Occurrence of Megakaryocytes in Various Vessels and Their Retention in the Pulmonary Capillaries in Man

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A total of 17 patients with hypertension undergoing renal vein or adrenal vein catheterization were investigated in order to ascertain the number of megakaryocytes in blood from the inferior vena cava, the femoral artery and a cubital vein. On an average 11.9, 3.8, and 4.5 megakaryocytes per ml were found, respectively. In blood from the inferior vena cava, 30% of the megakaryocytes had copious cytoplasm, while megakaryocytes in arterial and cubital venous blood had sparse or no visible cytoplasm. It was demonstrated that 2/3 of the megakaryocytes were retained in the pulmonary circulation and that at least 70% of the platelets could derive from megakaryocytes in central venous blood or the pulmonary circulation. It was found that megakaryocytes pass through a life cycle in which the differentiation take place in the bone marrow, platelet release occurs mainly in central venous blood and in the pulmonary circulation and the destruction of the megakaryocyte nucleus take place outside the bone marrow, especially in the pulmonary circulation.

Key words: blood – megakaryocyte – pulmonary capillaries

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As early as 1893 Aschoff observed the presence of megakaryocyte embolisms in the lung capillaries and a few megakaryocytes (MK) in organs such as the liver, kidneys, and heart in human autopsy material. Aschoff thought that MK were transported from the bone marrow to the lung capillaries via central venous blood and that the greater part of the MK were retained in the lung capillaries, only a few passing on to other organs. This MK circulation from bone marrow via central venous blood to the lung capillaries has been firmly established by experiments in dogs (Kaufman et al 1965) and rats (Tinggaard Pedersen 1971, 1974).

In human material a greater number of MK has been found in central venous blood than in arterial or peripheral venous blood. Results are highly variable, which may be due to differences in technique and material. Kaufman et al (1965) and Kallinikos-Mani-
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Atis (1969) employed heart catheterization on human patients with various cardiac defects, while Hume et al. (1964), Scheinin & Koivuniemi (1963) and Melamed et al. (1966) took blood samples for MK counts during surgery for various malignant conditions. Increased numbers of MK have been demonstrated in infectious and malignant conditions and after surgery by Scheinin & Koivuniemi (1963), Hume et al. (1964) and Breslow et al. (1968), while Jackson (1962) was unable to find greater numbers of MK in malignant conditions.

A systematic, comparative investigation of the number and morphology of circulating MK in blood from the inferior vena cava, the femoral artery and a cubital vein, respectively, in homogeneous patient material not exposed to surgery and in the absence of malignancy, has not been undertaken previously.

The aim of the present study has been to establish the quantity and morphology of MK in the inferior vena cava, femoral artery and cubital vein, respectively, in order to determine the capacity of the pulmonary circulation for retaining MK, and how much of the platelet production can occur outside the bone marrow. Further an attempt has been made to ascertain whether MK pass through the same life cycle as in the rat (Tinggaard Pedersen 1976), where MK originate and mature in the bone marrow, liberate platelets in the bone marrow, central venous blood and pulmonary circulation and, in the vast majority of cases, finally perish in the pulmonary circulation.

MATERIAL

The material comprised 16 patients referred to the Department of Clinical Physiology to undergo renal vein and one patient for adrenal vein catheterization. At the end of the diagnostic procedure the occasion was used to obtain simultaneous samples of blood from the inferior vena cava, femoral artery and a cubital vein for an MK count.

All patients were hypertensive and the large majority was in antihypertensive treatment. According to the records of the patients the values for thrombocytes and leucocytes were within normal range. There were 16 males and 1 female and the age was between 27 and 67 years. All blood samples were taken between 9 and 12 a.m.

METHOD

2.0 ml blood were taken from the inferior vena cava, the femoral artery and a cubital vein, respectively. Blood from the inferior vena cava was sampled through a catheter (Surgimed, DK-3650 Ølstykke, no. 08706252, length 70 cm, tubing 08) placed across the middle of the corpus of the 3rd lumbar vertebra identified radiologically. In pilot investigations no loss of MK could be established in this catheter. The blood from the femoral artery and the cubital vein was drawn off through a Stille® cannula (70 x 1.45 mm). Prior to sampling the catheters were flushed with heparin solution (25,000 IU per l), and 4 ml blood were drawn off in another syringe, before the 2.0 ml blood for counting were drawn off into a 5-ml plastic syringe, the inner side of which was coated with heparin solution (25,000 IU per l).

