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Pathogenesis of hereditary hemolytic jaundice

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THE PATHOGENESIS OF HEREDITARY HEMOLYTIC JAUNDICE

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INTRODUCTION

The statement of the problem which this thesis concerns may best be prefaced by a quotation from E. Muelengracht made in 1939 (1).

"Take chronic hereditary hemolytic icterus or similar conditions, in which hematology has achieved such triumphs. We have its description down to the finest details, and the most effective treatment; but thus far the innermost nature of the disease has remained hidden and has defied all attempts to probe it."

This thesis does not propose to settle the problem suggested by the above quotation, but a consideration of the attempts at solution of the problem will be made. If, after compilation and consideration of these efforts, conclusions can be drawn as to a logical course of events in the pathogenesis of hereditary hemolytic icterus which will be helpful to the writer this thesis will be justified.

Hereditary hemolytic icterus is an inherited familial disease; its clinical manifestations of recurrent or prolonged attacks of jaundice, anemia of varying grades, and splenomegaly all reflecting the underlying hyperhemolysis which is their basis.

Historically, the disease was observed as early as 1885, and was described in detail in 1895 by Hayem. Minkowski in 1900 pointed out the familial characteristics of the disease, and in 1901 Chauffard demonstrated the increased fragility of the red blood cells, the microcytosis, and the reticulocytosis which are present. (2) (3)

It is from these basic observations that the familial type of hemolytic icterus is known as the Chauffard-Minkowski type.

ETIOLOGY

The etiology of the disease is considered only incompletely at this point, since the basis etiologic factors are concerned with the discussion of pathogenesis as developed in the body of this thesis. There are hereditary and etiological factors which may be discussed at this point.

It is generally agreed that the familial type in inherited as a true dominant Mendelian characteristic. The main proponents of this have been Naegeli, Meulengracht, Gansslen, and Hattensen. Maegeli states the inheritance is as definite as in hemophilia, and in the average family about one-half of the off-

spring will show clinical evidence of the disease (2).

The transmission to succeeding generations is marked by an increase in virulence of symptoms, and the transmission of increased virulence is limited by the death of those affected with the severer types. (2) (4) Members of affected families may carry the abnormality in minor degree throughout life and never develope clinical symptoms, and yet be as capable of transmitting the disease as those clinically affected. This may explain apparently isolated cases, or so called "acquired" hemolytic jaundice in which no toxic etiological factor can be found.

Hemolytic jaundice has no sex or racial characteristics; it may be found at any age although clinical evidence usually appears in young adults. At this time the hemopoietic system of the young attains its less active adult proportions, and the anemia is more likely to appear. If this period is passed without the appearance of anemia there will likely be no symptoms unless strain is placed on the hemopoietic system from some other source. (2) (3)

The entire discussion of pathogenesis is deferred, and etiological considerations will be completed with its discussion.

PATHOLOGY

The pathology of hereditary hemolytic icterus may be described under the headings of: (1) bone marrow, (2) blood, (3) spleen, (4) liver, (5) jaundice, and (6) miscellaneous findings.

Bone Marrow

The bone marrow shows a picture of great overactivity, (4) and this in brief is the entire picture seem. Guizetti first described the marrow picture in 1912; he found evidence of great hyperactivity, the yellow marrow of long bones being largely replaced by erythroblastic marrow. He found excessive numbers of the erythroblastic series of cells to be present, with relative diminution of the myeloid series. (5) Since that time others have readily confirmed the excessive hyperactivity of the erythroblastic marrow, but it has also been confirmed that erythroblastic predominance is not usual, instead there is an increase in both erythroid and myeloid elements, their ratio remaining about normal. (5)

Blood

Examination of the blood reveals pathological variations are chiefly related to the red blood cells. Other changes will be noted.

The usual red cell count is between 3 and 4 million. It may be normal in some cases, and during crises it may fall to 1.5 - 2 million. Lower counts than this have been recorded during crises. (5)

The hemoglobin is found to be decreased in accordance to the red cell count. It is usually found to be from 60% to 80%. During crises, like the red cell count, it may be depressed to 20-'30%. (5)

The color index from these figures will be seen to remain about normal. It varies on the average from 0.8 to 1.2, and the usual index is very close to 1.0. (5)

Microcytosis is usually readily observable when smears of the red cells are examined. There is considerable anisocytosis, and the cells causing this appearance are smaller than normal, and stain evenly throughout their substance. These small, dense red cells were first observed by Chauffard in 1907, and are considered characteristic of the disease. Measured in their own plasma, the average red cell diameter of a patient is found to be about 7.2u to 7.3u, while that of a normal individual is about 7.8u. Average diameters as low as 5.7u have been recorded. Price Jones curves on dry smears of patients show the peak of the curve not only at a lesser diameter, but also a broadening of the curve from its normal rather sharp characteristic. That these cells not only are smaller, and appear more dense, but are actually thicker cells, can be told by the volume index of the cells which average about 1.0. (.80-1.2) From this characteristic the cells have been termed microspherocytes, or spherocytes. (5) Naegeli, Von Boros, and Gansslen have all reported the mean corpuscular volume in cases of chronic hemolytic icterus as being very close to that of a normal cell - from 85 cubic microns to a maximum of 111 cubic microns. This is further evidence of the sphericity of the small cells. (6) Haden states that the diameter of the cell is decreased out of proportion to the change in volume, as the cell

must tend to be spherical instead of biconcave in shape. (6)

Chauffard in 1907 pointed out the next important feature of these spherocytes. They have decreased osmotic resistance to hypotonic salt solutions. The red cells of a normal person show beginning hemolysis in a solution of .44% NaCl, complete hemolysis at .32% NaCl. Cells from a patient with chronic hemolytic icterus show hemolysis at higher concentrations of hypotonic saline indicating that the cells are more fragile than normal cells. Meulengracht (5) found in a series of 35 patients that beginning hemolysis occurred at concentrations ranging from .88% to .52%. Occasionally values may be nearly normal, but this probably is a transitory phenomenon.

Reticulocytosis in varying degrees is also a constant feature. These cells are usually found to be present in numbers from a few percent to 30 percent. Reported cases have run as high as 92 percent. With this increase in reticulocytosis is also found varying degrees of polychromasia which would be expected. (5) (3)

Nucleated red blood cells, normoblasts, are an inconstant feature. They may be absent, be found only occasionally, or be present in numbers as high as a reported case with 22.5 %. (5) Their presence might serve as an index of the strain being placed on the erythroblastic process by the hyperhemolysis.

The white blood cells show some increase in total count. This may show a count of from 10,000 to as high as 20-28,000.

The higher white counts are associated with crises, and the increase is polymorphonuclear. (5)

There have been no definite deviations from normal in regard to the platelets recorded.

Spleen

Splenic tumor has been mentioned in the physical findings. Meulengracht states that marked enlargement is a constant finding. (5) Elliott and Kanavel in a review of splenectomies performed up to 1915 for the disease (4), stated the average weight was 1000 grams--4 to 5 times enlarged over normal. The cases which are most severe show the greatest splenic enlargement, and an instance of one weighing 3500 grams is reported. (5)

The pathologic anatomy has been well investigated, for material has been plentiful. The first American investigators of splenic pathology were Tileston and Griffin in 1910. Their description was accurate, and their findings were a marked engorgement of the pulp with blood, the sinuses containing little blood, little connective tissue proliferation, and follicles in the main were normal. They found more or less pigment, usually in the endothelial cells. (3)

Meulengracht gives the best description of splenic pathology available today. (5) The cut surface of the spleen gives a picture of enormous congestion, borne out in microscopic examination. The predominant feature in the picture of the pulp is an enormous engorgement with blood most conspicuous in the

spaces between the reticulum (the pulp Proper), while the sinuses are relatively empty of blood. In contrast to the splenic pulp the sinuses are empty of blood, appear collapsed, irregular, with slit like lumina. The endothelial lining of these sinuses, instead of being composed of long filamentous cells with but scattered nuclei appearing, is lined with closely packed, large, round nuclei protruding into the lumen. These cells must be shorter than normal, and appear quite different.

