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THROMBOCYTOPOIESIS IN MAN

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Introduction

The orderly delivery of platelets to the blood is dependent upon the integrity and normal functioning of the megakaryocytic series of cells[1,2] in man.* Normally the bulk of the megakaryocytes are found in the hematopoietic bone marrow. Examination of tissues of cases of "sudden death" by Smith and Butcher[3] revealed that megakaryocytes were located both intra—and extravascularly in bone marrow, spleen and lymph nodes. In the lung, liver, kidney, heart, adrenal, pituitary, cerebrum, cerebellum, pancreas, striated muscles and wall of the aorta the megakaryocytes were intravascular only. In the lungs about three fourths and in the spleen about half of the megakaryocytes were "naked nuclei" without cytoplasm. Although "naked nuclei" were not uncommon in kidney, liver or heart, adult megakaryocytes were unusually so situated. Howell[4] found extensive platelet formation from megakaryocytes in the pulmonary vessels of the dog.

The youngest member of the megakaryocytic series is termed the megakaryoblast,[5] which forms the promegakaryocyte,[5] which in turn forms the megakaryocyte[5] or platelet-forming cell. Ordinarily the megakaryoblast is derived from the myeloblast (hemocytoblast)[6,7,8,9] or from the reticulum cell (mesenchymal cell).[6,8,9,10,11]

Megakaryoblast (Fig. 1-4)

As observed in dry-fixed smears or imprints stained with Romanowsky methods (Wright, May-Grunwald Giemsa) this cell measures 15 to 50 mu which is larger than the myeloblast which it somewhat resembles. The nucleus is round, oval, reniform or indented, and centrally or eccentrically situated. This cell is rarely multinucleated. The nuclear membrane is thin. The chromatin pattern presents small chromatin particles arranged in a fine, sieve-like or regularly stippled pattern which stains somewhat more darkly than the myeloblast pattern. The parachromatin appears as abundant and distinct pink (not blue) granules in between the chromatin meshwork. Nucleoli may be 0 to 10 in number, are pale blue and well circumscribed when present.

The nuclear:cytoplasmic ratio favors the nucleus. The narrow band of cytoplasm is of varying shades of blue, usually zoned in lighter areas near the nucleus and darker more peripherally. In the area of diminished density (Fig. 4), as the megakaryoblast enlarges, a few azurophil granules first make their appearance. These cells do not produce platelets. Viewed with the phase microscope[12] numerous mitochondria occupied the perinuclear zone, the remainder of the cytoplasm was hyaline.

Promegakaryocyte (Fig. 5-7)

Under similar conditions as described for the megakaryoblast this cell measures from 20 to 80 mu and is thus larger than the megakaryoblast. The nucleus, too, is

*All workers cited in the bibliography subsequent to 1906, with the exception of Levy, adhere to this concept. All descriptions and references, unless otherwise stated, concern conditions in man.

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larger than that of its predecessor. The nucleus may be oval but polylobulation begins at this stage. (Fig. 6, 7) The nuclear membrane is still thin but the chromatin pattern stains more heavily than in the megakaryoblast. The regular, distinct network of chromatin remains but the chromatin threads are coarser and may show areas of clumping. The parachromatin shows as smaller pink areas between chromatin strands where they are not clumped. Nucleoli are usually less numerous than in the megakaryoblast but may still be very numerous as in fig. 6. During this stage, growth of cytoplasm proceeds and the nuclear:cytoplasmic ratio is reversed in favor of the latter. The "functional area" of granulopoiesis[13] grows rapidly with the cytoplasm until the entire cell body is filled with azurophilic granulation except for a narrow peripheral transparent blue border. The cytoplasmic background is blue in color but becomes paler blue as maturation approaches. Budding of small and large lobules from the convexity of the nucleus (Fig. 11) may occur. The heavily granulated larger promegakaryocytes have been termed *"Intermediate forms"* by Dameshek and Miller.[10]

An unusual, but normally occurring, variant of this stage possessing a relatively small, distinctly lobulated nucleus imbedded in deeply basophilic cytoplasm almost devoid of granules has been described by the latter authors[10] as *"lymphoid megakaryocyte."* (Their Fig. 6, 7)

Platelet formation may or may not be present at the periphery of the intermediate forms, when viewed with the phase microscope[14] agglutination of cytoplasmic granules formed units resembling granular platelets at the periphery of the cell.

Megakaryocyte (Fig. 8-10)

The megakaryocyte is the largest member of this series and measures from 30 to 160 u. The nucleus is large, polymorphous and complicated in outline. The chromatin strands are so thickened that they form heavy, dark-staining clumped masses with either a few small distinct areas of parachromatin or no parachromatin visible. The nuclear membrane is thick and nucleoli are not visible. The abundant cytoplasm is pale blue to pink in color. The full component of azurophilic granulation extends to the periphery of the cell (active form of Frey[15] and Schwarz[16]) where the granules gradually form groups surrounded by clear areas of cytoplasm. (Fig. 9) The cell body itself begins to show intracytoplasmic dissolution about granular groups. Pseudopodial processes frequently contain platelet-like aggregates of azurophil granules surrounded by clear areas of cytoplasm. For characteristics of the corresponding elements as observed in tissue sections the reader is referred to the excellent illustrations of Downey.[17] For comparison of the other cells described herein as observed in tissue sections please see our earlier report.[18]

Recently Schwarz[16] has deservedly directed attention to a normal variant of the megakaryocyte, the *reserve megakaryocyte* of Frey[15] and Downey.[7] Although similar in granular content to the active form, the reserve or resting cell is spheroid in outline and surrounded by a continuous hyaline envelope itself devoid of any structure or granulation. (cf. Schwarz,[16] Fig. 1-3)

Platelet Formation

Although the intermediate forms are capable of some platelet formation, the active form of the adult megakaryocyte furnishes most of the platelets to the blood. Two forms of platelet production are now recognized, the first or pseudopodial process is

best outlined by its discoverer.[2] "All of the blood platelets are detached portions or fragments of the cytoplasm of the megakaryocytes, which are in such relation to the blood channels in the marrow that detached portions of their cytoplasm are quickly carried by the blood current into the circulation. The breaking up of the cytoplasm into the platelets occurs only in cells which have reached a certain stage of growth and development, and is probably rapidly completed when once begun. It takes place in various ways but usually by the pinching off of small rounded projections or pseudopods from the cell body or from larger pseudopods, or by the segmentation of slender pseudopods, or by pinching off of longer or shorter pseudopods which may or may not undergo segmentation later. All, or most of the cytoplasm of the giant cell is given off to the blood stream and the nucleus degenerates. The more or less naked nucleus if often carried by the blood stream to the lungs where it lodges in the capillaries. Before the separation of a platelet takes place, the red to purple staining granules, in the portion of the cytoplasm which is to form the platelet, are separated from the rest by a zone of hyaline cytoplasm and arranged in a more or less sharply outlined, rounded, or oval mass. The line of cleavage is through this zone of hyaline cytoplasm and this sharply outlined mass of granules becomes the central granular mass of the blood platelet which has been regarded by some observers as a nucleus."

