

ACTA STEREOLOGICA 1983; 2: 413-417
STEREOLOGY AND MORPHOMETRY IN PATHOLOGY
Proc 2nd Symp Morphometry in Morphol Diagnosis
Collan Y. et al., eds.
Kuopio University Press, Kuopio 1983

MORPHOMETRIC AND STEREOLOGIC STUDIES ON BLOOD PLATELETS FROM
FIVE DIFFERENT MAMMALIAN SPECIES §

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ABSTRACT

The present paper deals with the stereologic processing of intrathrombocytic localized, electronmicroscopically discernible alpha-granules, dense bodies (DB) and mitochondria, and their distribution in platelets from five species including man, dog, pig, sheep, and cat. It is pointed out that volume-, surface-, and numerical density of the three cytoplasmic organelles show a remarkable variation among the various mammals studied. In earlier investigations differences were also found for the same species concerning their functional thrombocytic behaviour. By means of the DB - i.e. specific platelet storage sites of the non-metabolic adenine nucleotides - it is indicated exemplarily that simple relations cannot be established between the stereology of these organelles and the cellular reactivity upon exogenous stimulation in vitro.

INTRODUCTION

The fundamental involvement of blood platelets in diseases of the blood circulatory system is largely accepted. In the civilized world more than 50% of all cases of death are directly a result of circulatory disturbances, the vast majority of which is caused by thrombotic and thromboembolic events. Therefore it is small wonder that many clinicians and pharmacologists make every effort to detect and develop substances which are capable to control the thrombocytic function. Usually, the typical experimental animals are employed in preclinical tests of such drugs. In

the past years, however, it was emphasized repeatedly that a direct extrapolation of observations on the platelets from one mammal to the other is not allowed without strong reservations (e.g. Addonizio et al., 1978; Grabowski et al., 1977; Hawkey, 1977). Earlier investigations on the in-vitro reactivity also have revealed considerable differences existing between the blood platelets from several mammals (Tosch and Benner, 1979). The present study was designed to analyze and quantitate the ultrastructural composition of the blood platelets from these species and to compare these data with the functional results of the study quoted.

MATERIALS AND METHODS

For ultrastructural morphometry and stereology of the platelet organelles - alpha-granules, dense bodies (DB), and mitochondria - the blood samples from man (n=8), dog (n=5), pig (n=4), sheep (n=3), and cat (n=2) were stabilized immediately after their withdrawal and prepared following the method of Stockinger et al. (1969). 5 specimens randomly were chosen out of a total number of 10 embedded blocks from each individuum. 10 sections were selected from each of these 5 blocks and electronmicrographs of random platelet fields were taken at a magnification of 28,500 x. The subcellular structures were measured planimetrically on a test area of 8.16 cm² - corresponding to a true platelet volume of 1 μm³ - using a manual-optic-picture system (MOP AM-02; Kontron, Eching, FRG). The primary values were stored on-line with a desk top computer (HP 9835; Hewlett-Packard, Frankfurt/M., FRG) for stereological and statistical processing (e.g. analysis of variance). The distributions of the total platelet section areas studied were found not to differ statistically significant from the normal distribution (p>0.05; Kolmogoroff-Smirnoff test). The three stereological parameter: volume-, surface-, and numerical density (Vv, Sv, Nv) were calculated for the platelet organelles with the formulae reviewed by Weibel (1969).

RESULTS AND DISCUSSION

Figures 1 to 3 give the stereological results obtained on the three platelet organelles from the five mammals. Obviously there are reasonable discrepancies existing interindividually as well as between the five species; this is true for all cellular organelles investigated. The highest

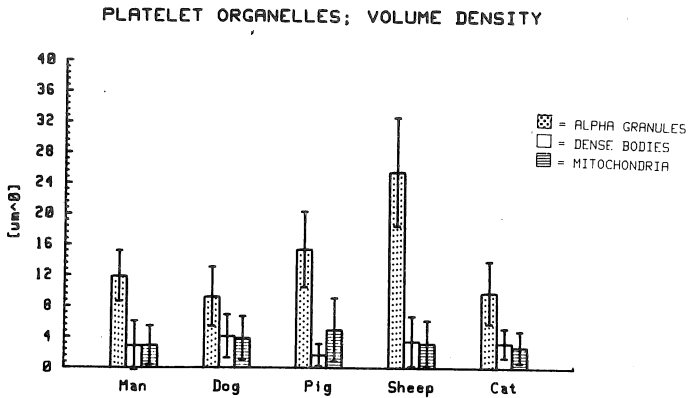


Fig 1. Comparative calculations of the volume density of platelet organelles; mean values and s.d..The primary planimetric data were harvested from electronmicrographs (magn. x 28,500) of platelet samples derived from five mammalian species.

