Normal Functions of the Spleen Relative to Red Blood Cells: A Review

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The words hyposplenism and hypersplenism have enjoyed a long vogue. Hyposplenism, which may have been used first by Eppinger in 1913, has come to mean a pattern of changes that are due to or related to absence or atrophy of the spleen. Hypersplenism, coined by Chauffard in 1907, is more commonly used, but it describes a condition that is considerably less well defined. Hypersplenism is usually intended to mean a pathologic exuberance of splenic function which is manifested by a lack of circulating blood cells. Hypersplenism is usually associated with splenomegaly, but it is not essential that it be. The mark sterling of the hypersplenic syndrome is cure by removal of the spleen. Hyposplenism and hypersplenism describe the spleen's errors of omission and errors of commission. Now a word is needed to describe the honest, daily toil of a healthy spleen in a healthy body. The word "splenism" was introduced into the hematologists' vocabulary by Dameshek who said that it means "normal functional activity of the spleen," but he has added, with characteristic caution, that it is not definitely known whether splenism exists. However, for purposes of discussion let us assume that the spleen has normal functions, that certain of these functions pertain to the erythrocytes, and that some of the normal functions of the spleen may involve abnormal situations. For example, when the spleen is torn it may heal itself. The injury is abnormal, but healing is a normal function. When pathogenic bacteria or abnormal red cells appear in the splenic pulp, the spleen may destroy them—a normal splenic function. This short discussion will restrict itself to those normal splenic functions which relate to the erythrocyte: splenism and the red cell.

Of the methods used to study splenic function, splenectomy probably heads the list. Ligation of splenic blood vessels also has been used and spleens have been transplanted with and without maintaining the integrity of the blood supply. Parabiosis has been used, joining the circulations of a splenectomized animal with one whose spleen is intact. Other technics have been tried, too numerous to mention. In this field of splenic function, as in every other, there are pitfalls set and waiting. For years the results of splenectomy in dogs were distorted by the presence of unsuspected bartonella infections which caused hemolytic disease when the protecting spleen was gone. Experiments with parabiotic rats have been interpreted upon the assumption that blood cells are not exchanged by parabionts but actually two-thirds...
per cent of their red cells are exchanged each minute. Experiments designed to exclude the splenic venous blood from the portal circulation by ligation of the splenic vein are quickly thwarted by collaterals. All of these are physiologic technics. Anatomic methods have also played an important part in developing our knowledge of splenism. This is well demonstrated in the first normal function of the spleen to be considered.

Erythropoiesis is a function of the spleen during intrauterine life. The embryonal spleen derives from primitive mesenchyme. During the fifth fetal month certain fixed mesenchymal cells in the spleen give rise to so-called hemocytoblasts which in turn produce erythroblasts. At this time the production of red cells is a normal function of the spleen. After the fifth month this activity diminishes and it is practically absent in the sixth month. But the mesenchymal tissue of the spleen has a long memory. When the spleen is removed and a portion of it is transplanted into the donor animal, the fragment becomes a functioning splenunculus. However, the tissue of the new spleen is not the same tissue that was transplanted. That piece of tissue became necrotic except for a few mesenchymal cells at the surface, and these cells proliferated to become the new spleen, a spleen with follicles, trabeculae, red pulp, sinusoïds, a spleen that is indistinguishable from the parent organ.

As the new spleen grew it repeated the ontogenic development of the embryonic spleen. At one point in the development erythroblasts appeared and for a brief moment the spleen was again an erythropoietic organ.

When the erythroblasts of the bone marrow have been obliterated by x-rays, an injection of normal splenic tissue apparently is able to provide the seeds for a new erythropoietic system. The animals so treated live; the controls die. Present evidence favors the interpretation that some sort of stem cell is transferred to the marrow and reproduces and differentiates. From studies of regenerating spleens it is evident that cells are present which have this capability.

The normal human spleen evidently retains throughout life a potential capacity to form blood cells. Myeloid metaplasia may develop in certain types of anemia, but it cannot be said that this is a normal function of the spleen. These diseases involve such serious disorders of the blood-forming organs that one must suspect that the spleen also is diseased.

Splenic control of erythropoiesis in the bone marrow is a topic on which good friends fall out. The reason for this is, of course, a lack of valid information which leaves us our hypotheses to argue over and ambivalent data to support them. The bone marrow is not the organ of erythropoiesis; the bone marrow is its housing. The organ of red cell production is the erythroblast. The process of red cell production is largely one of putting proteins together in an orderly fashion to make a cell for delivery that can pass inspection and survive in the bloodstream for 120 days. The manufacture of proteins by erythroblasts or any other cell is an enzymatic process whose rate can be altered by withholding substrate materials or by providing or failing to provide enzyme inhibitors.