Although continuous observation of this process has not as yet been observed, Wright himself[2] noted in supravital preparations: "In this connection I may state that I have seen a few megakaryocytes change their form very markedly, sending out the withdrawing pseudopods, such as are seen in the sections. This seems to show that the presence of pseudopods and protoplasmic prolongations of megakaryocytes in blood vessels, as I have seen in the sections, is not a passive act, due to local conditions of pressure in the tissue, but is a manifestation of vital activity."

Under the phase microscope Bessis[12] found sperm-like cytoplasmic tongues at the cytoplasmic periphery, the tongues themselves were surmounted by long filamentous projections. (His Fig. 79, 80, 82) In unstained preparations under the dark-field, Saltzman[19] observed that amid all the bone marrow cells, fibrin accumulation occurred only about the megakaryocytes and platelets.

A second mode of platelet formation is one in which the platelets are set free by simultaneous disintegration of large portions of the entire cytoplasm. (Fig. 10) Sabin's supravital studies supported disintegration as a regular process of platelet formation, a process seriously entertained by Schwarz.[21] Recently Pisciotta, Stefanini and Dameshek,[14] utilizing the phase microscope for their studies, described fragmentation of the cell into platelet units by aggregation of the granules toward the periphery of the heavily granulated adult megakaryocytes. "At certain points on the surface, the cell membrane appeared ruptured with an outpouring of numerous granular aggregates or platelets." (Fig. 2, 3, 23) Downey and Nordland[7] observed platelet formation by multinucleated megakaryocytes which possessed small dark nuclei; a similar cell is shown in our fig. 12.

Degenerating Megakaryocytes

Having performed their platelet-producing functions, degeneration is the ultimate fate of the megakaryocytes. Limarzi and Schleicher[22] found 28% degenerating forms

in normal marrow, Japa[23] 36%. The latter author found signs of degeneration beginning at the 8-nuclei stage. The chromatin pattern is coarsened to the stage of pyknosis, pseudohyperlobulation or conversely lobular massing may ensue. The cytoplasm is usually lost to a deeply basophilic rim about the structureless nuclear mass; in the last stage only the naked nuclear remnants are seen. The degenerating megakaryocytes attract granular leukocytes,[24] a condition which may be mistaken for a phagocytic function on the part of the megakaryocyte.[25]

Cellular Division

The megakaryoblasts and promegakaryocytes undergo a type of cellular division in which the nuclei divide by mitosis (fig. 13) but cytoplasmic division is customarily lacking.[4,13,23,26] In addition amitotic forms have been described.[6,27] Schwarz[13] believed that mitoses ceased when the megakaryocyte contained its full complement of granules.[4] The resultant megakaryocytes may be polymorphonuclear and multinuclear cells.[10,26,28] Dameshek and Miller[10] and Epstein[28] believed that the polymorphonuclear forms predominated in normal marrows over the uncommon multinucleated megakaryocytes. (Fig. 14) Japa[23] presented experimental evidence for his concept that all megakaryocytes beyond the early megakaryoblast (1 nucleus) stage are in reality multinucleated. He described megakaryocytes with 2, 4, 8, 16 and 32 nuclei respectively. Instead of describing the process as pluripolar[21] or multipolar mitosis as in common usage he observed that each of the component nuclei formed a bipolar spindle synchronously. Most authors follow the broader views of Dameshek and Miller[10] and Epstein.[28]

The Polykaryocytes

When in 1890, Howell[29] differentiated the polymorphonuclear "megakaryocyte" from the other marrow giant cells, he designated the remaining multinucleated giant cells of the marrow as "polykaryocytes." Di Guglielmo[30] expressed the view that platelet-forming polykaryocytes arose from the fusion of primitive mononuclear cells. As we have seen above (Fig. 14) multinucleated megakaryocytes, although uncommon, may be found in normal marrows. Rather than arising by fusion of mononuclears Japa[23] has presented the alternative and more attractive explanation of their presence, that is the occurrence of nuclear division without cytoplasmic division and without subsequent fusion of the daughter nuclei. Previously we[18] have described, depicted and differentiated, both in tissue sections and marrow aspirates, the normal nonmegakaryocytic multinucleated giant cells of the marrow: osteoclasts, multinucleated plasma cells, and the rare multinucleated erythroblasts. Our fig. 15 again depicts an osteoclast from a marrow aspirate because it is this cell that is most frequently confused with the platelet-forming megakaryocyte and indeed with metastatic carcinoma. The many round or oval, monotonously patterned nuclei, all of a kind, resemble histiocytic or reticulum cell nuclei. One to three deeply basophilic nucleoli are invariably present amid a nuclear pattern of angular distinct chromatin particles irregularly distributed throughout the nucleus. The abundant cytoplasm is a mixture of blue and colorless components. Occasionally the cytoplasm contains small fine numerous or sparse angular large purple inclusions (bone salts).

Leukemic Megakaryocytes

In megakaryocytic leukemia it is possible to observe the bizarre megakaryocytic forms resulting from the leukemic process.[18,31] In figs. 16 and 17 we have reproduced

two luekemic megakaryocytes as seen in tissue sections from a previous report.[18] One can compare their structure with both the definitive megakaryocytes and the Reed-Sternberg cells of Hodgkin's disease. Their nuclei may undergo extreme hyperlobulation, with the formation of massive albeit vesicular nuclei, two or three giant lobes may contain large basophilic nucleoli more prominent than in the definitive megakaryocytic series, but never as large as in Reed-Sternberg cells. (cf. Fig. 18, 19) Recently Schwarz[16] has described abnormal, giant nucleolated megakaryocytes in chronic granulocytic leukemia terminating in a myeloblastic crisis. (cf. his Case 2)

Differentiation of Megakaryocytes from Reed-Sternberg Cells

In sectioned material, differentiation of these two series may pose a difficult problem. In aspirate material however (Fig. 18, 19) the two cell lines show marked nuclear disparity. The Reed-Sternberg cell has increasing giant, bizarre nuclear polylobulation which is characterized by retention of the fine reticular chromatin network with its chromatin-parachromatin distinction. The nucleoli which may reach diameters up to 7 mu are a deep blue color. The cytoplasm may be scant or abundant, is composed of blue and colorless components and is poor or lacking in azurophil granules. If aspirate material is not available for differentiation, Fisher and Hazard[32] have reported that the cytoplasm of the megakaryocyte is strongly positive for the periodic acid-Schiff method in contrast to the Reed-Sternberg cell in sectioned material.

