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HEMOGLOBIN BIOSYNTHESIS IN BONE MARROW AND PERIPHERAL BLOOD ERYTHROID CELLS OF THE SNAKE *WAGLEROPHIS MERREMII* (REPTILIA, OPHIDIA, COLUBRIDAE)*

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ABSTRACT: Bone marrow and peripheral blood erythroid cells of the snake *Waglerophis merremii* were ultrastrùcturally analysed. To enhance the entry of immature cells to the peripheral blood, erythropoiesis was stimulated through the induction of a hemolytic anemia, by injecting saponin. Typical hemosomes, very similar to those observed in mammalian and avian immature erythroid cells were found, as well as some of their precursor forms. These organelles are taken as possible sites where the integration of heme into the four globin polypeptide chains occur, on account of their content of hemoglobinic nature. Such entities are a specialyzed indirect derivation from mitochondria, through successive transformations to lamellated bodies, prehemosomal vesicles, prohemosomes and finally to hemosomes.

KEY WORDS: Hemoglobin biosynthesis; snake hemoglobin; snake hemosome.

INTRODUCTION

It has been proposed that hemoglobin (*Hb*) biosynthesis could occur in hemoglobinized organelles termed hemosomes, or where heme is integrated into the four polypeptide globin chains[1]. Such organelles have a highly dense matrix, due to their *Hb* content, their narrow intralamellar space has a longitudinal disposition and in general their diameters are smaller than the diameters of mitochondria. These studies began by the examination of

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rabbit-embryo peripheral blood erythroblasts and reticulocytes. Further investigations were carried out in order to ascertain the hypothesis on the functional role of hemosomes. Several experimental and analytical works showed circumstantially a constant close relationship between *Hb* biosynthesis and hemosome formation[2-5,7,8]. Hemosomes were also found in mouse[11,12,14], other rodents[13], chicken[15], toads (*Bufo ictericus* and *Bufo paracnemis*)[10], as well as in the fish *Thymallus thymallus* erythroid cells[14]. It has been pointed out that this characteristic organelle is an indirect derivation of the mitochondrion[7,9], that occurs through successive modifications to lamellated body, prehemosomal vesicle, prohemosome, and hemosome.

This paper shows that *Waglerophis merremii* erythroid cells contain hemoglobinized organelles, through cell fractionation and electrophoresis of the organellar lysate supernatant. Results of electron microscopic studies on some stages of hemosome formation are presented, as already partially shown[19], comparatively to the findings in erythroid cells of other vertebrates.

MATERIAL AND METHODS

Normal adult snakes (*Waglerophis merremii*) newly received by the Instituto Butantan were submitted to hemolytic anemia. The snakes were subcutaneously injected, each day with saponin in 0.75% saline solution, 3 mg/kg body weight, for three days; blood was harvested (72 h), and reticulocyte countings reached approximately 30%, as examined through a modified new methylen blue technique[18]. For electron microscopy, blood was fixed initially in 1% glutaraldehyde in 0.20 M Millonig's buffer, for 1 h at room temperature, in order to concentrate the immature forms in the course of normal sedimentation. After this, erythroid cells were fixed in 3% glutaraldehyde, for approximately 2 h at room temperature, followed by osmic tetroxide fixation, 1% in the same buffer for 1 h at 4°C, uranyl acetate contrastation, 40 min at room temperature, dehydration, and embedding in Polylite 8001[9]. Thin sections were obtained in a Porter-Blum MT-1 microtome, contrasted by lead citrate and examined in the Elmiskop I and Zeiss-EM 109 electron microscopes, at 60 and 80 kV, from × 7,000 to × 50,000 magnification.

For the demonstration of *Hb* within the hemosomes through electrophoresis in polyacrylamide gel[16], blood cells were fractionated, the hemosomes isolated, washed and osmotically lysed, following Brunner *et al.*[1] with some modifications: Centrifugation at 4,000 xg, instead of 1,350 xg, for a complete nucleus and cell debris sedimentation without repetition of this step; centrifugation at 12,000 xg, instead of 10,000 xg, for the organellar fraction sedimentation. The supernatants of the organellar lysate and of the last washing were concentrated ten fold, or lyophilized, and *Hb* was diluted to 1:50. A 2.5mA current was applied for 40 min at 5°C, and *Hb* bands were identified by benzidine or tolidine reaction.

