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Morphology of the Pars Intermedia and the Melanophore-Stimulating Cells in *Xenopus laevis* in Relation to Background Adaptation

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The melanophore-stimulating hormone (MSH) cells of the amphibian pars intermedia secrete the peptide α -melanophore-stimulating hormone (α -MSH), which induces pigment dispersion in dermal melanophores. The purpose of the present study was to determine (1) whether prolonged activation of the secretory activity of the pars intermedia is associated with hypertrophy, hyperplasia, or both and (2) whether the MSH cells function as a homogeneous or heterogeneous population in meeting the physiological demand for MSH. The demand for MSH was manipulated by adapting animals for at least 3 weeks to white, two shades of grey, or black backgrounds. Morphometric analysis showed that the intermediate lobe volume was positively correlated with the degree of pigment dispersion in the melanophores. The number of MSH cells per lobe was not affected by the degree of pigment dispersion. Therefore, we conclude that enlargement of the tissue associated with MSH cell activation involves hypertrophy rather than hyperplasia. Ultrastructural examination indicated that the majority of MSH cells in black-adapted animals are biosynthetically active, whereas the cells of white-adapted animals are relatively inactive and show granule storage. The pars intermedia of grey-adapted toads contained both active and inactive cells, indicating that MSH cells respond as a heterogeneous cell population in meeting the endocrine demand imposed by background. © 1990 Academic Press, Inc.

In general, activation of endocrine cells may involve hypertrophy, hyperplasia, or both. Furthermore, two patterns of cell responses can be distinguished during activation: either all cells become activated or only subpopulations of cells become activated. An example of the former are the oxytocin-producing neuroendocrine cells that respond as a homogeneous population to the suckling stimulus (Poulain and Wakerley, 1982). An example of the latter are the vasopressin-producing neuroendocrine cells that respond as a heterogeneous population to osmotic stress (Poulain and Wakerley, 1982). Morphometric and biochemical evidence has been advanced that the rat gonadotropes (Neill *et al.*, 1986) and lactotropes (Lucque *et al.*, 1986) as well as the β cells of the pancreatic islets (Schuit *et al.*, 1988) and thyroid follicular cells in mice (Gerber *et al.*, 1987) form heterogeneous

populations with regard to their secretory response to demand for hormone.

In the present investigation we studied the response of amphibian melanophore-stimulating hormone (MSH)-producing cells to a physiological stimulus. Two major questions were addressed. First, does the activation of the pars intermedia involve hypertrophy, hyperplasia, or both? Second, do the endocrine cells of this tissue function as a homogeneous or as a heterogeneous cell population in response to a demand for MSH? The MSH cell of *Xenopus laevis* provides a good model to study these questions because the pars intermedia activity can be easily manipulated. The MSH cells of animals placed on a black background release α -MSH, which stimulates pigment dispersion in dermal melanophores, thereby darkening the skin. Conversely, MSH cells of animals on a white back-

ground are relatively inactive, which results in a light skin. To manipulate the pars intermedia activity, we adapted *X. laevis* 3 weeks to white, light grey, dark grey, or black backgrounds. Plasma MSH levels were measured to confirm that the treatment altered secretory activity of the pars intermedia. Analysis of intermediate lobe activity included morphometric determinations at the light microscopic level and ultrastructural characterization of the MSH cells.

MATERIALS AND METHODS

Animals. *X. laevis* were obtained from laboratory stock and fed on ground beef heart and trout pellets (Trouvit) once a week. Lights were on for 12 hr a day. The water temperature was 22°. All animals were reared and held on a grey background before the experiments started. Consequently, they showed a melanophore index (MI) of 3 or less, according to the protocol of Hogben and Slome (1931). A MI of 1 indicates full aggregation of melanin in dermal melanophores, a MI of 5 indicates full dispersion. During experiments they were fed trout pellets only and were kept in lights for 24 hr. Males and females of approximately the same weight (25 g) were used for the experiments. They were adapted to white, light grey, dark grey, and black backgrounds in 10-liter buckets for 3 weeks. These backgrounds give consistent MI values of 1.0 ± 0.1 , 1.9 ± 0.1 , 3.5 ± 0.1 , and 4.9 ± 0.1 , respectively.