The lymph follicles are nearly always scanty in number, and are relatively small. There is no increase in the amount of connective tissue in the pulp, in contrast to splenic anemia in which the increase in size is due to proliferated connective tissue.

Elliott and Kanavel (4) as well as Muelengracht (5) emphasize that erythrophagic cells are not found in the spleen. This is in agreement with other workers. The fact that erythrophagic cells are found exceptionally or not at all is a very important point and should be remembered. It precludes phogocytic destruction of red cells, and the process must be extra cellular.

The presence of hemoglobinogenous pigment in the spleen is not entirely settled, but it certainly is not present in any considerable amount. Tileston and Griffin (3) found "more or less" pigment within endothelial cells, Elliott and Kanavel (4) found an "unusually small amount of iron containing pigment",

while Meulengracht (5) has found the amount of iron containing pigment to be "insignificant", and believes that its presence or absence depends upon staining reactions employed. The important point is its general insignificance in amount in spite of the hemolytic process.

Liver

The liver shows no pathological changes of note. It is not enlarged; the presence of iron containing pigments in excess has not been confirmed. Tileston and Griffin (3) report its presence, but Meulengracht (5) finds no changes in hepatic cells or in Kupffer cells except for occasional yellowish brown pigment in the cells of Kupffer.

The biliary system does reflect changes due to the hyperhemolysis. The bile is dark and contains a large amount of pigment. It is also noteworthy that gallstones of the pigment type are extremely common, being reported in 60% of the cases. (5)

Jaundice

The jaundice is associated with certain findings which distinguish it from abstructive jaundice. The term "acholuric jaundice" has long been associated with the disease because of these findings. They are, briefly, bilirubinemia with urobilinuria, but without bilirubinuria. This may be found in moderate protracted jaundice of other forms, so it is not pathognomonic. The icteric index ranges from 10-40 units, may be higher during crises. The Van Den Bergh reaction is delayed,

or indirectly positive. The urobilin excretion is greatly increased above normal. It has been investigated by Robertson (7), Goldschmidt, Pepper, and Pearce (8), and McKelvey and Rosenbloom (9) and by many others. They find urobilin and urobilinogen excretion increased from ten to twenty times, their determinations being made on fecal urobilin. Its urinary excretion often imparts a reddish tinge to the urine. Meulengracht (5) concludes that the great increase in fecal urobilin means that there is a corresponding increase in red cell destruction, and that the jaundice is a reflection of the inability of the excretory apparatus to deal with increased biliary pigment occasioned by this destruction. Robertson (7) concurred, concluding that the amount of urobilin in the stool was a measure of the hemolysis going on in the body.

Miscellaneous

Miscellaneous pathological variations have been described by various authors. Of those worth mentioning because of their occurance in the literature are the brachycephaly, protruding eyes, persistant pupillary membrane, elevated palate, prognathism, and protruding teeth, which are often ascribed to the "hemolytic constitution". Their occurance is probably an accidental coincidence. (5)

SYMPTOMS

The symptoms of the disease depend upon the degree to which the individual is affected. Many of the cases are of such a mild character as to live to old age and demand no therapy.

In mild cases there may be no complaint of jaundice, and the chief complaints may be weakness, fatigue, and lassitude from an apparent anemia. There may be a dragging sensation of weight from the splenomegaly associated with the disease. Added to these symptoms in others will be definite histories of hemolytic crises. These are very often precipitated by an unrelated acute illness, or even vigorous excercise. In the more severe cases they occur spontaneously. These crises are characterized by fever, headache, upper abdominal pain, jaundice, often dark colored urine at the height of a crises. The patient does not complain of pruritis during the icteric periods. A definite history of gall bladder trouble may be given, or be associated with the acute attacks. This is of importance for there is a high incidence of gall stones associated with the disease. (2) (4) Some patients are as Chauffard remarked "more yellow than sick". (2)

PHYSICAL FINDINGS

The physical findings may be divided into those of the anemia, the icterus, and the splenic tumor. The anemia may be reflected by the pale mucous membranes and general wan appearance of the patient. The jaundice is peculiar to itself, and does not resemble that of the more common obstructive type. When well marked it is a distinct lemon yellow color, but may be so slight as to give the patient only the suggestion of an icteric color. No pruritis is associated with the icterus, there

are no petechia, and there is no bradycardia as in the more common type. (2) (4) The splenic tumor may be one of the most striking findings of the disease. It is present in nearly every case, and may be so large as to fill the entire left side of the abdomen. (3)

LABORATORY FINDINGS

The laboratory findings have all been discussed previously under the pathology encountered. Those of importance will be briefly mentioned again.

The first is anemia of a moderate grade, 3-4 million total count, hemoglobin correspondingly low, with a color index of approximately normal. From the stained smear the anisocytosis will be evident, with its tendency to microspherocytosis, which is of prime importance. The reticulocytosis and occasional nucleated red cell may also be seen.

The hypotonic saline fragility test will show increased fragility as described, which is of diagnostic importance. The icteric index determines the degree of icterus present, and the Van Den Bergh is indirectly positive. Examination of urine shows urobilinuria, and the Gmelin test for bilirubin is negative. Stool examination shows great increase in urobilin excretion.

COURSE AND TERMINATION

Tileston and Griffin (3) stated that the disease often dates from birth, or is noticed in adolescence and persists throughout life, yet in spite of long duration the patient experiences

little inconvenience from his complaints and may attain an advanced age. Elliott and Kanavel (4) agreed with this. They add that in certain individuals the process becomes so marked as to give rise to a typical picture demanding treatment. This may be described as chronic, mild icterus, with or without associated weakness or malaise, upon which are superimposed at intervals the periods of crises. At these intervals there is a marked increase in size and tenderness of the spleen, malaise, headache, slight fever, accompanied by a period of intense hemolysis with anemia, urobilinuria, deepening of the jaundice, and bile pigment in the blood. During these crises the patient may become severely prostrated.

The majority of cases do not reach the severe crises, and probably many go through life undiagnosed, or the hemolytic crises appear only at some late date in conjunction with some unrelated serious illness which places strain on the heretofore compensated erythropoietic process. Indications for treatment, and treatment, will be discussed under that heading.

COMPLICATIONS

The important complication of hereditary hemolytic icterus is cholelithiasis with its attendant problems. Of 44 patients in whom splenectomy was performed, Mayo found that in 60% the presence of gall stones was an indication for operation. These may be single or multiple, show a preponderance of pigment, and reflect the increased pigment excretion attendent to hyperhemolysis. (5)

There have been several reports of typical cases of arthritis uratica with tophi around the joints in patients with chronic hereditary hemolytic jaundice, and isolated cases of renal calculi are reported. (5)

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The diagnosis of a patient jaundiced since adolescence, with recurrent attacks of hemolytic crises, anemic in appearance, and with a palpable spleen is not difficult. The two cardinal laboratory findings of microcytosis and increased red cell fragility clinch a presumptive diagnosis. In sub-clinical cases, the diagnosis is not as evident, but laboratory findings still provide the key. A history of other members of the family suffering similar conditions is important but not necessary to establish the disgnosis. If the family history is carefully gone into, other cases can be frequently found since, as Tileston and Griffin state, "The disease is an exquisitely hereditary affection, frequently involving three or even four generations".

The differential diagnosis of familial hemolytic icterus from other hemolytic syndromes may be difficult at times. An outline of hemolytic anemias suggested by Haden (10) is helpful. The hemolytic anemias are divided into two groups, with subdivisions.

I. Those due to increased hemolysis from damage to normal cells by foreign agents.

a. Chemical poisons--phenylhydrazine, and sulfonamides.

b. Bacterial toxins--gas gangrene.

c. Parasitic injury--malaria.

d. Amboceptor and complement reactions--transfusion with incompatible blood, and paroxysmal hemoglobinuria.

II. Those due to activity of the spleen.

a. Normal activity on abnormal or imperfect cells--congenital hemolytic icterus, sicklocytosis, ovalocytosis, and erythroblastosis.

b. Abnormal action of the spleen.