RESULTS AND DISCUSSION

The interpretation given for the several structural aspects found in *Waglerophis merremii* erythroid cells synthesizing *Hb* and involved in hemosome formation, is basically fundamented on studies carried out in mammalian erythroid cells[9]. A general view of a maturing bone marrow erythroblast is shown in Fig.1, corresponding to a still highly immature stage, as evaluated by the high number of polysomes and single ribosomes. Some organelles modifying continuously from prohemosomes to transitional stages followed by transformation to the hemoglobinized hemosomes, are seen. A long organelle constituted by the three developmental stages is shown in Fig.2, presenting a great structural similarity to the hemosomes and their precursors found in rabbit-embryo[1], adult rabbit[6,8], guinea-pig[3], human[2], and chicken[15] erythroid cells. A typical hemosome of a peripheral blood immature erythrocyte is seen in Fig.3; for comparison, an obliquely sectioned mitochondrion is presented in the inset.

As to *Hb* biosynthesis dynamics it begin by iron uptake via transferrin which constitute complexes through an interaction with glycoprotein receptors bound at numerous sites of the cell surface, probably as happens in mammalian erythroid cells[17]. This occurs through rhopheocytosis, consisting in a progressive invagination process of the plasma membrane (Fig.8), giving rise to endocytic vesicles (Fig. 6). These vesicles fuse among themselves resulting large, highly electron-dense free ferruginous particle inclusions, due to the disappearance of the limiting membrane. Afterwards, such ferruginous inclusions, constituting an iron source for heme biosynthesis, are involved by an initially single membrane (Fig.4); the vesicle content becomes amorphous and less electron-dense. The membrane which involves the ferruginous material results from the unfolding of double lamellae originated from a lamellated body that in turn is a mitochondrial derived structure (Fig.5), as shown in tissue culture cells[7] and in rabbit erythroblasts[9]. This lamellated body is a structural as well as probably an enzymatic complex catalysing heme and the final *Hb* biosynthesis reactions. From this stage onwards the captation of iron containing compounds, and of the globin chains occur through double lamellae expansions. These expansions return constituting prehemosomal vesicles which, possibly after a rotational movement, approaches the ferruginous material and the globin chains one another, giving rise to condensed prehemosomal vesicles (Fig. 6) that afterwards modify successively to prohemosomes (Fig. 7) and hemosomes[9]. At this stage the integration of heme into the four globin chains could take place. When hemosomes attain a high *Hb* concentration, their membranes disrupt and *Hb* spread throughout the cytoplasm. This happens repeatedly until the final cytoplasmic *Hb* concentration is reached for the constitution of a new mature erythrocyte. Claussen *et al.*[13] and Claussen[14] studying erythropoiesis in the rodents *Apodemus sylvaticus* and *Micromys minutus,* in the mouse *Mus musculus* and in the fish *Thymallus thymallus* found very interesting ultrastructural aspects of *Hb* biosynthesis similar to those found in the course of red blood cell maturation in other vertebrates. However, there exists a little disagreement between Claussen's[14] diagram of *Hb* biosynthesis and the interpretation of Brunner *et al.*[9] as to the integration of the globin chains

