruction. Performing a thorough history and physical (including family and drug history), examining the peripheral blood film, and ordering a few inexpensive laboratory tests, such as the direct antiglobulin (Coombs') test for autoantibodies directed against the rbc mem-brane antigens and the hemoglobin electrophoresis, will lead you into Diagnosisland in 95+% of the cases. Rare cases will require labor-intensive, costly tests that have to be sent away somewhere like King of Prussia, Pennsylvania, or that are batched for six months in a dusty, coffee-stained research lab tucked away in a closet into which the Medicare inspector has yet to stumble.

III. Specific Conditions

Let us consider selected hemolytic anemias individually. These particular diseases are covered either because they are common or because they illustrate important pathophysiologic features (or both).

A. Mechanical hemolytic anemias

These are certainly the easiest to understand, even to the most concrete of thinkers. Red cells are destroyed due to hydrodynamic turbulence when they are forced over gross obstructions (such as an artificial heart valve) or "clotheslined" by innumerable fibrin strands in such microangiopathic condi-tions as disseminated intravascular coagulation (better known by the macho-nym "DIC," covered later in the heme bloc) or thrombotic thrombocytopenic purpura (TTP), an uncommon and mysterious disease of unknown etiology. The hallmark of microangiopathic hemolytic anemia is the presence of schizocytes on the routine blood film.[28]

B. Immunohemolytic anemias

In **autoimmune** hemolytic anemias, the body discourteously mounts an im-mune attack against its own rbc membrane antigens. This condition not sur-prisingly tends to occur in states characterized by systemic autoimmunity,

----

[28]At the risk of sounding like a tenured senior full professor, I really do recommend that you personally look at the peripheral blood film on all of your seriously ill or problem admissions. Even an inexperienced examiner can, armed with the clinical history, turn up very clever diagnoses that would otherwise be missed.

such as lupus erythematosus. If the autoantibody is of the IgG class, hemolysis will usually occur at any temperature ("**warm autoimmune hemolytic anemia**"). Several drugs are known to produce warm autoimmune hemolytic anemia which goes away after withdrawal of the drug. Typically the antibody in warm hemolysis is one directed against a universal component of the Rh system absent only in individuals (usually of native Australian blood) with the extremely rare Rh-null rbc membrane phenotype

Autoantibodies of the IgM class typically produce **cold agglutinin syndrome**, in which the patient is at greater risk of symptoms in a low-temperature environment. Cold agglutinin syndrome may occasionally occur transiently in cases of *Mycoplasma* pneumonia and rarely infectious mononucleosis. Most cold autoagglutinins are directed against the I antigen, found in almost all adults. The rare infectious mono cold agglutinin has been characterized as anti-i.

Paroxysmal cold hemoglobinuria is a very rare syndrome in which intravascular hemolysis is produced upon exposure to cold temperature by an IgG autoantibody directed against the P antigen found on the red cells of nearly all individuals.[29]
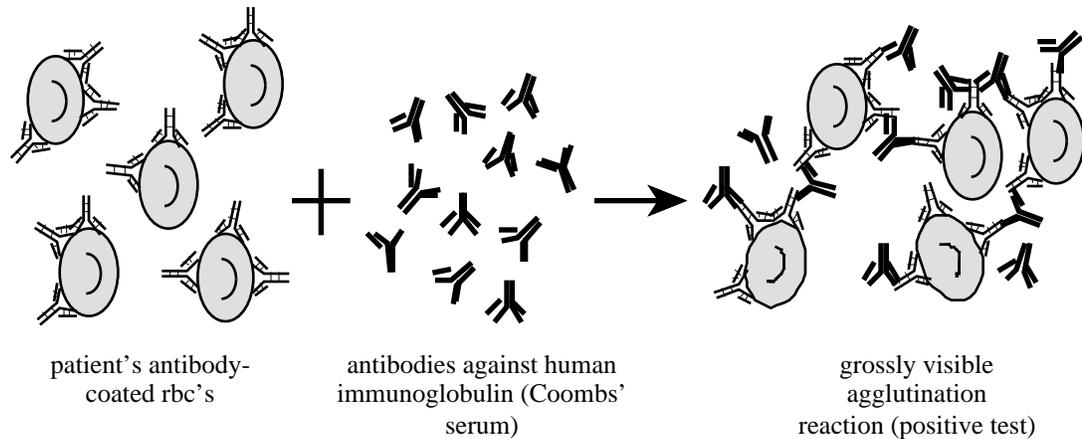
In **alloimmune**[30] hemolytic anemia, the body synthesizes antibodies against red cell antigens foreign to the host. These antibodies may be *naturally occurring* (such as those directed against ABO blood group antigens) or *acquired* as a result of blood transfusion (including that from a fetus to its pregnant mother). Acquired antibodies include those directed against the Rh, Kell, Duffy, and Kidd system antigens. Clinical hemolysis occurs 1) when maternal antibodies send a raiding party across the placenta to raise a little hell in Fetusville (to produce hemolytic disease of the newborn — "erythroblastosis fetalis"), and 2) when host antibodies destroy transfused red blood cells in a hemolytic transfusion reaction (which fortunately is very rare with modern blood banking practices).

Diagnosis of immunohemolytic anemia is made by demonstrating (after having proved hemolysis is occurring, as discussed above) a positive result on a simple agglutination test to demonstrate that antibodies are present on the surface of the patient's rbc's. This test is properly called the **direct antiglobulin test**. All but the most pedantic eschew this term, preferring the eponymous designa-

---

[29]This cold-reacting anti-P antibody is called the **Donath-Landsteiner** antibody, a classic question in the roundsmanship trivia game. Remember you heard it here first.

[30] "Alloimmune" pertains to antibodies made against antigens of *another* individual; "isoimmune" is a special case of "alloimmune" that pertains specifically to antigens and naturally-occurring antibodies of the ABO blood group system. Compare these terms with "autoimmune," which pertains to antibodies made against the host's *own* antigens.

tion **direct Coombs' test**.[31] Another term for immunohemolytic anemia is, therefore, "Coombs'-positive hemolytic anemia." The diagram below illustrates the principle of the Coombs' test:

| patient's antibody-coated rbc's | antibodies against human immunoglobulin (Coombs' serum) | grossly visible agglutination reaction (positive test) |

A blood sample from the patient has the plasma poured off and the cells washed. The rbc's are resuspended in an aqueous medium. Antiserum containing antibodies against human immunoglobulin (prepared by diabolically injecting cute, innocent little animals with doses of human immunoglobulin) is then added to the patient's cells. A positive result is indicated grossly (or occasionally microscopically) by a visible agglutination reaction.