Phagocytic Functions of Megakaryocytes

This subject has long been one of contention. Frank[33] described megakaryocytic erythrophagocytosis as well as phagocytosis of granular leukocytes. Downey, Palmer and Powell[6] and Schwarz[24] similarly described phagocytosis of granular leukocytes. Kabelitz[35] has emphasized that the younger megakaryocytes are especially phagocytic for leukocytes and other cells. Saltzman[25] in contrast has observed in supravital preparations migration of granular leukocytes to the megakaryocytes and occasional destruction of degenerating megakaryocytic forms by the leukocytes.

Time and Spatial Relationships of Megakaryocytes

Characteristically in cases of idiopathic thrombocytopenic purpura, thrombocytopoiesis on the part of the megakaryocytes comes to a standstill. Subsequent to splenectomy, the minimum time that elapsed until resumption of platelet formation by the adult megakaryocytes affords an important clue as to the time needed for the performance of this phase of thrombocytopoiesis. Dameshek and Miller[10] reported well-defined platelet production at the edges of the megakaryocytes within 24 hours. Valentine[35] depicted massive platelet production by mature megakaryocytes within 48 hours.

Downey and his associates[6] and Schwarz[16] have commented on the inferiority of extramedullary megakaryocytic platelet-production compared to intramedullary production.

Histochemical Studies of Megakaryocytes and a Comparison of Comparable Histochemical Studies in Platelets

Our table I outlines the histochemistry of the megakaryocytes and platelets as reported in the literature. All the tests listed were performed on human material except

those noted for RNA.[44] which were performed on the marrows of rhesus monkeys and guinea pigs. The importance of true polysaccharides in the metabolism of the megakaryocytes is attested to by the first 11 tests listed. Furthermore the phosphatases underline not only carbohydrate metabolism but are importanly related to nucleoprotein metabolism[43] as well. The presence of a positive plasmal reaction[39] indicates the presence of glyceryl-phosphoryl-ethanolamine and possible related compounds. The 13th to 17th tests are related to mitochondrial activity. Bloom and Wislocki[40] in commenting on the sudanophobia of human platelets suggested that the platelet mitochondria had become swollen and had lost some of their capacity to stain. The material submitted in table I strongly affirms the histochemical identity of megakaryocytic cytoplasm and platelets.

Megakaryocytic Nuclear Budding (Fig. 11)

Downey[17] observed in his early studies that the megakaryocytic nucleus gave off chromatic material to the cytoplasm and felt that this material was in some way associated with the development of cytoplasmic granules, Schwarz[21] felt that the bud-like excrescences of the nuclei were limited to seriously injured cells. Davidson and Smith[45] depicted a similar projection in neutrophilic leukocytes which was a manifestation of a structural difference in cells from the human female. Levy[26] proposed that disturbances of mitosis could lead to formation of small accessory nuclei which would have fewer chromosomes than the main nuclei.

Abnormalities in Thrombocytopoiesis in Pernicious Anemia

Although abnormalities in thrombocytopoiesis occur in many disorders of the marrow,[3,6,7,10,14,16,18,27,31,47,48] three types of such disturbances will be surveyed in this work because of the light they shed on the fundamental mechanisms of platelet formation. The first disorder to be considered is an investigation into the causes of the thrombocytopenia usually to be found in deficiencies of B_{12} leading to pernicious anemia. In the megakaryocytic series this deficiency leads primarily to disturbances of nucleoprotein synthesis. Gasper[46] noted that megakaryocytes were diminished in numbers and that their nuclei stained more intensely. Jones[47] confirmed these findings but in addition encountered pathologic megakaryocytes with intensely basophilic cytoplasm devoid of azurophilic granulation. Rohr[48] found similar cytoplasmic defects but was impressed by the marked nuclear segmentation. Paseyro[49] also described increased nuclear segmentation without separation. Japa[23] in contrast described an increase in his 2- and 4-nucleated stages and a marked decrease in megakaryocytes with 8 and 16 nuclei. Epstein[28] found 49.8 to 68.4% of the megakaryocytes in relapse to be multinucleated megakaryocytes (megakaryocytic polykaryocytes). After specific therapy only 0.0 to 11.2% of multinucleated megakaryocytes were present. In 4 out of 5 patients prior low platelet counts were increased and in 3 out of 5 patients remission brought about an increase in the number of megakaryocytes exhibiting apparent platelet formation. Pisciotta, Stefanini and Dameshek[14] using phase microscopy confirmed the earlier findings as seen with the light microscope. They commented on the "honeycomb" appearance of the nuclear chromatin characteristic of megaloblasts in some of the megakaryocytes before specific treatment was instituted. No platelet formation was found and the cytoplasm was either devoid of granules or granules were limited to the perinuclear area. In our own experience the basic defect is apparent in the disturbance of nuclear maturation. Hyperlobulation is present precociously in the pro-

megakaryocytic stage (Fig. 20); in addition there is abnormal retention of the fine, reticulated chromatin pattern of the nuclei even as late as the 8-nuclei stage. (Fig. 21) Excess number of multinucleated megakaryocytes as reported by Epstein[28] are present. (Fig. 21 and 22) Failure of normal nuclear growth and differentiation leads in turn to dissociation of development between nucleus and cytoplasm with subsequent inhibition of azurophilic granule formation, retention of primitive cytoplasmic basophilia and failure of platelet production.[50] Structural and functional integrity of the megakaryocytic system of cells has been accomplished within a week after institution of specific therapy.[14]

Abnormalities in Thrombocytopoiesis in Heat Stroke

Malamud and his associates[51] have uncovered an almost specific injury of the megakaryocytic series in heat stroke in man associated with temperatures above 106° F. Six hours after the onset of hyperpyrexia a reduction in numbers of megakaryocytes was visible. Severe damage of the nucleus was evidenced by pyknosis, karyorrhexis and disappearance of the nuclei before cytoplasmic dissolution.

