JOURNAL OF MORPHOLOGY 168:137–149 (1981)

Reptilian Bone Marrow. An Ultrastructural Study in the Spanish Lizard, *Lacerta hispanica*

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ABSTRACT The ultrastructure of hemopoietic bone marrow of the Spanish lizard, *Lacerta hispanica*, has been studied for the first time. The organ consists of a stroma formed by venous sinuses and reticular cells. Erythropoiesis takes place in the lumen of blood vessels, while granulopoiesis is extravascular. Pluripotent stem cells are structurally differentiated into erythrocytes and granulocytes. Two types of granulocytes, heterophils and acidophils, have been found, and a third granular cell type is tentatively identified as granular leukocyte. Remarkably, plasmacytopoiesis occurs in the bone marrow of *Lacerta hispanica*. The possible functional significance of these results is discussed with emphasis on their importance for the reptilian immune system.

An actively hemopoietic bone marrow arises evolutionarily for the first time in the lungless salamanders of the family Plethodontidae (Barret, '47). However, this bone marrow, as in the frog (Campbell, '70), shows granulopoietic and lymphopoietic function but lacks erythropoietic activity (Curtis et al., '79). In some reptiles, the bone marrow acquires a full hemopoietic capacity producing all blood cell lines. In the horned toad, *Phrynosoma solare*, the spleen is the primary blood-forming organ, but in most lizards it is the bone marrow. In turtles, there is an almost equal division of the process of red cell formation between spleen and bone marrow (Jordan, '38). Reptiles appear to represent a transitional group between amphibians, where the spleen is the chief erythropoietic organ, and birds, where the bone marrow is practically the exclusive blood-forming tissue of the adults.

There are no studies on the ultrastructure of reptilian bone marrow, although the morphology of their blood cells has been known for a long time (see reviews by Jordan, '38; Andrew, '65), and their ultrastructure has been studied in some species (Taylor et al., '63, in *Pseudemys scripta elegans*; Kelényi and Nemeth, '69, in *Lacerta agilis* and *Emys orbicularis*; Efrati et al., '70, in *Agama stellio*). This work examines the ultrastructure of bone marrow from *Lacerta hispanica*, the hematopoietic process and the possible implications of lymphocytes and plasma cells for the reptilian lymphoid system. In previous investigations, we analyzed other lymphoid organs of *Lacerta hispanica* (Zapata and Fernández, '79; Solas and Zapata, '79; Zapata and Solas, '79) as a morphologic basis for further studies on reptilian immunology.

MATERIAL AND METHODS

Bone marrow from tibia and femur of fifteen adult *Lacerta hispanica*, collected in Toledo (Spain) during the spring, were used in this study. The pieces, including the bone, were fixed *in toto*, by immersion into 2.5% glutaraldehyde buffered to pH 7.3 with Millonig's buffer and postfixed in 1% osmium tetroxide, and dehydrated in acetone for embedding in Araldite. Ultrathin sections were obtained with a Reichert OM-U3 ultratome, double-stained with lead citrate and uranyl acetate, and examined with a JEOL 100-B electron microscope. Semithin sections approximately 1–2 μm in thickness were cut and stained with an alkaline solution of Toluidine blue.

RESULTS

The hemopoietic bone marrow of *L. hispanica* consists of a reticular cell network and abundant blood sinuses. Reptilian granulopoiesis is an extravascular phenomenon, but erythropoiesis occurs in the blood vessel lumen (Fig. 3).

Reticular cells

Reticular cells are the dominant elements of marrow stroma. They cover the outside surface

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of the venus sinus endothelium and branch into the surrounding hematopoietic spaces. They show a light nucleus and cytoplasmic processes containing electron-dense mitochondria, ribosomes, some profiles of rough endoplasmic reticulum and pinocytotic vesicles (Fig. 1). Sometimes, lysosome-like dense bodies, Golgi complexes, and filaments have also been observed in these cells. Moreover, in the bone marrow of *L. hispanica*, there are free macrophages containing abundant cell debris together with rough and smooth endoplasmic reticulum and dyctiosomes (Fig. 2). Extracellular space is filled by connective tissue fibers exhibiting unclear banding (Fig. 1). Some pigment cells were observed.

Lining cells

Venous sinus walls are formed by elongated electron-dense lining cells, containing abundant cytoplasmic membranous organelles and some filaments (Fig. 3). These cells are joined together by tight and gap junctions and lacked a basement membrane.

Hemopoietic stem cells

Only a few primitive cells have been found in the bone marrow of *L. hispanica* (Fig. 4). They show scant condensed chromatin and membranous organelles and large amounts of cytoplasmic monoribosomes.

Erythroid cells

The erythrocytic line of *L. hispanica* consists of proerythroblasts, basophil, polychromatophil and orthochromatophil erythroblasts, reticulocytes, and mature erythrocytes. Proerythroblasts are large, round, or polygonal cells, with scant condensed chromatin, a prominent nucleolus, and a very electron-dense cytoplasm filled with polyribosomes. Small electron-dense mitochondria are abundant and some ropheocytic vesicles appear at the cell surface (Fig. 5). In basophilic erythroblasts, there is an increase of condensed chromatin in contrast to the decrease of polysomes. The nucleolus and mitochondria remain normal (Fig. 6).