Radioimmunoassays. To determine the plasma MSH content, animals were decapitated and trunk blood (1 ml) of individual animals was collected in 250 μ l ice-cold saline (0.9%) containing $\text{Na}_2\text{-EDTA}$ (2 mM) and trasylol (45 KIU/ml). After centrifugation (10 min, 10,000g, 4°), the supernatant of each sample was applied to a octadecyl C-18 column (J. T. Baker, Chemical Co), pretreated with 3×1 ml buffer (0.5% formic acid and 0.14 M pyridine) and 3×1 ml 1% polypep (Sigma) in the same buffer. The column was washed with 6×1 ml 8% propanol in buffer. MSH was eluted from the column with 3×1 ml 26% propanol in buffer. The eluents were pooled and $20 \mu\text{g} \cdot \text{ml}^{-1}$ bovine serum albumin (BSA; Sigma, fraction V) was added. The sample was vacuum dried (Savant Speed Vac) and redissolved in assay buffer (0.02 M Na-barbital, 0.2 g/liter Na-azide, and 0.3% BSA, pH 8.6) for the radioimmunoassay. MSH plasma levels were not corrected for losses during the extractions and were therefore relative (percentage recovery was 40–50%).

The MSH antiserum used in the radioimmunoassay was produced by injecting rabbits with synthetic α -MSH (Sigma) coupled to thyroglobulin with carbo-

diimide. In all our assays the MSH antiserum, obtained by the ninth bleeding (L9), was used at a final dilution of 1:30,000. The cross-reactivity of the MSH antiserum for des-acetyl- α -MSH and for α -MSH was 100% and for ACTH and ACTH(1–24) less than 0.5%. Free and bound ^{125}I - α -MSH was separated by precipitation of the immunocomplex with polyethylene glycol (BDH)/ovalbumine (Sigma) (15%/2.4%). The MSH detection limit was 2.5 pg per tube.

Light microscopy. Freshly dissected brain-pituitary complexes of *Xenopus* were fixed overnight by immersion in Bouin-Hollande, dehydrated, and embedded in Paraplast. Serial sections (5 μm) were cut. Every 10th section was mounted on a microscope slide and stained with Mayer's haematoxylin/eosin.

Electron microscopy. Freshly dissected neurointermediate lobes were prefixed in 3% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.4) for 15 min and fixed in a mixture of 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, 2% osmium tetroxide (OsO_4), and 5% potassium dichromate (1:1:1) for 1–1.5 hr (Wendellar Bonga *et al.*, 1984). After fixation the tissues were block stained by 2% uranyl acetate in water (1 hr). Subsequently, the tissue was dehydrated and embedded in Spurr's resin. Ultrathin sections (50 nm) were collected on 150 mesh copper grids, stained for 5 min with lead citrate (Reynolds, 1963), and examined with a Jeol JEM 100CX II microscope. Semithin sections (1 μm) of the same blocks were cut for the determination of surface areas of MSH cells and their nuclei.

Immunogold labeling. Neurointermediate lobe tissue was fixed in Karnovsky's mixture (2% paraformaldehyde and 2% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4) and embedded in Spurr's resin. Sections (90 nm) were collected on 150 mesh nickel grids and immunogold labeled with protein A gold (ϕ 16.2 nm; Janssen Biotech NV) essentially as described by Van Putten and Kiliaan (1988). One modification was introduced by omitting etching with H_2O_2 . The α -MSH antiserum was used in a dilution of 1:1000. Replacement of the α -MSH antiserum with preimmune rabbit serum or preadsorption of the antiserum with excess antigen served as control procedures.

Morphometry of the intermediate lobe volume, cellular and nuclear cross-sectional surface areas. Cross-sectional surface areas of sagittal intermediate lobe sections, of MSH cells, and of their nuclei were estimated with a light microscope connected with an X-Y tablet and Kontron MOP integration equipment. By determining the intermediate lobe surface areas of every 10th Paraplast section, the intermediate lobe volume was calculated.

The number of cells per intermediate lobe was estimated by determining the average cell cross-sectional surface area of 100 MSH cells in semithin sections. Only cell profiles that showed a nuclear section were scored. From these data the average cell volume was

calculated. *In vitro* studies on MSH cells showed that the cells are approximately spheroidal in shape. This information has been used in the calculation of the average cell volume. Consequently, the cell surface area is directly related to cell volume. By dividing the intermediate lobe volume by the average cell volume, the number of MSH cells per intermediate lobe was assessed. The nuclear cross-sectional surface area was determined in semithin sections for a sample of 100 MSH cell nuclei per experimental group.

Statistical tests. Differences between means of plasma MSH levels, of intermediate lobe volumes, and of cell numbers were tested with Student's *t* test. Differences in the relative numbers of MSH cell types were tested with nested ANOVA (Sokal and Rohlf, 1981). In both tests statistical significance was accepted for $P < 0.05$.

