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EVIDENCE FOR A CORRELATION BETWEEN THE NUMBER OF MARGINAL BAND MICROTUBULES AND THE SIZE OF VERTEBRATE ERYTHROCYTES

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SUMMARY

In 23 species of vertebrates the dimensions of erythrocytes and the number of their marginal band microtubules were examined. A positive correlation was found between the size of erythrocytes and the number of microtubules. The absence of microtubules in diskoid erythrocytes of mammals – Camelidae – is discussed.

INTRODUCTION

The erythrocytes of non-mammalian vertebrates are nucleated, ellipsoidal and biconvex. Among mammals only Camelidae have been described as possessing ellipsoidal erythrocytes. A constant feature of ellipsoidal red blood cells is their 'marginal band', first described by Ranvier (1875). Meves (1904, 1911) reported that the marginal band of *Salamandra* erythrocytes was composed of fibrillar subunits and suggested that it was elastic and could be involved in the maintenance of the diskoidal and biconvex shape of the cell. Electron-microscopic investigations confirmed the fibrillar nature of the marginal band (Fawcett & Witebsky, 1964; Maser & Philpott, 1964; Sekhon & Beams, 1969; Weinreb & Weinreb, 1965), which is composed of microtubules encircling the cell just inside the plasma membrane and lying in a plane parallel to the flat surface of the cell. If Meves' hypothesis is right, there should be a correlation between the size of cell and the number of marginal band microtubules, since bigger erythrocytes would need a thicker marginal band (i.e. more microtubules) to maintain their shape, than smaller ones. The studies of this point have so far not yielded unequivocal results: Maser & Philpott (1964) have not found any correlation, whereas recent observations (Goniakowska-Witalińska, 1974) suggest that such a correlation might in fact exist.

In order to clarify the problem, an attempt has been made to compare erythrocytes of 23 various species of vertebrates.

MATERIALS AND METHODS

Adult males of 23 vertebrate species were used for the experiments. All the poikilothermic specimens were kept under natural conditions at room temperature (about 20 °C) for a few days. Blood was collected from subcutaneous vessels (camel, llama, crocodile) or directly from the heart, mixed with sodium citrate and centrifuged 3 times at 12 **g**. Erythrocytes were resuspended in the appropriate isotonic physiological saline. All erythrocytes studied have an oval shape. For each species the long and short axes (length and width) of 100 mature erythrocytes were measured using a micrometer eyepiece. If specimens possessed erythrocytes of different sizes, the ranges were determined, and not the average dimensions.

For electron-microscopic observations, small blocks of various tissues (lung, gills, liver) or the blood itself were fixed at 20 °C in 2 % glutaraldehyde buffered to pH 7·4 with 0·1 M Na-cacodylate, postfixed in osmium tetroxide, dehydrated in ethanol and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate. The number of microtubules was estimated by counting the circular profiles in cross-sections of 50 mature erythrocytes of each species.

RESULTS AND DISCUSSION

The results obtained in the present study as well as those reported by other authors are summarized in Table 1. The available data confirm the existence of a close correlation between the length of erythrocytes and the number of the marginal band microtubules (Fig. 1).

The largest erythrocytes are found in the lungfishes and some tailed amphibians (*Amphiuma*, *Necturus*, *Proteus*), whereas the smallest ones occur in teleost fishes, birds and mammals. The middle-size class includes elasmobranch fishes, anurans and reptiles. Of the species examined the erythrocytes of *Lepidosiren paradoxa*, a lungfish, are the largest and contain the greatest number of microtubules: 330–427. Other large red blood cells, e.g. those of *Salamandra salamandra* and *Triturus alpestris* have approximately 200 and 90 microtubules respectively. On the other hand, the smallest erythrocytes (fishes: *Cyprinus carpio*, *Esox lucius*; and birds: *Passer montanus* and *Columba livia*) possess a marginal band composed of only 5–12 microtubules.

The question of the occurrence of microtubules in red blood cells of mammals (family Camelidae) seems controversial, since Barclay, (1966) reported their presence in camel red blood cells without providing photographic documentation or any data concerning the number of microtubules. Our investigations do not confirm Barclay's results. After examining over 2000 camel and llama erythrocytes we found a few barely visible microtubules in only 3 cells. It is relevant here to note that Yamamoto & Iuchi (1975) found a reduction in the number of microtubules in *Salmo* from 70 to 20 during erythrocyte maturation. Barrett & Dawson (1974) observed the same process during erythropoiesis in the chicken. Furthermore, they reported that destruction of the marginal band in young erythrocytes of chicken caused a change of cell shape from diskoidal to spheroid, whereas in mature red blood cells it had no effect (Barrett & Dawson, 1974; Behnke, 1970). Barrett & Dawson conclude that 'microtubules play little or no role in the maintenance of mature red blood cell shape, however, they play an important role in the development of flat diskoid shape of avian erythrocytes during maturation'.

