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THE VALUE OF BONE MARROW BIOPSY IN THE DIAGNOSIS OF ESSENTIAL THROMBOCYTOPENIC PURPURA

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Senior Thesis

Presented to the College of Medicine,
University of Nebraska
Omaha, 1948

THE VALUE OF BONE MARROW BIOPSY IN THE DIAGNOSIS OF ESSENTIAL THROMBOCYTOPENIC PURPURA

The first written reference to the disease we now know as essential thrombocytopenic purpura is that of Paul Gotlieb Werlhoff. In his book Opera omnia, published in 1775, he described a disease characterized by bleeding into the skin and mucous membranes which he called morbus maculosus haemorrhagicus.

Little more is to be found in the world's medical literature about this disease until 112 years later when, in 1887, Denys (1) recognized the great diminution in the number of platelets in the peripheral blood.

J. H. Wright (2-3) in 1906 and 1910 layed the corner stone for subsequent investigation by his researches upon the histogenesis of the blood platelets. It was he who first determined that the megakaryocytes in the bone marrow were the precursors of the blood platelets.

Acting upon Wright's theory of platelet genesis, Frank (4), postulated a marked decrease in quantitative platelet formation by the megakaryocytes as the basic pathology in essential thrombocytopenic purpura and suggested that this was due to a selective inhibitory function of the spleen.

Kaznelson, in 1916, assumed an analagous situation between hemolytic anemia and essential thrombocytopenic purpura and

performed the first successful splenectomy for this disease. The results of surgery were most dramatic despite the erroneous conceptions which fathered them.

The dramatic recovery so commonly observed following splenectomy definitely implicated the spleen in the pathogenesis of this disease, and in the years following Kaznelson's first report, many investigators worked long and hard to prove in their laboratories what the empiric use of splenectomy was proving daily in the hospital wards. Troland and Lee (5) in 1929, were first to produce the picture of essential thrombocytopenic purpura in experimental animals by injection of extracts of splenic tissue. Hobson and Witts (6), Rose and Boyer (7), Atanasek and Lee (8), and Cronkite (9), have all confirmed the presence in the spleen of a substance which depresses megakaryocyte platelet formation. This confirmation of Frank's (4) observation places essential thrombocytopenic purpura within that group of diseases to which Dameshek (10) has given the name "hypersplenism".

CLINICAL ASPECTS

Essential thrombocytopenic purpura is a disease of unknown etiology, which is apparently caused by hypersecretion of a specific splenic factor which acts to depress the formation of platelets in the bone marrow.

The disease is characterized by a generalized bleeding into the skin, mucous membranes and body cavities. The clinical

picture is not clear cut in the sense that there are no characteristic pathognomic signs or symptoms. Fortunately, however, the laboratory findings are characteristic and the diagnosis is usually proved or disproved in the clinical laboratory.

Chief in the differential diagnostic "musts" are those diseases such as the aplasias, leukemias, the splenomegalic syndromes, and the toxic secondary purpuras, any one of which can produce the clinical and laboratory findings of essential thrombocytopenic purpura.

Since Kaznelson's first splenectomy in 1916, this operation has been the treatment of choice in essential thrombocytopenic purpura. At various times there have been attempts at conservative management. Oil of turpentine, vitamin C, sesame oil, progynon, testosterone, snake venom and antivenom, rutin, and multiple transfusions have all been used. None of these methods have produced more than a transient benefit.

Numerous writers have recorded evaluations of splenectomy. R. H. E. Elliott (11) reported 41 cases of essential thrombocytopenic purpura in 1939. Twenty-one of this group were treated by splenectomy and 20 by various other methods. Twenty of the first group showed improvement with 1 death, while 13 of the second group showed no improvement and 1 died with only 2 showing complete arrest of the disease. In a later study, Elliott (12) presented a detailed follow-up of 90 cases, some of which had been followed

as long as 27 years. He found the disease to occur most frequently in the adolescent or young female, with 36 occurring in the pediatrics age group. It was his experience that the prognosis for the pediatric group was far more favorable than for the series as a whole. Sixty-two cases were operated without surgical mortality. The results are presented in tabular form below:

