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THE LUMINOUS ORGANS OF *PROCTOPORUS* (SAURIA, REPTILIA) — A RE-EVALUATION

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INTRODUCTION

The herpetological literature contains two reports describing the first luminous organs in a terrestrial vertebrate. The two papers discuss identical specimens of the Trinidad lizard *Proctoporus shrevei* Parker. Sanderson (1939, and observations cited by Parker, same date) claimed that light was emitted by black bordered ocelli on the sides of a male, and Parker (1939) supported this on the basis of his histological examination of the preserved animal.

No new observations have been published since that time, but a number of workers have commented upon the original observations. Thus Pope (1955, p. 306) remarked that "other teiids have spots somewhat like those of *P. shrevei*, so it is highly probable that they, too, can light up." In contrast to this, Harvey (1952, p. 494) in his monograph on bioluminescence stated that he believed "all reports of luminescence in higher vertebrates to be false or spurious due to reflection of light or infection by luminous bacteria."

The divergence of opinion on this interesting point prompted a re-examination of this question. The present paper reports a few additional field observations, and includes as well a detailed examination of the histological structure of the ocelli. Since *Proctoporus shrevei* is very rare, this re-examination had to be carried out on two related and superficially similar forms.

OBSERVATIONS ON LIVING SPECIMENS

Parker (1939, p. 659) mentioned that Sanderson's field notes contained only a brief reference to color pattern involving "five, black spots each containing a small, vivid white, sometimes luminous bead." Parker further stated that Sanderson in conversation informed him "that the animal was kept alive in captivity and could be stimulated to emit light from the lateral spots; the light was of a pale greenish hue, similar to that produced by the hands and figures of a luminous watch. Excitement produced by flashing an intermittent beam of light on the lizard was found to be a very effective stimulus to light production."

A more specific first-hand report was given by Sanderson (1939, pp. 41-43) in his popular, considerably amplified and slightly different, account. When initially observed the lizard "turned its head away from me and both its sides lit up for a few seconds like the portholes of a ship." "After one brilliant display on the night of its arrival in camp it refused to shine with full brightness though the beadlike spots remained plainly discernible in a darkened box when the rest of the animal was invisible." A "loud whistle, sudden winds, and flashes of light greatly agitated our lizard, causing it to switch on its "portholes". . . . The light was much brighter the first time it was switched on after the animal had been quiescent for a period, and more especially after it (the lizard) had previously been subjected to intense illumination."

We have been able to obtain four sets of further observations on live specimens of the genus *Proctoporus*.

Julian S. Kenny (V. C. Quesnel, personal communication) some time ago repeated Sanderson's experiments on the original species (*P. shrevei*) with entirely negative results. Kenny's field notes also indicate that the lizards are diurnal and inhabit relatively open spaces on El Tucuche, Trinidad.

Dr. Janis Roze (*in litt.*) states that a specimen of *Proctoporus achlyens* Uzzell (M.C.Z. 53128, later used for histological examination reported herein) did not glow when placed in a darkened room after capture. He adds that exposure to ultraviolet light did cause the spots to shine faintly. The test was carried out in an incompletely darkened room, and the results seem to be open to some question.

Harold Heatwole and Owen J. Sexton (personal communication) performed a number of experiments at a field station in Venezuela. They tested one adult male of *P. luctuosus* (Peters)

and two adult males of *P. achlyens* for a period of one month. The spots of the first species were yellow and those of the second were red in life, both series of spots bleaching to white after formalin preservation. The specimens were repeatedly moved from light to dark environments and were observed at night. The animals were disturbed. No luminescent effect was ever noted. Ultraviolet illumination was not attempted.

The most extensive series of observations on live animals was made in Ecuador by James A. Peters (*in litt.*). Specimens of *Prionodactylus vertebralis* (O'Shaughnessy) and *Neusticurus ecpleopus* Cope were observed while free in the field, during the collecting process, and for several weeks in the laboratory. He reported that neither luminescence nor any other kind of light could be noted in broad sunlight, dim or artificial light, or in the complete absence of light. No reflection could be noted under various types of lighting (sun, fluorescent and incandescent), in quiescent, active or deliberately disturbed animals. The evidence is most valuable because Peters was aware of the lizards' reputation and was deliberately testing the hypothesis of luminescence.