Table 1. *The average or range of dimensions of erythrocytes and the range of the number of microtubules in the marginal band*

Species	Dimensions of erythrocyte, length × width, μm	Range of no. of microtubules
Agnatha		
Eptatretus stoutti	26·4 × 18·3* (Wintrobe, 1934)	30–35 (Sekhon & Maxwell, 1970)
Pisces		
Carassius auratus		6–10 (Weinreb & Weinreb, 1965)
Cyprinus carpio	9·2 × 6·2 – 13·2 × 6·9	8–11
Fundulus heteroclitus	9·7 × 7·2 (Srivastava & Griffith, 1974)	5–6 (Maser & Philpott, 1964)
Esox lucius	11·8 × 6·5	5–7
Gasterosteus aculeatus	13·4 × 7·5	8–10
Salmo gardnerii irideus	16·0 × 10·0	30–40 (Sekhon & Beams, 1969)
		20 (Yamamoto & Iuchi, 1975)
Perca fluviatilis	16·4 × 9·0	10–15
Opsanus tau		24–28 (Fawcett & Witebsky, 1964)
Misgurnus fossilis	17·8 × 9·9	11–16
Lepidosiren paradoxa	45·5 × 28·0 (Gulliver, 1875)	330–427
Amphibia		
Hyla arborea	20·6 × 15·1	35–40
Rana esculenta	25·7 × 14·6	38–55
R. temporaria	24·6 × 16·9	34–40
Bombina variegata	29·0 × 18·5	56–62
Triturus alpestris	38·3 × 21·5	90 (Goniakowska, 1973)
T. viridescens		35–125 (Wolfe, 1967)
T. cristatus	39·3 × 22·3	93–157 (Bertolini & Monaco, 1976)
Salamandra salamandra	41·6 × 26·9	200 (Goniakowska-Witalińska, 1974)
Reptilia		
Lacerta vivipara	16·7 × 11·6 – 18·9 × 10·8	22–32
L. muralis	19·5 × 10·1	21–35
Natrix natrix	19·5 × 11·2	22–35
Gymnodactylus kotschyi	17·8 × 9·0 – 22·0 × 9·5	22–36
Pseudemys scripta elegans	20·9 × 12·3	23–38
Caiman crocodilus crocodilus	22·1 × 12·0 – 27·2 × 13·7	25–50
Aves		
Passer montanus	11·9 × 7·3	5–6
Columba livia	11·4 × 6·7 – 15·2 × 6·1	5–12
Gallus domesticus	15·5 × 7·7	10 (Barrett & Dawson, 1974)
		10–16
Mammalia		
Camelus dromedarius	8·4 × 4·8	? (Barclay, 1966)
Lama glama	8·6 × 4·9	?

* Of Myxine glutinosa.

It has been found that in llama red blood cells, microtubules do occur in the course of the erythropoiesis but only temporarily (Goniakowska-Witalińska & Witaliński in preparation). Therefore it is tempting to suggest that they induce the oval shape of the cell at the time of their occurrence. From that point of view the physical features of the smallest mature erythrocytes (Camelidae) might permit them to maintain their shape without the presence of microtubules.

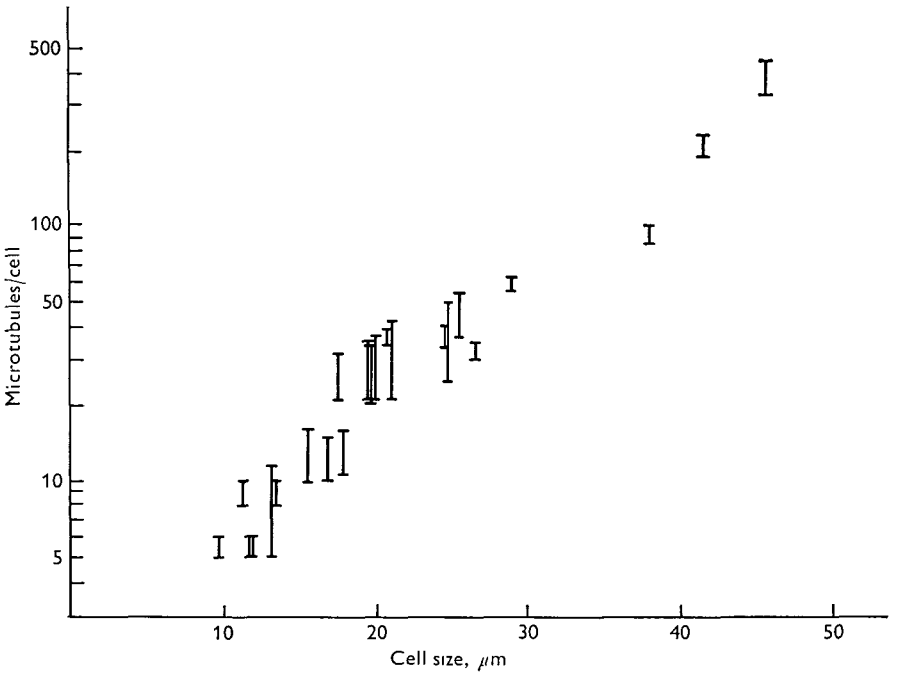

Fig. 1. Correlation between average erythrocyte length and range of the number of microtubules per erythrocyte (logarithmic scale). Other authors' results have been included in the data for this figure.

In lower vertebrates the marginal band can perform some additional functions like helping big erythrocytes to regain their usual shape after leaving narrow capillaries, where they undergo considerable deformation (Goniakowska, 1973). Moreover, in the case of amphibians and lungfishes the erythrocytes are exposed to drastic osmolality changes, so the marginal band can protect them from excessive swelling (Goniakowska, 1973; Goniakowska-Witalińska, 1974).

The correlation we have found between the size of red blood cells and their number of microtubules basically seems to confirm Meves' (1904, 1911) hypothesis concerning the role of the marginal band in maintenance of the ellipsoidal and biconvex shape of erythrocytes, but on the other hand this function of the band seems to decrease in importance with decrease in cell size.

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