Treatment of immunohemolytic anemias is aimed at reducing the activity of the body's misdirected immune system. **Glucocorticoids** are the mainstay of therapy, although refractory cases can be treated with other immunosuppressive drugs and by **splenectomy**. This latter may be of benefit since immunohemolytic anemias most commonly are due to *extravascular* destruction of the auto-opsonized cells, at least some of which may occur in the spleen. Transfusion is of limited or no benefit (unless you can find a few Australian aboriginal blood donors), since the patient's autoantibodies very willingly go to work on the transfused red cells with as much relish as they do on the patient's own.

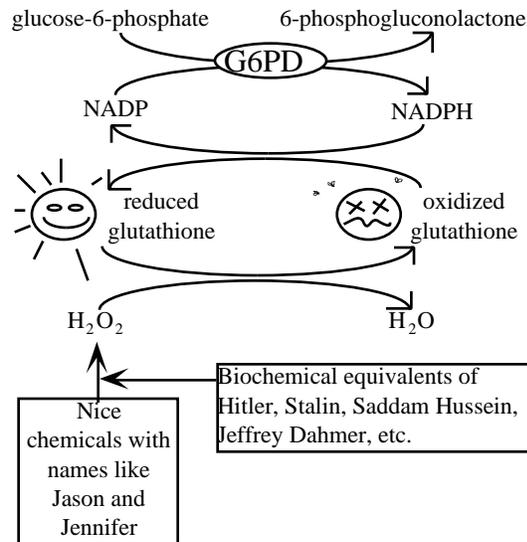## C. Paroxysmal nocturnal hemoglobinuria

This is sort of analogous to the Holy Roman Empire in that PNH is often neither paroxysmal, nor nocturnal, nor productive of gross hemoglobinuria. What does happen is that the patient's rbcs develop an acquired somatic mutation that affects the structure of the cell membrane and makes it more sensitive to nonspecific attachment and activation of complement (sounds pretty vague doesn't it? At least there's no complicated mechanism to memorize). Intravas-

---

[31]The **direct** Coombs' test should be distinguished from the **indirect** Coombs' test. While the former detects antibodies *on patient's rbc's,* the latter detects antibodies directed against rbc's *in a patient's serum.* The indirect Coombs' test is a specialized blood banking tool; it is rarely required in clinical hematology.

cular hemolysis is the result, producing anemia if not always hemoglobinuria. In such cases of suspected chronic intravascular hemolysis without a positive urine hemoglobin test, a good thing to try is staining the urine sediment with Prussian blue reagent; the **hemosiderinuria** thus detected will lead you to the correct diagnosis.

## D. Glucose-6-phosphate dehydrogenase deficiency

The vicissitudes of life as a red cell include the potential for harm brought about by the accumulation, through natural metabolic activities, of hydrogen peroxide (H_2O_2). Accumulation of peroxide is especially pronounced in exposure to certain "oxidative" substances in foods (such as fava beans) and the pharmacopœia (such as sulfonamide antimicrobials, nalidixic acid [NeGram®, a urinary tract antimicrobial quinolone], nitrofurantoin [Furadantin®, another UTI drug], and certain antimalarials). Fortunately, the cell has means by which peroxide can be turned into harmless water, as illustrated on the next page. The enzyme which is responsible for reduction of the peroxide to water is **glutathione peroxidase**. It relies on a supply of reduced glutathione to act as a reducing agent. The exhausted, oxidized glutathione must be reduced by NADPH, the only source of which is the first step in the ever-hated-by-med--students-suffering-through-biochemistry **hexose monophosphate shunt** (a.k.a., pentose phosphate pathway). The enzyme that presides over this first step is glucose-6-phosphate dehydrogenase. In an **X-linked recessive** genetic variant, the activity of G-6-PD is depressed, so that exposure to the oxidative substances overwhelms the weakened compensatory mechanism, and hemolysis, often severe and intravascular, results. Obviously, the vast majority of these patients are males (since it is X-linked). Unlike sickle cell disease, the condition may be present from birth, although there is great variation in the clinical severity of the various mutations. For instance, the G-6-PD deficiency found in eleven per cent of African-Americans is usually subclinical throughout the life of the patient, while some variants seen in Mediterranean Caucasians may be catastrophic at birth. The incidence of G-6-PD deficiency of one type or another varies from 0.1% in Germanic/Slavic/Baltic types to 50% in Kurdish Jews.

glucose-6-phosphate   6-phosphogluconolactone

G6PD

NADP   NADPH

reduced glutathione   oxidized glutathione

H_2O_2 H_2O

Nice chemicals with names like Jason and Jennifer

Biochemical equivalents of Hitler, Stalin, Saddam Hussein, Jeffrey Dahmer, etc.

Favism, the catastrophic condition caused when someone with the severe Mediterranean variant eats fava beans (which you can find at Fiesta, if any of you are getting any ideas) is rare, and you may never see it. However, milder degrees of G-6-PD deficiency should be kept in mind when you see an unexplained hemolytic anemia in a black, Asian, or Mediterranean male. The treatment is aimed at prevention using the Hee Haw Clinic method.[32] Patents are instructed to avoid oxidant substances and to always make their condition known to any medical personnel they see in the future.

E. Pyruvate kinase deficiency

Since the erythrocyte has no mitochondria, it has no Krebs cycle. Its only source of ATP is through the Embden-Myerhof pathway (the hexose monophosphate shunt does not generate any high-energy phosphate bonds, thus no ATP). ATP is needed primarily for the maintenance of the ATP-dependent potassium/sodium pump (see illustration).

Na^+

ATP

ADP

K^+
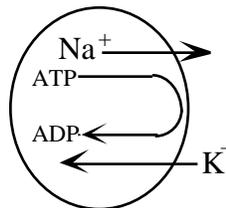
The pump is necessary to keep potassium in the cell and sodium out. If this does not happen, everything goes haywire and the cell lyses. Since this pump activity flies in the face of entropy, energy must be added to the system. As you might expect in a publish-or-perish academic system, someone has described

---

[32]Buck Owens: "Doc, it hurts whenever I do *this*" [abducts shoulder] Archie Campbell [striking Buck]: "Well, don't *do* that!"

genetic deficiencies of just about all of the Embden-Myerhof pathway enzymes. Fortunately for medical students in a memorize-or-perish academic system, the only one ever found on the National Boards is **pyruvate kinase deficiency**. All of the others are extraordinarily rare. PK deficiency differs somewhat from G-6-PD deficiency. The mode of inheritance is **autosomal recessive**. Also, the hemolysis is chronic and ongoing, unlike G-6-PD deficiency, which characteristically is episodic and related to environmental exposures, as noted above.

F. Spherocytosis

Spherocytes are seen in a variety of hemolytic anemias, specifically those in which the RES is involved in removing the cells. Examples would be immuno-hemolytic anemias (see above) and hypersplenism (see below). In such cases the spherocytosis is a *secondary* change due to escape and repair of a partially damaged rbc's from the RES.