Abnormalities in Thrombocytopoiesis in Idiopathic Thrombocytopenic Purpura

In idiopathic thrombocytopenic purpura (I.T.P.) of the chronic type megakaryocytes are usually paradoxically increased in numbers. Megakaryoblasts and promegakaryocytes are increased. Degenerated forms are increased but usually consist of degenerative changes in the afore-mentioned younger cells. Platelet productivity on the part of the megakaryocytes is significantly diminished. The numerous structural defects described in members of the megakaryocytic series of cells in I.T.P. can now be re-evaluated in the light of newer knowledge of the fundamental mechanisms of the disease by establishment of (a) the thrombocytopenic activity of plasma from patients with I.T.P. when injected into normal controls[52] and (b) megakaryocytic inhibition by potent platelet agglutinins in a patient with I.T.P. or induced in recipients with previously intact megakaryocytic systems by administration of such platelet agglutinins.[14,53]

Frank[33] early had described megakaryocytes in I.T.P. as possessing a hyoline cytoplasm, agranular and vacuolated. (cf. his Abt. 10, Fig. 3-6) These changes were reaffirmed by Limarzi and Schleicher[22] who reported in addition that many of the promegakaryocytes were forming nongranular pseudoplatelets. Dameshek and Miller[10] and Valentine[35] also described precocious nongranular platelet production by the promegakaryocytes but emphasized the marked reduction in platelet production from adult megakaryocytes. De La Fuente[27] after careful quantitation of this latter defect was able to state that in severe cases of I.T.P. mature or immature megakaryocytes containing more than 10 platelet units in their cell bodies were absent. Fig. 23 depicts a megakaryoblast in the marrow from a patient with I.T.P. The pseudopodial projections of the cytoplasm are a common finding in the increased numbers of this stage. The first azurophil granule have made their appearance near the nucleus. Fig. 24 is a promegakaryocyte from a similar patient. Azurophilic granulation is lacking. There is intense peripheral cytoplasmic vacuolation. Four large nongranular, vacuolated pseudopodia are present. Figs. 25 and 26 are adult megakaryocytes in the same condition. Fig. 25 shows unusual juxta-or intranuclear vacuolation. The large cell body is completely devoid of granules. An outer hyaline cytoplasmic zone is clearly

demarcated from the inner zone of abnormally deep basophilia, platelet formation is lacking. Fig. 26 depicts intense peripheral cytoplasmic vacuolation, presence of central azurophilic granulation, but the customary deletion of platelet productivity.

In the patient of Pisciotta and his associates[14] with a circulating high-titer platelet agglutinin the megakaryocytes showed generally intact nuclei, but there was pronounced vacuolization of the cytoplasm, with some cells showing peripheral "bare areas" and with little platelet formation in evidence. Two hours after the administration of the same plasma, the megakaryocytes of the recipients showed complete loss of platelet formation and some cells showed some loss of granule formation as well. Loss of granules was at times confined to the cell periphery. All megakaryocytes showed intense cytoplasmic vacuolation. Nuclear changes were minor. In such recipients the changes in the megakaryocytes were largely reversible. These findings suggest that in I.T.P. structural and functional defects in the megakaryocytes are brought about by direct injury to the cell. The increased proliferation of the younger forms would be compensatory in type. There remains unsettled the problem as to whether prolonged extrinsic injury of the cellular system ultimately can lead to intrinsic maturation defects. The demonstration of antibodies against nuclear materials, cytoplasmic organelles, and submicroscopic particulates in turn, as has been done for similar leukocytic components,[54] may render unnecessary further invocation of intrinsic maturation defects to explain megakaryocytic pathology in I.T.P.

Quantitative Studies of the Megakaryocytic System

Pizzolato[55] reviewed the literature of the many technics available up to 1948 for counting megakaryocytes. The results obtained varied greatly, chiefly because accurate total nucleated cell counts per unit of volume are not at present obtainable from the hematopoietic organs of man. Yoffey[56] has recently described a technic suitable for such quantitative total nucleated cell studies of the bone marrow in guinea pigs, but unfortunately this is not applicable to man. Semiquantitative methods in the hands of individual groups have shown a rewarding degree of reliability. Among such latter technics may be listed the thin-smear method of Dameshek and Miller,[10] the thick smear method of Osgood[57] and the various histologic section studies of Smith and Butcher[3] Berman[58] and Bowman and his associates.[59]

A Note on the Formation of Pathologic Platelets

From a consideration of the processes of normal and abnormal megakaryocytopoiesis as outlined in the preceding material, an understanding of many features of abnormal platelet formation can be obtained. Platelet release, before full granular aggregation has occurred, is the most common abnormality encountered and leads to the formation of large but otherwise unaffected platelets. Failure of regular hyalomeric cleavage may lead to release of pseudopodial forms in the case of pseudopodial production or to release of irregular undissociated platelet aggregates when production is by cytoplasmic disintegration. Nongranular platelets can be produced either by precocious production on the part of the megakaryoblasts and promegakaryocytes which cannot be expected to have attained their full component of granules or by pseduopodial formation on the part of older forms whose proper granulation has been inhibited or destroyed. Unduly basophilic nongranular forms with or without hyaline zones and vacuolation are the product of cells similar to those depicted in figs. 24 and 25. Platelet

release after complete granulation but before granular aggregation or hyalomeric cleavage (Fig. 12) can lead to either unusually large, completely granular, platelets or conversely very small ones, depending upon the size of the cytoplasmic fragments. For detailed descriptions and illustrations of these abnormal platelet products the reader is referred to work of Downey and Nordland.[7] Finally, the existence of normal platelet counts in man within fairly regularly assigned limits, implies the existence of a regulator or regulators determining megakaryocytic differentiation, maturation and platelet release.

