Vesicles Carrying Nuclear Material in Mature *Cyprinus carpio* Erythrocytes

The ultrastructural constituents of mature *Cyprinus carpio* erythrocytes are similar to those found in chick[1,2], some bothropic species[3], amphibian[1,4–7] and toadfish[4] erythrocytes. One of the purposes of investigations at our Laboratory is the as yet unknown role of vesicles frequently found in variable number in the mature erythrocytes of each of all vertebrates from chicks to fishes. The aim of this communication is the description of such vesicles and their mitochondrial origin in carp erythrocytes.

Material and methods. Blood samples were obtained by cardiac puncture from adult carps, free of hemoparasites, with the use of a 2% EDTA solution adjusted to pH 7.3–7.4 by the addition of a 4% $NaHCO_3$ solution; final anticoagulant concentration was 0.15%.

To verify whether the content of erythrocyte vesicles, or part of it, could be DNA, thin blood smears were submitted to the FEULGEN reaction according to LISON[8]. As controls, other smears were previously treated by 0.5% DNase in 0.1 *M* phosphate buffer (pH 7.2), for 90 min at 37 °C, and by the buffer only, under the same conditions.

For thin sectioning, fixation proceeded as follows[9]: to 15 drops of blood, 15 drops of 2% glutaraldehyde in 0.2 *M* MILLONIG's buffer[10] were added drop by drop, each followed by slow agitation; after 30 min, the suspension was diluted with 2–3 volumes of 1% glutaraldehyde in the buffer and fixed for 2 h. Erythrocytes were washed 3 times, and fixed for 20 min in 1% osmium tetroxide in the same buffer. After staining in an 1% aqueous uranyl acetate solution for 30 min, the cells were dehydrated in the alcohol series, and embedded in Polylite 8001[11]. Thin sections were obtained in an MT-1 Porter-Blum microtome, stained by lead citrate[12], and examined in an Elmiskop I electron microscope at 60 Kv, from ×2,500 to ×20,000 magnification.

Results and discussion. Positive Feulgen reaction was hardly visible at points distant from the erythrocyte nuclei. Generally, positive reactions were seen near the nuclei, which, however, could be taken for nuclear protrusions.

Commonly, mature erythrocytes present typical mitochondria of paranuclear disposition. They attach at points of the nuclear membrane, more or less distant from the pores, where chromatinic material is juxtaposed. Through the pores of the nuclear membrane, penetration of hemoglobinized cytoplasm takes place (Figure 1a), as just in all vertebrate erythrocytes[1–7] and in mammalian erythroblasts[13–15].

It can be admitted that digestion of the nuclear membrane occurs at the points where mitochondria contact the nucleus, since chromatin gradually enters the mitochondria (Figures 1, b and c). These organelles suffer modifications characterized by a gradual disappearance of their double lamellae, or an alteration occurs before chromatin penetration ceases. After the loss of their inner structure, mitochondria begin to swell (Figure 1 d), giving rise to the presumable Feulgen positive vesicles of variable dimensions. They detach from the nucleus and displace themselves through the cytoplasm, carrying myelin figures, granulated, fibrous and dense amorphous material of nuclear and membranous origin (Figure 2a). Autoradiographic studies with ^{3}H-tymidine in *Bufo ictericus*

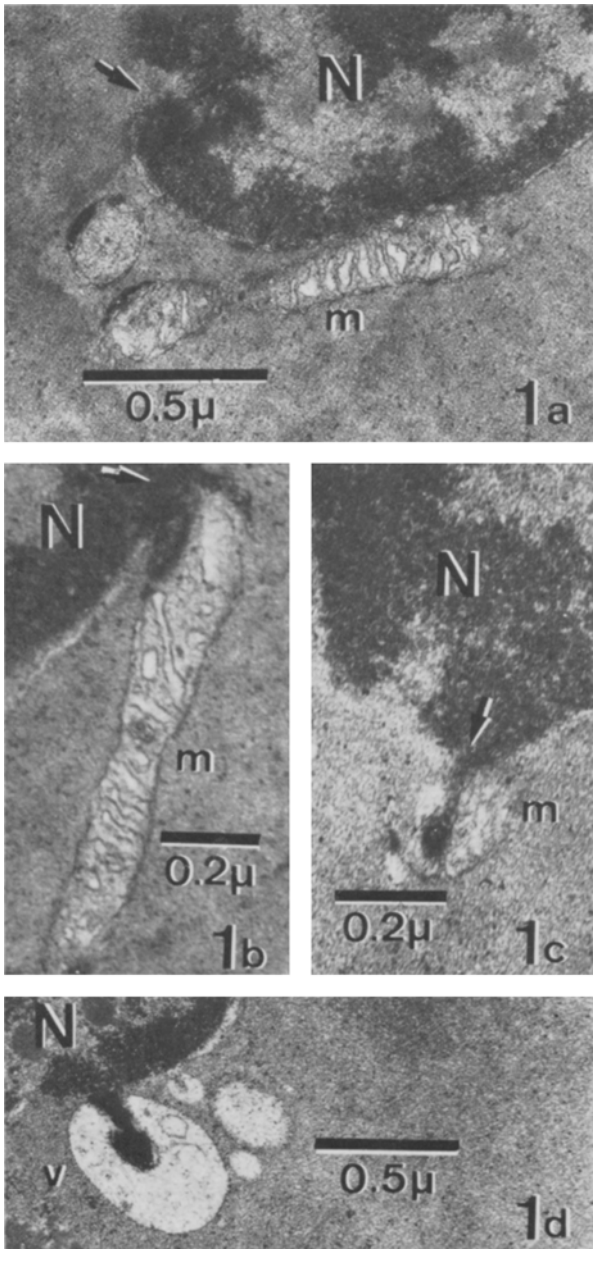

Fig. 1. Thin sections of mature erythrocytes. a) N, nucleus; m, mitochondrion; arrow, penetration of hemoglobinized cytoplasm into the nucleoplasm. b, c) N, nucleus; arrows, passage of chromatin to the mitochondria (m). d) N, nucleus; V, vesicle recently risen from a mitochondrion, containing chromatinic material.