The maturing of erythroblasts results in an increase in condensed chromatin, while the amount of polyribosomes decreases as cytoplasmic haemoglobin appears. This produces a higher electron density, which in polychromatophilic erythroblasts is already very evident (Fig. 7). In orthochromatophils, condensed chromatin has considerably increased and the cytoplasm is filled with haemoglobin. Nevertheless, there are still numerous polysomes (Fig. 8). Reticulocytes or immature

Abbreviations

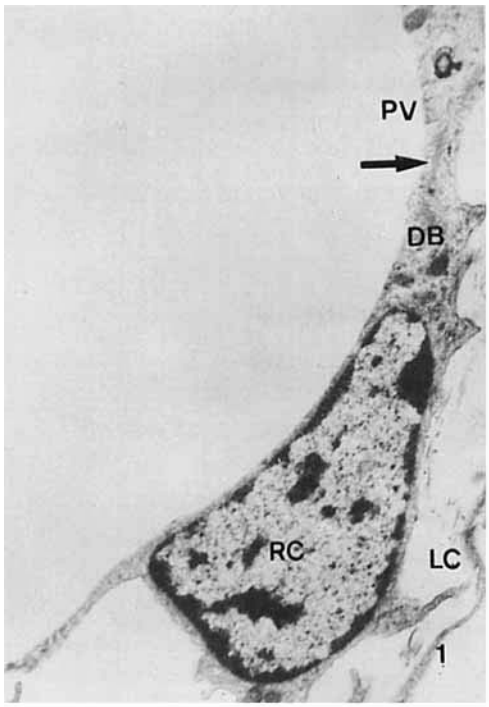
AR	Adventitial reticular cells	M	Mitochondria
C	Centriole	N	Nucleus
CD	Cell debris	NU	Nucleolus
DB	Dense bodies	PV	Pinocytotic vesicles
ER	Endoplasmic reticulum	R	Ribosomes
G	Golgi complex	RC	Reticular cell
GP	Granulopoiesis	RER	Rough endoplasmic reticulum
GR	Granules	RV	Ropheocytic vesicles
IE	Erythoid elements	V	Vacuoles
LC	Lining cells or endothelial processes		

Fig. 1. Reticular cell. This is a very ramified cell, showing low electron density, pinocytotic vesicles (PV), some filaments (arrow), and dense bodies (DB). Note the connective tissue fibres between the reticular cell (RC) and endothelial processes (LC). × 12,500.

Fig. 2. Phagocytic cell. Free macrophages are frequent in the marrow stroma of *L. hispanica*. They contain abundant cell debris (CD), a well-developed Golgi complex (G), and endoplasmic reticulum (ER). Note the cytoplasmic projections engulfing cell debris (arrows). × 8,300.

Fig. 3. Lining cell (LC) of the wall of a venous sinus. The cell appears to be elongated by thin very electron-dense processes. In the nuclear area, there are dense bodies (DB) and some coated vesicles contacting with cell surface. Note the absence of a basement membrane, the discontinuous layer of adventitial reticular cells (AR), and the extravascular disposition of granulopoiesis (GP) and intravascular of erythroid elements (IE). × 13,500.