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Table I

Histochemistry of Megakaryocytes and Platelets

	HISTOCHEMICAL TESTS																							
	Iodine	36	Best's Carmine	36	Bauer's	37, 38	Gomori	37, 38	Hotchkiss	36, 37, 38	Saliva + Hotchkiss	38	Sulfite blockade + Hotchkiss	38	Pyridine + Hotchkiss	38	Acetylation + Hotchkiss	38	Acetylation + KOH + Hotchkiss	38	Metachromasia	38	Plasmal reaction	39	Acid hematein	40
Megakaryocyte Cytoplasm	+		+		+		+		+		+		+		+		−		+		−		+		
Granules					+		+		+		−		+		+		−		+		−				+
Inclusions					+		+		+		−		+		+		−		+		−				
Nucleus																									−
Nucleoli																									
Platelets	+		+		+		+		+		−		+		+		−		+		−		+		+

	Pyridine Control	40	Sudan black B	40	Janus Green	40	Neutral Red	20, 40	Alkaline phosphatase	42, 43	Acid phosphatase	42, 43	RNA	44	Ribonuclease + RNA	44
Megakaryocyte Cytoplasm									±		+		+		−	
Granules	−		+		+		+									
Inclusions																
Nucleus	+								+		+					
Nucleoli									+		±					
Platelets	−		−		+		+		+				±			

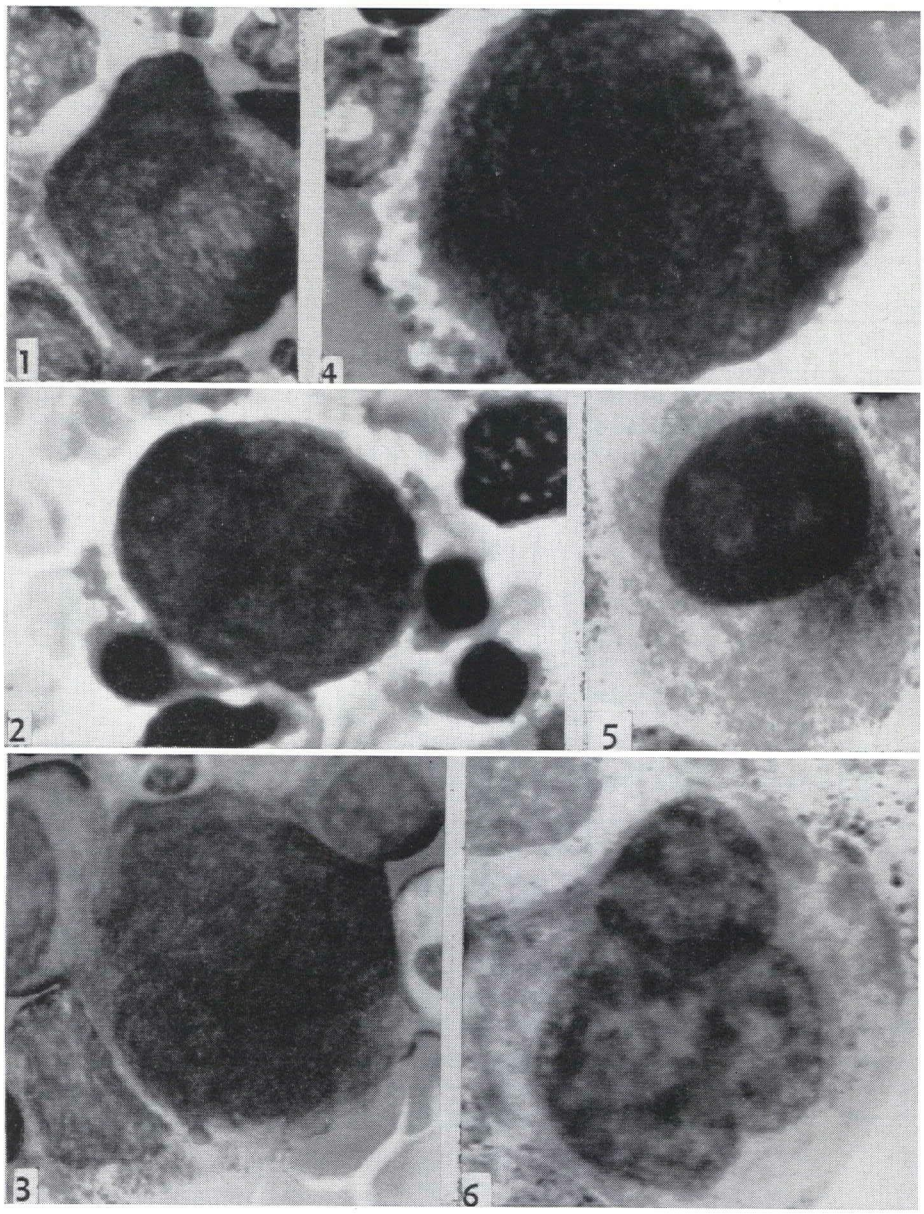


Fig. 1. Megakaryoblast; sternal bone marrow aspirate. May-Grunwald Giemsa stain. x 2000 AFIP Neg. 96993.

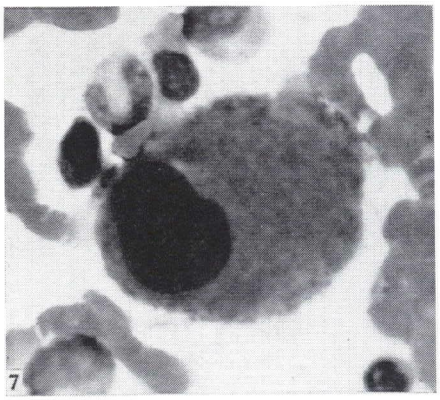
Fig. 2. Megakaryoblast; bone marrow aspirate. May-Grunwald Giemsa stain. x 1800.

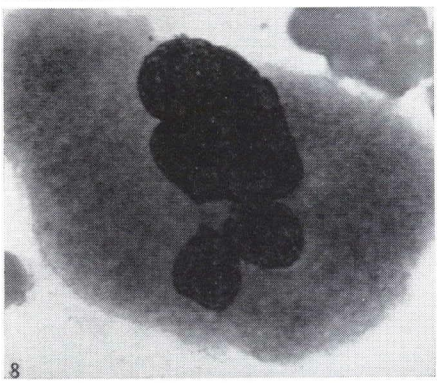
Fig. 3. Megakaryoblast; bone marrow aspirate. May-Grunwald Giemsa stain. x 1500.

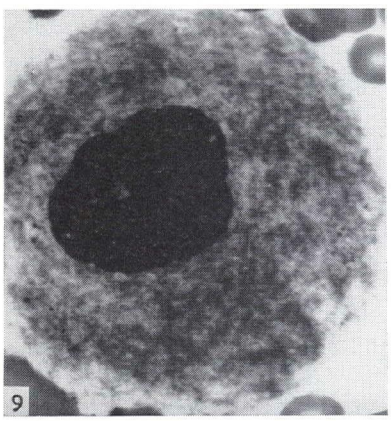
Fig. 4. Late megakaryoblast, granulation at right of nucleus; bone marrow aspirate. May-Grunwald Giemsa stain. x 1800.