RESULTS

Plasma MSH levels. Plasma MSH levels of black-adapted animals were all above 100 pg/ml, whereas those of white-adapted animals were below the linear part of the standard curve of our RIA. Plasma MSH levels of dark grey- and light grey-adapted animals fell between the values of black and white ones. As shown in Fig. 1 the MI is positively correlated with the logarithm of the MSH levels in the blood plasma ($r_0 = 0.8263$; $n = 12$; $P < 0.001$).

Intermediate lobe volume. The volume of the intermediate lobe of black-adapted animals was almost twice the volume of that of white-adapted animals (Table 1). Intermediate lobe volumes of dark grey- and black-adapted animals had significantly increased compared to white-adapted toads ($0.02 < P < 0.05$ and $P < 0.001$, respectively) and the difference in lobe volume of dark grey- and black-adapted animals was also significant ($0.002 < P < 0.01$).

The number of MSH cells. Table 1 gives the number of cells in the intermediate lobes of animals adapted to white, light grey, dark grey, and black backgrounds. No significant differences in cell numbers among these groups were found ($P > 0.05$; Student's *t* test).

Ultrastructure of MSH cells. On the basis of ultrastructural and immunocytochemical (see below) criteria, two types of MSH

cells were distinguished. The first cell type (type I; Fig. 2a) contained numerous round-to oval-shaped secretory granules, which were often electron translucent. The Golgi complex in this cell type was poorly developed and dilatations of the rough endoplasmic reticulum (RER) were rarely observed. The other cell-type (type II; Fig. 2e) contained less secretory granules, which were round in shape and contained mostly electron-dense material. The Golgi areas in this cell type were well developed and large amounts of RER were present. The type II cell was much larger than the type I cell. Nearly all MSH cells in the intermediate lobes of white-adapted animals were identified as type I (about 95%). In black-adapted toads type II cells were the predominant type (about 80%). The pars intermedia of (dark) grey-adapted *Xenopus* consisted of nearly equal numbers of type I and II cells. This is illustrated in Fig. 2c. Immunocytochemistry at the EM level showed that both cell types contained MSH-positive granules (Figs. 2b, 2d and 2f). The electron micrographs also showed some immunostaining on RER areas. Control sections were essentially devoid of immunostaining (Fig. 2g).

No signs of increased apoptosis or necrosis of MSH cells were found.

Cell cross-sectional surface area (cellular surface) and nuclear cross-sectional surface area (nuclear surface). In Fig. 3 the relative abundance of MSH cells has been plotted against cellular surface (Fig. 3A) or nuclear surface (Fig. 3B). The cellular surface was categorized in groups by $0.1 \times 10^{-4} \text{ mm}^2$ steps, the nuclear surface by $0.46 \times 10^{-5} \text{ mm}^2$ steps. Figure 3A shows that MSH cells can be divided into two main groups on the basis of the cellular surface: one group below a surface area of $0.9 \times 10^{-4} \text{ mm}^2$ and the other group above this value, particularly evident in the dark grey-adapted animals. The cellular surface of most cells of white-adapted animals was below $0.9 \times 10^{-4} \text{ mm}^2$. In light grey-adapted animals the surface of the majority of cells