Fig. 4. Pluripotent stem cell. Note the absence of condensed chromatin and cytoplasmic membranous organelles. The cytoplasm is filled by free ribosomes (R), a nucleus (N), rough endoplasmic reticulum (RER), and mitochondria (M). × 12,800.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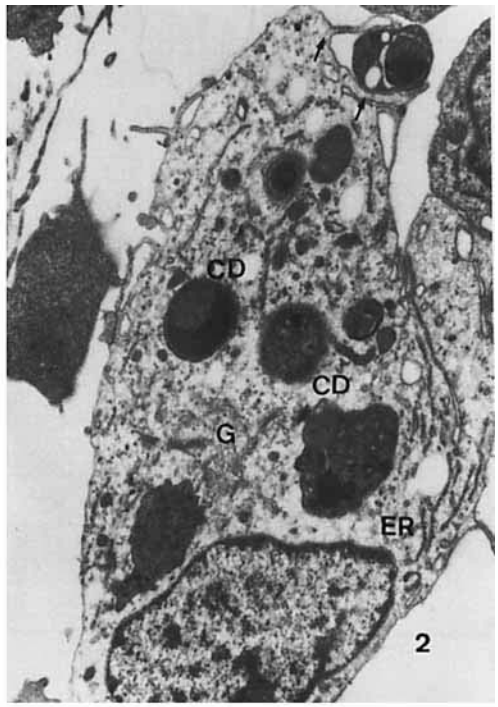
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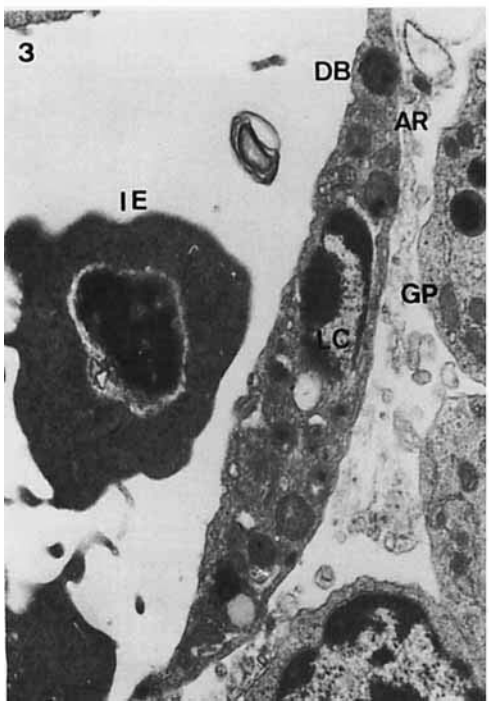
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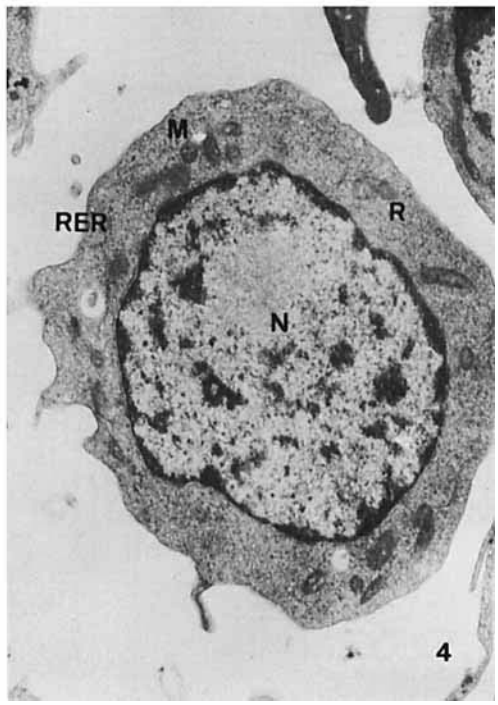
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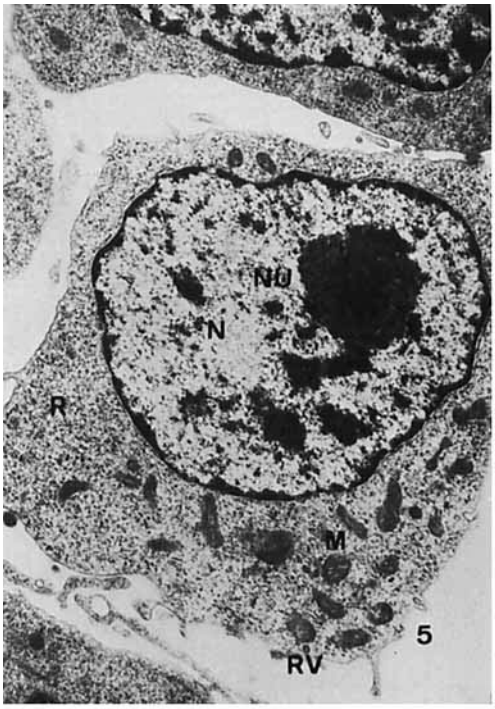
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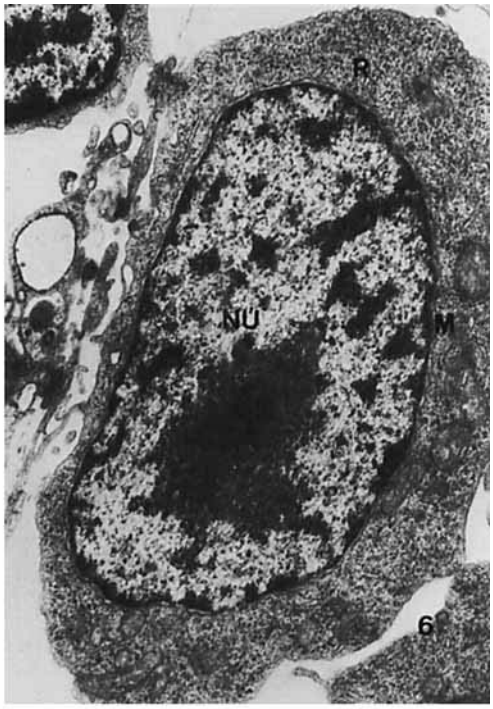
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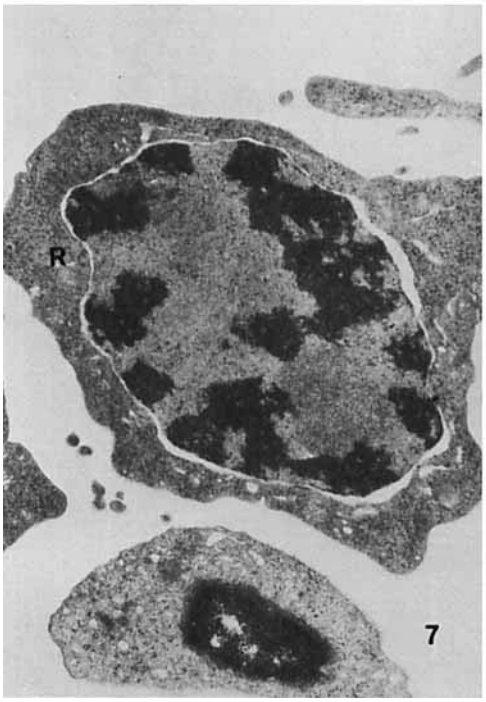
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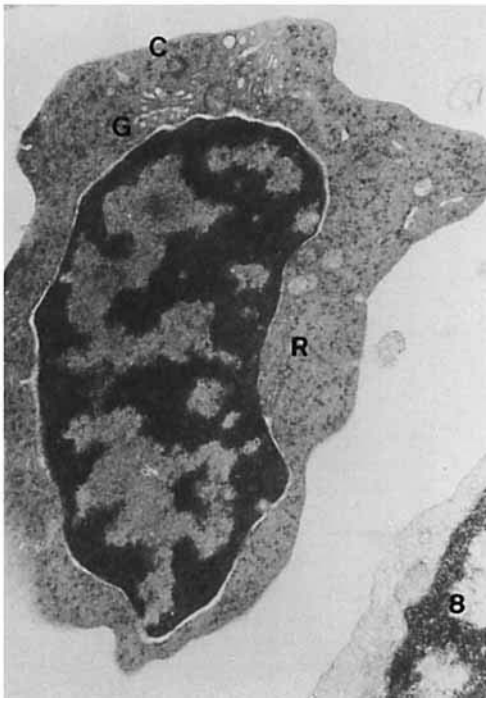
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erythrocytes are elongated, very similar to the mature cell, although still showing polysomes, some membranous organelles and ropheocytic vesicles (Fig. 9). All of the above erythroid stages display microtubular marginal bands formed by parallel peripheral bundles of 25–30 units. Several cytoplasmic organelles show degenerative processes during differentiation, culminating with the release of myelin figures. In late developing stages, the nuclear interchromatinic areas show haemoglobin.

Granulocytic cells

Heterophilic granulocytes were easily characterized, but the presence of basophils and acidophils was confused. A third granular cell type was tentatively identified as granular leukocyte.

A myeloblast apparently common to all granulocytic lines was found. It is a large cell with a light electron-dense nucleus, showing condensed chromatin surrounding the nucleolus and in the nuclear periphery. The cytoplasm contains many free ribosomes, some rough endoplasmic reticulum, and scattered electron-dense granules (Fig. 10).

The first type of cytoplasmic granules occur in the heterophilic promyelocytes, along with a considerable increase of the rough endoplasmic reticulum and Golgi complex. Also, the condensed chromatin has increased though the nucleolus is still present (Fig. 11). Golgi complexes, rough endoplasmic reticulum, and primarily granules are very abundant in myelocytes (Fig. 12). Granule formation occurs in relation with Golgi dyctiosomes. At least two distinct types of heterophilic granules can be identified: (1) Round or lightly elongated small granules associated with the Golgi area; and (2) larger round or polygonal granules with electron-dense cores. The relationship between the two granular types is uncertain, but they seem to be independent (Fig. 13). In mature cells, the abundance of granules is higher, the rough endoplasmic reticulum and Golgi complexes are practically absent, and the nucleus may appear lobulated (Fig. 13).