There are three ways in which the spleen might conceivably exert control
over erythropoiesis. (1) The spleen might hasten or impede the enzymatic synthesis of hemoglobin or stromal proteins. While this is a possibility, there is no evidence to suggest that spleen can do this. (2) The spleen might increase or decrease the number of erythropoietic units in the bone marrow. Hemolytic disease induced by the spleen can cause an erythroid hyperplasia of the marrow, but this is not control of erythropoiesis. Ferrata,\textsuperscript{13} who believed that the spleen exerts a normal inhibitory effect upon bone marrow activity, proposed that progressive atrophy of the bone marrow might in some cases be due to hypersplenism, but this is not a normal function. (3) The spleen may determine the age at which the young erythrocytes are released from the bone marrow. When the young red cell has lost its nucleus it pauses as a reticulocyte to wait its turn to go into the circulation. The reticulocyte stage of a red cell's development may last two days. Perhaps half of this time is spent waiting in the marrow. When this is so, the peripheral reticulocyte count reflects only the last day of the reticulocytes' existence. If the cells were to leave the marrow one day earlier they would live two days in circulation and the peripheral reticulocyte count would be doubled, yet the output of the marrow would not have increased.\textsuperscript{14} This phenomenon is sometimes seen in patients when splenectomy fails to cure a hemolytic anemia. Red cell survival is unchanged, pigment production is unchanged, the amount of circulating red cells is unchanged, but the reticulocyte count is doubled.\textsuperscript{15} Removal of the spleen seems to permit earlier delivery of the young erythrocytes. This may or may not indicate a splenic control of red cell delivery.

It has been reported and confirmed that anemia in rabbits occurs when the splenic vein is ligated so that the spleen is drained by collaterals emptying into the vena cava rather than through the portal system.\textsuperscript{5,16} This anemia is due partly to increased hemolysis and partly to inhibition of hematopoiesis.\textsuperscript{9} It has been explained on the basis of a hypothetical inhibitory hormone originating in the spleen. When the splenic blood flows through the portal system and liver the hypothetical hormone is detoxified. If there is such a hormone it is not necessarily a normal one. It may be an abnormal substance provoked by the chronic passive congestion which is incident to ligation of the splenic vein, because the anemia disappears when the collaterals that drain the spleen enlarge, and anemia never develops in dogs when the splenic vein, rather than ligated, is transplanted to drain directly into the vena cava.\textsuperscript{17}

It has been stated that the red cell count may be elevated above normal after splenectomy, and the phenomenon has been interpreted as manifesting a release from splenic inhibition of the bone marrow.\textsuperscript{4} "Postsplenectomy polycythemia" is often seen in patients with hereditary spherocytosis, but it does not occur when the spleen is removed from normal humans\textsuperscript{18} or from monkeys.\textsuperscript{19} But in any event, the phenomenon, when it occurs, should not be interpreted as reflecting a splenic effect upon the bone marrow, but rather as an effect on the mechanism that controls blood volume or the red cell volume.

A splenic effect upon maturation of the red cell's surface can be demon-
The surface of a red cell is composed predominantly of lipids, and most of the lipid in the cell is at the surface. Its molecular arrangement contributes to the enormously great zeta potential which surrounds the red cells and acts as an electrostatic shield to protect them in their many collisions. Because of their electrostatic charge mature red cells are not sticky. As reticulocytes become mature red cells they undergo many changes in addition to losing the reticulum. They shrink in diameter and also in volume. Most of the loss of volume is due to loss of water, so the hemoglobin in the cell becomes more concentrated. The surface of the cell also changes as it matures: (1) the total surface area decreases from about 250 \( \mu^2 \) to 135 \( \mu^2 \). (2) The amount of lipid diminishes in direct proportion to the loss of surface area. (3) The relative concentration of essential fatty acids and cholesterol increases. (4) Reflecting an increase of zeta potential the isoelectric point of the cell changes from about 3.5 to less than 2. The somewhat sticky reticulocyte loses stickiness as it becomes mature.

After splenectomy the surface area of the red cells is larger than before, but the cellular volume is normal which means that the cells are thinner. They appear on stained smears as thin target cells. The change is not an abrupt one. Loss of the spleen does not increase the surface area of the cells already present, but by the process of normal attrition and replacement a population of thin cells appears \(^{21} \) (See fig. 1). The reticulocytes of this new population mature and lose volume and water as they did when the spleen was present, but they do not lose so much surface area and surface lipids. The proportion of lipid to surface area is the same after splenectomy as before and so is the ratio of essential to nonessential fatty acids. \(^{22} \) One normal function of the spleen apparently controls to some extent the normal maturation of the red cell surface.

Because reticulocytes are slightly sticky and surface stickiness determines
to some degree the time when young cells are released from the marrow, the
effect of the spleen on the red cell surface may influence the age at which
reticulocytes may enter the circulation.