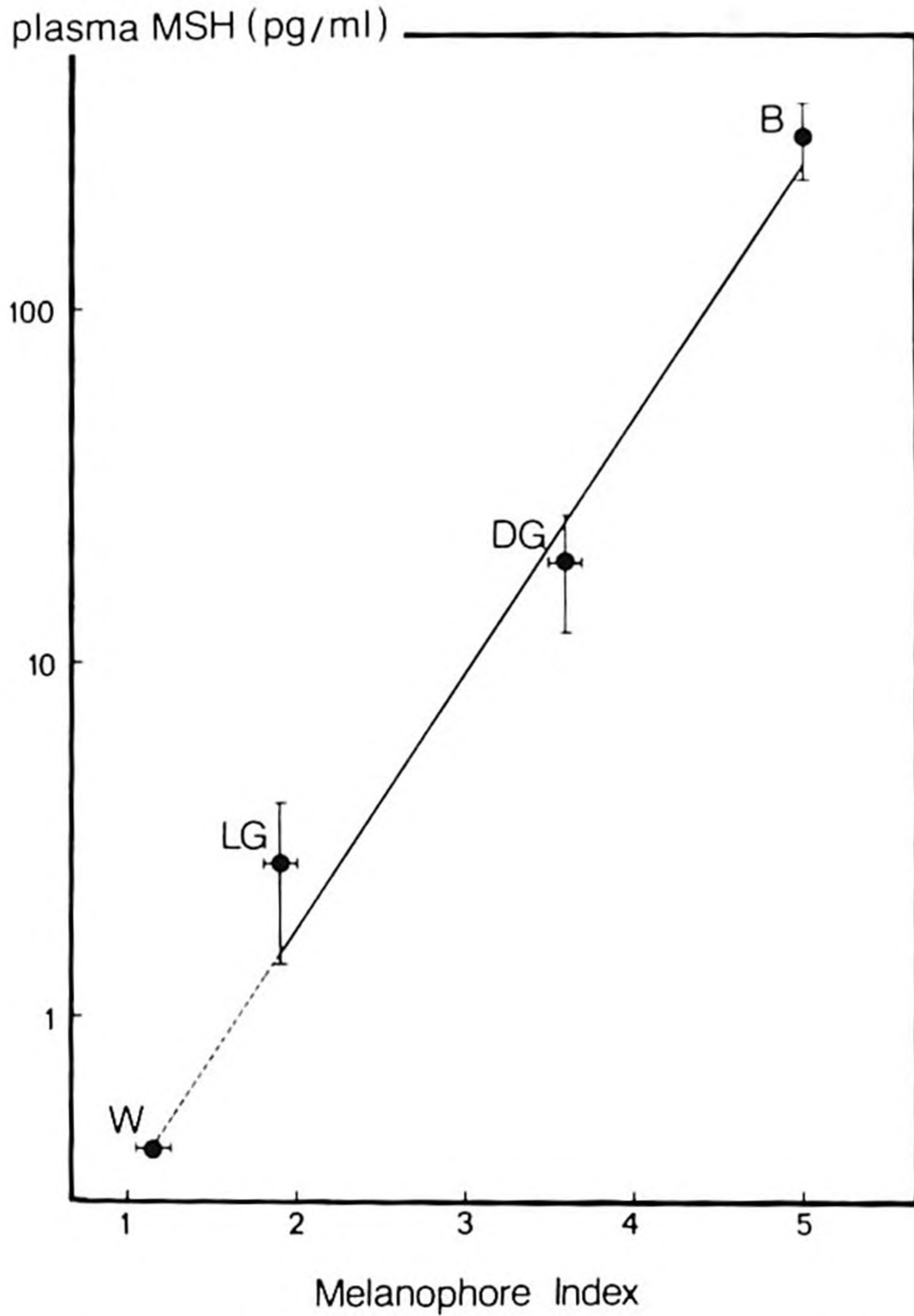


FIG. 1. Effects of long-term adaptation to different backgrounds (W, white; LG, light grey; DG, dark grey; B, black background-adapted animals) on the MI and the α -MSH levels in the blood plasma in *X. laevis* (means \pm SEM; $n = 4$)

was also below this value, although a small population of cells with a surface larger than $0.9 \times 10^{-4} \text{ mm}^2$ was present. The latter group comprised the majority (75%) of MSH cells in black-adapted animals. The distribution of nuclear surfaces followed the same trend but no clear segregation into

two populations was observed. The differences in distribution of cell and nuclear surface of the different groups were significant ($P < 0.001$; nested ANOVA).

DISCUSSION

We found a positive correlation between plasma MSH values and the degree of pigment dispersion in dermal melanophores, which indicates that our manipulation of backgrounds indeed alters the demand for MSH. These results were in line with those of Wilson and Morgan (1979) who concluded that in long-term background adaptation the circulating level of MSH is the primary factor determining the degree of pigment dispersion in *Xenopus* kept on black, grey, and white backgrounds for long periods.

Our morphometric analyses demonstrate that the volume of the intermediate lobe of black-adapted *Xenopus* almost doubled compared to white-adapted animals. We found that the mean cell size is directly related to the intermediate lobe volume and that there was essentially no difference in the number of cells in the pars intermedia of animals adapted to the different backgrounds. Therefore, we relate this difference in pars intermedia volume to an increase in volume of the individual MSH cells. Both hypertrophy and hyperplasia have been shown in pars intermedia cells of some fish species (Thornton and Howe, 1974; Malo-Michele, 1977; Van Eys, 1980; Wendelaar Bonga *et al.*, 1986), while in the pars intermedia of the rat, only hyperplasia was observed after giving the animals a treatment which leads to activation of MSH cells (Chronwall *et al.*, 1988). Apparently there is considerable species difference in the response of the intermediate lobe tissue to endocrine cell activation.