SUPERFICIAL APPEARANCE AND PHYLOGENETIC DISTRIBUTION OF THE OCELLI

The supposedly luminous ocelli (Fig. 1) are rather similar in the species of *Proctoporus* and *Neusticurus* here discussed. They are arranged in a single row along the side of the animal; each ocellus always shows a light-colored, sharply-defined, black-bordered, circular center. They may be restricted to adult males, with juvenile specimens and females showing only traces. There is usually a size decrease of the ocellar center posteriorly along the series. There may be a marked irregularity in the width of the black border. There is no correlation between the ocellar and the scale patterns.

While sharply-defined ocelli are commonly well developed only in Boulenger's (1885, p. 332) teiid group II, a check of the more than 130 species of teiid lizards as well as forms of other families represented in the collection of the Museum of Comparative Zoology at Harvard College indicated that patterns with sharply contrasting light and dark colors are extremely common. A complete morphological series may be demonstrated in the Teiidae. This series ranges from patterns with alternating light and dark stripes, through those in which the stripes alternately fuse and break up, to patterns with well-defined light circles on

a dark background. Such spots may be found over the entire body or may be restricted to the sides. The condition found in the males of *Proctoporus* and *Neusticurus* represents only one extreme development of color variation. Similar conditions may also be observed in certain geckonids and iguanids. Here the ocellar pattern may occur all along the side, with the color contrast emphasized around a limited number of spots.

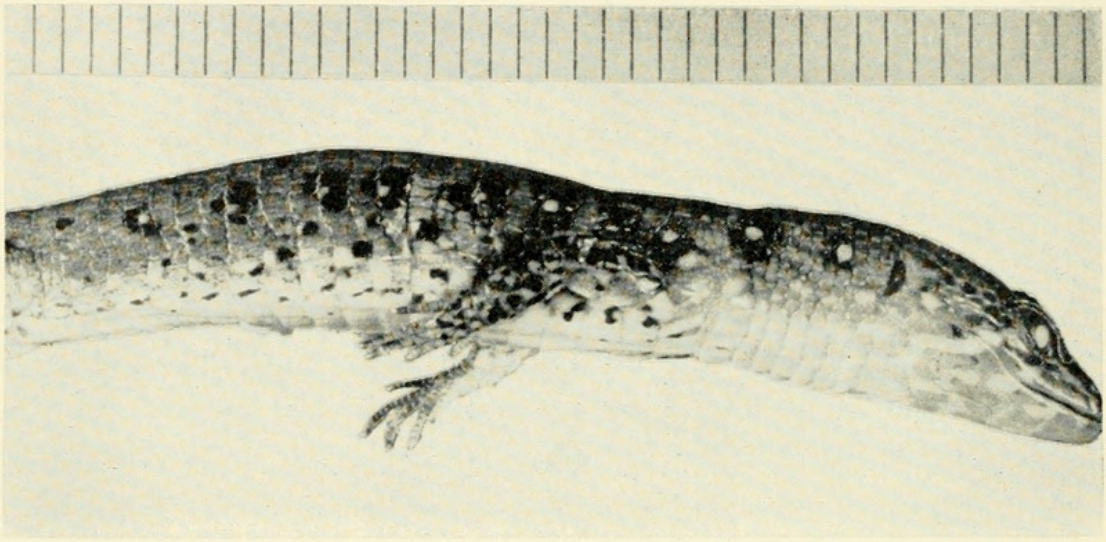

Fig. 1. *Proctoporus achlyens* Uzzell. Lateral view to show shape, size and arrangement of ocelli. The scale is graduated in mm. (M.C.Z. 53128 — C.G. photo.)

HISTOLOGICAL AND HISTOCHEMICAL EXAMINATION OF THE SKIN

Methods

Two specimens were used for histological examination. These were M.C.Z. 43764, *Neusticurus ecpleopus ocellatus* Sinitzen from Hacienda Pampayacu, Departamento de Huanuco, Peru, and M.C.Z. 53128, *Proctoporus achlyens* Uzzell from Choroni, Estado de Aragua, Venezuela. The museum specimens were reported to have been fixed in 10 per cent formalin and transferred to 70 per cent alcohol for storage.