In **hereditary spherocytosis** (HS), on the other hand, a genetic variant of the structure of a cytoskeletal protein (probably **spectrin**, whatever that is) results in the marrow producing spherocytes *de novo*. These rotund cells are your basic spleen-fodder, and extravascular hemolysis is the result. The mutation is in-herited as an **autosomal dominant**. The diagnosis is made by the use of the **osmotic fragility test**, in which it is demonstrated that spherocytes are more fragile when placed in a hypo-osmotic environment than are normal rbc's. Also, it is necessary to demonstrate a negative direct Coombs' test to rule out immu-nohemolytic anemia. An interesting feature of hereditary spherocytosis seen al-most nowhere else is *increase* in the **MCHC**. Spherocytes may be easy to see on the peripheral smear of some patients, but in others the abnormality is so sub-tle, that HS cannot be ruled out based on normal RBC morphology.

Hereditary spherocytosis can be cured by **splenectomy**, making HS one of the very few curable hematologic conditions, and one of the only three (with some iron-deficiency anemias and a few cases of megaloblastic anemia) that can be cured by surgery.

G. Hypersplenism

Any condition which causes enlargement of the spleen turns it into a labyrinth of horrors for even the most normal corn-fed, Oklahoma-bred, antibody-free, enzyme-stuffed, glutathione-replete erythrocyte. Thus even normal rbc's can undergo hemolysis when sufficiently jacked around with. The condition is termed, somewhat vaguely, hypersplenism. You should think of this when a patient with chronic liver disease, leukemia/lymphoma, and congestive heart failure (all of which may produce splenomegaly) turns up with a hemolytic ane-mia and a negative direct Coombs' test.

H. Infections of the RBC

In some infections, hemolysis dominates the clinical picture. **Malaria**, **Carrión's disease**, and **babesiosis** are covered elsewhere. ***Clostridium perfringens* sepsis** may produce a severe episode of acute hemolysis, with virtual opacity of the patient's plasma due to hemoglobin released by the brisk intravascular hemolysis.

I. Hemoglobinopathies and Thalassemias

These extremely important conditions are covered in the final section. It should be noted that not all hemoglobinopathies or thalassemias produce hemolysis, but in some of them, especially sickle cell anemia and hemoglobin C disease, hemolysis dominates the clinical presentation.

# Part Five: Hemoglobinopathies and Thalassemias

I. Introduction

These conditions comprise a very large number of genetic biochemical/ physiological entities, most of which are academic curiosities whose major effect on medicine is to add to the surfeit of useless scientific information. However, several of these conditions (e.g., sickle cell anemia, hemoglobin SC disease, and some thalassemias) are common major life-threatening diseases, and some others (*e.g.*, most thalassemias, hemoglobin E disease, and hemoglobin O disease) are conditions that produce clinically noticeable — if not serious — effects and can cause the unaware physician a lot of frustration and the hapless patient a lot of expense and inconvenience. We will study a few hemoglobinopathies and thalassemias of special importance. It should be kept in mind, though, that there are literally *hundreds* of diseases in these categories.

II. Definitions

Hemoglobinopathy: *A genetic defect that results in abnormal structure of one of the globin chains of the hemoglobin molecule.* Although the suffix "-pathy" would conjure an image of "disease," most of the hemoglobinopathies are not clinically apparent. Others produce asymptomatic abnormal hematologic laboratory findings. A very few produce serious disease. The genetic defect may be due to substitution of one amino acid for another (as with the very common Hb S and Hb C and the great majority of the other abnormal hemoglobins), deletion of a portion of the amino acid sequence (Hb Gun Hill), abnormal hybridization between two chains (Hb Lepore), or abnormal elongation of the globin chain (Hb Constant Spring). The abnormal chain that results may be the   chain (Hb G$_{Philadelphia}$),   chain (Hb S, Hb C),   chain (Hb

F$_{Texas}$), or   chain (Hb A2$_{Flatbush}$).[33] These abnormal hemoglobins can have a variety of physiologically significant effects, discussed below in greater depth, but the most severe hemoglobinopathies (Hb S and Hb C diseases) are characterized by hemolysis.

Thalassemia: *A genetic defect that results in production of an abnormally low quantity of a given hemoglobin chain or chains*. The defect may affect the , , , or   chain, or may affect some combination of the , , and   chain in the same patient (but never the   and   chain together). The result is an imbalance in production of globin chains and the production of an inadequate number of red cells. The cells which are produced are hypochromic/microcytic and contain a surfeit of the unaffected chains which cannot stoichiometrically "mate" with the inadequate supply of thalassemic chains. These "bachelor" chains can produce adverse effects on the red cell and lead to destruction of the red cell in the marrow (ineffective erythropoiesis) and in the circulation (hemolysis). Note that these two definitions are not mutually exclusive — some hemoglobinopathies may also be thalassemias, in that a structurally abnormal hemoglobin (hemoglobinopathy) may also be under-produced (thalassemia).[34] Some, but not all, hemoglobinopathies and thalassemias are hemolytic anemias. These nosologic concepts are summarized by the Venn diagram below.
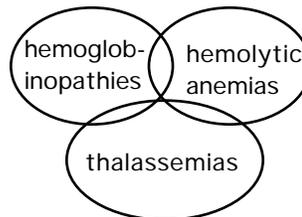
III. Pathophysiology of hemoglobinopathies

Messing around with the amino acid sequence of a globin chain has something of a red kryptonite effect. While some positions on the protein chain can tolerate a lot of substitutions without compromising the physiologic integrity of hemoglobin, other positions are very sensitive to amino acid substitutions. For instance, substitution of valine or lysine for glutamate at position 6 of the   chain produces hemoglobins S and C, respectively, which form intraerythrocytic tactoids (see below) and crystals (again respectively) that cause premature destruction of the rbc (hemolysis). On the other hand, substitution of glutamate, asparagine, and threonine for lysine at position 59 of the   chain produces, respectively, hemoglobins I$_{High\ Wycombe}$, J$_{Lome}$, and J$_{Kaoshiung}$, all of which are physiologically indistinguishable from normal Hb A.

---

[33]I might fan the fires of regional rivalry by pointing out that there is a Hb Memphis, Hb Little Rock, Hb Jackson, Hb Austin, Hb G$_{Galveston}$, Hb Mobile, Hb Cowtown, even (God forbid!) a Hb Baylor, but there is no "Hb Houston."

[34]This is true in the case of Hb Lepore.