At the ultrastructural level we found that the granular cells in the intermediate lobe of white-adapted animals could be categorized as secretory inactive (type I) cells. The pars intermedia of black-adapted toads con-

TABLE 1

THE INTERMEDIATE LOBE VOLUME AND THE NUMBER OF MSH CELLS IN THE PARS INTERMEDIA OF WHITE-, LIGHT GREY-, DARK GREY-, AND BLACK-ADAPTED *X. laevis* (MEANS \pm SEM; $n = 5$)

Background	Intermediate lobe volume (mm ³)	Number of MSH cells ($\times 10^{-4}$)
White	0.036 \pm 0.002	7.3 \pm 0.7
Light grey	0.040 \pm 0.004	6.9 \pm 0.8
Dark grey	0.045 \pm 0.003*	5.9 \pm 0.5
Black	0.063 \pm 0.003**	6.2 \pm 0.5

* $0.02 < P < 0.05$; ** $P < 0.001$.

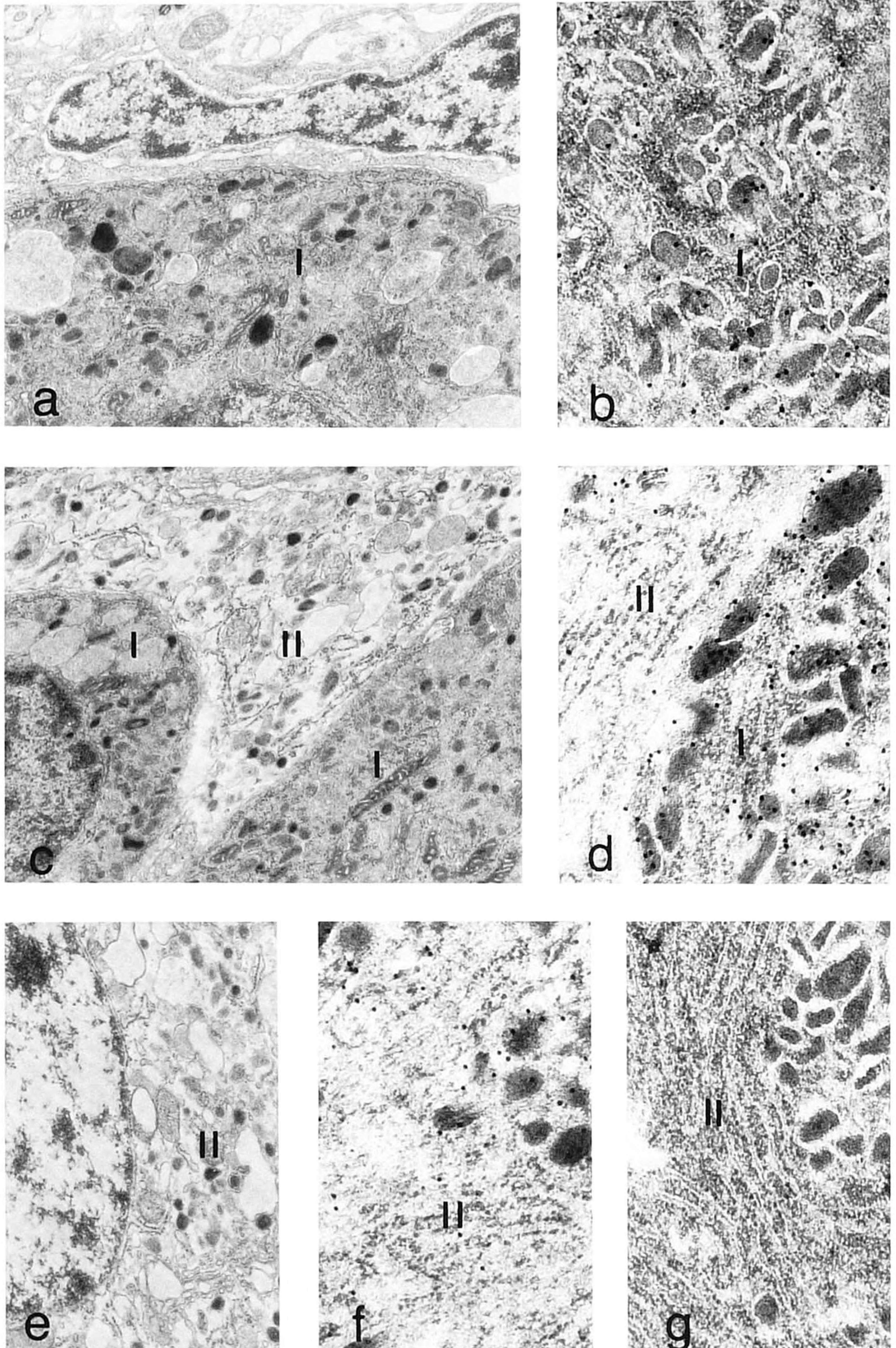


FIG. 2. Electron micrographs of MSH cells from the pars intermedia of *X. laevis*, adapted to white background (a), dark grey background (c), and black background (e) ($\times 10,900$). Immunocytochemical staining with anti- α -MSH/immunogold of sections from animals adapted to white (b), dark grey (d), and black backgrounds (f). In controls (preimmune rabbit serum), no labeling was observed (g) ($\times 39,000$). (I, type I; II, type II)