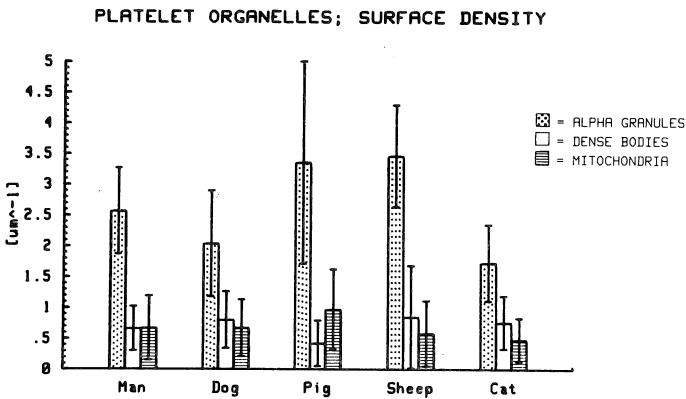


Fig 2. Comparative calculations of the surface density of platelet organelles in five different species (see Fig 1.).

Vv- and Sv-values e.g. of alpha-granules ($25.7 \pm 7.1 \mu\text{m}^0$; $3.5 \pm 0.8 \mu\text{m}^{-1}$) were observed in sheep platelets, maximum Nv-values ($12.3 \pm 9.8 \mu\text{m}^{-3}$) for the same structure, however, were found in human thrombocytes. In contrast, feline

platelets exert minimum values for Vv ($9.8 \pm 4.1 \mu\text{m}^0$), Sv ($1.7 \pm 0.6 \mu\text{m}^{-1}$), and Nv ($3.4 \pm 1.7 \mu\text{m}^{-3}$). Since the alpha-granules are assumed to be storage sites

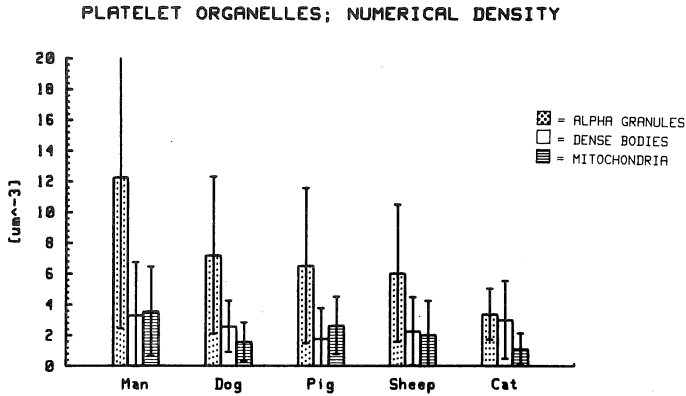


Fig 3. Comparative calculations of the numerical density of platelet organelles in five different species (see Fig 1.).

for platelet specific proteins and lysosomal enzymes (among others) one could suggest that human and sheep platelets contain relatively high levels and feline platelets the lowest concentrations of those substances. (That this conclusion cannot be drawn easily is subject of a second contribution).

Table 1 shows a comparison between the results from stereologic calculations and functional thrombocytic studies. Stereology was applied on the DB, the storage sites of the non-metabolic adenine nucleotides; aggregability as an indicator for in-vitro platelet function was tested by the addition of ADP ($17 \mu\text{mol/l}$) as well as collagen, adrenaline, and serotonin to samples of platelet-rich-plasma (PRP). The extent of aggregation response was registered nephelometrically and in the table is given as maximum transmission value (Tmax) recorded 5 min after the addition of ADP to the agitated PRP.

Again remarkable differences in the geometry of DB are obvious to exist among the mammalian platelets; discrepancies are also recorded in the functional tests. However, stereological and functional data show no evident correlation, no matter whether thrombocytic reactivity is

Table 1. Comparison between the results of the stereological evaluations (volume-, surface-, and numerical density) of platelet dense bodies (DB) and functional data obtained with platelets from five different mammals. Platelet function was tested nephelometrically; Tmax=maximum light transmission recorded in a platelet-rich-plasma sample after the addition of ADP.

SPECIES	Vv DB (μm^0)		Sv DB (μm^{-1})		Nv DB (μm^{-3})		Tmax (%)
man	2.9+/-3.2		0.7+/-0.7		3.3+/-3.4		89
dog	4.1	2.8	0.8	0.5	2.6	1.7	58
pig	1.7	1.4	0.4	0.4	1.8	2.0	12
sheep	3.5	3.2	0.9	0.8	2.3	2.2	57
cat	3.3	1.9	0.8	0.4	3.0	2.6	17

faced with the volume fraction of alpha-granules, DB, or mitochondria. In other words, the functional status of platelets cannot be predicted on the basis of electronmicroscopically visuable (and discernible?) storage granules alone.

REFERENCES

- Addonizio VP, Edmunds LH, Colman RW. The function of monkey (*M. mulatta*) platelets compared to platelets of pig, sheep, and man. *J Lab Clin Med* 1978;91:989-997.
- Grabowski EF, Didisheim P, Lewis JC, Franta JT, Stropp JQ. Platelet adhesion to foreign surfaces under controlled conditions of whole blood flow: human vs rabbit, dog, calf, sheep, pig, macaque, and baboon. *Trans Am Soc Artif Intern Organs* 1977;23:141-149.
- Hawkey C. The importance of comparative studies. *Thrombos Haemostas* 1977;38:395-397.
- Tosch U, Benner KU. Comparative nephelometric aggregation studies with platelets from various species. *Microvasc Res* 1979;17:S50.
- Weibel ER. Stereological principles for morphometry in electron microscopic cytology. *Int Rev Cytol* 1969; 26:235-302.

§ supported by a grant from the Deutsche Forschungsgemeinschaft (Be 625/5)