Reticuloendotheliosis, Hodgkin's, Leukemia.

None of these syndromes will give the diagnastic picture of spherocytosis with increased fragility of the red cells, although chemical toxins may induce a hemolytic picture which is very similar. The family history and patients history should serve here to differentiate.

PROGNOSIS

The prognosis may be very well arrived at by the course and termination outlined. As Chauffard stated, many cases are "more yellow than sick" (2), and in a great many cases be so mild as to require no therapy, and the patients live to old age unhindered. (4)

Those cases which are more severe, with definite periods of crises, have definite and complete relief of symptoms by means of specific surgical therapy. In this case the prognosis is that of the surgical risk involved. This will be more fully discussed in the next section.

TREATMENT

The treatment may be divided into two classes--one palliative, and the other specific and curative.

Palliative treatment consists of the usual general measures, and transfusions if the degree of anemia warrants. That transfusions should be used at all may be in some doubt. Doan, Curtis, and Wiseman (11) report a series of acute cases in which splenectomy was performed. The accustomed measures in acute cases had been supportive treatment with transfusions until the crisis passed, then to perform splenectomy in the interim. This is the path advised by Kracke. (2) The results of Doan, et al, were spectacular, and they advise emergency splenectomy as a life saving proceedure during a serious crisis.

Splenectomy is the one sure curative therapy for the disease. It is followed by permanent remission in every case. (2) (5) (11) The anemia subsides in a few weeks. The microcytosis subsides after splenectomy, but not completely. This may be of considerable importance. Whitcher (12) reports a complete, or very marked, return in cases observed, but the majority of cases report only a partial improvement in the microsytosis. The fragility of the cells follows the same course in general thet the spherocytosis does. According to Haden this would be expected, since fragility is a direct function of the spherocytosis. (10) The reticulocytosis disappears very quickly after operation, and marked improvement may be noted by the time the

patient leaves the operating table. (11) The jaundice disappears as a rule within a few weeks. (5) The loss of urobilin in the feces was estimated by Goldschmidt, et al, (8) to be one-ninth the amount post operatively to that found preoperatively. The bone marrow has also been found to no longer show hypertrophy and hyperactivity. (5)

The clinical effect of splenectomy is astonishing, and within a very short time after the operation, the patients look and feel perfectly well, and the effect is lasting. (5)

PATHOGENESIS

A brief note on the historical aspect of the pathogenesis is of interest. Among those theories first advanced, and quickly discarded as the pathology became better understood, are the theories of Hayem, Minkowski, and Pick. (3) Hayem, 1898, first advanced the theory that the icterus and splenic enlargement were due to chronic cholangitis. How this fitted into a hereditary picture was not explained. He later, 1908, advanced the thought that it was due to syphilis. Minkowski, 1900, stated that he believed the disease was due to a congenitally perverted function of liver cells, with the bile being excreted into the lymphaties in part instead of into the bile capillaries. Pick, 1903, believed that the manifestations of the disease were due to a congenital communication between bile passages and lymphatics in the liver. This was an obvious effort at explanation of the symptom of icterus on a hereditary basis.

The above theories are attempts to explain the icterus commonly found in the disease on a hepatogenous basis. Since this origin of jaundice is the commonest, it is reasonable that these theories should be among the first to be advanced.

Chauffard in 1907 (2) (3) who first noted the red cell abnormalities of spherocytosis and increased fragility was the first to assign a reasonable course of pathogenesis to the disease. He believed that both of these features were evidence of lessened vitality of the cells, and that the red cells, being susceptible to the action of hemolyzing agents, were readily destroyed. His theory of pathogenesis explains many of the phenomena observed. The anemia is the result of the constant red cell destruction; the bone marrow picture of hyperplasia with nucleated and reticulated red cells in circulation is due to the red cell deficiency; the icterus is the result of increased red cell destruction with the liberation of great amounts of hemoglobin from which the bile pigments are made. Chauffard's theory was the first stepping stone to the understanding of the disease process.

A general consideration of the problem at hand shows certain phenomena to be prominent and well substantiated. The one around which a discussion of pathogenesis must be built is the rapid change of the blood which occurs. That this occurs has been definitely proven, and the urobilin excretion is one of the best indicators of this activity. As mentioned previously

The urobilin excretion has been found to be ten to twenty times increased (7) (8) (9), and the amount of urobilin excreted serves as an approximate measure of the amount of hemolysis going on in the body. The life of red blood cells has been variously determined to be from about forty days by some authors to as much as one hundred and fifty days. If the rate of destruction is increased from ten to twenty times, the average life of the red blood cells at the extremes would be from two to fifteen days. This reflects the great turn over which occurs.

Additional evidence of the phenomenon of red cell destruction is the hyperplastic marrow picture found in the disease, and the reticulocytosis which is constantly present and may reach considerable proportions. These are evidence of a great increase in the rate at which the cells are being supplied to the peripheral circulation, and yet in spite of this obvious compensative activity there is present an anemia.

From this central fact of increased red cell destruction, which as we see may be considerable in amount, arises the obvious question with which any discussion of pathogenesis must deal--how is this increased destruction brought about.

For purposes of analysis the pathogenesis of the disease is best studied by dividing it up into the schools of thought represented. As presented here they will be considered in the following order.

1. The key pathogenic factor is a bone marrow defect, producing abnormal red cells.

2. The key factor is splenic pathology, acting in a mechanical manner by hyperactivity of the reticutoendothelial system.

3. The combined view of abnormal red cell production with an associated hypersplenism.

4. Hyperhemolysis due to the action of hemolysin, normal or pathological, and its relation to hemolytic jaundice.

Chauffard, as just previously outlined, was the first to note the increased fragility and microcytosis of red cells in the disease, and was the first to explain the pathology on the basis of these defects.

Naegeli (13) believes that the presence of erythrocytes of decreased diameter and increased thickness is a constant feature and fundamental variation from normal in patients with congenital hemolytic icterus, and that it represents the inherited feature of the disease indicating a distinct type of human species. In stating his stand in this manner, he places the entire responsibility for the disease on inheritance of a defective blood cell type. He is probably the most modern of the workers to take this view on the pathogenesis.

Gansslen, in 1922, (14) stated a belief along the same line as the preceeding paragraph. He believed tha primary defect was in the bone marrow, making it incapable of supplying cells

of normal size and resistance.

The view that red blood cell defect was the primary defect received its biggest set back very soon after its inception when it was found that splenectomy offered a complete clinical cure for the disease. It is for this reason that there are few modern writers who hold the red cell, or bone marrow, defect is the sole pathogenic factor on which the disease hinges. This statement is made in spite of the common textbook statement that the disease is due to a congenitally defective bone marrow, or red blood cell.

To seriously consider pathogenesis in a hemolytic syndrome the normal physiological method by which red cells are removed from the circulation and broken down must be considered.

The mechanism of disposal in the physiological process by which red cells are removed from the circulation is not known. It is very probable that the spleen is the graveyard for the red cells in this process, and plays a major role in their destruction. Experimentally they are destroyed by two means:

1. In hypotonic solutions the membrane is stretched until it breaks by osmosis of fluid into the cell.

2. Chemical lysins alter the permeability of the cell membrane, make it more permeable, and the cell ruptured by the fluids collecting within the cell as above.

The physiological process which accomplishes this, however,

remains a mystery. The thoughts along this line are chiefly: (10)

1. Rous fragmentation hypothesis, in which the red cell is fragmented and phagocytosed by the reticuloendothelial system. There should be evidence of this process if this is true, and microscopic examination of the spleen does not support this view. (10)

Doan, et al, (16) do not agree that there is no evidence of this process. They state that in stages of active hemolysis hemosiderin is found abundantly in the reticuloendothelial cells of the sinuses and clasmatocytes of the parenchyma. They agree that there is some difference of opinion as to the prominence of red cell phagocytosis and the role of the reticuloendothelial cells in the hemolytic process, but they hold that in hemolytic anemias the evidence increasingly points to a destruction of red blood cells chiefly within this group of phagocytic cells. They call attention to the fact that, while they are most abundant in the spleen, they are also found in the liver, bone marrow, and general tissues.