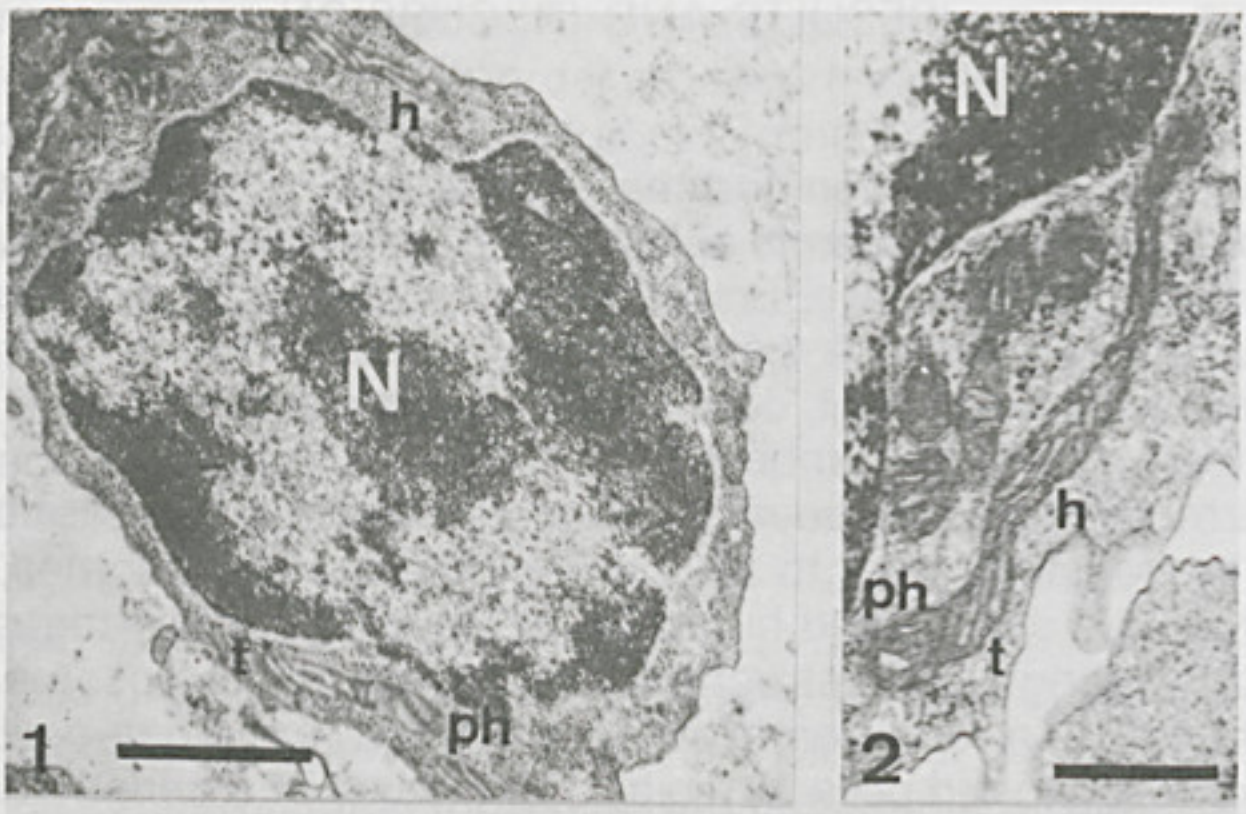


Fig. 1 — Bone marrow erythroblast showing a prohemosome *ph* continuous to a transitional stage *t*. At the upper region of the picture, the continuity of this stage *(t)* with the hemosome *h* can be seen; *N* — nucleus. Bar equals 1.0 μm.

Fig. 2 — Larger magnification of an erythroblast showing a long organelle constituted by a prohemosome *ph* followed by a transitional stage *t* and a hemosome *h; N* — nucleus. Bar equals 0.5 μm.

Fig. 3 — A typical hemosome *h* is seen. For comparison a mitochondrion *m* is shown in the inset; *N* — nucleus.