AGE	NUMBER UNSUCCESSFUL	PERCENT UNSUCCESSFUL	NUMBER SUCCESSFUL	PERCENT SUCCESSFUL	TOTAL
Before 31	2	4.9	39	95.1	41
After 31	12	57.1	9	42.9	21

Twenty-five of the 28 cases which were treated by non operative methods were evaluated with the following results:

		No. Cases	Per Cent Cases
SUCCESSFUL:	Complete arrest	2	8
	Marked benefit	7	28
	Total	9	36
UNSUCCESSFUL:	Dubious benefit	7	28
	Failure	9	36
	Total	16	64

A comparison of the figures for operative and non operative therapy reveals:

	NUMBER UNSUCCESSFUL	PERCENT UNSUCCESSFUL	NUMBER SUCCESSFUL	PERCENT SUCCESSFUL	TOTAL
With surgery	14	22.6	48	77.4	62
Without surgery	16	64.0	9	36.0	25

Thus, one may expect to at least markedly benefit about 75 per cent of all cases treated surgically and about 95 per cent of all treated surgically who are in the younger age group.

Many other reports confirm the findings of Elliott, although no other series has been followed as closely as his. Rosenthal (13) reported 153 cases with the same general results revealed in Elliott's series. S. L. Vaughn and Wright (14) followed a small group for from 10 to 15 years, all of whom remained symptom free, although some showed continuously low platelet counts.

THE MECHANISMS OF ESSENTIAL THROMBOCYTOPENIC PURPURA

Platelets have been thought to arise from (1) the plasma itself; (2) from the endothelium of the blood vessels; (3) from erythrocytes or their nuclei; (4) from the nuclei of degenerated leukocytes or the granules of eosinophiles; (5) from the lymph nodes; (6) in the spleen; or (7), from the megakaryocytes of the bone marrow. (15) Evidence is available to support only the last named theory.

MORPHOLOGY AND ORIGIN OF MEGAKARYOCYTES

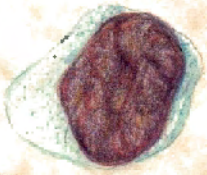
The megakaryocytes are always present among the free cells of the bone marrow. They are usually extremely large cells, measuring from 35.0 to 40 microns. The nucleus is enormous and may occupy the greater portion of the cell. It is multilobulated resembling that of a polymorphonuclear leukocyte but with much larger nuclear bridges. It stains a deep purple and is reticular

and contains a few small nucleoli. The cytoplasm is finely granular and platelets can be seen within it.

There are, according to current views, several alternative methods of origin of the megakaryocyte. It is generally accepted that the predominant mode of origin is from the pluripotential histiocyte (hemohistioblast) from which is evolved the stem cell (megakaryoblast). (16) The megakaryoblast is a large cell twice the size of a myeloblast. The cytoplasm is a deep blue and agranular. The nucleus is not lobulated and shows many nucleoli. These cells do not produce platelets. The promegakaryocyte is formed from the megakaryoblast. This cell is similar to the megakaryoblast except that platelets are seen in the cytoplasm which may or may not be granular. The promegakaryocyte develops into the so-called intermediate forms which are intermediate in size between the promegakaryocyte and the adult megakaryocyte. Their cytoplasms are highly granular and platelet formation may or may not be present. The intermediate forms develop into the adult megakaryocyte which has been described.

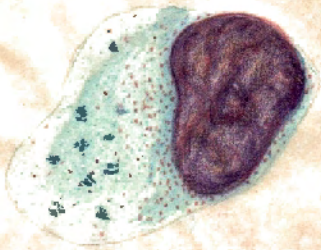
One alternative method of megakaryocyte development is through the formation of lymphoid megakaryocytes by the megakaryoblast. The lymphoid forms are large cells with basophilic non granular cytoplasms and a small distinctly lobulated nucleus.

Figure 1 demonstrates these two forms of megakaryocyte genesis.