The blood was immediately transferred to siliconized centrifuge tubes (50 ml), containing ¾ ml 1 % EDTA in 0.9 % NaCl; 15 ml "working solution" (Tinggaard Pedersen 1971) were then added, and after about 2 min mixing, the red blood corporcles were haemolyzed by gradual addition of Saponin 1 % (Kaufman et al 1965). After haemolysis was complete (Mood translucent), 15 ml 4 % buffered neutral formalin were added and after 2 h fixation the solution was centrifuged at 200 g for 35 min. The sedimented cells were suspended in 15 ml 0.9 % saline, retained on Millipore® filters with a pore diameter of 5 μm, and stained with haematoxylin-eosin as previously described (Tinggaard Pedersen 1971). The Millipore filters were then systematically screened at 100 x magnification and all large cells evaluated at 400 x magnification.
RESULTS
Criteria for identification of MK were: large polylolular nucleus (larger than or equal to 20 μm), surrounded by eosinophilic cytoplasm, if present.
MK may be divided into two groups:
Group 1 comprising MK with copious cytoplasm (thrombocytogenic), which may be in the form of long processes, and Group 2 comprising MK with sparse or no visible cytoplasm (naked nuclei).

In the blood from the inferior vena cava 405 MK were found in 34 ml, equivalent to 11.9 ± 1.2 (SEM) MK per ml, with a range of 6–24 MK per ml; 30% of MK had copious cytoplasm and about 15% long cytoplasmic processes. The 95% confidence limit for MK concentration is thus 9.4–14.4 MK per ml blood.

In blood from the femoral artery 130 MK were found in 34 ml, equivalent to 3.8 ± 0.54 (SEM) MK per ml, with a range of 2–9.5 MK per ml. A single MK had so much cytoplasm that it was classified in Group 1. The 95% confidence limit for MK concentration is thus 2.7–5.0 MK per ml blood.

In blood from the cubital vein 152 MK were found in 34 ml, equivalent to 4.5 ± 0.56 (SEM) MK per ml, with a range of 2.5–8.5 MK per ml. A single MK had so much cytoplasm that it was classified in Group 1. The 95% confidence limit for MK concentration is thus 3.3–5.7 MK per ml blood.

No significant difference in numbers of MK could be demonstrated between blood from the cubital vein and blood from the femoral artery using Wilcoxon’s test for paired differences (P > 0.10). A significant difference in numbers of MK could be demonstrated between the inferior vena cava on the one hand and the femoral artery and cubital vein, respectively, on the other, using the Wilcoxon test for paired differences (P < 0.05).

A comparison between caval blood and arterial blood with respect to the number of MK present reveals that 21–56% of MK, mean 32%, can pass the pulmonary circulation in man and thereby recirculate; 30% of MK from the inferior vena cava had copious cytoplasm (Figure 4), and

![Figure 1. Histogram showing the number of megakaryocytes in 2 ml blood from the inferior vena cava, the femoral artery and a cubital vein, respectively. The hatched part of each column indicates MK with copious cytoplasm.](image)
Figure 2. Megakaryocyte with sparse cytoplasm located at the pole of the nucleus, cubital vein sample. (1500 x)

Figure 3. Megakaryocyte with sparse cytoplasm located at both poles of the nucleus, femoral artery sample. (1500 x)

Figures 4 and 5. Megakaryocytes with copious cytoplasm and a long flagelliform process, respectively, are sampled from the inferior vena cava. (1500 and 700 x)
with sparse cytoplasm pass through the lung capillaries, and can be demonstrated in arterial (Figure 3) and cubital venous blood (Figure 2). Only one MK with copious cytoplasm in 34 ml of arterial and peripheral venous blood, respectively, has been demonstrated, equivalent to 0.5–1 % of MK.

**DISCUSSION**

In human material on an average, 11.9 MK per ml blood from the inferior vena cava and 3.8 and 4.5 MK per ml blood from the femoral artery and the cubital vein, respectively, have been found; 30 % of MK from the inferior vena cava possessed copious cytoplasm, half of which was in the form of long cytoplasmic processes, while MK from arterial and peripheral venous blood were naked nuclei or possessed only a thin border of cytoplasm. No significant difference could be demonstrated between cubital venous blood and arterial blood with respect to numbers of MK, whereas there was a significant difference in the MK count between the inferior vena cava and the arterial and cubital venous blood, respectively. Thus there is a great loss of MK in the pulmonary capillaries.