The reservoir function of the spleen refers to the sequestration of red cells
in the pulp and splenic sinuses. The spleens of some animals are enormously
expansile and it is possible for them to remove a large proportion of the red
cells from active circulation, holding them perhaps against the time when
the animal must fight or run. This is not an important function of the human
spleen. In an average-size adult the spleen holds 30 or 40 ml. of red cells.
In its normal way the human spleen does not expand or contract to any great
extent. In its capsule there are no muscle fibers.

Nevertheless, the concentration of erythrocytes in the spleen is high. There
are parts of the splenic circulation through which the red cells move slowly,
and of the 120 days of a human red cell’s life, about two days are spent in
the spleen. During periods of splenic sequestration, the red cells may undergo
certain changes. It is known, for example, that the erythrocytes in the blood
from the splenic vein are more fragile when exposed to hypotonic saline than
are the red cells from the splenic artery. A mild degree of spherocytosis has
developed. The red cells loitering in the spleen have taken up a little water
which increases their fragility. The same thing happens to red cells when
they are incubated in a test tube. By direct microscopy of living tissues, the
Clarks found that extravasated red cells are at first immune to attack by
wandering phagocytes. When a phagocyte encounters freshly extravasated
red cells it shoulders them aside. But after about 18 hours, if it encounters
them again, the phagocyte stops and eats them. The sequestered cells have
somehow changed, and since phagocytosis depends to a great extent upon
the surface qualities of the phagocyte and its victim, we may suspect that
the surface of the sequestered red cell has undergone some change to make
it appetizing to the passing scavenger. This may be one of the risks of stopping
too long in the spleen.

The “culling function” of the spleen describes the ability of this organ to
scrutinize the passing red cells and to remove from circulation those which
do not meet certain minimum requirements. The erythropoietic bone marrow
is an efficient organ and the red cells it produces are marvellously similar in
size and shape. But the marrow is not perfect. Some of the cells it makes are
deformed or are otherwise abnormal. Perhaps 10 per cent of red cells pro-
duced by the marrow are incapable of surviving in the circulation. One reason
for their failure to survive is the presence of a normal spleen.

The behavior of the spleen in hereditary spherocytosis is a good example
of its ability to destroy misshapen red cells. When blood from such a patient
is transfused into a normal recipient the red cells rapidly disappear; their
average survival may be less than 15 days. When blood from the same donor
is transfused into a recipient who has no spleen the hereditary spherocytes
may survive almost normally. In hereditary spherocytosis the spleen destroys
the red cells prematurely, but this is not an example of a spleen behaving
abnormally. It is a normal spleen behaving in a normal fashion toward a population of abnormal erythrocytes.

The spleen probably accomplishes its culling function by means of phagocytes. Wandering phagocytes are present in the red pulp and fixed phagocytes form the walls of splenic sinusoids. These latter cells are hypertrophic in hereditary spherocytosis. If this hypertrophy is due to hyperfunction it may indicate that these are the cells that destroy the spherocytes. In the splenic circulation erythrocytes are shunted into the red pulp and move sluggishly in the direction of the barrel-shaped sinusoids. Cells of normal thickness slide easily between the barrel staves and enter the lumen of the sinus and return to the blood stream. The thickened spherocytes are restrained. The “barrel staves” that bar the way are cytoplasm of the phagocytes which line the sinus wall. In order to return to the circulation the spherocytes must squeeze between two phagocytes. If the spherocyte has loitered in the red pulp, its surface may be changed and attractive to the phagocytes.

The role of the spleen in iron metabolism is related to its destruction of red cells. Hemoglobin from the red cells is degraded and the iron is returned to the erythroblasts to be re-used. Other organs than the spleen are sometimes involved in hemoglobin breakdown. Not all of them are able to dispose of the hemoglobin iron. The epithelial cells of renal tubules are able to destroy hemoglobin, but they cannot dispose of the iron efficiently. They become filled with hemosiderin and while some of the iron is returned to the blood, much is spilled into the urine. Thus hemosiderinuria occurs when the kidney handles hemoglobin. When blood is extravasated into the lung the red cells are picked up by phagocytes. These pulmonary phagocytes seem completely incapable of returning the iron to the body. In the disease, pulmonary siderosis, a child may develop a severe iron-deficiency anemia while iron accumulates in the pulmonary parenchyma as a consequence of repeated extravasations.

The spleen by comparison readily disposes of its iron. In hereditary spherocytosis 150 mg. per day of hemoglobin iron may be turned over by the phagocytes that line the sinusoids. The wandering phagocytes of the pulp cords seem not to be so efficient. In other hemolytic diseases where erythrocytes are damaged and liable to destruction by any phagocyte, one finds macrophages in the splenic pulp crusted with hemosiderin which they seem unable to dispose of. In such conditions the spleen grows siderotic and becomes a storage depot for unused iron.