Without venturing too deeply into tedious stereochemistry, we can say that abnormal globin structure can functionally manifest itself in one or more of the following ways:

A. Increased O_2 affinity

These hemoglobins tend to result when mutations affect the portions of the amino acid sequence that compose 1) the regions of contact between   and chains, 2) the C-terminal regions, and 3) the regions that form the pocket which binds 2,3-DPG. The hemoglobin eagerly scarfs up the O_2 from the alveoli but then only stingily gives it up to the peripheral tissues. The kidney, always compulsively vigilant for hypoxia, cranks out the erythropoietin thinking that a few extra red cells might help out matters. Erythropoiesis then is stimulated, even though there is no anemia, and **erythrocytosis** (increased total body rbc mass, increased blood hemoglobin concentration, increased hematocrit) is the result.

It is important to know that these rare increased O_2 affinity hemoglobins exist to prevent diagnostic errors from occurring in working up patients presenting with erythrocytosis (which is much more commonly caused by other conditions, including polycythemia vera [a neoplasm], cigarette smoking, psychosocial stress, chronic residence at high altitudes, and chronic lung disease). Examples of these include Hb Chesapeake and Hb J$_{Capetown}$.

B. Decreased O_2 affinity

This is the other side of the coin. These hemoglobins are reluctant to pick up O_2 from the lung. The result is a decreased proportion of hemoglobin that is oxygenated at a given Po$_2$. The remainder of the hemoglobin is, of course, deoxygenated and is blue. If the level of blue hemoglobin exceeds 5 g/dL in capillary blood, the clinical result is **cyanosis**, a bluish discoloration of skin and mucous membranes.

Again, it is important to know about these hemoglobins and keep them in the back of your mind when working up cases of cyanosis, a condition much more commonly caused by pulmonary dysfunction or right-to-left cardiovascular shunts. Examples of low O_2 affinity hemoglobins include Hb Seattle, Hb Vancouver, and Hb Mobile.

C. Methemoglobinemia

These hemoglobins are a special class of low O_2 affinity hemoglobin variants that are characterized by the presence of heme that contains iron in the ferric (Fe^{+++}) oxidation state, rather than the normal ferrous (Fe^{++}) state. These *methemoglobins* are all designated "Hb M" and further divided by the geographic site of their discovery, e.g., Hb M$_{Saskatoon}$ and Hb M$_{Kankakee}$. The affected patients have **cyanosis**, since the methemoglobin is useless in O_2 binding.

Methemoglobinemia due to hemoglobinopathy should be distinguished from methemoglobinemia due to other causes, such as **NADH-diaphorase deficiency**. This enzyme is needed for the reduction (to heme) of metheme that accumulates as a result of normal metabolic processes. Congenital absence of NADH-diaphorase causes an accumulation of metheme, despite the fact that the structure of the globin chain is normal. **Toxic methemoglobinemia** occurs in normal individuals exposed to certain oxidizing drugs and other compounds in the environment, even though these individuals have normal hemoglobin structure and a normal complement of NADH-diaphorase. In such victims, the oxidizing power of the toxin overwhelms the normal antioxidant defenses.

Since methemoglobin is a brown pigment, patients with clinically severe methemoglobinemia have *obviously brown blood*. This observation allows one to make a clever and memorable diagnosis at the bedside during the patient's first venipuncture.

D. Unstable hemoglobin (Heinz body anemia)

Certain abnormalities in the globin chain sequence produce a hemoglobin that is intrinsically unstable. When the hemoglobin destabilizes, it forms up into erythrocyte inclusions called Heinz bodies. It is important to know that *Heinz bodies are not visible in cells stained with the routine Wright stain*. It is necessary for the cells to be stained with a supravital dye (such as brilliant cresyl blue, which can also be used to demonstrate reticulocytes) to be visible. These inclusions attach to the internal aspect of the rbc membrane and reduce the deformability of the cell and basically turn it into spleenfodder. The result is hemolytic anemia. All of these hemoglobins are rare; inheritance is autosomal dominant. Homozygotes have not been described. Examples of unstable hemoglobins are Hb Gun Hill, Hb Leiden, and Hb Köln.

E. Sickling and crystallization

These phenomena occur respectively in Hb S and Hb C, the most important of the abnormal hemoglobins. We will deal with these in greater depth next.

IV. Specific hemoglobinopathies

A. Hemoglobin S and sickle cell disease

1. Epidemiology and genetics

The Hb S gene is found primarily in populations of native tropical African origin (which include most African-Americans). The incidence of the gene in some African populations is as high as 40%; in African-Americans the incidence is 8%. The gene is also found with less frequency in non-Indo-European aboriginal peoples of India and in the Middle East. Rare cases have been reported in Caucasians of Mediterranean descent. The gene es-

tablished itself in the tropical African population presumably because its expression in heterozygotes (**sickle cell trait**) affords some protection against the clinical consequences of *Plasmodium falciparum* infestation. Unfortunately, homozygous expression produces **sickle cell disease**, which is a chronic hemolytic anemia and vaso-occlusive condition that usually takes the life of the patient.

Hemoglobin S has the peculiar characteristic of expressing its biochemical instability by precipitating out of solution and forming up into long microtubular arrays called **tactoids**. The erythrocytes which contain the Hb S stretch around the tactoids to form the characteristic long, pointed, slightly curved cells called (with somewhat liberal imagination) "sickle cells."[35] Only the deoxygenated form of Hb S (deoxy-Hb S) makes tactoids. The greater the proportion of Hb S in the cell, the greater is the propensity to sickle. Therefore, persons with 100% Hb S (being homozygotes) sickle under everyday conditions, while typical heterozygotes (who usually have about 30–40% Hb S) do not sickle except possibly under extraordinary physiologic conditions.[36] Since Hb S is a β chain mutation, the disease does not manifest itself until six months of age; prior to that time the Hb S is sufficiently "watered down" by Hb F ($\alpha_2\gamma_2$), which of course has no β chain.

In post-infancy individuals homozygous for the Hb S gene, 97+% of the hemoglobin is Hb S, the remainder being the normal minor hemoglobin, Hb A_2 ($\alpha_2\delta_2$). Several coexisting genetic "abnormalities" (actually godsends) prevalent in African populaitons may ameliorate the course of the disease:

1) α-**thalassemia** carriers (which comprise 20% of African-Americans!) have a lower MCHC than normal individuals. It has been suggested that a low MCHC is beneficial in decreasing the vaso-occlusive properties of sickled cells. These sickle cell patients live longer and have a milder disease than do non-thalassemic patients. Thalassemia is discussed in greater detail below.

2) **Hereditary persistence of fetal hemoglobin** (HPFH) has established itself in the black population and allows Hb F to so dilute the Hb S that sickling does not occur or is less prominent. In these people the Hb F gene does not "turn off" in infancy but persists indefinitely.

---

[35]Sickle cells were first observed microscopically around 1910 by James Herrick who found them while examining the blood of an anemic African American medical student. Herrick is also credited with the first description of the clinical syndrome of coronary thrombosis.