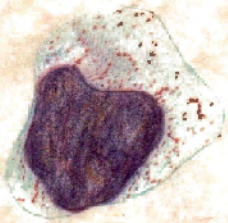


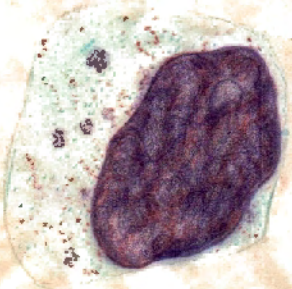
MEGAKARYOBLAST

LYMPHOID
MEGAKARYOCYTE

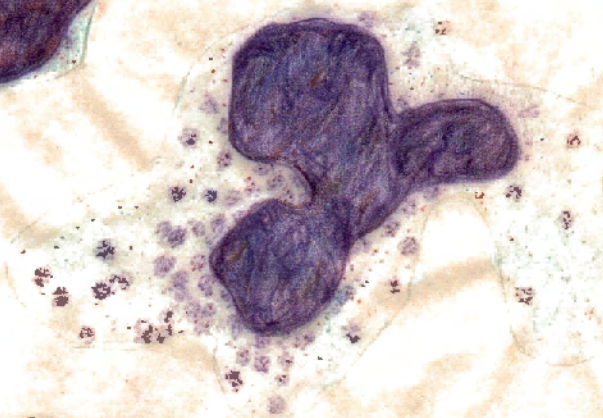


PROMEGAKARYOCYTE

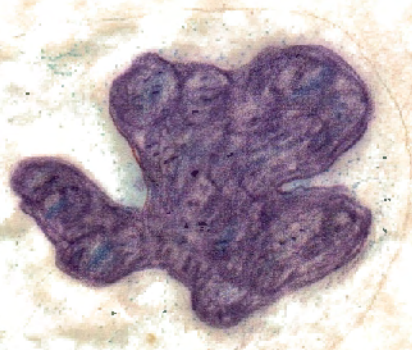




INTERMEDIATE FORMS



AOULT MEGAKARYOCYTE



DEGENERATED FORM

There has been much discussion in the literature in regard to the formation of megakaryocytes by fusion of many histoid cells to form a polykaryocyte and thence the megakaryocyte. This view was first advance by DiGuglielmo in Italy who worked with kittens. Rosenthal (17) repeated this work in this country and confirmed DiGuglielmo's findings but suggested that this phase of development is not found in adult mammals.

Figure 2 presents a diagramatic schema of the three methods of platelet production.

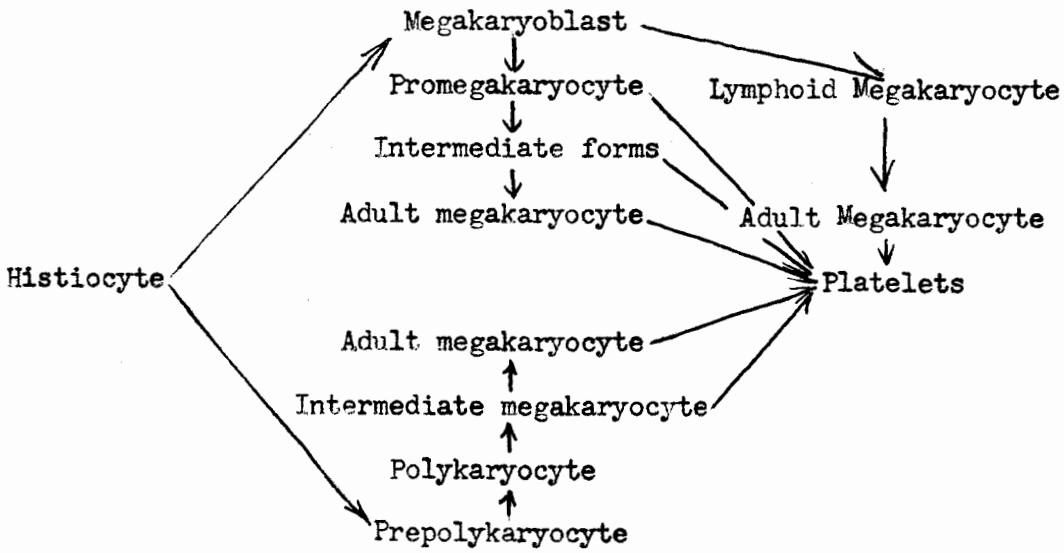


Figure 2 (after Dameshek) (10)

MEGAKARYOCYTES IN NORMAL STERNAL MARROW

Limarzi and Schleicher (18), Dameshek and Miller (10), and Valentine (19) have all studied the megakaryocytic elements in normal bone marrow. Their results are compared in the table

below. Because of the difficulty in accurately classifying many of the younger forms, the megakaryoblasts, promegakaryocytes, and the intermediate forms are classified as young forms, the adult and degenerated forms being recorded separately.