The second type of cytoplasmic granules occurs in cells with frequently lobulated nuclei and round homogeneous electron-dense granules (Fig. 14). Although in some granules a certain noncrystalline substructure can be described, most of them are homogeneous. Possible precursors of these cells show a large development of rough endoplasmic reticulum and some smaller electron-dense granules. The condensed chromatin is infrequent and the nucleolus is very prominent (Fig. 15).

Occasionally, there are other irregular granular cells showing many lightly electron-dense lipid droplets and electron-dense granules with inclusions (Fig. 16). Precursors of these cells have not been clearly identified in the bone marrow of *L. hispanica*.

Lymphocytes

Rare lymphoid cells, mainly small and median lymphocytes, appear in the bone marrow of *L. hispanica*. They display a high nucleocytoplasmic ratio, a large nucleus with much condensed chromatin, and a large amount of ribosomes (Fig. 17).

Plasma cells

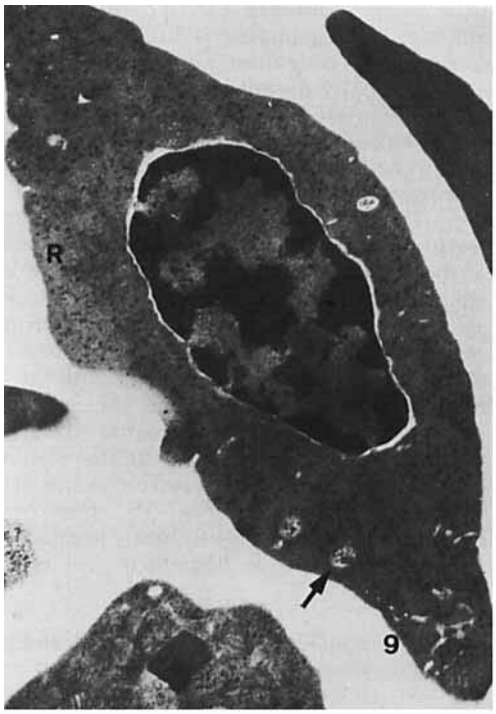
Mature plasma cells, lacking cytoplasmic granules, abundant rough endoplasmic reticulum, and prominent condensed chromatin with nucleolus, are numerous in reptilian bone marrow (Fig. 18). The presence of immature plasma cells demonstrates plasmacytogenetic capacity in reptilian bone marrow. In plasmablasts, condensed chromatin is scarce and the rough endoplasmic reticulum is poorly developed (Fig. 19). In proplasmatocytes, the condensa-

Fig. 5. Proerythroblast. The nucleus (N) shows some condensed chromatin and a prominent nucleolus (NU). In the cytoplasm, electron-dense mitochondria (M) and free polyribosomes (R) are abundant. Ropheocytic vesicles (RV). × 10,200.

Fig. 6. Basophil erythroblast. Note the prominent nucleolus (NU) and the absence of condensed chromatin. In the cytoplasm, polyribosomes (R) are fewer than in proerythroblasts. Mitochondria (M) are present. × 11,300.

Fig. 7. Polychromatophilic erythroblast in prophase. The number of polysomes (R) has decreased and hemoglobin fills the cytoplasm, increasing its electron density. × 11,800.

Fig. 8. Orthochromatophilic erythroblast. The condensed chromatin has notably increased and the nucleolus has disappeared. In the cytoplasm, polysomes (R) have decreased in relation to an increase of hemoglobin. Some cytoplasmic organelles are still present, i.e., Golgi complex (G) and centriole (C). × 16,000.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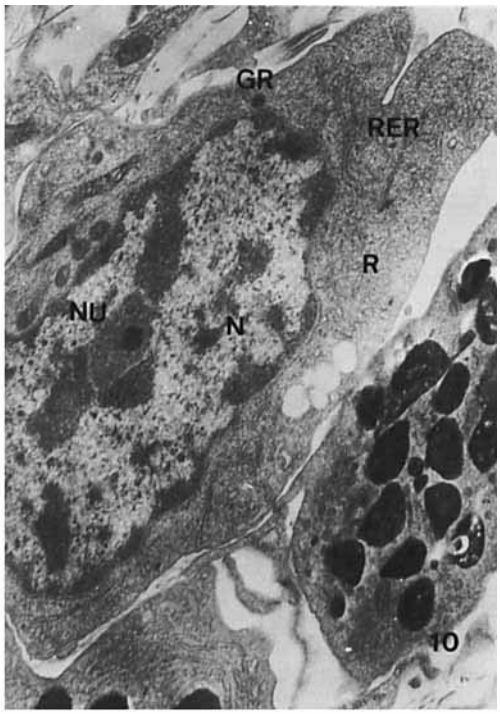
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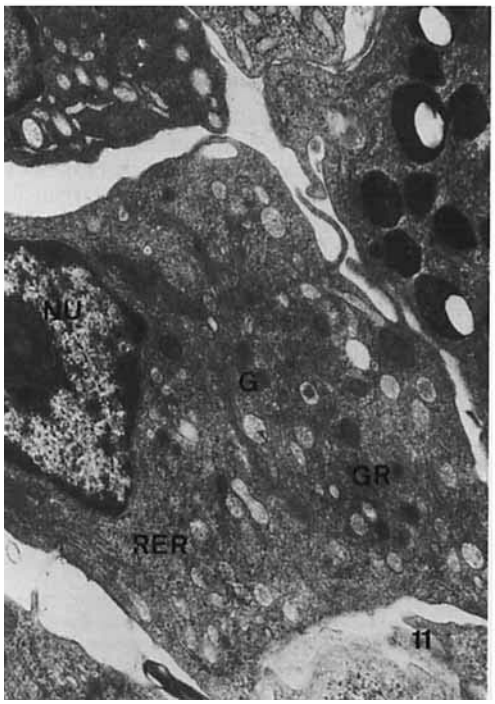
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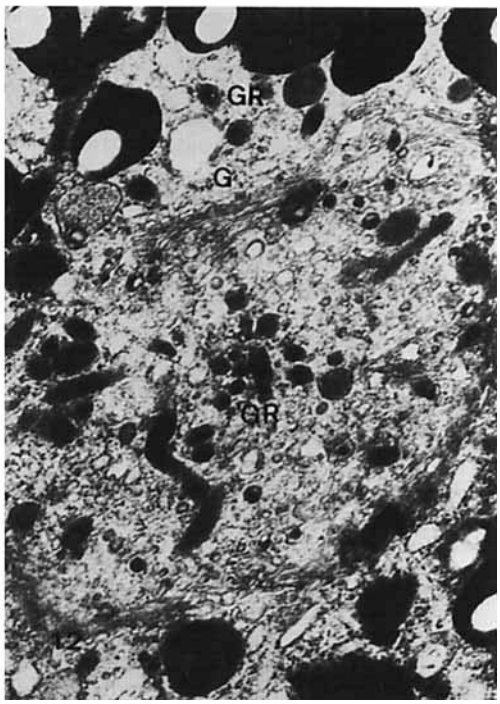
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tion of chromatin increases and rough endoplasmic reticulum is better developed, although it does not appear to be dilated, but is packed in parallel rows (Fig. 20).