Sections were cut from the second (and largest) ocellus of the left side of each specimen. A sample of faintly pigmented skin from the ventral surface of M.C.Z. 53128 was sectioned for comparison. Small blocks of tissue, including both the center and the black margin, in the case of the ocelli, were excised. These tissue blocks, including the epidermis, dermis, and a small amount of underlying skeletal muscle were hydrated through a

descending alcohol series, post-chromated in saturated aqueous potassium dichromate at room temperature for three days, washed overnight in running tap water, dehydrated in ethanol, cleared in chloroform, and embedded in tissue mat (56-58°). Five-micron sections were mounted individually so that various staining techniques could be carried out on adjacent sections.

The techniques used were: Harris hematoxylin and eosin and the paraldehyde fuchsin method as modified by Halmi (Halmi, 1950) for general morphology, Wilder's modification of the Bielchowsky silver impregnation method (Romeis, 1948, p. 355) for nerve fibers and endings, Sudan black B with acetone controls for lipid compounds, the periodic acid-Schiff technique for 1-2 glycol linkages, the azo dye methods for protein bound sulfhydryl and disulfide groups (Barrnett and Seligman, 1952, 1954), dilute methylene blue at pH's 4 to 9 and dilute light green at pH's 3 to 8 for a rough approximation of pH signature of proteins (Singer, 1952) and buffered toluidine blue and thionine for metachromasia.

GENERAL MORPHOLOGY OF THE SKIN AND OCELLUS

On the basis of the appearance of nuclei, blood cells, striated muscle fibers, small nerves, and the cells of the epidermis, the fixation was judged to be good. Presumably the external location of the tissue with the consequent immediate exposure to the formalin had been advantageous. In addition, the tanning with dichromate seems to have been successful in preventing the shrinkage that normally results from paraffin embedding of formalin-fixed tissues.

As revealed by hematoxylin and eosin (Figs. 2, 3), the epidermis is composed of a very thin stratified squamous epithelium showing a prominent basement membrane. The epithelium consists of a single-layered cuboidal stratum germinativum covered by only one or two layers of flattened squamous cells. The surface is covered by dense keratinous scales.

The dermis can be subdivided into two layers. Superficially, it is composed of a rather loose fibroelastic tissue which contains a dense accumulation of melanin pigment except in the region of the ocellus. The pigment appears to be contained in chromatophores, but this cannot be stated categorically for all of it. Often fine strands of pigment granules extend into the epidermis. In some instances these granules seem to be in processes between the epidermal cells, but in others they seem to occur within the

cytoplasm of the epithelial cells. Possibly they occur in both locations. The deep layer of the dermis is composed of typical dense collagenous connective tissue with a rather regular orientation parallel to the surface of the skin. Both layers of the dermis contain an extensive network of elastic and reticular fibers.

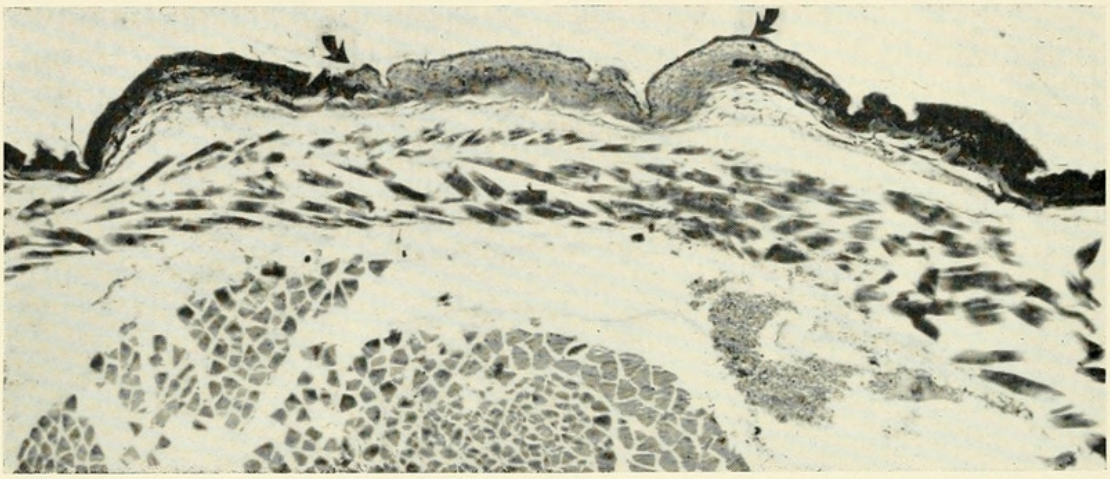

Fig. 2. Cross-section through the center of ocellus (between the arrows) and surrounding pigmented skin taken from *Proctoporus achlyens* Uzzell. Hematoxylin and eosin. 50X.