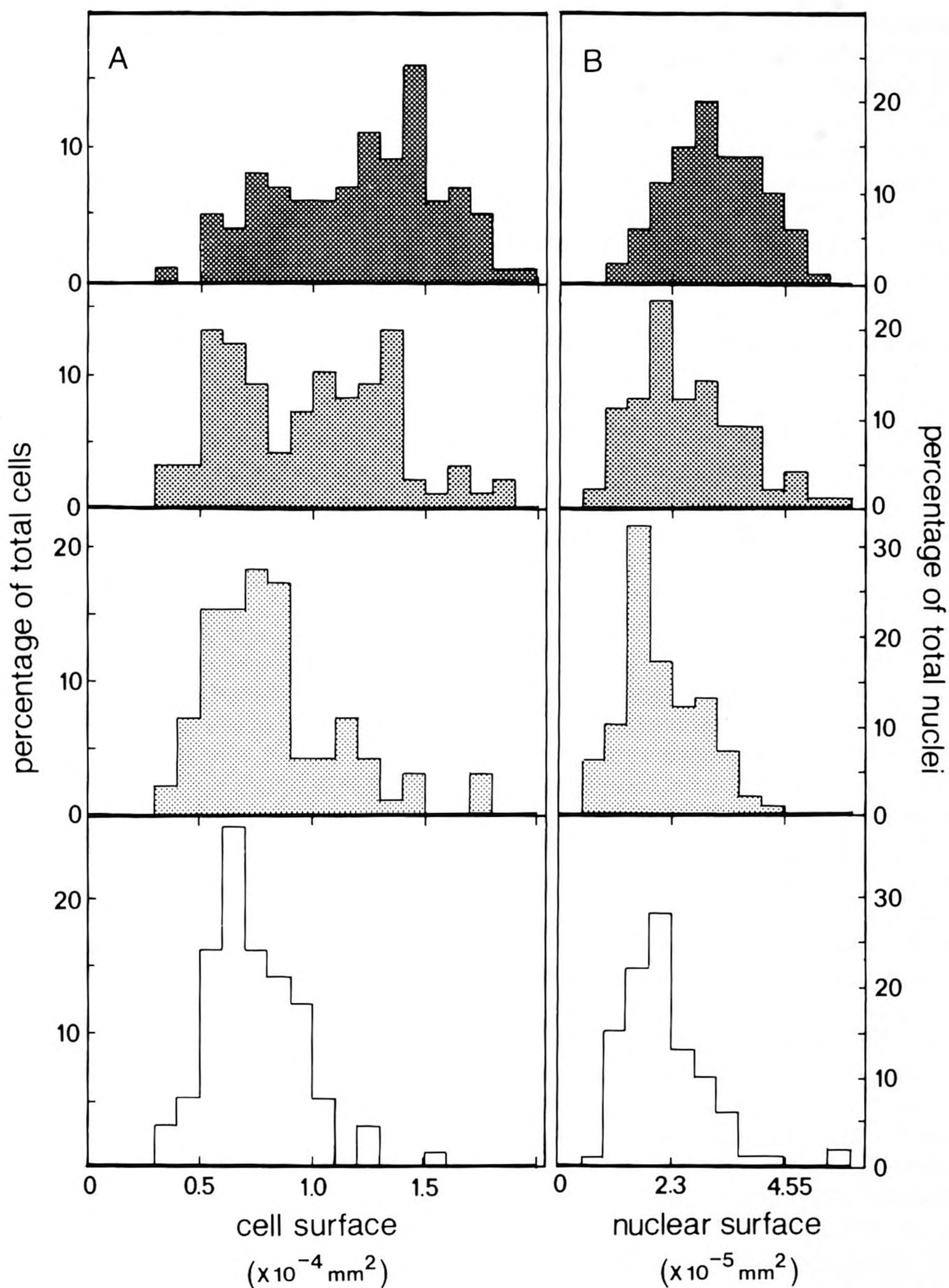


FIG. 3. Effects of long-term adaptation to white, light grey, dark grey, and black backgrounds on the distribution of MSH cell surface areas (A) and MSH cell nuclear surface areas (B) in *X. laevis* ($n = 100$). The degree of shading of the bars corresponds to the background colour.

sisted primarily of secretory active (type II) cells. Similar observations have been made in earlier studies and we conclude that cells with characteristics of our type I are stor-

age type cells while the type II cells are probably very active, producing and secreting α -MSH (Hopkins, 1970; Weatherhead and Whur, 1972; Van Helden, 1980). This

conclusion has been supported by *in vitro* studies showing that the pars intermedia of black-adapted animals is biosynthetically active while that of white-adapted toads is relatively inactive (Thornton, 1971; Whur and Weatherhead, 1971; Hopkins, 1972; Jenks *et al.*, 1977, 1985). Application of the protein A gold technique with a specific α -MSH antiserum showed that both types were α -MSH-producing cells.