Kaufman et al (1965) and Kallinikos-Maniatis (1969) have in studies involving heart catheterization of patients with various cardiac defects found 2.4 MK and 1.8 MK per ml blood, respectively, from the right atrium, about 50 % of which had cytoplasm. The higher MK count in the inferior vena cava than in the right atrium may be due to the fact that blood from the whole organism is pooled in the right atrium. But it may also be due to the fact that the patients subjected to heart catheterization had cardiac defects with left-right shunt and other turbulence of the blood, resulting in a depression of the MK count in the right atrium. Detailed specification of the catheters used is not given in these papers.

It was found in the present investigation that the pulmonary capillaries in man retained about two thirds of the circulating MK: MK release their cytoplasm in the pulmonary circulation to form blood platelets and can only be detected in arterial blood as naked MK nuclei or MK with a thin border of cytoplasm (nonthrombocytogenic). The investigations in rats support that MK nuclei perish in the lung circulation and the MK cytoplasm is fragmented to platelets. Tinggaard Pedersen (1975) found that the pulmonary capillaries in rats were a more effective filter for incoming MK, because almost all of the MK were retained, and there was a higher number of platelets in blood from aorta than in blood from the inferior vena cava. The occurrence of a few naked MK in capillaries in various organs in man must therefore be regarded as without pathological significance.

Kallinikos-Maniatis (1969) found about six times as many MK in central venous blood as in arterial blood; Scheinin & Koivuniemi (1963) and Melamed et al (1966) found from three to five times as many MK in central venous blood as in blood from the pulmonary veins in patients subjected to thoracotomy for malignant pulmonary disease. There is thus good agreement between the literature and the present investigation with respect to retention of MK in the pulmonary circulation.

In an earlier investigation Hansen & Tinggaard Pedersen (1978) found 5.8 MK per ml blood from the cubital vein in healthy individuals as compared to 4.5 MK...
per ml in the present case. Statistically no significant difference could be demonstrated between the two values at the 95% significance level. The present MK values can therefore be equated with MK values for healthy persons.

In peripheral venous blood, the MK were almost exclusively naked or had merely a thin border of cytoplasm, only 0.5–1% having copious cytoplasm. This is in accordance with observations of Herbeuval et al. (1962a), Kallinikos-Maniatis (1969) and Hansen & Tinggaard Pedersen (1978). The presence of more than 1% MK with copious cytoplasm in peripheral venous blood is taken as an expression of a pathological condition (Herbeuval et al. 1962b, Guerci 1965).

Calculation of extramedullary platelet production

If the number of MK in the inferior vena cava is representative for the pooled venous blood in the organism and the number of MK in the arterial blood is a figure for the passage through the pulmonary capillaries, an estimation based on these values can give an assumption for how great a part of platelet production taking place in the central venous blood and pulmonary circulation. The flow through the heart is about 5,000 ml blood/min in man. To the lung circulation is then carried ((11.9–3.9) × 5,000) ~ 40,000 new MK per min, of which about 18,000 MK had copious cytoplasm (thrombocytogenic). If each MK can form about 4,000 platelets, and the platelet requirement per min is 1 × 10⁸ (Kaufman et al 1965), 7.2 × 10⁷ platelets per min, equivalent to about 70% of the requirement, can be formed by MK on their way to or in the pulmonary capillaries. It is possible that some of the MK have given off cytoplasm to form platelets before they reach the place for blood sampling in the inferior vena cava, so it may be concluded that at least 70%, perhaps 100%, of the blood platelet production in man takes place in central venous blood and in the pulmonary circulation. Kaufman et al (1965) found that 7–17% of platelet production was extramedullary. The present investigation, which shows an entry of about 40,000 MK to the pulmonary circulation per min and, when related to the requirement for the maintenance of platelet production of about 25,000 MK, suggests that all MK nuclei could be destroyed outside the red bone marrow, while Kaufman et al (1965) found that only 20–50% pass out into the bloodstream. The present findings are supported by the fact that only a few naked MK can be demonstrated in the red bone marrow.

It can thus be concluded that in man MK pass through a life cycle similar to that in the rat (Tinggaard Pedersen 1976) where MK are formed and mature in the bone marrow, blood platelet production occurs mainly in central venous blood and in the pulmonary circulation, and the MK nucleus is destroyed outside the bone marrow, especially in the pulmonary circulation.

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