	TOTAL	PER CENT YOUNG	PER CENT ADULT	DEGENERATED
Limarzi & Schleicher	58.8*	24	48	28
Dameshek & Miller	182.5*	46	40.5	13
Valentine	____	24.7	75.3[1]	____

* per million nucleated cells.

[1] degenerated classified with adults.

Various descrepancies may be noted upon examination of the table above. As these three are the only attempts to establish normal differential values which have been published to date, the presence of such a wide range of values in the control series of these investigators means that we do not have a firm basis with which pathological specimens may be compared. Some observations were made in common, however. All three agree that megakaryoblast forms are rare, and that about two-thirds of all promegakaryocytes and adult megakaryocytes show platelets in their cytoplasms.

MEGAKARYOCYTES IN THE BONE MARROW FROM CASES OF ESSENTIAL THROMBOCYTOPENIC PURPURA

The same three investigators named above have published their findings in cases of essential thrombocytopenic purpura.

The table below compares these findings.

	TOTAL	PER CENT YOUNG	PER CENT ADULT	DEGENER-ATED
Limarzi & Schleicher	850.2*	43	36	21
Dameshek & Miller	523.7*	27.4	55.4	14.4
Valentine	____	26	74	7% of adults

* per million nucleated cells.

In these series the total megakaryocytes per million nucleated cells varied from three times greater than normal in Dameshek's series to fourteen times greater in Limarzi's series.

The young forms showed an increase of 19% over normal in Limarzi's group, a decrease of 18.6% in Dameshek's, and an increase of 1.3% in Valentine's group.

The adult forms showed a decrease of 12% below normal in Limarzi's series, and increase of 14.9% in Dameshek's, and a decrease of 1.3% in Valentine's group.

In each case the normal values which those from cases of essential thrombocytopenic purpura were compared was that established by author in question.

All observers agreed that a lesser number of megakaryocytes produced platelets, that there was an increase in number of megkaryocytes seen, and that there was no increase in the number of degenerated forms.

DISCUSSION

Splenectomy, as has been shown, fails as a therapeutic measure in about 25 per cent of the instances in which it is utilized. The purpose of this paper is to determine the causes of these failures and to determine the role which study of the bone marrow may play in bringing about a higher incidence of successful therapy. It is the author's opinion that there are two major causes of failure; incorrect diagnosis and the presence of accessory spleens.

The frequency with which accessory splenic tissue is found is greater than is ordinarily realized, and the existence of this tissue must be responsible for many of the failures of splenectomy. Maingot (20) found accessory spleens to be present in 7 of 13 operated cases. J. H. Vaughn (21) reported 17 per cent failures in 303 cases, and felt that the presence of accessory spleens was responsible for many of them. McLaughlin (22) states that accessory splenic tissue was found in approximately 20 per cent of his splenectomies.

Clinical determination of the presence of accessory splenic tissue is not possible at the present time. If further study should prove the specificity of bone marrow changes in essential thrombocytopenic purpura, bone marrow examination should reveal the effects of continued splenic influence on the marrow megakaryocytes. The only attempt to make this diagnosis by bone marrow study with which the author is familiar is a case of Schenken's (23) which has not been published. In this case the

patient failed to respond to splenectomy. Further marrow studies were carried out. The marrow revealed the presence of many megakaryocyte forms which were not producing platelets. The patient was explored surgically but no splenic tissue was found.

Analysis of Elliott's report reveals that 85.7 per cent of the failures occurred in patients over 31 years of age. This is the age group in which we see the highest incidence of those disease which are most apt to produce secondary thrombocytopenias and purpuric hemorrhages. It is felt that many incorrect diagnoses of essential thrombocytopenic purpura are made in this group when adequate diagnostic study would reveal the true etiology of the thrombocytopenia and the purpuric manifestations.