To determine whether all cells or only a subpopulation are activated during activation of the pars intermedia, we paid special attention to the ultrastructural characteristics of MSH cells in animals adapted to grey backgrounds. To our knowledge, the pars intermedia of such animals has never been examined at the EM level. When all MSH cells are activated during adaptation to grey backgrounds (as in the case of a homogeneous cell population), one would predict that all MSH cells show structural characteristics intermediate between those of type I and type II cells discussed above. In the case of the activation of subpopulations, one would predict that some cells remain in a relative inactive form (type I) while others are activated (type II). The latter situation was clearly observed in grey-adapted animals. These data on the ultrastructure of the intermediate lobes combined with the data of plasma α -MSH of animals kept at different backgrounds support our conclusion that high plasma MSH levels correlate with the appearance of increased numbers of type II cells in the intermediate lobe. We conclude that MSH cells respond as a heterogeneous cell population to meet the demand for MSH.

The distribution of the cell surface area of the MSH cells in white-, light grey-, dark grey-, and black-adapted toads further supports the presence of two populations of MSH cells, different in size and activity. Our results demonstrate that the pars intermedia of white-adapted animals is composed of small cells, while black-adapted animals possess primarily large cells. These

results are consistent with the ultrastructural observations of type I and type II cells. Analysis of the size of cells in the pars intermedia of grey-adapted animals showed a distribution into at least two cell populations. This was particularly evident in dark grey-adapted animals, where one of these populations had an average surface area similar to that observed in white-adapted animals, while the other population had a surface distribution comparable with that displayed by black-adapted animals. The distribution of MSH cells in grey-adapted animals into two populations is supported by the nuclear surface area measurements, although the differences observed between the two populations were not as dramatic as those found in the cell surface area analysis.

In conclusion, we present evidence that the response of the pars intermedia of *X. laevis* to the demand for MSH in the long term involves hypertrophy rather than hyperplasia. Moreover, during such activation the MSH cells respond as a heterogeneous population, with progressively more cells activated as the physiological demand for α -MSH increases. The regulation of the secretory activity of the MSH cells of this species is very complex, involving both stimulatory and inhibitory hypothalamic input, conveyed by both classical neurotransmitters and neuropeptides (Jenks *et al.*, 1988). A difference in the sensitivity of individual MSH cells to these regulatory factors might form the basis for the heterogeneity observed in the response of the MSH cell population to changes in background.