DISCUSSION

The ultrastructure of reptilian bone marrow has not been previously described, and, in general, ultrastructural data on bone marrow of lower vertebrates and birds are scarce. Campbell ('70) described the bone marrow of *Rana pipiens,* and recently, Curtis et al. ('79) examined the bone marrow of *Plethodon glutinosus.* Moreover, the architecture of the marrow vasculature was investigated in three amphibian species in relation to its significance in hematopoietic development (Tanaka, '76). Several authors (Andrew, '65; Efrati et al., '70; Taïb-Cazal, '73) have pointed to bone marrow as the most important hemopoietic locus in reptiles.

In many lizards, erythro- and granulopoiesis occur in the bone marrow, while the spleen is mainly a lymphoid organ. However, in the primitive lizard *Phrynosoma solare*, erythropoiesis occurs in the spleen, but the granulopoiesis is in the bone marrow (Jordan, '38), as in amphibians (Campbell, '70; Curtis et al., '79). Our own results show than in *L. hispanica*, the bone marrow is the chief blood-forming organ. In turtles, bone marrow and spleen share the hemopoietic capacity (Jordan, '38). In this sense, as Jordan ('38) already noted the reptiles constitute a transitional group between amphibians and birds. In the former, erythropoiesis is a splenic activity, while in birds, all hemopoiesis occurs in the bone marrow (Jordan, '38; Campbell, '67). In *L. hispanica*, granulopoiesis is an extravascular process, while erythropoiesis occurs in the lumen of venous sinuses. Other authors have described the same findings in the bone marrow of reptiles (Maximow, '27) and birds (Campbell, '67). In lower vertebrates, erythropoiesis generally occurs intravascularly, whereas granulopoiesis is extravascular.

The morphology of *L. hispanica* marrow stroma is similar to that described for lower vertebrates (Campbell, '70; Curtis et al., '79) and birds and mammals (Campbell, '67, '72; Tavassoli, '77; Hoshi and Weiss, '78). The walls of venous sinuses are formed by electron-dense endothelial cells joined together by tight and gap junctions. We were not able to observe a basement membrane beneath these cells; at most, some connective tissue fibers and a substance indistinguishable from ground substance were found. Campbell noted this absence in the bone marrow of chicken and pigeons (Campbell, '67) and frog (Campbell, '70), but Campbell ('72) and Weiss ('65) described a basement membrane in rats, mice, and guinea pigs. However, the basement membrane is generally considered to be absent between endothelial cells and adventitial reticular cells in mammalian bone marrow (Tavassoli, '77). In *L. hispanica*, tight and gap junctions have been found joining the lining cells, as in the bone marrow of the frog (Campbell, '70) and in rats, mice, and guinea pigs (Campbell, '72).

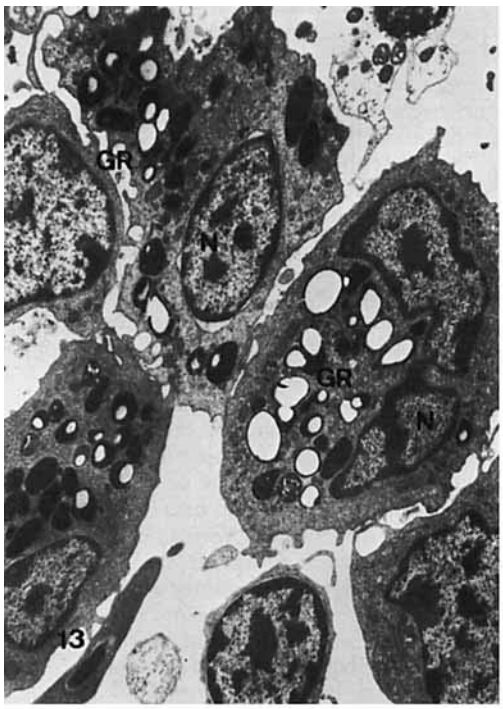
Beneath the lining cells, there is a discontinuous layer of adventitial reticular cell processes. In *L. hispanica*, these processes are always present. They do not make contacts between each other nor with the lining cells. In accordance with these results, a considerable proportion of endothelium is covered by adventitial cells, considered to be an integral part of the sinus wall in the rat (Weiss, '70; Campbell, '72), mouse (Campbell, '72), and rabbit (Tavas-

Fig. 9. Reticulocytes. Note the highly electron dense cytoplasm and the amount of condensed chromatin, as well as the existence of intranuclear haemoglobin. In the cytoplasm, there are some polysomes (R) and degenerated membranous organelles (arrow). × 14,800.

Fig. 10. Myeloblast. The cell shows a lightly electron-dense nucleus (N), with perinuclear condensed chromatin and a prominent nucleolus (NU). The cytoplasm contains many ribonucleoproteins (R), profiles of rough endoplasmic reticulum (RER), and some scattered electron-dense granules (GR). × 12,300.