2. Bergenhem and Fahraeus identified a lysolecithin in normal serum, a very active hemolysin, formed in the spleen, and cells exposed to its action became spherical in shape. (21)

3. Heilmeyer found that red cells of the splenic vein are more spherocytic than those of the splenic artery, and he thinks the spleen contributes something causing spherocytosis. None of

these methods of physiologic action on red cells is completely explained, but all center around splenic activity on the red cell. If the problem of physiologic red cell removal could be completely answered, it would very likely provide a key to the answer of pathogenesis in hemolytic icterus. The phagocytic activity of the reticuloendothelial cells is an understandable process, but by what process the selection of red cells is made is not explained.

That the spherocyte characteristic of the disease is actually a more fragile cell cannot be denied. Chauffard and his co-workers noted the association in 1907. Modern workers have not only proven the fragility of the spherocytic cells, but have shown that there is a direct relationship between the two factors. (10) (14) (35)

In 1934 Haden (6) made some interesting observations on the mechanism of fragility of the red cells in chronic hemolytic icterus. Prior to these observations no one had proven the direct relationship between spherocytosis and fragility. He showed that increased fragility is dependent upon the spherocytosis or altered shape of the cells, and believed that it offered a possible explanation for the increased hemolysis characteristic of the disease.

He tabulated the cell changes as to glabular shape in different species of animals, and found that the resistance of the red blood cells to hypotonic saline solution decreases in direct proportion to the increase in globularity. From this a definite

relation between the spherocytic tendency and fragility in lower animals seems evident. (Chart I)

	Diameter	Thickness	Volume	Relation Thickness To Dia.	Resistance % NaCl
MAN	7.8 μ	1.84 μ	88 μ^3	1:4.2	.42 – .48
DOG	7.2	1.70	69	1:4.2	.50 – .54
RABBIT	6.6	1.84	63	1:3.6	.52 – .54
CAT	5.6	1.75	43	1:3.2	.60 – .66
GOAT	4.0	1.95	25	1:2.1	.72 – .74

CHART I

The relation of sphericity to fragility. (6)

Another chart which shows visually the relation of sphericity to fragility in human red cells illustrates the same point. (Chart II)

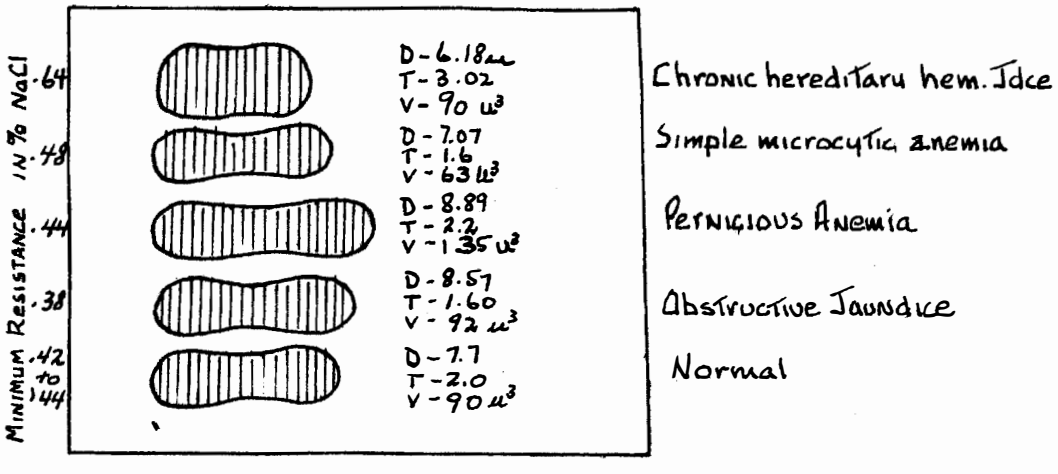


CHART II

The relation of sphericity to fragility in human red cells (6)

Both of these last two charts illustrate that increased fragility and sphericity go hand in hand. If red cells are placed in hypotonic solutions of graded dilutions up to the hemolysis point, it is found that the cells gradually become globular before being hemolyzed. (Chart III)

Test Tube & 5c.c. Blood	c.c. Dist H_2O Added	Mean Vol. of R.B.C.	Diameter	Calculated Thickness
1	0	86	7.6 u	1.9 u
2	1	98	7.4	2.3
3	2	110	7.7	2.35
4	3	128	7.2	3.15
5	4	141	7.3	3.4

CHART III

Red Cell Shape in increasingly hypotonic Solutions (6)

Since the characteristic variation from normal of the erythrocyte in chronic hereditary icterus is decreased diameter with increased thickness, it is apparent that much less dilution is necessary to bring the cell to the shape at which hemolysis occurs. The cells in this disease are nearer their hemolysis point by reason of their shape. Since the one fundamental variation from normal in the disease is this spherocytosis, the anemia, jaundice, reticulocytosis, and increased fragility are all secondary to the globular form of the erythrocyte. (6) (36)

The preceeding paragraphs have covered the essence of the

evidence used to build the hypothesis that the abnormal red cell is the essential defect, and responsible for the disease because of this defect. The evidence, pro and con, will be summarized along with a summary of the information to be advanced by proponents of the other views. In this way we can evaluate their worth with better understanding.

Hypersplenism, or the view that the hemolytic anemia is caused by hyperactivity of the splenic phagocytes, or by some abnormal splenic action, has many strong proponents. Its backers have strong evidence in their behalf in the clinical effect of splenectomy, and it was on this basis that the theory first arose. In 1915, in reviewing all cases of splenectomy performed for the disease to that time, Elliott and Kanavel (4) concluded that, although the pathogenesis was not definitely known, operative results set aside hepatogenous theories which did not ascribe to the spleen the principal role. They thought it was safe to say that if the spleen was not the sole cause it was at least an essential factor in the disease. Prior to this, in 1909, Eppinger had stated that altered splenic function was the essential etiological factor, and ascribed the name "hypersplenism" to the view.

In evaluating the action of the spleen two mechanical factors in its action on red cell destruction are called to attention by Barcroft (17) and Knisley (18), and later were elaborated on by Ham and Castle (20). Barcroft called attention

to the fact that the spleen acts as a reservoir for blood, and termed this as "erythrostasis". Knisley pointed out that during this period of erythrostasis there is a filtering off of plasma resulting in a concentration of red cells, or "erythro concentration".

That these two factors may play a role in erythrocyte destruction was brought out by the experiments of Ham and Castle. They found that, in vitro, erythrostasis of normal red cells caused an increase in their volume, an increase in sphericity and fragility, until the red cells would hemolyze in isotonic salt solution. They found that erythroconcentration, in vitro , would accelerate the above process. Erythrostasis in vivo in experimental animals caused variable increases in fragility of red cells from the spleen, and produced some degree of hemolysis in every case. Erythroconcentration in vivo produced a marked increase in red cell fragility in the spleen and circulating blood. The relation of acute infections to the onset of hemolytic crises is suggested by the fact that in such processes the authors found an increase in blood viscosity averaging 42% above normal. This increased viscosity, leading to an increase in erythrostasis and rouleau formation (erythroconcentration), may precipitate the hemolytic attack.

Among the strongest supporters of the splenic view of pathogenesis have been Doan and his co-workers. In 1934 Doan, Wiseman, and Erf (16) in reviewing a series of cases of chronic

hemolytic icterus which had undergone splenectomy came to definite conclusions as to the guilt of the spleen in causation of the hemolytic process. They stated that the spleen was the chief organ involved in the hemolysis, and that the bone marrow, with the spleen removed, had proven its competence to bring about prompt recovery from severe anemia and to maintain the red cell count and hemoglobin within normal limits. They base these statements on the appearance of the spleen (evidence of hyperhemolytic activity as described in this paper under discussion of physiologic hemolysis in the spleen), and the clinical result of splenectomy. They point to the return of erythrocyte fragility completely to normal in one case of 25 years duration (post splenectomy) as evidence against bone marrow defect in the syndrome.