[36]For instance, the spleen, a sludgy environment at best even for normal red cells, may infarct in sickle cell trait individuals at aeronautical altitudes. Therefore, these persons are advised not to travel in unpressurized aircraft cabins.

3) **G-6-PD deficiency** has been suggested as an ameliorative condition for sickle cell disease. This is controversial; the pathophysiologic basis of any such effect must be pretty obscure.

2. Clinical findings

Sickle cell anemia is a particularly bad disease in that not only is it a hemolytic anemia, but also a vaso-occlusive condition. The clinical findings can then be divided into one of these two groups:

a. Effects of chronic hemolysis

1. **Anemia**. Pretty much self-explanatory

2. **Jaundice**, due to rapid heme turnover and subsequent generation of bilirubin

3. **Cholelithiasis**. It has been classically taught that sickle cell patients are prone to the formation of calcium bilirubinate gallstones due to excess bilirubin secretion into the hepatobiliary tree.

4. **Aplastic crisis**. Many of us have brief episodes of marrow aplasia as a result of common viral infections. With a normal erythrocyte life span of 120 days, no anemia results from an unnoticed marrow shut-down of a few days. However, the sickle cell patients, with their markedly abbreviated rbc life span, can have a precipitous fall in hematocrit (and retic count) under such conditions. This may be life-threatening.

5. **Hemolytic crisis**. Most sickle cell patients establish a stable, tonic level of hemolysis. Rarely, for obscure reasons, they experience a catastrophic fall in hematocrit, increasing intensity of jaundice, and increasing reticulocyte count. This is called a "hemolytic crisis."

b. Effects of vaso-occlusion

1. **Dactylitis**. Resulting presumably from infarction or ischemia of the bones of the hands and feet, this is often the presenting manifestation of sickle cell disease in a six-months-old infant. The hands and feet are swollen and painful.

2. **Autosplenectomy**. In childhood, the spleen is enlarged due to excess activity in destruction of the sickled erythrocytes. Gradually, the spleen infarcts itself down to a fibrous nubbin.[37]

------

[37]Actually I think it just goes away. I have never been able to find the nubbin on my autopsies of these patients. I think medical writers confabulate the nubbin so as not to appear to violate the Law of Conservation of Mass.

3. **Priapism**.[38] This refers to a painful and sustained penile erection, apparently due to sludging of sickled cells in the corpora cavernosa. Sometimes the penis has to be surgically decompressed. Repeated episodes of priapism cause the spongy erectile tissues to be replaced by fibrous tissue, with impotence being the end result.

4. **Renal papillary necrosis**. The physiologic function of the loops of Henle make the renal medulla an eldritch, unbodylike area of high hematocrit, high osmolarity, low pH, hemodynamic stasis, and low P_{O_2}. All of these conditions predispose to sickling and infarctive loss of the papillae of the pyramids. The result is inability to concentrate and dilute urine. Even sickle cell *trait* individuals may experience episodes of hematuria, presumably due to this mechanism.

5. **Infarctive (painful) crisis**. Increased sickling activity may be brought about by any general stress on the body, especially infection. Almost any organ may suffer acute infarction (includinmg the heart), and pain is the chief symptom.

6. **Sequestration crisis**. This occurs mostly in infants and young children and is characterized by sudden pooling of sickled erythrocytes in the RES and vascular compartment. This produces a sudden fall in hematocrit. Sequestration crisis may be the most common cause of death in sickle cell patients in the youngest age group.

7. **Leg ulcers**. After all of the disasters mentioned above, this seems trivial. However, the deep, nonhealing ulcers of skin and tela subcutanea (classically around the medial malleolus) may be the only clinical manifestation of sickle cell disease in an otherwise well-compensated patient. These may be the only bugaboo standing between the patient and a productive, financially solvent life.

B. Hemoglobin C

The gene for Hb C is also prevalent in the African-American population but with less frequency (2-3%) than that of the sickle cell gene. Hb C does *not* form tactoids, but intracellular blunt ended **crystalloids**. The result is decreased rbc survival time; however, hemolysis is not as severe as in sickle cell disease, and the vaso-occlusive phenomena, so devastating in sickle cell disease, are not generally noted. Like sickle cell trait, the Hb C trait is asymptomatic. Homozygotes (and some heterozygotes) for Hb C often have many target cells (codocytes) in the peripheral smear, but the crystals, although pathognomonic,

---

[38]From Priapus, archaic Greek god of the phallus (predecessor of Eros), the offspring of Dionysus and Aphrodite. Worship of Priapus persisted until the Middle Ages, and his images were even regarded by some as saints.

are only occasionally seen. The prognosis of homozygous Hb C disease is excellent.

An individual may inherit a Hb S gene from one parent and a Hb C gene from the other. The result of this double whammy is **Hb SC disease**. The clinical severity of this condition is intermediate between that of sickle cell disease and Hb C disease, except that *visual damage due to retinal vascular lesions is characteristically worse in SC disease than in sickle cell anemia*. The intracellular bodies that occur upon hemoglobin destabilization in SC disease are curious hybrids of the blunt-ended crystalloids of Hb C and the sharp-pointed tactoids of Hb S — they often have one pointed end and one blunt end, thus vaguely resembling arrowheads.
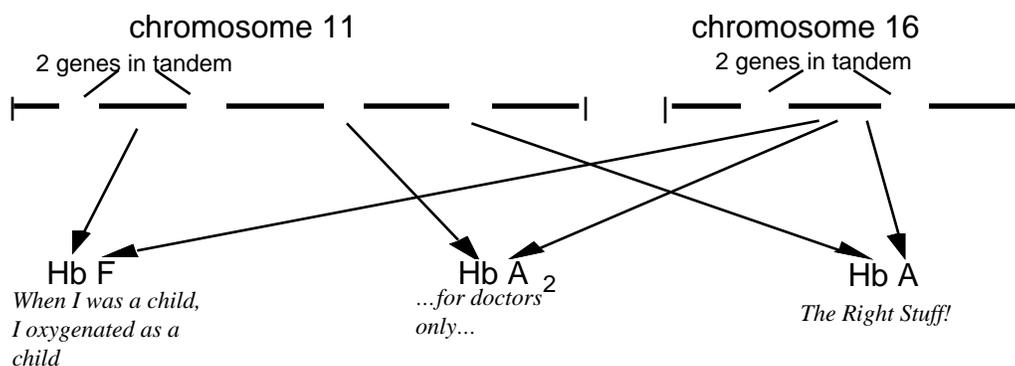
C. Hemoglobin E

This is a very common   chain mutation among Southeast Asians. The Thai and Khmer groups have the highest frequency, followed by Burmese and Malays, then Vietnamese and Bengalis. The gene does *not* occur in ethnic Han Chinese[39] or Japanese. The heterozygous state is asymptomatic but causes **microcytosis without anemia**, thus resembling some cases of   thalassemia minor (see below). The homozygous state has more severe microcytosis and hypochromia, but little, if any, anemia (this is also reminiscent of thalassemia minor). Hemoglobin E should always be considered working up an unexplained microcytosis in a member of one of the affected ethnic groups.