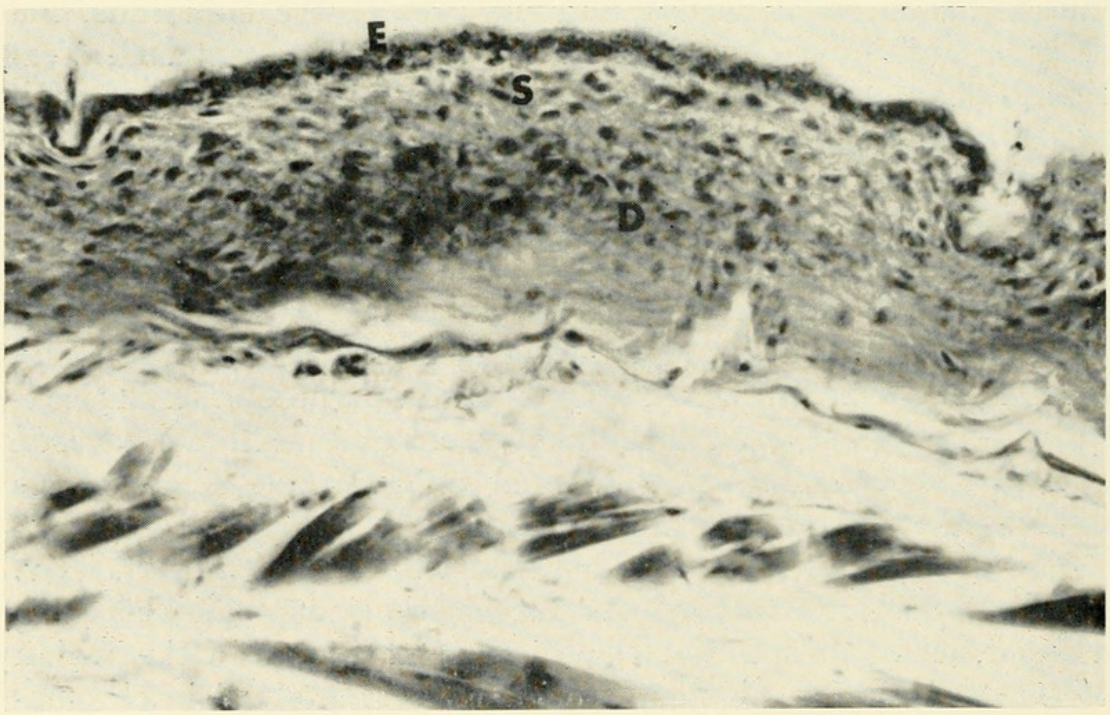

Fig. 3. Cross-section through the center of the ocellus shown in Fig. 2. Note the thin epidermis (E), the vacuolated appearance of the superficial layer of the dermis (S), and the dense collagenous deep layer of the dermis (D). Hematoxylin and eosin. 400X.

The dermis is underlain by typical loose connective tissue containing small blood vessels, fat cells, and small peripheral nerves. *The white center of the ocellus, which is the prime object of this study, differs histologically from the rest of the skin only in the fact that no melanin pigment occurs in the superficial layer of the dermis or epidermis.* The sections through the ocellus do not show any obvious differences in thickness or arrangement of skin structures as compared with the normal pigmented areas.

It should be noted especially that no nerve fibers or specialized nerve endings were recognized in the dermis of either the ocellar or the pigmented regions of the skin, although distinct nerve fibers could be seen in the subcutaneous tissue and in the underlying muscle bundles. It should be noted further that no extensive or unusual vascular arrangement occurs in the region of the ocellus.