Fig. 11. Heterophil promyelocyte. Note the increase of condensed chromatin and prominent nucleolus (NU). In the cytoplasm, Golgi complex (G) and rough endoplasmic reticulum (RER) are enlarged in comparison to myeloblasts, and the first granules (GR) appear. × 12,300.

Fig. 12. Golgi area of a heterophilic myelocyte. Note the different types of granules (GR) and their origin from Golgi complex (G). × 70,000.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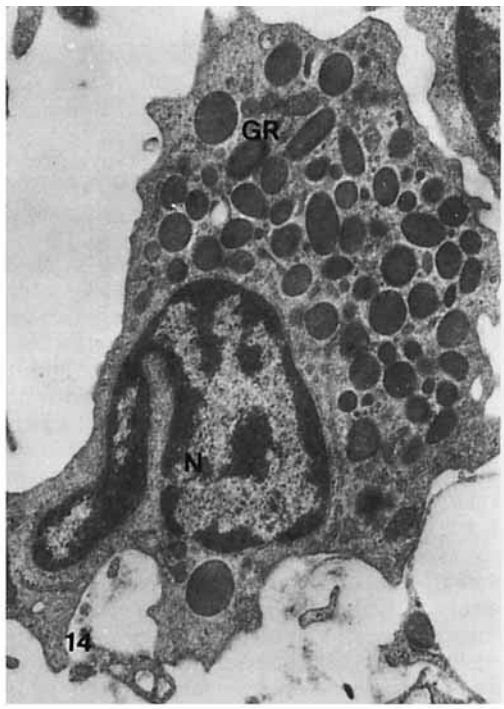
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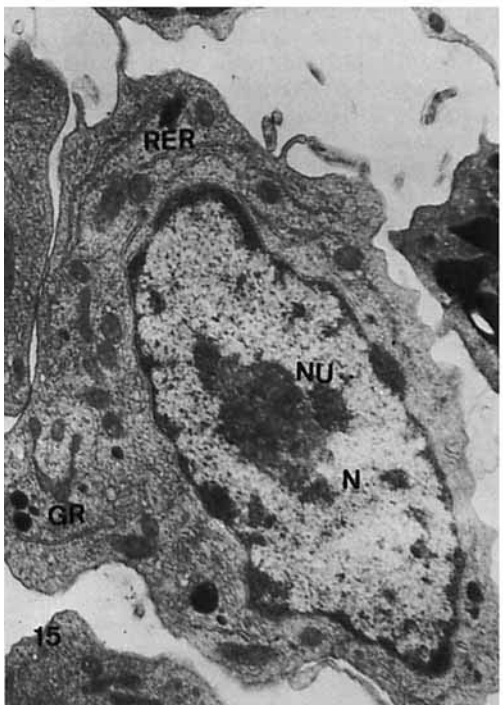
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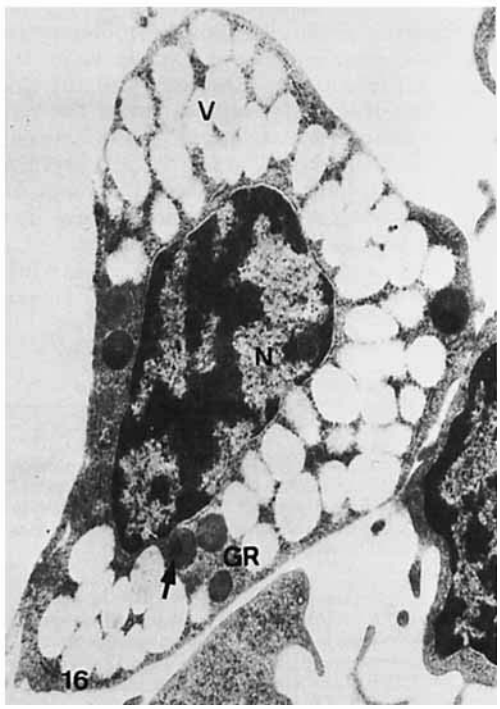
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soli, '77; Hoshi and Weiss, '78). However, adventitial cells have not been described in avian bone marrow (Campbell, '67). Tavassoli ('77) did not find junctions between the adventitial cells or endothelium and adventitial cells, but Weiss ('76) reported gap junctions in both cases. The importance of an adventitial layer for cell migration through the endothelium was investigated by Campbell ('72) and Tavassoli ('77) in mammalian bone marrow. They suggested that the extent of endothelium coverage correlates negatively with the amount of cell movement across the wall.

The morphology of reticular cells of *L. hispanica* is similar to that described in other vertebrates (Campbell, '70, '72; Biermann and Graf von Keyserlingk, '78; Hoshi and Weiss, '78). These cells are lighter than endothelial cells and appear to be very ramified where developing hemopoietic cells are located. They have been considered to be fibroblasts, because of their intimate association with reticular fibers and because of their ultrastructure (Hoshi and Weiss, '78). In *L. hispanica*, many sections suggest a functional relationship between reticular cells and developing blood elements. Chen and Weiss ('75) noted that these relationships may constitute an important element in the hematopoietic microenvironment, inducing the differentiation of stem cells into a particular cell line. Besides, the capacity of human fetal marrow to trap stem cells from the blood appears to depend upon its stromal reticular cells (Chen and Weiss, '75). With respect to a phagocytic function of lining cells and fixed reticular cells (Maximow, '27; Bloom and Fawcett, '68) in the bone marrow of *L. hispanica*, both cells types show lysosome-like dense bodies, but nevertheless this function appears correlated with free macrophages in the stroma. Campbell ('70) also thought that phagocytosis in frog bone marrow is the function of free cells which occur in and around the sinuses.