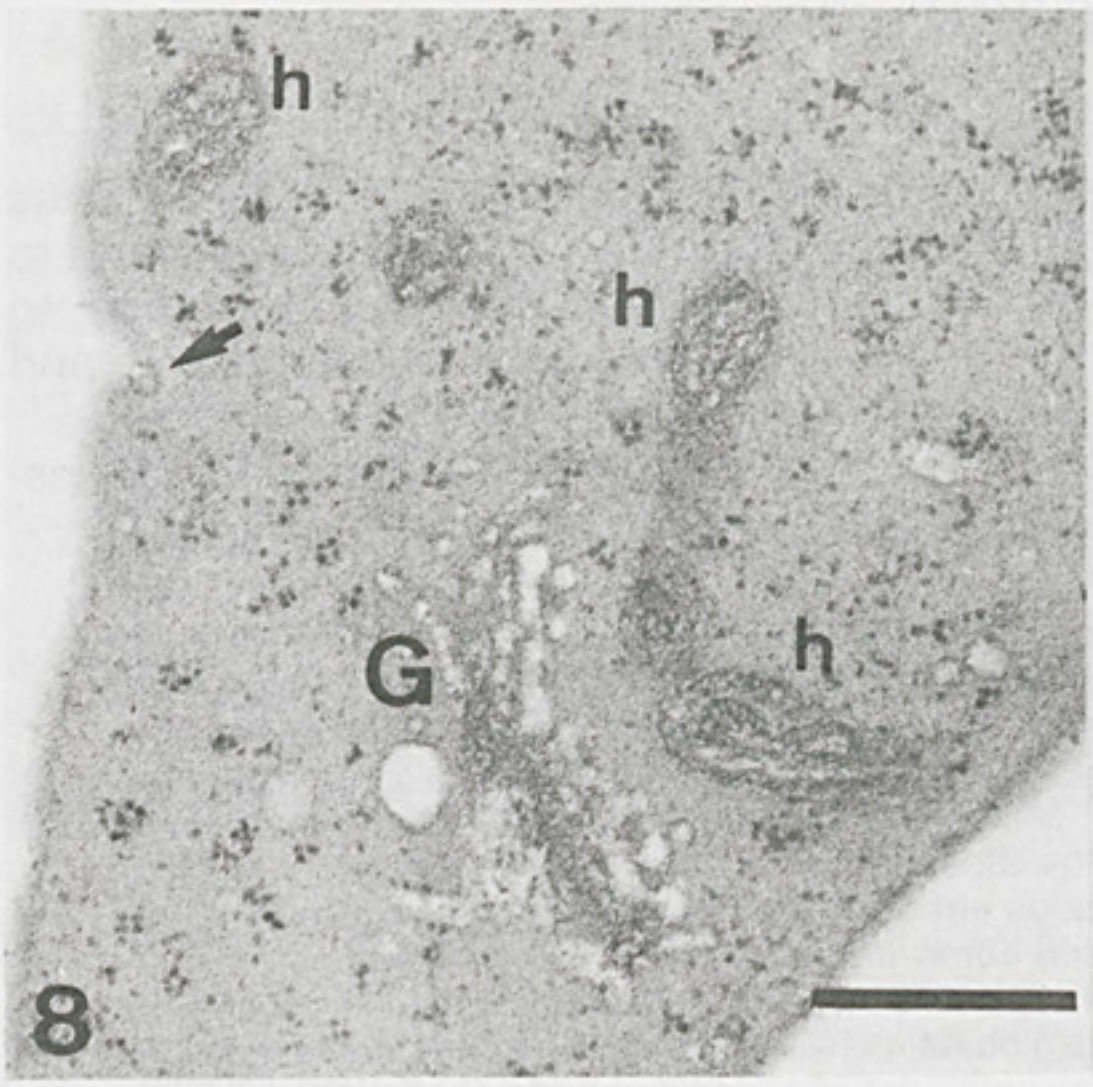
Fig. 4 — Vesicle *v* containing a still granulated ferruginous material.

Fig. 5 — A lamellated body *lb* apparently rising from a mitochondrion *m* is shown.

Fig. 6 — Condensed prehemosomal vesicle *hv* constituted by three concentric double lamellae; within the double lamellae some ribosomal particles can be observed. *Arrow* points to an endocytic vesicle.

Fig. 7 — Condensed prehemosomal vesicle *hv* modifying to prohemosomes *ph*.

Bars in Figs. 3-7 correspond to 0.25 μm.