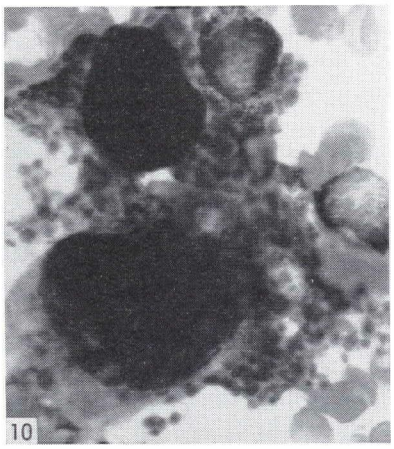
Fig. 5. Promegakaryocyte; sternal bone marrow aspirate. May-Grunwald Giemsa stain. x 2000. AFIP Neg. 97006.

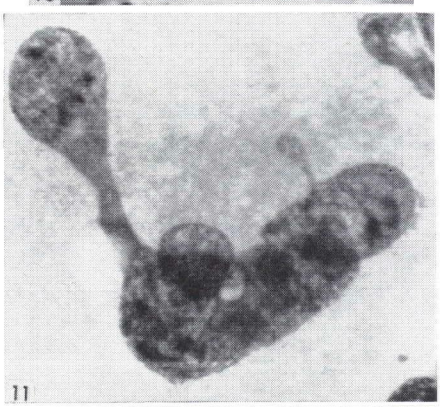
Fig. 6. Promegakaryocyte; sternal bone marrow aspirate. May-Grunwald Giemsa stain. x 2000. AFIP Neg. 97005.











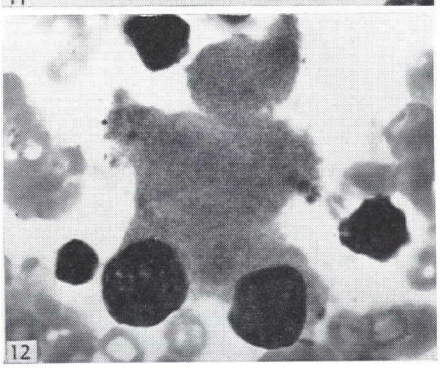


Fig. 7. Late promegakaryocyte (intermediate form). Bone marrow aspirate. May-Grunwald Giemsa stain. x 1500.

Fig. 8. Adult megakaryocyte; bone marrow aspirate. May-Grunwald Giemsa stain. x 1500.

Fig. 9. Mature megakaryocyte showing granular aggregation; sternal bone marrow aspirate. May-Grunwald Giemsa stain. x 2000. AFIP Neg. 98047.

Fig. 10. Megakaryocyte showing platelet formation; bone marrow aspirate. May-Grunwald Giemsa stain. x 1500.

Fig. 11. Megakaryocytic nuclear budding; bone marrow aspirate. May-Grunwald Giemsa stain. x 1800.

Fig. 12. Multinucleated megakaryocyte; bone marrow aspirate. May-Grunwald Giemsa stain. x 1500.