They hold that evidence points strongly to an inherent tendency of overactivity of the phogocytic mechanism--reticuloendothelial system--of the body, centered normally in the spleen, as the basic fundamental etiology in the clinical and pathologic entity. Many factors, including trauma, infection, dietary habits, and other disturbances condition the clinical manifestations of the disease and cause its recurrent, relapsing course.

In a later work Doan, Curtis, and Wiseman (11) list the following reasons for their firm belief that the spleen is the major pathologic agent in chronic hemolytic icterus.

1. Microcytosis, increased fragility, and increased reticulocytosis are not necessarily present, even in acute cases.

2. Anemia, when present, is always accompanied by acholuric jaundice or other evidence of excessive hemolysis.

3. The more severe the anemia the greater the bone marrow activity as measured either by increased reticulocytosis or by direct observations on the erythroblastic hyperplasia of the bone marrow.

4. Splenomegaly, and the number and activity of the splenic phogocytes, fluctuates directly with the severity of the anemia and the icterus.

5. The splenic artery, in several instances of sampling just prior to ligation, contained more cellular elements than the splenic vein.

6. The epenephrine test reveals an excessive cellular sequestration capacity of the spleen in congenital jaundice. The data accumulated following splenectomy further supports their thesis in that:

7. Hemoclostic crises, spontaneous or precipitated, may be terminated instantly by successful removal of the spleen, and do not recur.

8. The total volume of circulating red blood cells becomes immediately increased, as much as 77% of the original volume.

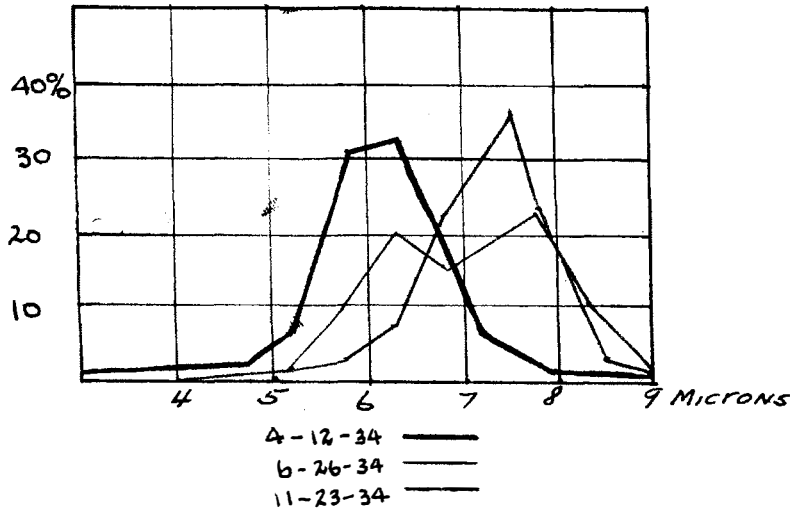
10. Restitution of a relatively normal bone marrow as reflected by reduction to normal of erythroblastic hyperplasia, disappearance of high peripheral reticulocytosis, recovery from

myeloid hypoplasia with correction of peripheral neutropenia (?), the elimination of megakaryocytic hypoplasia with reversal of the peripheral thrombopenia and the maintainance of elevated thrombocytosis, the return of erythrocyte diameters (Price-Jones curves) to more nearly the established limits for normal, a decrease in the erythrocyte volume thickness (Haden) index, and the increase in erythrocyte resistance sometimes to an entirely normal range, and finally--

11. The clinical recovery is complete and permanent so far as the hemolytic tendency is concerned.

In 1935 Levi and Bairati (19) investigated the Price-Jones distribution of red cells in two patients which were splenectomized. They found that the curve shifted to a higher average diameter (Chart IV) after splenectomy, and before establishing this form there was immediately post operatively a biapicality to the curve. The second peak of greater diameters was due to reticulocytes present in considerable number, the curve of lesser diameter to microcytes still present in the blood. They hold that the spleen has a central position in the pathogenesis of hemolytic anemias. They believe that the rapid stabilization of biapicality of Price-Jones curves, and also the increase in osmotic resistance which they observed, showed the ability of the bone marrow to produce normal cells, at least from the view point of their diameters. To them it means that with the cessation of hyperhemolysis the state of affairs disappears which transforms into an actuality the potential disposition of the bone marrow to form pathologic

erythrocytes. To these authors, then, the cells are pathologic and formed in the bone marrow, but only because of the stress caused by hyperhemolysis in which the spleen is the causal agent. This is an idea not commonly stated but worth remembering.



Splenectomy on 4-21-34

CHART IV

Price Jones Curves before and after Splenectomy. (19)

Haden (10), although not entirely in accord with the splenic hypothesis, makes the following statements in regard to removal of red cells from the circulation by the spleen. "The immediate cause of the anemia is hyperactivity of the spleen since the symptoms of the disease are all relieved by splenectomy.--It is apparent from the beneficial results of splenectomy that the anemia is due to the activity of the spleen in picking spherocytes. The exact mode of action of the spleen is unknown. The spleen might make the cells more spherocytic and thus more susceptible to hemolysis, perhaps through the action of lysolecithin as suggested by Bergenhem and Fahraeus. Recently I measured the

diameter of the cells on a film of blood obtained from the pulp of a spleen immediately after removal for congenital hemolytic jaundice. The mean cell diameter was 5.8u, while the mean diameter of the circulating cells at the same time was 6.1u. The spleen might also affect the cells in some other was so as to injure the cell envelope and thus make it more susceptible to hemolysis."

Whitcher (12) studied the blood picture before and after splenectomy and found by careful measurements of the red cells that there was a distinct return toward normal. He believes that because of the disappearance of microcytosis after splenectomy, in contrast to their abundance prior to operation, that the microcytosis is a concomitant manifestation of the anemia of the disease rather than an inherent erythrocyte character.

Meulengracht (5) has considerable to say regarding the pathogenesis of the disease, and regards the spleen as the foremost factor. He believes that the histological picture of the spleen with over crowded pulp spaces in contrast to relatively empty sinuses means that there is something going on which has to do with destruction of red blood cells. He states Eppinger's idea that red cells which pass through the splenic pulp do not necessarily have to be hemolyzed there, but may be prepared for destruction which can take place elsewhere, is entirely hypothetical but plausible.

He feels that microcytosis and increased fragility are secondary to splenic activity, because it would be reasonable to expect they would be even more pronounced after the removal of the spleen if this were not so, since the least resistant red cells would then be saved from destruction. He agrees that in spite of clinical recovery after splenectomy there still remains some degree of these defects present in the red cells. He mentions a cardinal fact, however, when he says that in splenic removal not all of the reticuloendothelial system is removed, only its main concentration. This seems to me to be very important in explaining a partial instead of complete recovery from the cell defects characteristic of the disease after splenectomy.

In summation Meulengracht points out that the central phenomenon is a marked increase in change of the blood with the appearance of a hyperhemolytic syndrome. This may be due to a primary condition of hyperactivity of the spleen and other parts of the reticuloendothelial system, or it may be due to a primary abnormality of the red blood cells. The evidence however indicates that the hyperactivity of the spleen is the primary and fundamental factor in pathogenesis of the disease.