The existence of hematopoietic stem cells was established in mammals without knowledge of their morphology by irradiated mice (Becker et al., '63). The morphology identification of pluripotent stem cells is difficult. They have been considered to be transitional elements between the small lymphocytes and the blasts of different blood cell lines (Yoffey, '57; Hudson and Yoffey, '63). They are characterized by the absence of cytoplasmic organelles and condensed chromatin, and by having a prominent nucleolus and free monoribosomes. We have found morphologically similar cells in the bone marrow of *L. hispanica*. Previously, Thierry-Wirth ('72) commented on the existence of a primitive cell, difficult to identify in reptilian bone marrow. Also, in teleosts (Zapata, '79a), urodeles (Grasso, '73), and the anura (Campbell, '70), similar cells have been found. This apparent morphological identity between the hemopoietic stem cells of all vertebrates suggests a homologous evolutionary mechanism for vertebrates hemopoiesis.

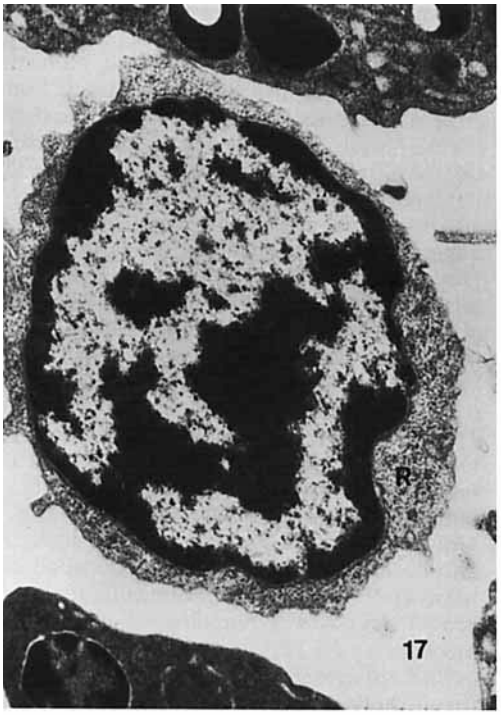
The intravascular differentiation of erythroid cells of *L. hispanica* consists of five stages—proerythroblasts, basophil erythroblasts, polychromatophilic erythroblasts, orthochromatophilic erythroblasts, and reticulocytes—before the mature stage is reached. In other reptiles, various authors (de Villiers-Piennar, '62; Thierry-Wirth, '72; Taïb-Cazal, '73) have studied erythroid differentiation showing the same stages as we have, although some of them have used a different nomenclature. The ultrastructural results described are coincident with those pointed for all vertebrates, suggesting a common morphology of erythropoiesis for all vertebrates (see review by Sinclair and Brasch, '75). The changes exhibited by the developing erythroid cells of *L. his-*

Fig. 13. Mature heterophils in the bone marrow of *L. hispanica*. The lobulated nucleus (N) shows peripheral condensed chromatin. The cytoplasm contains different granule types (GR) and the rough endoplasmic reticulum and Golgi complexes have almost disappeared. × 7,000.

Fig. 14. Mature acidophil. Note the lobulated nucleus (N) and the homogeneous electron-dense cytoplasmic granules (GR). × 12,100.

Fig. 15. Acidophilic promyelocyte. This cell shows a lightly electron-dense nucleus (N) with scant condensed chromatin, a well-developed nucleolus (NU), and a cytoplasm containing developing rough endoplasmic reticulum (RER) and a few homogeneous granules (GR). × 13,400.

Fig. 16. Presumptive mature globular leukocyte. Note the high nuclear electron density (N) and the presence of light vacuoles (V) and electron-dense cytoplasmic granules (GR) showing a certain content (arrow). × 13,000.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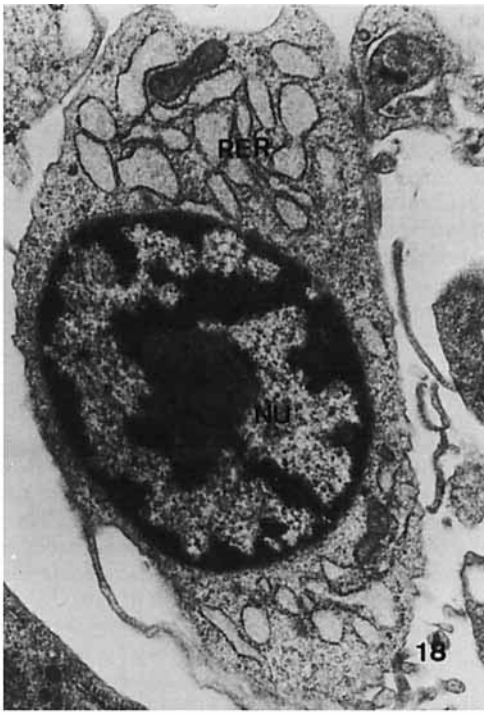
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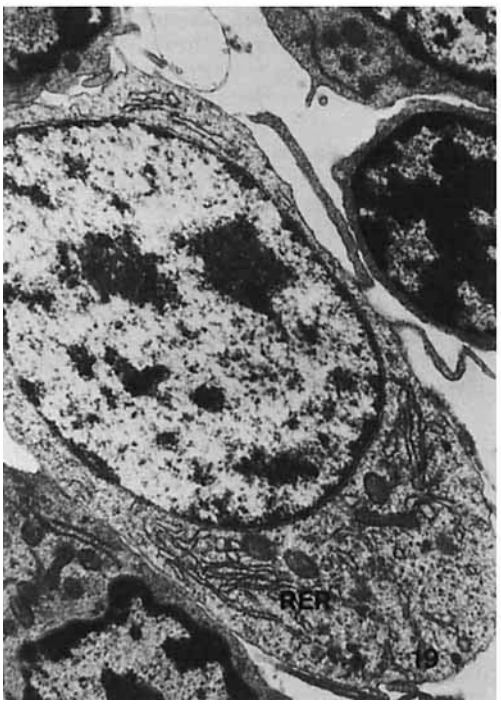
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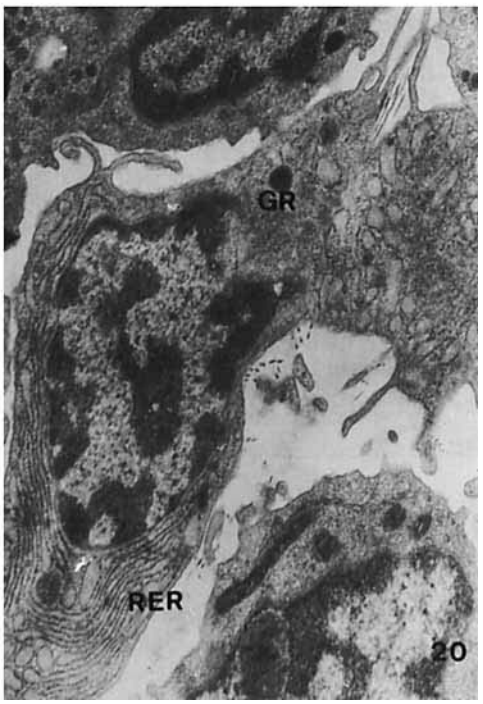
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panica, as well as the rest of the vertebrates, are related to the condensation of nuclear chromatin and the disappearance of cytoplasmic ribosomes in relation to haemoglobin storage. From the proerythroblast to reticulocyte, there is a gradual increase of chromatin condensation, while ribosomes disappear with an increase of hemoglobin storage. These changes are evidently a demonstration of the gradual inactivation of the erythroid genome, as has been pointed out for other vertebrates (Mac Rae and Meetz, '70; Brasch et al., '74; Ringertz and Bolund, '74).