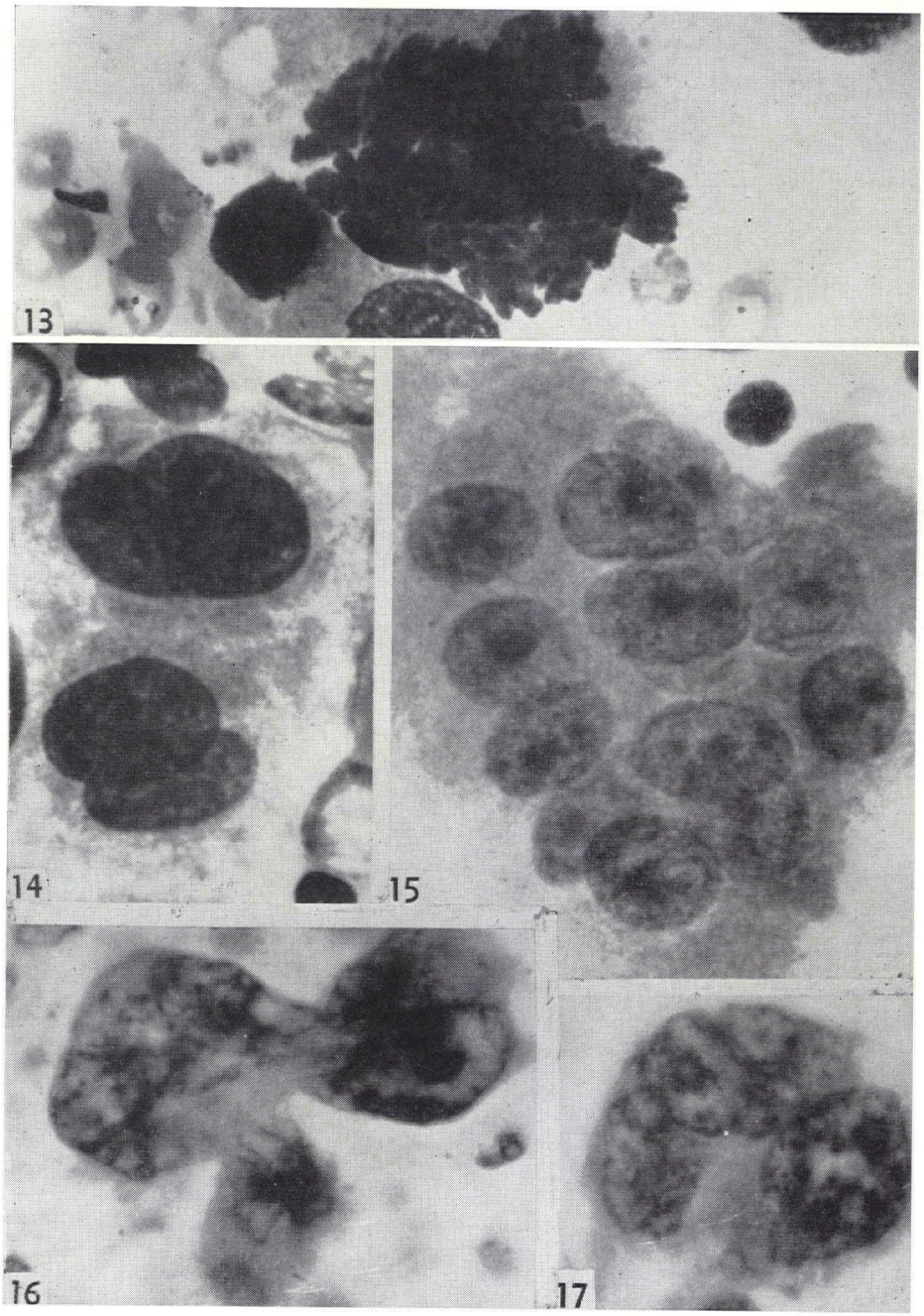


Fig. 13. Multipolar mitosis in developing megakaryocyte; bone marrow aspirate. May-Grunwald Giemsa stain. x 1550. Fig. 14. Multinucleated megakaryocyte; sternal bone marrow aspirate. May-Grunwald Giemsa stain. x 2000. AFIP Neg. 96991. Fig. 15. Osteoclast; sternal bone marrow aspirate. May-Grunwald Giemsa stain. x 2000. AFIP Neg. 98023. Fig. 16. Leukemic megakaryocyte. Section of spleen in megakaryocytic leukemia. Hematoxylin and eosin stain. x 2000. AFIP Neg. 97896. Fig. 17. Leukemic megakaryocyte. Section of bone marrow in megakaryocytic leukemia. Hematoxylin and eosin stain. x 2000. AFIP Neg. 97604.

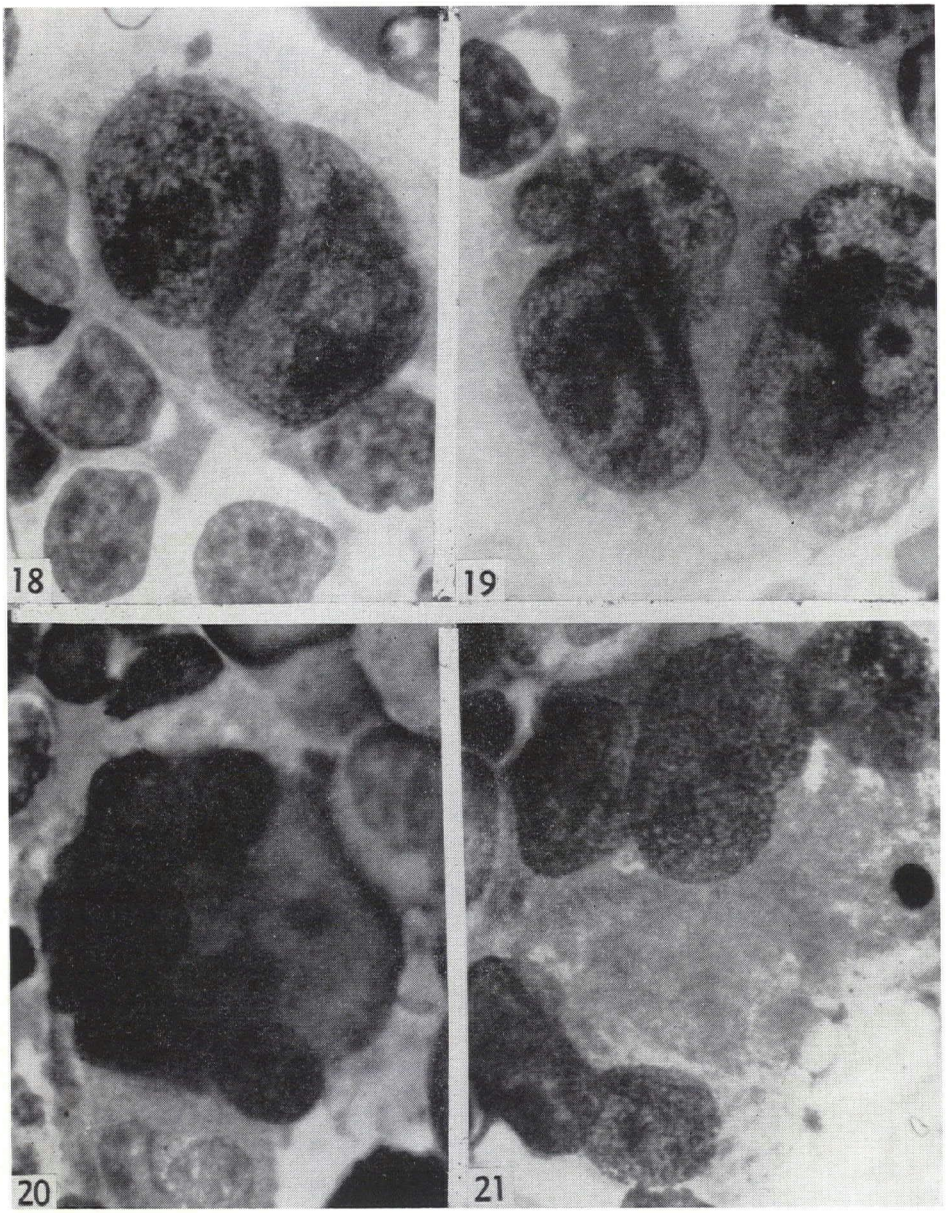


Fig. 18. Small Reed-Sternberg cell. Imprint of lymph node in Hodgkin's disease. Wright-Giemsa stain. x 2000. AFIP Neg. 98050.

Fig. 19. Reed-Sternberg cell. Imprint of lymph node in Hodgkin's disease. Wright-Giemsa stain. x 2000. AFIP Neg. 97901.

Fig. 20. Hyperlobulation of nucleus in promegakaryocyte of pernicious anemia; bone marrow aspirate. May-Grunwald Giemsa stain. x 1500.

Fig. 21. Failure of nuclear maturation in promegakaryocyte of pernicious anemia; bone marrow aspirate. May-Grunwald Giemsa stain. x 1550.