The two most commonly held views as to pathogenesis are the two which have been discussed so far. That another view should be held which combines these two viewpoints is not surprising. This combined view, that the pathogenesis is due to an abnormal red blood cell associated with hypersplenism, arises

as a result of the fact that there are still remaining after splenectomy degrees of spherocytosis, increased fragility, reticulocytosis, and other pathological evidences of the disease process in spite of the clinical cure accomplished. (37)

The strongest proponent of this view is Haden in an article published in 1939. (10) He believes that the persistant fragility and spherocytosis after splenectomy with no clinical evidence of the disease shows that the bone marrow is still supplying cells thicker than normal. The fact that prior to splenectomy the cells were more fragile, more spherocytic, and if subjected to heat hemolyzed more readily, than after splenectomy seems to prove that the spleen in some way affects these cells. The splenic action may be only changing its shape by affecting the constituents of the cell so that it becomes more spherocytic and less resistant. He states the splenic action on the spherocytic cells may only be the normal physiological action on any cell, and in congenital hemolytic icterus this is sufficient to cause destruction of the anemia. This sounds as if he still considered the congenital defect of bone marrow to be the major factor, as he originally stated in 1934. (6)

In his classification of hemolytic anemias previously cited he classes congenital hemolytic icterus as a disease caused by normal splenic action on abnormal or imperfect red blood cells. This bears evidence also as to his belief that the marrow defect is primary. From his conclusions however we find that following

Statements in regard to his "conbimed" pathogenesis--"In congenital hemolytic icterus the cells are always spherocytic in varying degrees. A sphere has a smaller surface area in relation to volume, so can undergo less stretching before hemolysis occurs than a biconcave disc. The increased fragility characteristic of the red blood cells in contenital hemolytic icterus is due to the spherocytosis. The spherocytosis is due partly to the congenital anomoly in shape of the red cell, partly to the activity of the spleen."

There is little more to be said for the combined pathogenesis theory. The evidence is maily that hematologically the patient is not cured even though he is clinically by splenectomy, therefore the bone marrow and spleen must both be at fault. This contention will be considered in the summary.

Considerable work, most of it quite recent, has been done in investigating the possibility that hemolysins, normal or abnormal, may play a part in the pathogenesis of congenital hemolytic icterus. An investigation of this evidence is interesting.

In 1936 two Danish workers, Bergenhem and Fahræus (21) isolated an hemolysin from normal blood which was produced from plasma and cellular lipoid. They termed this hemolysin lysolecithin. It is apparently produced by enzyme action, can be extracted from plasma or serum, and is known not only to inhibit rouleau formation and retard sedimentation, but also to convert discoidal erythrocytes into spheres. If present in sufficient

amount it produces hemolysis. (22) They felt that spherocytosis in hemolytic anemia might be due to the presence of lysolecithin in the blood which acted chiefly in areas of stasis or sluggish circulation. Because of these characteristics in splenic circulation they felt the spleen might be the organ responsible for the process. (23)

In 1940 Singer (24) extracted lysolecithin from serum, and used it as a means of testing red blood cell fragility. He found a very interesting fact, the spherocytes of congenital hemolytic jaundice are very much less resistant than normal erythrocytes toward this lysin, and the spherocytes of various acquired hemolytic syndromes other than congenital hemolytic jaundice show normal fragility when tested by lysolecithin. If tested in hypotonic saline they show increased fragility however. Singer believes spherocytosis is a morphologic expression of alteration in the structure of the erythrocytes due to action of different types of hemolytic substances.

In 1941 Singer (25) again published investigations on lysolecithin. He found that there was an increase in lysolecithin in blood which was incubated unmolested, but no increase if it was moved about while incubated. From this he theorized that in the body there is less lysolecithin in moving than in stagnant blood. He found increased lysolecithin in the splenic vein, over that in the splenic artery, indicative of splenic stagnation (dogs). In congenital hemolytic anemia the lysolecithin quotient

(a mathmatical index) was normal, or the same as that found in normal persons studied. Because of this he thought the significance of lysolecithin as the physiologic hemolysin involved in the mechanism of normal blood destruction was inconclusive. Singer thinks it further unlikely that lysolecithin is responsible for increased hemolysis in congenital hemolytic anemia, since he could demonstrate no increase above normal in that disease. He does observe that, since splenectomy has such a remarkable curative effect in congenital hemolytic anemia, it would seem necessary to compare the lysolecithin content of splenic pulp and venous blood at operation with that of other conditions before it could be denied that lysolecithin did not play a role in pathogenesis of the hemolytic process.

In 1938 Dameshek and Schwartz (26) were led to the experimental investigation of hemolysin activity in hemolytic anemias. They had three clinical cases of acute non-familial jaundice under observation in which active serum isohemolysins were discovered. It should be noted they called these cases non-familial before we proceed. It is presumed no hereditary factors could be found.

The isohemolysins found had the following properties:

1. Inactivation by heat.
2. Reactivation by addition of complement.
3. Increased activity after storage for few hours.
4. Diminished activity after prolonged storage.
5. Positive Ehrlich-Morgenroth phenomenon.

The findings in these cases suggested to the investigators that acute hemolytic anemias, and possibly other hemolytic syndromes may be due to the action of hemolysins. They wondered if differences in hemolytic reactions might be due to different "dosages" of hemolysin, and if spherocytosis and increased fragility might not be due to hemolysins also.

They developed a hemolytic serum in rabbits for guinea pig red cells. They used this in varying dosages to produce varying degrees of hemolysis in their guinea pigs. The results obtained were hemolytic anemias remarkably like the clinical syndromes. The fragility, spherocytosis, reticulocytosis, and pathological anatomy found were all typical.

Reasoning by analogy with their experimental syndromes they concluded that the various differences in clinical syndromes were due to the degree of hemolysis taking place. The most outstanding feature of the experimental blood picture was the developement of typical small, thick spherocytes which exhibited increased fragility. They were led to the view that spherocytosis might be the result of a hemolytic agent in the serum. In one of their observed clinical cases the serum titer of hemolysin diminished as the red cell diameter and fragility approached normal when the patient recovered from his acute hemolytic phase.

A very important observation on a clinical case of hemolytic icterus is also reported. They found that the nucleated red cells in the marrow, and the nucleated and reticulated red cells in the

peripheral circulation were all of average normal diameter. This was in spite of an average red cell (mature) diameter of 6.18u, (reticulocytes averaged 8.16u). These findings demonstrated that immature red cells when delivered to the circulation are of normal diameter, and microspherocytosis of the mature cells must be a phenomenon occuring after the cells reach maturity in the peripheral circulation. This would free the bone marrow of implication in producing defective cells.

They concluded that spherocytosis is formed outside the bone marrow, that it developes only in mature red cells, and that a hemolytic agent is responsible for their formation. In congenital hemolytic icterus they felt that spherocytosis may be due to more or less continued action of an hemolysin, and that crises may be caused by sudden liberation of large amounts of hemolysin.

This is an interesting viewpoint, and in 1940 the same authors published a very complete review of the literature with further discussion of the relation of hemolysins to the hemolytic syndrome. (23)

First lets look at a diagram which presents graphically their premise that the varying degrees of clinical hemolytic syndromes may be explained on the basis of the amount of hemolysin present. (Chart V)