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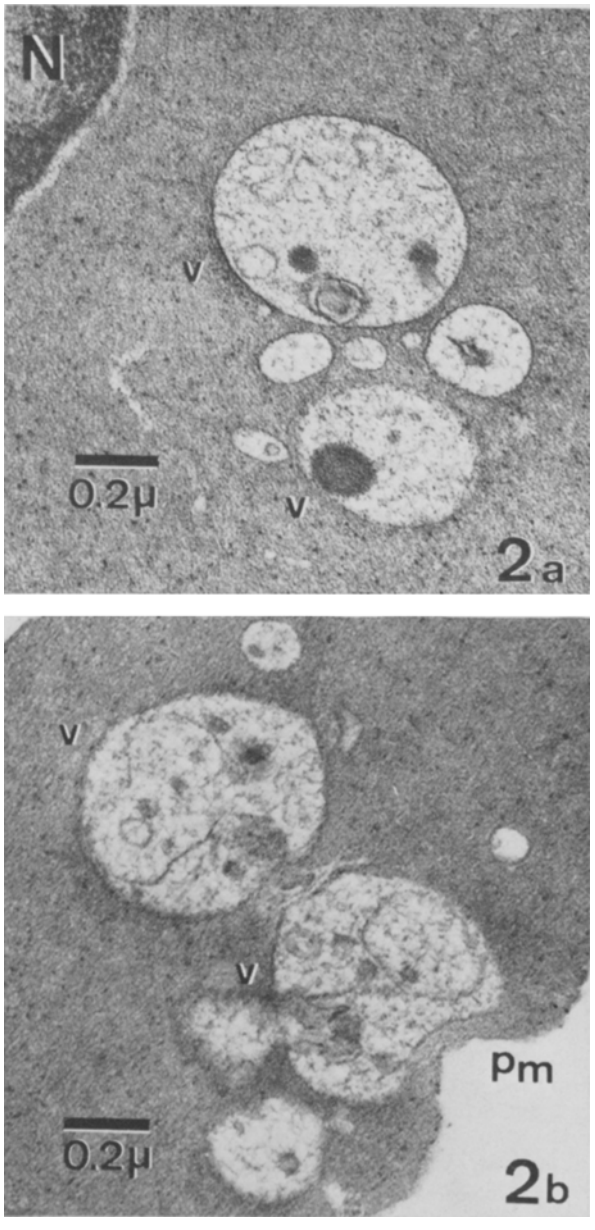

Fig. 2. Thin sections of mature erythrocytes. a) N, nucleus; V, free vesicles containing dense material. b) V, vesicles approaching the invaginated plasmic membrane (pm).

erythrocytes revealed the presence of labelled vesicles[16]. Often vesicles fuse among themselves, approaching the plasmic membrane which shows an invagination (Figure 2b); this suggests that vesicles or their content may be expelled from erythrocytes. Besides this, small Golgi complexes, and little of the smooth endoplasmic reticulum were also found. This peculiar mechanism of chromatin extrusion through vesicles of mitochondrial origin occurs only in the mature erythrocytic forms, i.e. those cells in which globin synthesis has ceased. These erythrocytes may still contain simple ribosomes, but no polysomes, which disintegrate after the globin molecule chain synthesis.

The functional relationship between the vesicles of mitochondrial origin, carrying nuclear material, and structures such as the Golgi complex, is still unknown. Further studies with labelled material will probably provide more information on this question, as well as on the significance of this phenomenon in the final erythrocytic maturation. The possibility of a correlation between this event and the nuclear extrusion, occurring in the orthochromatic erythroblasts of all mammals, may be considered.

Zusammenfassung. Chromatinhaltige feulgenpositive Bläschen wurden im Cytoplasma der Erythrocyten von *Cyprinus carpio* gefunden. Sie haben ihren Ursprung in den unmittelbar an der Kernmembran anliegenden Mitochondrien, die während der Chromatinaufnahme allmählich ihre Struktur verlieren.

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Physical Evidence of a Plasmid in *Rhizobium japonicum*

Many genetic observations, but little physical data, suggest the existence of plasmids in the genus *Rhizobium*. The transfer of clover infectivity from *Rhizobium trifoli* to *Rhizobium phaseoli* and a loss of infectivity upon treatment of the recipient culture with acridine orange has been observed[1]. Recombination has been demonstrated in conjugation experiments using strains of *Rhizobium lupini*[2] and *Rhizobium leguminosarum*[3], but could not be found in strains treated with acridine orange. Treatment of the antibiotic resistant strain 10324 of *Rhizobium japonicum* with acridine orange has been reported to increase its antibiotic sensitivity[4]. This presumptive R-factor was transmissible to *Agrobacterium tumefaciens*. The similarities between this observation and R-factor mediated antibiotic resistance in enteric bacteria, and the suggestion of plasmid control of the symbiotic nitrogen-fixing property[5] led us to examine *Rhizobium japonicum*, ATCC strain 10324, for the presence of plasmid DNA.

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