The “pitting function” of the spleen refers to its ability to remove a solid particle from the cytoplasm of a red cell without destroying the cell itself, much as a housewife plucks the stone from a cherry without crushing the fruit. Like the culling function, this is an example of the spleen behaving as an inspector of erythrocytes and taking action when cells are found that do not meet the standard. But in this instance the spleen does not destroy the offending red cell; it takes corrective action.

After splenectomy in certain types of hemolytic anemia a portion of the red cells in the circulating blood contain granules of hemosiderin. For many years it was supposed that these siderocytes were absent from the blood before splenectomy because the spleen destroyed them as fast as they were produced.
The results of splenectomy in hereditary nonspherocytic hemolytic anemia indicate that this is not the case. The spleen does not destroy siderocytes because of their iron granules. If it did, the sparing of the affected red cells should result in some improvement of the anemia after splenectomy. But there is no improvement. Forty per cent of the red cells are siderocytes and the anemia remains unchanged.

Experiments with transfusions of siderocytes have demonstrated the behavior of the spleen toward these red cells and their iron granules. Blood containing a high proportion of siderocytes was transfused into two recipients, one with a spleen and one without (fig. 2). The red cells were tagged with radioactive chromium so that it was possible to determine the rate at which they were destroyed after the transfusion. At intervals the transfused siderocytes in the recipient's blood were counted. The count fell rapidly in the subject with a spleen. In the splenectomized subject the siderocyte count remained elevated throughout the 24-hour period of observation. In neither case was there any great loss of transfused red cells, as determined by their radioactivity. These studies show definitely that the iron granules were removed by the spleen without destroying the red cells that contained them.

In the absence of a spleen many sorts of red-cell inclusions are apt to be more numerous: red-cell nuclei, Howell-Jolly bodies, Heinz bodies, malarial plasmodia and bartonella organisms. The reason for this difference is not known, but the pitting function of the spleen may not be specific for siderocytes.

The final normal function of the spleen relative to erythrocytes is to destroy...
DURATION OF DAILY BLEEDINGS (MONTHS)

Fig. 3.—Effect of hemorrhage on the weight of rabbit spleen (de Lagen, 1943). Splenic atrophy develops during prolonged anemia due to hemorrhage.

them when they reach the end of a normal life cycle. Many years ago Rous and Robertson34 proposed that worn-out red cells were destroyed by fragmentation, and they found that the fragments accumulated in the spleen. The formation of stromal lipids is the function of one of the enzyme systems in the red cell. Even after the cell loses its nucleus this continues,35 and it probably represents a reparative process that maintains the integrity of the cell's surface. Activity of the enzyme systems of the red cell gradually decays36 and presumably the enzymatic ability to repair the stroma also declines. The aging cell grows thinner37; apparently the stroma relaxes and the surface area increases. The cell becomes brittle, mechanically fragile38 and hence more susceptible to fragmentation. The fragments of the old red cells are finally destroyed in the spleen. Finch39 has shown with isotopic iron that the spleen is the graveyard of the red cells. Dogs were given Fe59 and the radioactive element was incorporated into red cells. Then the dogs were given an excess of nonradioactive iron, so that any iron released by degradation of hemoglobin would not be needed and therefore would be stored at the site of red cell destruction. When the radioactive red cells were finally destroyed, almost all of the radioactivity was found to be in the spleen.

When the spleen is deprived of this hemolytic function, it seems not to prosper. Consider the case of deLangen's rabbits.40 Each day for many months these animals were bled enough to keep them moderately anemic without producing iron deficiency or other malnutrition. On this regimen their spleens
became atrophic (fig. 3). The pathogenesis of the atrophy is not known, but it is possible to compute that, because of the bleeding, less than one per cent of the red cells would die of old age. In such an animal the hemolytic function of the spleen would be almost nonexistent.

**SUMMARY**

During all the stages of a red cell’s life the normal spleen exerts a normal function. Eight of these functions have been considered: (1) erythropoiesis; (2) an effect upon red cell production; (3) an effect upon maturation of the red cell surface; (4) the reservoir function; (5) the “culling function”; (6) iron turnover and storage; (7) the “pitting function”; (8) destruction of old red cells.

**SUMMARIO IN INTERLINGUA**

In omne le stadios del vita del erythrocyto, le splen que es normal exerce normalmente certe functiones. Octo tales es discutite: (1) Erythropoiesis; (2) effecto super le production de erythrocytos; (3) effecto super le maturacion del superficie de erythrocytos; (4) rolo de reservoir; (5) elimination de erythrocytos imperfecte; (6) metabolismo de ferro; (7) “excision” de particulas ab erythrocytos alteremente perfecte; e (8) destruction de erythrocytos ancian.

**REFERENCES**

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