V. Thalassemia

A. Genetics

Understanding the thalassemias can be facilitated by reviewing the genesis of the normal post-embryonal hemoglobins:

chromosome 11 — 2 genes in tandem

chromosome 16 — 2 genes in tandem

Hb F
When I was a child,
I oxygenated as a
child

Hb A2
...for doctors
only...

Hb A
The Right Stuff!

Chromosome 16 contains the genes for the all-important   chain. The genes for all of the other important globin chains are on chromosome 11, where they are

---

39Han Chinese represent the overwhelming majority (>95%) of the population of the People's Republic of China and Taiwan.

closely linked. The linkage means (if you will briefly abuse yourself by recalling basic genetics) that the genes tend to be inherited as a group, as opposed to non-linked (or distantly linked) genes which assort independently due to crossing over during gametogenesis. Because of the linkage, a mutation that affects the rate of production of the β chain not uncommonly affects rate of production of the adjacent δ chain. An individual carrying such a mutation would then have a gene for "$\delta\beta$ thalassemia." He or she could pass on the thalassemia gene to offspring but would essentially never, say, pass a β thalassemia gene to one child and a δ thalassemia gene to another. Conversely, since the genome for the α chain is on a completely different chromosome than the genes for all the other chains, one would expect no mutation in a chromosome 11 chain gene (β, γ, δ) to affect α chain production. Moreover, if some poor schlimazel happened to inherit an α thalassemia gene from one parent and a β thalassemia gene from another, he would not tend to pass both abnormal genes on as a unit to his or her offspring. One kid (out of a representative Mendelian sibship of four) would get the abnormal α gene, one would get the abnormal β, one would get neither, and one would get both.

B. Biochemistry and pathophysiology

But enough of Mendel! We're in med school to learn about hemoglobin, right? Whatever the genetics, the clinical problem in the thalassemias is the inability to maintain a balance between the synthesis rate of one type of globin chain vis-à-vis that of its mate. Even though thalassemias have been described for all four of the above chains, we will consider only those that involve the β chain (the β thalassemias and $\delta\beta$ thalassemias) and the α chain (α thalassemias). It will be useful to review what kind of hemoglobins you can build by mixing and matching globin chains:

Hemoglobin	Globin chain composition	Notes
A	$\alpha_2\beta_2$	The only physiologically important adult hemoglobin in normal individuals. Includes the post-translational glycosylated hemoglobins A_{1a}, A_{1b}, and A_{1c}, the last being important in monitoring diabetics.
F	$\alpha_2\gamma_2$	The major physiologic hemoglobin in post embryonal fetuses. Adapted best for lowered intrauterine O_2 tension because of its left-shifted Hb-O_2 dissociation curve (allowing O_2 to be more readily picked up from maternal circulation). Production normally turns off in early infancy. Proportion of circulating Hb F fades to insignificance at about 6 months of age.

A$_2$	$_2$ $_2$	Medical philosopher's Platonist proof of the existence of God (and God's love of physicians). Apparently put here solely as a marker for doctors trying to figure out whether a patient has iron deficiency anemia or thalassemia. Normally less than 3% of circulating hemoglobin (thus physiologically insignificant), Hb A$_2$ is slightly elevated in most thalassemias, but normal or decreased in iron deficiency, thus making it a nifty marker for evaluating microcytic, hypochromic anemias.
Gower 1	$_2$ $_2$	Very early normal embryonal hemoglobins that disappear after 8 weeks of gestation. The only one of clinical importance is Hb Portland, which may be seen at birth in cases of the severest form of thalassemia (see below).
Gower 2	$_2$ $_2$	
Portland	$_2$ $_2$	
H	$_4$	Abnormal hemoglobin produced in cases of thalassemia, when excess chains decide to get it on with each other, there not being enough chains to go around. Intrinsically unstable, Hb H produces Heinz bodies in the erythrocytes and subsequent hemolysis.
Bart's	$_4$	Delinquent youth gang analogue of Hb H. This abnormal hemoglobin is found in *infants* with thalassemia. Detecting presence of Hb Bart's in cord blood may be the only practical way to screen for the very large number of individuals who are silent carriers of one type of thalassemia (see below).

C. Beta thalassemia

Although this is the classic form of thalassemia it is not the most common. The first description was written by Dr. Thomas Cooley in 1925. The term "Cooley's anemia" has been used synonymously with clinically severe forms of thalassemia, although the remainder of Cooley's career was so undistinguished as to cause some to suggest that his name is not worthy of eponymous immortality. Cooley's anemia was a fatal microcytic anemia of children of Mediterranean descent. The name "thalassemia" was coined to reflect the original geographic home of the target population ("   " is the classical Greek name for the Mediterranean Sea). Over the years, it became clear that many other groups (Africans, African-Americans, Arabs, Indians, and Southeast Asians) are affected. In fact, thalassemias in general tend to affect races of people that hail from a tropical belt that girdles the Mediterranean and extends all the way through the Indian subcontinent to Southeast Asia.

There are a multiplicity of different   thalassemia genes that give rise to a clinically heterogeneous spectrum ranging from asymptomatic expression to classical, deadly Cooley's anemia.   It is convenient to group the various thalassemias into two groups, based on the amount of   globin chain production:

β^0 thalassemia

This abnormal gene allows no production of   chains. Individuals homozygous for this gene produce only Hb A$_2$, Hb F (and very little of that after six months of age), and unstable α_4 **tetramers** that trash the red cells