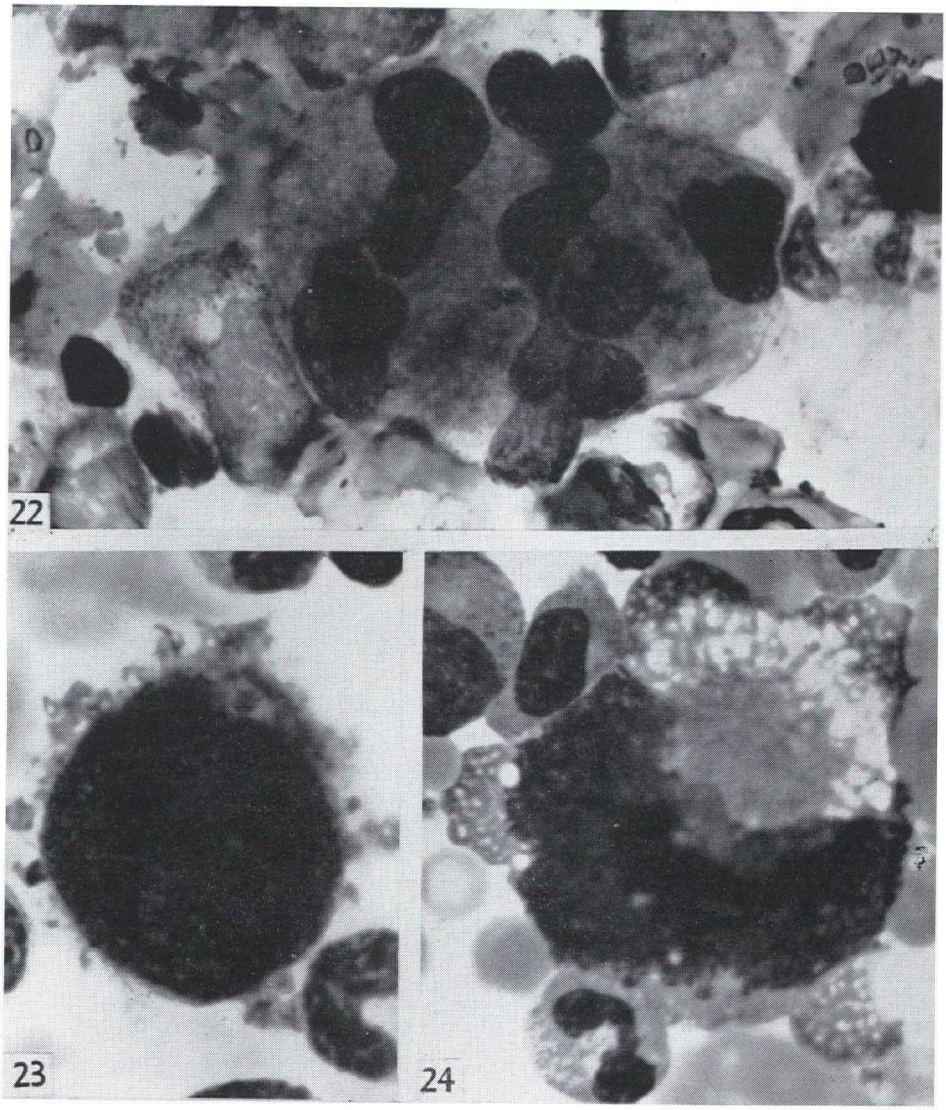
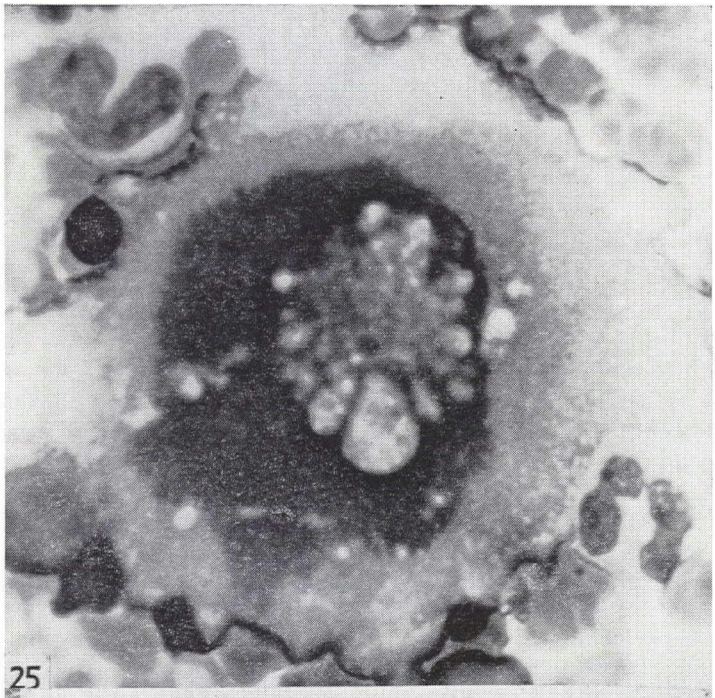


Fig. 22. Multinucleated megakaryocyte lacking platelet formation in pernicious anemia; bone marrow aspirate. May-Grunwald Giemsa stain. x 1550.

Fig. 23. Megakaryoblast showing precocious platelet formation in ITP; bone marrow aspirate. May-Grunwald Giemsa stain. x 1900.

Fig. 24. Promegakaryocyte, agranular with peripheral vacuolation in ITP; bone marrow aspirate. May-Grunwald Giemsa stain. x 1500.



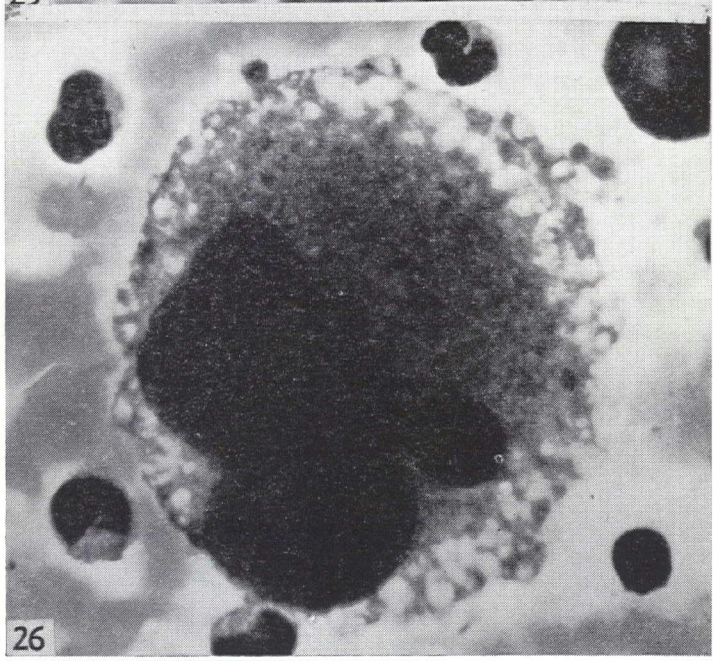


Fig. 25. Megakaryocyte, agranular with perinuclear vacuolation and lacking platelet formation in ITP; bone marrow aspirate. May-Grunwald Giemsa stain. x 1500.

Fig. 26. Megakaryocyte with peripheral vacuolation and lacking platelet formation in ITP; bone marrow aspirate. May-Grunwald Giemsa stain. x 1800.