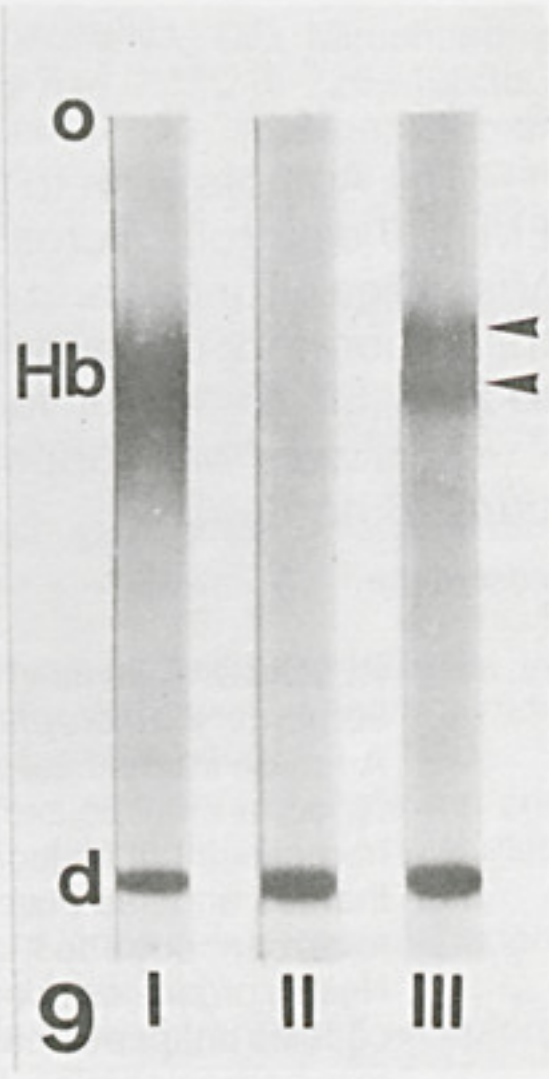


Fig. 8 — Reticulocyte showing obliquely sectioned hemosomes *h*, and a Golgi complex *G*. *Arrow* points to a rhopheocytic site. Bar equals 0.5 μm .

Fig. 9 — Electrophoretic *Hb* patterns (evidenced by tolidine) from the hemoglobinized cytoplasm (I), and from the supernatant of the lysed hemosomal fraction (III), showing two bands. The concentrated supernatant of the last washing of the fraction did not present any visible *Hb* band (II).

in the course of hemosomegenesis. According to these authors, the globin chains are enclosed in the prehemosomal vesicle, together with the ferruginous compounds, before prohemosome formation.

Typical hemosomes, showing similar structural characteristics as those found in mammalian and chicken erythroid cells, are seen in Fig. 8. The hemoglobinic nature of the hemosome content, determined through electrophoresis in polyacrylamide gel is seen in Fig. 9, showing two *Hb* bands. The hemosomal fraction isolated from erythroid cells homogenate and submitted to five washings, was osmotically lysed so that *Hb* had been released from the organelles. The supernatant of the last washing did not present any visible *Hb* band showing, therefore, that all cytoplasmic *Hb* had been discarded.

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RESUMO: Células eritróides de medula óssea e do sangue periférico da serpente *Waglerophis merremii* foram analisadas ultra-estruturalmente. A fim de intensificar a passagem de células imaturas para o sangue periférico, a eritropoiese foi estimulada através da indução de uma anemia hemolítica pela injeção de saponina. Hemossomos típicos, muito semelhantes àqueles observados em células eritróides de mamíferos e aves, foram encontrados, assim como algumas de suas formas precursoras. Nestes organelos ocorreria, provavelmente, a integração do hemo às quatro cadeias polipeptídicas de globina, considerando o seu conteúdo de natureza hemoglobínica. Estas entidades são uma derivação especializada indireta das mitocôndrias, através de sucessivas transformações para corpos lamelados, vesículas pré-hemossômicas, pró-hemossomos e, finalmente, para hemossomos.

UNITERMOS: Biossíntese de hemoglobina; hemoglobina de serpente; hemossomo de serpente.

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