while they are still in the marrow. As you might imagine, these people are in pretty dire straits unless some guardian angel has given them another, independent gene for hereditary persistence of fetal hemoglobin (HPFH). This prevents the Hb F spigot from turning down to a trickle at six months. Such persons can live to ripe old age and still be young at heart.
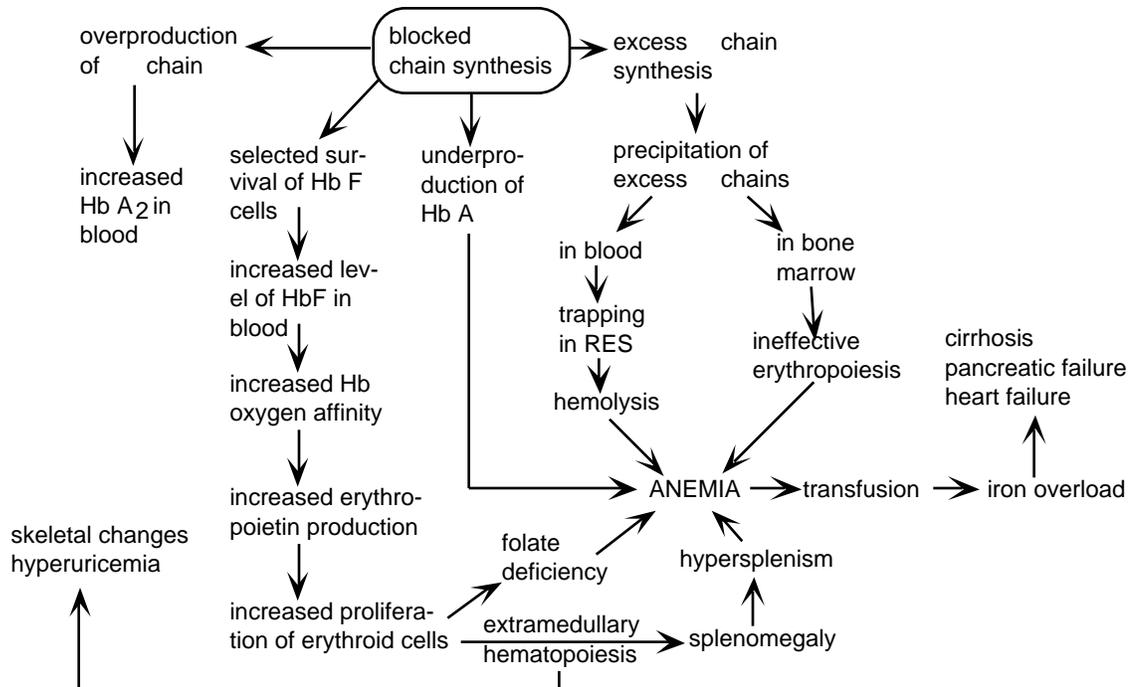
β^+ **thalassemia**

This abnormal gene allows some, but still subnormal, production of   chains. People homozygous for this gene will make a subnormal amount of Hb A but will still have trouble with the destructive effects of $_4$ tetramers on the erythrocytes and erythrocyte precursors in the marrow. The $^+$ genes can be further subdivided into the classic β^+ **(severe)** form, seen in Mediterranean Caucasians, and the mild β^+ **(Negro)** form seen in blacks. Nowadays this gene has its highest population concentration in Liberia.

Although these genes are remarkably varied in their effect on   chain synthesis rate, one can make up some useful rules of thumb:

1. Individuals heterozygous for any of the   thalassemia genes are either silent carriers or have minimal clinical effects, usually manifested as a **borderline anemia** (Hct   35 cL/L) with **disproportionate microcytosis** (MCV   60 fL) and a reciprocally **high rbc count** ($6 \times 10^6/\mu$L). The Hb A$_2$ is increased. This clinical presentation is called **thalassemia minor**. It makes for interesting wine-tasting party conversation if you have this condition, and all that your friends can muster is chronic fatigue syndrome. Your kids should have no problems if you just marry a Teuton, Slav, Balt, or Lapp.

2. Individuals homozygous for all of the   thalassemia genes [except the $^+$ (Negro) gene] have severe anemia and some or all of the pathophysiological consequences given in the diagram below. This is classic Cooley's anemia and is termed **thalassemia major**. This is bad news.

3. Individuals homozygous for the $^+$ (Negro) gene and several other miscellaneous types of mildly behaving genes have a relatively mild clinical anemia called **thalassemia intermedia**. These patients may require transfusion, but only later in life than is the case in the very sick children with thalassemia major.

The pathophysiology of   thalassemia major is best understood by going and getting yourself a beer (or politically correct beverage), watching a little TV, doing one or two other chores to postpone the inevitable, and then sitting down to study the next diagram.

overproduction of ⎯ chain ← blocked ⎯ chain synthesis → excess ⎯ chain synthesis

increased Hb A$_2$ in blood

selected survival of Hb F cells

underproduction of Hb A

precipitation of excess ⎯ chains

in blood → trapping in RES → hemolysis

in bone marrow → ineffective erythropoiesis

cirrhosis
pancreatic failure
heart failure

increased level of HbF in blood

increased Hb oxygen affinity

increased erythropoietin production

skeletal changes
hyperuricemia

increased proliferation of erythroid cells

folate deficiency

hypersplenism

ANEMIA → transfusion → iron overload

extramedullary hematopoiesis → splenomegaly

While studying the illustration, consider the following observations concerning thalassemia:

1. Since there is a decrease in the synthesis of ⎯ chains, there is a net decreased synthesis of Hb A. With less Hb A available to fill the red cells, the result is **microcytic anemia**. Whereas in iron deficiency microcytosis occurs because there is not enough *heme*, in thalassemia the same thing occurs because there is not enough *globin*.

2. Since the body cannot make enough ⎯ chains, it makes a feeble attempt to compensate by trying to churn out ⎯ chains. The result is **increased Hb A$_2$**, which can be measured easily and inexpensively by column chromatography. This is a pretty specific test for the diagnosis of ⎯ thalassemia. Pitfall: both ⎯ and ⎯ chains are decreased in $\delta\beta$ **thalassemia**, which is not rare and presents like ⎯ thalassemia, except that the Hb A$_2$ is *not* elevated. You would expect this since Hb A$_2$ contains ⎯ chains).

3. In some cases of ⎯ thalassemia, there is attempt at compensation by maintaining some production of **Hb F**. This has some pathophysiologic consequences (as shown above) and also provides a laboratory marker to assist in diagnosis. Retention of Hb F production is not as common as increased rbc Hb A$_2$ content.

4. In severe forms of thalassemia, the anemia due to failure to make adequate amounts of Hb A is compounded by the hemolysis, ineffective erythropoiesis, and extramedullary hematopoiesis brought on by precipitation of ⎯$_4$ tetramers (which are unstable). In classic Cooley's anemia, the ineffective erythropoiesis dominates the clinical picture by producing tremen-

dous expansion of the marrow space, manifested by the so-called "tower skull" with an x-ray showing innumerable vertical bony striae between the inner and outer tables of the calvarium. This radiographic feature is fancifully called the "hair-on-end appearance" by radiologists, and the "guy-who--accidentally-sat-on-a-Van-de-Graaff-generator appearance" by those wacky electrical engineers. Extramedullary hematopoiesis and hemolysis causes **splenomegaly**, which produces **hypersplenism**, and more hemolysis.

5. The high turnover state caused by the tremendous erythroproliferative activity causes wastage of folate and may produce a complicating **megaloblastic anemia**. Another effect of the high turnover state is **hyperuricemia** (due to catabolism of the purine content of cellular DNA).