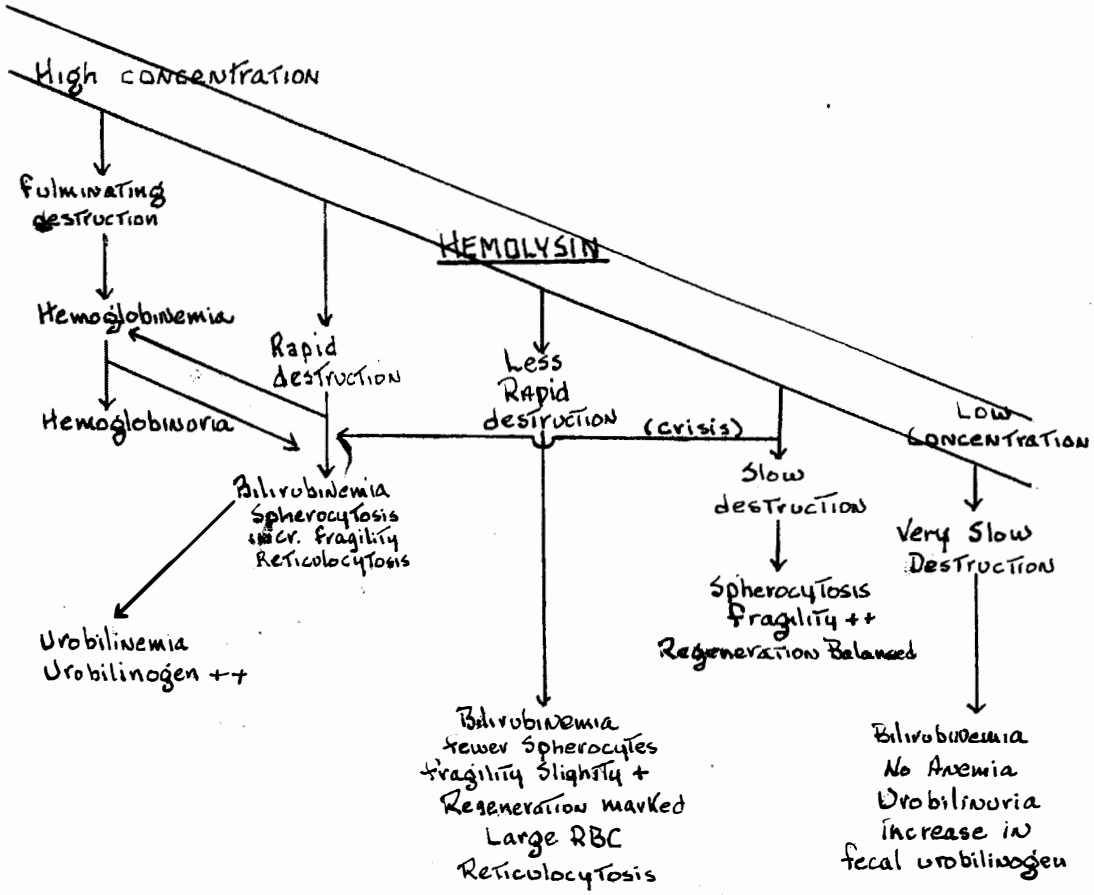


CHART V

Varying Concentrations of Hemolysin and Clinical Hemolytic Syndromes. (23)

The pathogenesis of hemolytic states with special reference to hemolysins is investigated thoroughly. The presence of hemolysins in hemolytic syndromes has long been substantiated. In 1906 Donath and Landsteiner discovered the cold hemolysin of

paroxysmal hemoglobinuria. Chauffard, et al, discovered hemolysins in acute hemolytic anemia in 1908 with the following characteristics.

1. Inactivated by heating to 56° C.
2. Reactivated by complement.
3. Hemolytic activity declined as patient improved.
4. Erlich-Morgenroth phenomenon positive.
5. Donath Landsteiner reaction negative.

In 1909 Chauffard and Vincent believed that this hemolysin had a definite pathogenic role, and that it was not secondary to red cell destruction. Confirmatory observations were made by Froisier in 1901, Dufourt in 1912, Widal in 1913, v. Stejskal 1909, and others. The authors first article (26) also was confirmatory evidence. Hemolysins have been demonstrated in other hemolytic syndromes--

1. In paroxysmal hemoglobinuria--by Donath and Landsteiner.
2. Transitory hemoglobinuria--Saten, 1935.
3. Paroxysmal Nocturnal hemoglobinuria--by Micheli, 1935, and Ham and Dingle, 1939. (27)

Tests for hemolysins in hemolytic icterus have rarely been made because of the emphasis on the mechanical or hereditary defect. In 1918 Beckman and Ludke found hemolysins in congenital hemolytic jaundice. Green (28) found that normal red cells, washed free of serum from a case of congenital hemolytic jaundice. That free hemolysins are not demonstrated in hemolytic processes does not

rule out their presence. It has been proven that injury insufficient to cause hemolysis in a test tube may cause hemolysis in the body, and hemolysin sufficient to lyse only a few cubic centimeters of blood in vitro may cause great destruction in vivo.

There are two possibilities as to the physiological sourse of the hemolysin. First it may be an overflow of an hemolysin normally present, or it may be a "precocious" hemolysin. The latter is not likely because it would postulate a purely chemical type of activity, the hemolysin would have no hemolysinogen, and those found behave in an orthodox immunological style.

The site of origin of the hemolysin has been long speculated. Minkowski originally indicted the spleen as a possible site. Other workers have found that splenic extracts possess hemolytic activity not due to amboceptor complement reaction. Banti (29) concluded that the spleen was the principal organ of hemolysis, probably through the medium of hypothetical cythhemolysins produced by splenic cells. v. Stejskal found that in congenital hemolytic icterus stasis of blood in arm veins increased the hemolytic activity, while elevation of arms to produce anemia decreased the hemolysis as observed in blood withdrawn. Because of the known splenic stasis of blood he concluded hemolysis might well occur there, and might be either chemical or physical in nature. Bergenhem and Fahraeus' (21) contributions to splenic hemolysin production (lysolecithin) have been discussed previously.

Dameshek and Schwartz themselves believed the spleen was definitely implicated. They think the hemolytic factor, whether

cellular or serological in type, causes changes inred cells--spherocytosis, increased fragility--rendering them more readily destructible by the normal organs of blood destruction. They believe that because hemolysin was demonstrated after splenectomy was performed, showed it was not entirely produced by the spleen, and perhaps the entire reticuloendothelial system was incolved. The chief site of its production however, undoubtedly is splenic, since clinically splenectomy is sucessful.

What the initiators of hemolysin activity are is only conjectured by Dameshek and Schwartz. The fundamental stimulus for its origin remains unanswered. Possibilities as to its origin which were suggested are--

1. Body may become sensitized to its own red cells.
2. Red cells may become sensitized to products of their breakdown.
3. Chronic splenic distension with erythrostosis and building up of considerable quanities of lipin is possible.

Ham and Dingle (27), previously mentioned, studied hemolysin relationship to chronic hemolytic jaundice with paroxysmal nocturnal hemoglobinuria, the Marchiafava--Micheli syndrome. They found that human complement, or a complement like substance, was necessary for in vitro hemolysis of the red cells, and that the cells did not show increased hemolysis to non immunologic hemolytic systems such as saponin, or hypotonic saline. The exact abnormality could not be defined, but they believed the

cells were sensitized by an hemolytic substance, conceivably an antibody. The serum factor necessary could not be distinguished from complement. That the hemolytic system could not be classified as strictly immunologic they agreed, since no antigen or antibody was demonstrable.

Tigert and Hill (31) investigated cases of hemolytic anemia in which the characteristics of microcytosis, spherocytosis, and increased fragility were absent. They could demonstrate no hemolysin, but believed that it was present and could not be demonstrated because of fixation to red blood cells. Investigation regarding the mode of action of hemolysin on the redcells is incomplete, but they believe there is some change in cell lipoid produced by this direct combination. The erythrocytes so altered resemble the cells of hemolytic anemia.

Tigertt, Duncan, and Hight (32) reported also that in hemolysis due to specific hemolysins the red cells approach a spherical form by diminution in diameter, but no increase in volume as in hypotonic hemolysis.

Dr. R. R. Kracke, (33) in discussion of the paper presented by Tigertt and Duncan just referred to, made the following interesting statements. "Undoubtedly hemolytic anemia is inhereted, but as the authors point out it is not the inheretance of an unusual type of hemolysin which circulates in the blood. If it is true that patients with hemolytic icterus have inherited hemolysins in their blood stream, then what is the role of the splean? After

the spleen is removed the patients are cured clinically speaking. It seems that instead of our old idea of the spleen being the graveyard for these fragile spherocyte cells, it has something to do with the production of this atypical hemolysin. When we remove the spleen we remove the seat of antibody formation which produces spherecity, and at the same time remove the organ that destroys the atypical spherocyte cells."

As the last example of thought or experimental work on hemolysins to be considered the work of Dacie (22) is looked into. He investigated cases of congenital hemolytic icterus in regard to in vitro hemolysis rate of their blood cells as compared to normal persons.

He found a great increase in all cases of congenital hemolytic icterus above normal. He tested by allowing the blood to stand undisturbed, noting at varied intervals that amount of hemolysis present. He also, with anticoagulant added, tested at intervals to find changes in cell volume by the hematocrit. The greater the hemolysis present the lower the cell volume that would be found. Chart VI is illustrative of the results he found.

Dacie also found that the following were true:

1. Hemolysis was slowed if cells were washed free of plasma and suspended in normal saline, although lysis was still more rapid than in normal washed cells.