THE HISTOCHEMISTRY OF THE SECTION

Histochemically, the epidermis shows nothing striking. Its surface gives a moderate reaction for sulfhydryl groups and an intense reaction for disulfide linkages, as would be expected if its

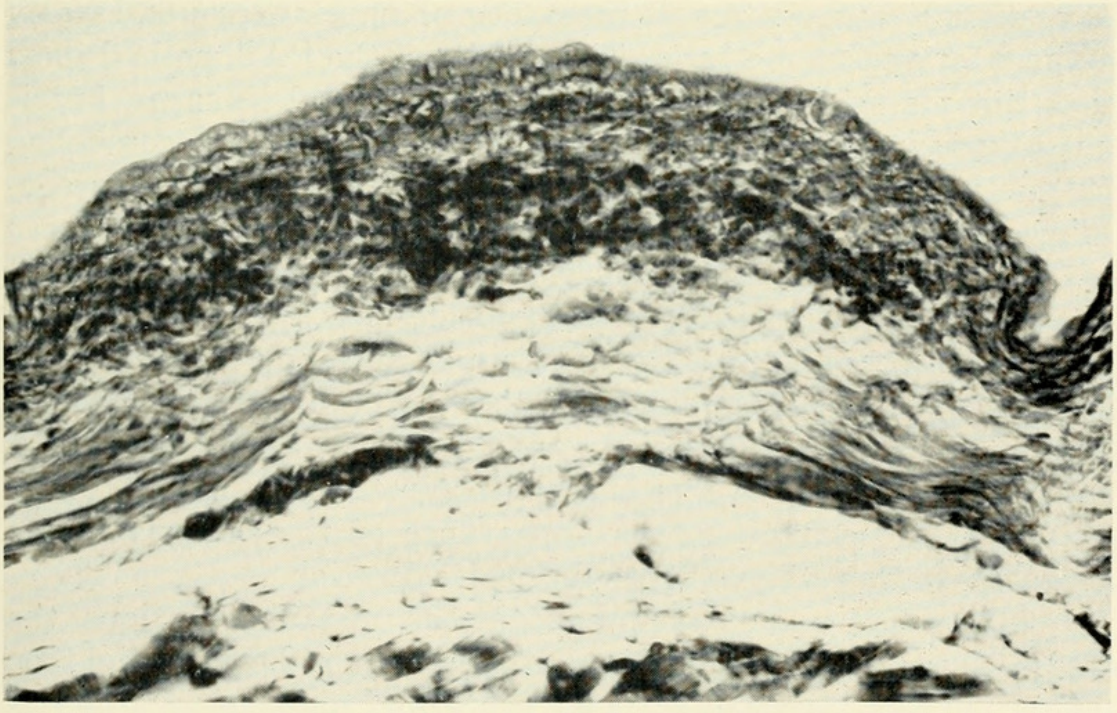

Fig. 4. Cross-section through the same ocellus as shown in Figures 2, 3. Note the intense PAS-positive reaction of the superficial layer of the dermis. Periodic-acid-Schiff reaction counterstained with hematoxylin 400X.

scales were keratinized. *The epidermis covering the white spot is identical with that covering pigmented dermis. The deep layer of the dermis also seems to be identical* below the white and pigmented skin. The reactions of both collagenous and elastic tissue present nothing novel. The superficial dermis of the white spot is of special interest, however. In hematoxylin and eosin preparations, it is much more lightly stained than the deep portion of the dermis, contains less collagen and is far more cellular. However, the cytoplasmic limits (and the cell boundaries) of these cells cannot be made out. The impression, therefore, is of tenuous, presumably branched cytoplasmic processes.

Histochemically, this superficial region of the dermis (the cell cytoplasm?) shows a number of characteristics. Thus it gives an intense positive PAS reaction which is diastase resistant (Fig. 4). Further, it shows a moderately strong reaction for sulfhydryl groups which is not greatly intensified with the disulfide test. With controlled pH staining it shows only a weak staining with light green even at low pH's, but a moderate (pH 5) to heavy (pH 8) staining with methylene blue or thionine. From these results it seems possible to conclude that the cells of this region are not vacuolated (as might appear from H and E sections), but contain a substance or substances not readily stained by routine methods. The results of the PAS method show that this material contains numerous 1-2 glycol linkages, but is not glycogen. The results of the sulphydryl and the controlled pH methods indicate a moderately basophilic protein which contains appreciable cystine or cysteine or both. A complete absence of staining with Sudan black B indicates that it is not a phospholipid, a glycolipid, or a lipoprotein. Thus these findings suggest the presence of a mucoprotein or mucopolysaccharide. A heavy accumulation of connective tissue ground substance would account for these observations except for the absence of metachromasia. While the fixation in this instance is not optimum for a critical evaluation, the appearance of the sections favors an intra- rather than extracellular localization.