6. Classically in thalassemia major, the treatment is the cause of death. The children are maintained by transfusions until about age ten years, at which time they start to show symptoms of excess iron loading. This happens because the transfusion bypasses the body's normal gastrointestinal mechanism of iron intake and excretion. The iron is poured into the bloodstream directly; the body cannot excrete it fast enough. First iron (as hemosiderin) fills the cytoplasm of the RES phagocytes and then starts to be deposited in the parenchymal cells of just about every organ of the body. The pancreas, liver, myocardium, adrenals, and gonads are among the organs most sensitive to iron toxicity. The clincial result is **diabetes mellitus**, **hepatic cirrhosis**, **congestive heart failure**, **adrenal insufficiency**, and **failure to undergo puberty**. Death usually occurs in the second or third decade of life, the most common immediate cause being complications of heart failure.

D. Alpha thalassemia

The   thalassemias include the most common of all hemoglobinopathies and thalassemias. One form of   thalassemia is very common in African-Americans. Fortunately this form is so mild that its very detection is almost impossible in adult heterozygotes, and even homozygotes are asymptomatic with mild laboratory abnormalities. Yet another form of   thalassemia, fortunately uncommon, produces the most severe disease of all the hemoglobinopathies and thalassemias and usually takes the life of its victim even before birth. Surely   thalassemia is a disease of extremes!

It is helpful to consider two concepts concerning   thalassemia:

1. Unlike   thalassemia,   thalassemia is present even before birth, since the   chain is integral to all hemoglobins past the very primordial Hb Gower 1 and Hb Portland. Thus, individuals, born and unborn, who carry one of these genes underproduce Hb Gower 2, Hb F, Hb A, and Hb A_2. Obviously

then, elevated Hb A$_2$ *cannot* be used as a diagnostic marker for thalassemia.

2. As noted in an illustration above, each individual has four genes for the α globin chain, which can be denoted as αα / αα. Each haplotype (αα) is inherited from a parent as an indestructible, non-crossoverable pair. In thalassemias, anywhere from one to all four of these genes of the diplotype (αα / αα) can be deleted (or transformed into some "bad guy" gene that doesn't do anything), producing a dose-related depression of α chain synthesis. Thus, the (α– / αα) diplotype produces the mildest condition, while (--/--) produces the fatal intrauterine disease. The (α–) gene is called the "α thalassemia-2" gene while the (--) gene is termed the "α thalassemia-1" gene. One can be homozygous or heterozygous for each of these, to give four possible diplotypes (αα / α– , α– / α– , α–/α– , --/--). An additional diplotype is the double heterozygote for α thalassemia-1 and α thalassemia-2 (α– / --). Since the α thalassemia-1 and α thalassemia-2 genes tend to occur in different races (Asians and Africans, respectively, between which there is a low rate of intermarriage), this combination is not common. Let us look at the various nefarious gene combinations individually:


normal genotype   alpha-thalassemia-2 trait (silent carrier)
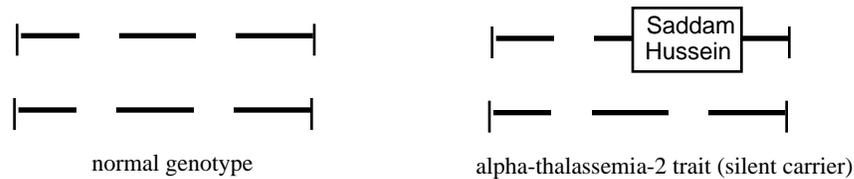
In the above diagram, the normal diplotype is compared with the heterozygous state for α thalassemia-2. The latter occurs in *20% of all African-Americans*. It produces no symptoms and no abnormalities on routine laboratory tests. The only practical way to detect it is to screen all black infants at birth for Hb Bart's (γ$_4$), and even this technique will miss some individuals. It is thought that the reason for the high prevalence of this gene in the black population is that it may be an "anti-sickle cell" gene. It turns out that Hb S homozygotes have a milder course of sickle cell anemia if they also are silent carriers for α thalassemia.

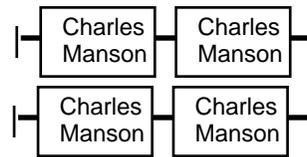alpha-thalassemia-2 homozygote   alpha-thalassemia-1 heterozygote
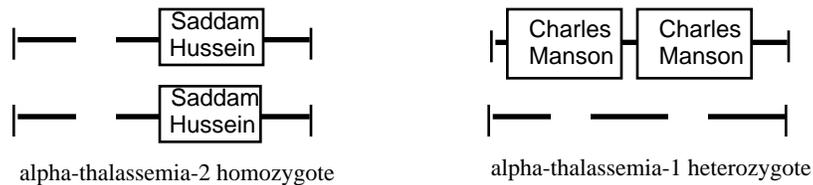
The the two diplotypes given above, two of the four α genes are trashed by "bad guy" mutations. Such individuals are usually not anemic but may have microcytosis. They become victims of the medical system when they are subjected to expensive and time consuming testing in a quixotic search for some

serious hematologic condition that does not exist. About the only way to make a diagnosis of one of these conditions is to eliminate other causes of microcytosis and/or check rbc indices on all available blood (no pun intended) relatives. Amidst all this shenanigans lurks a somber note — if an thalassemia-1 heterozygote finds his or her true love in another   thalassemia-1 heterozygote, one-fourth of the issue of such a union will suffer the always lethal homozygous state.
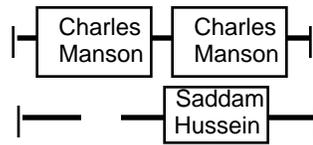
doubly heterozygous state for
alpha-thal-1 and alpha-thal-2:
hemoglobin H disease

alpha-thalassemia-1 homozygote:
hydrops fetalis, incompatible with
extended postnatal survival

The double heterozygote on the left has "**hemoglobin H disease**," so named because of the presence of a significant proportion of the hemoglobin composed of four   chains. Affected infants will, of course, show some Hb Bart's as well. These people have a hemolytic anemia which varies from very mild to that which clinically resembles   thalassemia major.

The   thalassemia-1 homozygote, on the right, is allowed *no* production of chains. The only hemoglobins present are Hb Bart's, Hb H, and Hb Portland. Most of the affected die *in utero* or within hours after birth. Autopsy shows massive extramedullary hematopoiesis in virtually every parenchymatous organ of the body. The severe anemia causes congestive heart failure and subsequent massive total body edema, termed "**hydrops fœtalis**." Parenthetically, it should be noted that hydrops (from which springs the term "dropsy") is not limited to thalassemia but is seen in any conditon that causes severe heart failure *in utero,* such as in the anemia due to alloimmune hemolytic disease of the newborn.