2. Rate of hemolysis unaltered if washed cells from acholuric jaundice suspended in normal plasma, although

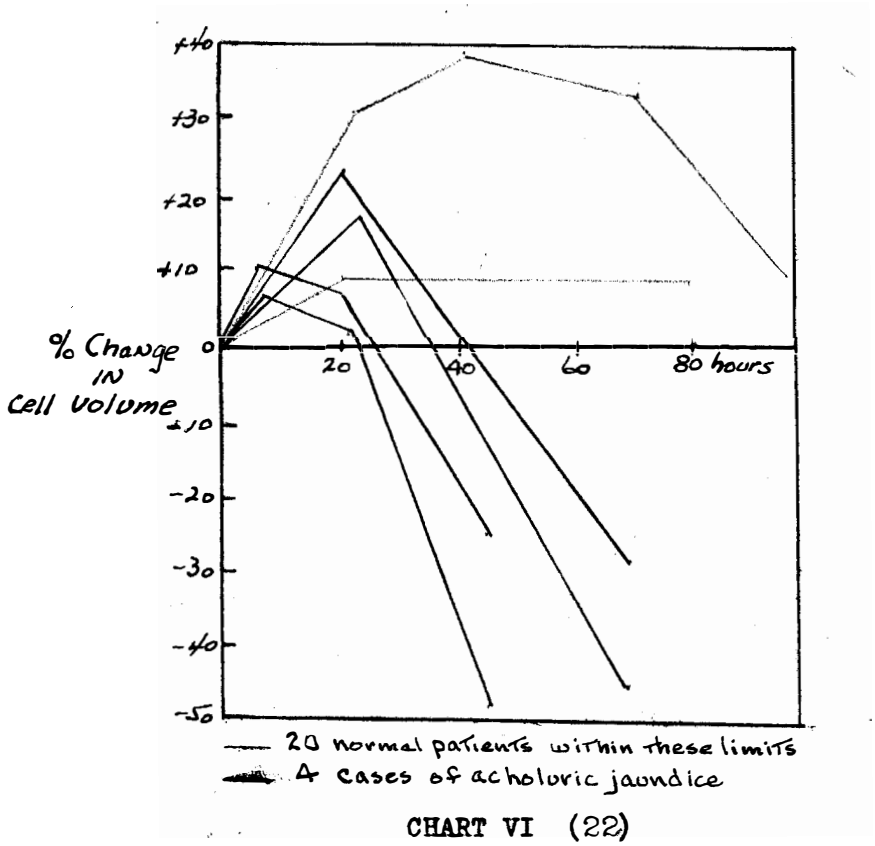


CHART VI (22)

Changes in Cell Volume of Normal and Acholuric Jaundice Oxalated Blood Produced by Incubation at 37° C.

diminished if heated to 57° C. for one half hour first.

3. Acholuric jaundice plasma added to normal cells does not increase their rate of hemolysis.

4. Anemia, per se, is responsible for acceleration in the rate of hemolysis due to a reduction in cell/plasma ratio.

Lysolecithin production is thought to be important in the results which Dacie found. The type of hemolysis observed was stated to be different than that described by Dameshek and Schwartz, and Donath and Landsteiner. It occurred without

autogenous plasma, is not inhabited by normal plasma, does not require complement, and is apparently determined by abnormalities in the red cells themselves. They offer the following possible interpretation of their findings. "The rapid onset of hemolysis in these cases is an exaggerated normal process, perhaps dependent on the activity of lysolecithin.--Perhaps, in acholuric jaundice, during circulation the red cells adsorb large amounts of lysolecithin, possibly during passage through the spleen, and increased fragility and retarded sedimentation of venous splenic blood may be evidence of this. When these cells are incubated more lysolecithin is produced, and cells hemolyze earlier than normal cells." The author found that the red cells of erythroblastic anemia were abnormally fragile to hypotonic hemolysis, but did not undergo in vitro hemolysis as those of acholuric jaundice. This may be supportive evidence of the hypothesis favoring lysolecithin which the author advanced.

Summary:

Evidence for the hypothesis that red cell (bone marrow) abnormality is the pathogenic factor in congenital hemolytic icterus:

1. Microspherocytes are characteristic of the disease.
2. Increased fragility is a function of spherocytes.
3. The anemia, jaundice, reticulocytosis, and increased fragility may all be logically considered secondary to the spherocytosis. (6)
4. Following splenectomy there is an incomplete recovery of spherocytosis and increased fragility.

Evidence in opposition to this viewpoint:

1. Splenectomy is clinically curative.
2. Definite improvement in spherocytosis and fragility follows splenectomy, complete recoveries have been reported. (12)
3. The bone marrow is not depressed, but on the contrary it makes a vigorous attempt to correct the sudden anemia as evidenced by high reticulocyte percentage, nucleated red cells, and young white clees seen in the blood. (34) (19)
4. The normoblasts in the bone marrow, and the reticulocytes newly delivered to the blood stream, are of normal diameter even in cases with marked microspherocytosis. (26)

5. The hemolytic process may be present without the characteristic picture of spherocytosis or cell abnormality. (11) (31)

6. The morphological blood picture, as well as clinical picture can be produced artificially in the presence of normal marrow. (23) (26)

Evidence supporting the hypothesis that the spleen is responsible for the anemia:

1. The curative effect of splenectomy, not only clinically, but the improvement of the red cell defects, and the change in the bone marrow picture to that of normal.

2. The physiological splenic action of hemolysis, and the known phagocytic action of the reticuloendothelial system.

3. The splenic picture of congestion, increased amount and size of reticulum cells, and erythrophagocytosis. (29) (16) (11)

4. Investigators have found more cellular elements in the splenic artery than the splenic vein. (11)

5. Hyperhemolytic syndromes caused by artificial means cause the characteristic picture of hemolytic jaundice. (23) (26)

Evidence opposing the splenic hypothesis:

1. The lack of complete recovery from reticulocytosis, spherocytosis and increased fragility following splenectomy.

This is the only reasonable evidence opposing the splenic hypothesis. It is not strong argument because the reticuloendothelial system, composing the phagocytic cells believed responsible for the hemolysis, is not entirely contained in the spleen alone.

Evidence for combined splenic and red cell defect as the pathogenic factor lies in the fact that in spite of the clinical cure of splenectomy the red cells still show alteration from normal. Assuming the reticuloendothelial system is the responsible system for the hemolysis in the theory of "hypersplenism", it is remembered that only a large concentration of this tissue, not all of it, is removed by splenectomy. This is the best evidence to be offered against a combined view.

Evidence for a hemolytic system composed of hemolysins as the pathogenic factor in congenital hemolytic icterus:

1. Hemolysins have been found in congenital hemolytic icterus, and other hemolytic syndromes.
2. These hemolysins do cause in vitro hemolysis.
3. Experimental syndromes of hemolytic anemia reproducing the clinical entity have been produced by hemolysins. The laboratory findings and pathological picture are characteristic.

Evidence opposing the hemolysin view:

1. Hemolysin (lysolecithin) has been isolated from normal serum, hemolytic in action when used in vitro.
2. There has been proven no increase in lysolicithin

in congenital hemolytic icterus.

3. Experimental hemolytic anemia production by means of immunologically prepared hemolysins, shows only that hemolytic icterus may be caused by substances toxic to red cells, and that the picture resembles the clinical entity.

Conclusion:

The weight of evidence explaining the pathogenesis of congenital hemolytic icterus seems to lie with those who support the splenic hypothesis. That splenic destruction of otherwise normal red cells, by reticuloendothelial activity, if excessive in amount, can produce the entire picture of the clinical, laboratory, and pathological findings, and is responsible for the disease, is I believe a true statement. That this "hypersplenisn" is inherited must be concluded because of the inheritable characteristic of the disease.

The investigation of the role of hemolysins in chronic hemolytic icterus is not new, but renewed interest has been recently displayed. There may soon be convincing evidence that they play an essential role in pathogenesis, but the view is not justified at present.

The common statement that chronic hemolytic icterus is due to an inherent defect in bone marrow, because of which it produces an abnormal red cell more susceptible to hemolysis, is not in accord with the weight of evidence.

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