Apart from granular leukocytes, the bone marrow of *L. hispanica* shows two classes of granulocytes. Taylor et al. ('63) described heterophils of *Pseudemys scripta elegans*, and Efrati et al. ('70) found heterophils in *Agama stellio* with two types of different electron-dense granules, some large, round, or fusiform, and others fine, round, or oval. Kelényi and Nemeth ('69) considered that fusiform granules arise from the fine round granules, mainly in immature heterophils, and appear to contain holes. In *L. hispanica*, heterophils have been clearly identified. Their morphology is similar to that described by the above authors, although three granule types were observed. The existence of holes in the larger granules, similar to those described by Kelényi and Nemeth ('69), is remarkable. These authors suggest that they may represent sites of interaction of the granules and cytoplasm.

With respect to the second type of granulocyte described in *L. hispanica*, they contain round, homogeneous, electron-dense granules, devoid of crystalline inclusions. Similar cells were identified as acidophils by Kelényi and Nemeth ('69) in *Lacerta agilis* and *Emys orbicularis*. However, Efrati et al. ('70) interpreted these cells as basophils, and others, not observed in *L. hispanica*, were considered to be the true acidophils. At first sight, we consider these cells to be acidophils, although some of them may be basophilic granulocytes.

The third type of granular cell of the bone marrow of *L. hispanica* has not been previously reported in reptiles nor in other lower vertebrates. Similar cells were observed in the intestine and identified as granular leukocytes by their ultrastructural resemblance with the cells described in mammals (Zapata and Solas, unpublished observations). Whether the presence of these cells in bone marrow indicates a possible origin in this hemopoietic organ is not known, because in our results the existence of marrow precursor cells is not clear.

Despite the lack of ultrastructural information on the mechanisms of granulocyte formation in reptiles, our descriptions in *L. hispanica* are similar to those of other vertebrates (Kelényi and Larsen, '76, in *Lampetra fluviatilis*; Zapata, '79b, in *Raja clavata* and *Torpedo marmorata;* Zapata, '77, in *Rutilus rutilus* and *Gobio gobio*; Curtis et al., '79 in *Plethodon glutinosus;* Campbell, '70, in *Rana pipiens*; Campbell, '67, in chicken and pigeon; Maxwell, '78, in the fowl and duck; Bainton and Farquhar, '66, '68, in mammals). Promyelocytic, myelocytic, and metamyelocytic stages and the existence of a common myeloblastic stage for the different granulocytic lines have been reported by all these authors. The differentiation includes the formation of characteristic granules of each type. In relation to granular appearance, there is an increase of rough endoplasmic reticulum and Golgi complex, and the nucleus forms lobes and gradually increases in electron density. These resemblances indicate that the cytologic mechanisms of granulocyte differentiation are present in lower vertebrates.

Fig. 17. Small lymphocyte. Note the high nucleocytoplasm ratio and the amount of condensed chromatin and free ribosomes (R). × 16,500.

Fig. 18. Mature plasma cell. The condensed chromatin exhibits a special arrangement in the nuclear periphery and surrounding the prominent nucleolus (NU). In the cytoplasm, the development of rough endoplasmic reticulum (RER) is remarkable. × 13,300.

Fig. 19. Proplasmatic cell. Precursors of mature plasma cells of bone marrow of *L. hispanica* show scant condensed chromatin and only some profiles of rough endoplasmic reticulum (RER). × 11,400.

Fig. 20. Immature plasma cell. Note the large development of rough endoplasmic reticulum (RER) and its arrangement in parallel structures without enlargement of its saccules. Some electron-dense granules (GR) appear in the cytoplasm. × 13,400.

Lymphocytes and plasma cells occur constantly in the bone marrow of *L. hispanica*. Lymphoid cells are common constituents of the marrow cell population of all vertebrates, and in lower vertebrates, they have been claimed to be present in the bone marrow of *Plethodon glutinosus* (Curtis et al., '79) and in the frog (Campbell, '70). In reptiles, Thierry-Wirth ('72) noted their presence, but other authors (Sidky and Auerbach, '68; Borysenko and Cooper, '72; LeFevre et al., '73) deny it. The morphology of these cells is similar to that reported in other reptilian lymphoid organs (see review by Borysenko, '78). A remarkable feature not reported by other authors is the existence of immature plasma cells in the bone marrow of *L. hispanica*, suggesting a plasmacytopoietic capacity. The immune function of the bone marrow of lower vertebrates is not known. Recently, some morphological and functional data in *Rana pipiens* (Cooper et al., '80) suggests the marrow as the source of hemopoietic stem cells and perhaps of mature lymphocytes. The presence of lymphocytes and plasma cells in the bone marrow of *L. hispanica* possibly indicates an immune function of reptilian bone marrow. Therefore, further investigations of the B cell origin and the immune memory in reptiles must take this data into consideration.

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