Wiseman, Doan, and Wilson (24) are of the opinion that meeting the following diagnostic criteria will greatly increase the accuracy of diagnosis of essential and secondary thrombocytopenic purpura: (1) spontaneous purpura must be present; (2) the platelet count must be below normal; (3) the clotting and prothrombin times must be normal and the bleeding time prolonged; (4) the anemia must not be out of proportion to the blood loss; (5) there must be no pathological white blood cells present in the circulating blood; (6) there must be no splenomegaly or unexplainable lymphadenopathy; (7) there must be no history of contact with toxic agents which are known to produce thrombocytopenia and purpuric hemorrhages, and (8) the bone marrow must be normal.

The diagnosis of the toxic secondary thrombocytopenic purpuras may be facilitated by marrow examination if Schwartz' (25) findings are verified by further study. He observed a great increase in the number of bone marrow eosinophiles in such cases.

Wiseman, Doan and Wilson examined the bone marrow before and after surgery in their series of cases and reported that there was no significant changes to be noted. As a result, they relegated bone marrow biopsy to the "negative side" in diagnosis, using it to rule out those causes of secondary thrombocytopenic purpura which can be diagnosed by this method. Valentine agrees in general with this opinion. In his series the marrow changes in proven cases of essential thrombocytopenic purpura were minimal and not, in his opinion, diagnostic.

Limarzi and Schleicher and Dameshek and Miller, on the other hand, feel that the bone marrow in essential thrombocytopenic purpura presents changes which are of diagnostic significance. Both groups of investigators report definite variations between their normal series and cases of essential thrombocytopenic purpura which they feel are significant. When their results are compared, however, they are found to be diametrically opposed. One reason for this variation may be found in Dameshek and Miller's statement to the effect that "the identification of megakaryocytes can be either very easy or quite difficult". Although this fact is no doubt operative in creating these discrepancies, the author

is of the opinion that the inherent technical difficulties met in performing total and differential megakaryocyte counts represent the greatest source of error leading to the production of these varying findings. Aspirated marrow was utilized by both groups, Dameshek and Miller using direct smears and Limarzi and Schleicher using smears made from a concentrated suspension of marrow cells. The total megakaryocyte count was expressed in terms of one million nucleated cells. It is impossible for methods based upon marrow aspiration to give satisfactory results because: (1) unavoidable dilution to varying degrees with sinusoidal blood; (2) irregular distribution of megakaryocytes on the slide, with concentration of the cells along the edge of the smear; (3) the nucleated cell count varies from patient to patient; (4) megakaryocyte values may vary independent of other cellular components of the marrow. The author feels that these technical factors preclude the use of marrow smears in determining the numerical megakaryocyte values. Various counting chamber methods have been used, but it is felt that these methods do not appreciably alter the factors enumerated above. It is the author's opinion that examination of the megakaryocytes for platelet production is the only procedure which might prove to be of value in light of our present day methods. Not all authors reviewed, however, agree that decreased platelet production by these cells is demonstrable. The eventual role of this procedure is in question at this time, but it may prove

to be of value in the light of subsequent investigation.

SUMMARY AND CONCLUSIONS

1. The literature of historical interest is reviewed.
2. Splenectomy is, without question, the treatment of choice in essential thrombocytopenic purpura.
3. The greatest incidence of failure of splenectomy occurs in the older age group. It is felt that this is because of faulty diagnosis of secondary thrombocytopenic purpura and the presence of accessory spleens.
4. The morphology and origin of the megakaryocytes are discussed.
5. Quantitative megakaryocyte studies by present methods are of no value as a diagnostic procedure.
6. Cytological study of the bone marrow is of questionable value in the diagnosis of essential thrombocytopenic purpura. It should be carried out in all cases, however, in order to rule out those causes of secondary thrombocytopenic purpura which can be recognized by this procedure.

ADDENDA

Since the preparation of this report, Berman, Axlerod, and Kumke (Am. J. Clin. Path. 18:898, 1948) have reported comparative studies of various methods of megakaryocyte counting. Their results bear out the comments upon this subject which are presented in this paper

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