Furthermore, upon the examination of unstained sections, the region shows no marked granulation under either the light or phase-contrast microscope. The reflecting pigment guanine is supposedly insoluble in all of the reagents used for fixation and embedding. Guanine crystals, if present, should therefore be evident in unstained sections, and their absence rules out this pigment as the source of the white appearance.

DISCUSSION AND CONCLUSIONS

Basically there are no important differences between the present more extensive histological description and that originally furnished by Parker (1939, p. 658). He accented the differences between the tissue underlying the glistening white spot and that below the remainder of the dermis. The points emphasized were five: (1) a reduction of the epidermis to one-half its normal thickness, (2) absence of the chromatophore layer, (3) presence of a mass of spongy mesenchymatous tissue, (4) large intra- or intercellular spaces in the spongy tissue, and (5) absence of nerve endings. Parker emphasized the poor preservation of the material. Neither the stains nor the method of preservation were specified, but the photomicrographs suggest that only H and E or a similar routine staining technique was employed.

We differ in failing to find either vacuolated spaces in the spongy tissue or a reduction in thickness of the overlying epidermis. It seems clear that the presence of "vacuolated spaces" could be accounted for entirely by the fact that Parker's material was poorly preserved for histological purposes and that these are presumably fixation or shrinkage artifacts. With regard to the variation in thickness of the epidermis, Parker's photographs indicate that such a reduction does not coincide with the absence of melanin. Our sections indicate similar and regional differences in the thickness of the epidermis of some of the pigmented scales.

On the basis of the additional evidence reported in this paper, the following statements may be made:

Sanderson's observations and Parker's comments thereon contain a number of inconsistencies. Sanderson reported that the light is (1) under the control of the animal and can be turned on and off, (2) much brighter the first time it is used after a period of quiescence, and (3) brightest just *after* the spot has been subjected to illumination. In spite of this Parker suggested that the organs are reflectors rather than truly luminous. A reflector might be capable of producing the first of the three effects, but the last two would be characteristic of true luminescence.

None of the subsequent field observers has reported similar results. Their experiments cover more specimens and a longer period than does Sanderson's report. However, in all but one case the species involved are different, though externally very similar and probably closely related to the form on which the original report had been based.

The lateral ocelli of *Proctoporus* might represent one of four types of structures: independent light-generating organs (either innervated or under hormonal control), receptacles for light-generating organisms, specialized reflecting structures, or simply nonpigmented "white" spots with a black margin.

The first of these possibilities is made unlikely by a number of factors. The center of the ocellus shows no gross difference from the surrounding skin and can be recognized only by the absence of melanin. The ocellus does not, in any manner, resemble previously described luminescent organs. The cells of the white spot do not have an epithelioid appearance and in no way resemble cells previously described for luminescent organs. There is neither special innervation nor vascularization. This is particularly important since Sanderson indicated that the light was turned on quite rapidly.

The absence of any vacuoles or staining reactions characteristic of bacteria seems to rule out the possibility of storage of luminous microorganisms.

There are certain difficulties in distinguishing between reflecting structures and plain white spots. It seems certain that the white spots do not represent one of the more complicated reflecting systems since specialized epidermal cells and similar structures are lacking.

It is, therefore, concluded that the white appearance is produced by an inter- or intracellular substance, which lies at the same level of the skin as the dermal melanophores, and which may or may not have special reflecting properties. The further possibility exists that a local accumulation of connective tissue ground substance, or a specific intracellular mucoprotein or mucopolysaccharide could be strongly reflective.

This conclusion is in good agreement with all reports of observations on live animals but Sanderson's. If any of the species of *Proctoporus* or related teiids are luminescent, they would seem to glow only under very special circumstances and by a yet undescribed mechanism. However, the inconsistencies of the initial reports by Sanderson and Parker and the completely negative result of the investigations presented here force us to reject, for the present, any interpretation of these "portholes" as bioluminescent organs.

Our findings, furthermore, suggest a quite different and interesting possibility. The location and appearance of the protein-containing cells in the superficial dermis suggests that they could be potential melanophores which have formed no pigment.

Clearly, proof of this would involve a study of the histochemistry of such "prepigmented" melanophores. Nevertheless, it seems possible to speculate that this color pattern in lizards might be achieved through a precise local control of the chemistry of the melanophore cells and hence, might prove an interesting area for the study of specified control of cellular differentiation.

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