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REFERENCES

- Chronwall, B. M., Hook, G. R., and Millington, W. R. (1988). Dopaminergic regulation of the biosynthetic activity of individual melanotropes in the rat pituitary intermediate lobe: A morphometric analysis by light and electron microscopy and *in situ* hybridization. *Endocrinology* 123, 1992–2002.
- Gerber, H. Peter, H. J., Bachmeier, C., Kaempf, J., and Studer, H. (1987). Progressive recruitment of follicular cells with graded secretory responsiveness during stimulation of the thyroid gland by thyrotropin. *Endocrinology* 120, 91–96.
- Hogben, L., and Slome, D. (1931). The pigmentary effector system: The dual character of endocrine coordination in amphibian color change. *Proc. R. Soc. London B* 108, 10–53.
- Hopkins, C. R. (1970). Studies on secretory activity in the pars intermedia of *Xenopus laevis*. 1. Fine structural changes related to the onset of secretory activity *in vivo*. *Tissue Cell* 2, 59–70.
- Hopkins, C. R. (1972). The biosynthesis, intracellular transport, and packaging of melanocyte-stimulating peptides in the amphibian pars intermedia. *J. Cell. Biol.* 53, 642–653.
- Jenks, B. G., Van Overbeeke, A. P., and McStay, B. F. (1977). Synthesis, storage, and release of MSH in the pars intermedia of the pituitary gland of *Xenopus laevis* during background adaptation. *Canad. J. Zool.* 55, 922–927.
- Jenks, B. G., Martens, G. J. M., Van Helden, H. P. M., and Van Overbeeke, A. P. (1985). Biosynthesis and release of melanotropins and related peptides by the pars intermedia in *Xenopus laevis*. In "Current Trends in Comparative Endocrinology" (B. Lofts and W. N. Holmes, Eds.), pp. 149–153. Hong Kong Univ. Press, Hong Kong.
- Jenks, B. G., Verburg-van Kemenade, B. M. L., and Martens, G. J. M. (1988). Proopiomelanocortin in the amphibian pars intermedia: A neuroendocrine model system. In "The Melanotropic Peptides" (M. E. Hadley, Ed.), Vol. 1, pp. 67–83. CRC Press, Boca Raton, FL.
- Lucque, E. H., Munoz, M., Smith, P. F., and Neill, J. D. (1986). Subpopulations of lactotropes detected with the reverse hemolytic plaque assay show differential responsiveness to dopamine. *Endocrinology* 118, 2120–2124.
- Malo-Michele, M. (1977). Réaction cytologiques de la pars intermedia de l'hypophyse de la saupe, *Boops salpa* L. (poisson téléostéen, Sparidae) aux variations de la couleur de fond et de l'illumination. *Gen. Comp. Endocrinol.* 31, 343–349.
- Neill, J. D., Smith, P. F., Luque, E. H., Munoz de Toro, M., Nagy, G., and Mulchahey, J. J. (1986). Analysis of hormone secretion from individual pituitary cells. In "Neuroendocrine Molecular Biology" (G. Fink, A. J. Harmar, and K. W. McKerns, Eds.), pp. 325–340. Plenum, New York.
- Poulain, D. A., and Wakerley, B. (1982). Electrophysiology of hypothalamic magnocellular neurons secreting oxytocin and vasopressin. *Neuroscience* 7, 773–808.
- Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17, 208–212.
- Schuit, F. C., In 't Veld, P. A., and Pipeleers, D. G. (1988). Glucose stimulates proinsulin biosynthesis by dose-dependent recruitment of pancreatic beta cells. *Proc. Natl. Acad. Sci. USA* 85, 3865–3869.
- Sokal, R. R., and Rohlf, F. J. (1981). "Biometry: The Principles and Practice of Statistics in Biological Research." Freeman, New York.
- Thornton, V. F. (1971). The effect of change of background colour on the melanocyte-stimulating hormone content of the pituitary of *Xenopus laevis*. *Gen. Comp. Endocrinol.* 17, 554–560.
- Thornton, V. F., and Howe, C. (1974). The effect of change of background colour on the ultrastructure of the pars intermedia of the eel (*Anguilla anguilla*). *Cell Tissue Res.* 151, 103–115.
- Van Eys, G. J. J. M. (1980). Structural changes in the pars intermedia of the cichlid teleost *Sarotherodon mossambicus* as a result of background adaptation and illumination. I. The MSH cells. *Cell Tissue Res.* 208, 99–110.
- Van Helden, H. P. M. (1980). "Aspects of Biosynthesis and Release of Peptides by the Pars Intermedia of the Pituitary Gland in *Xenopus laevis*." Thesis, University of Nijmegen.
- Van Putten, L. J. A., and Kiliaan, A. J. (1988). Immuno-electron microscopic study of the prolactin cells in the pituitary gland of male Wistar rats during aging. *Cell Tissue Res.* 251, 353–358.
- Weatherhead, B., and Whur, P. (1972). Quantification of ultrastructural changes in the 'melanocyte-stimulating hormone cell' of the pars intermedia of the pituitary of *Xenopus laevis*, produced by change of background colour. *J. Endocrinol.* 53, 303–310.
- Wendelaar Bonga, S. E., Van der Meij, J. C. A., Van der Krabben, W. A. W. A., and Flik, G. (1984). The effect of water acidification on prolactin cells and pars intermedia PAS positive cells in the teleost fish *Oreochromis* (formerly *Sarotherodon*)

- mossambicus* and *Carassius auratus*. *Cell Tissue Res.* 238, 601–609.
- Wendelaar Bonga, S. E., Van der Meij, J. C. A., and Flik, G. (1986). Response of PAS-positive cells of the pituitary pars intermedia in the teleost *Carassius auratus* to acid water. *Cell Tissue Res.* 243, 609–617.
- Whur, P., and Weatherhead, B. (1971). Rates of incorporation of ³H-leucine into protein of the pars intermedia of the pituitary in the amphibian *Xenopus laevis* after change of background color. *J. Endocrinol.* 51, 521–532.
- Wilson, J. F., and Morgan, M. A. (1979). α -Melanotropin-like substances in the pituitary and plasma of *Xenopus laevis* in relation to colour change responses. *Gen. Comp. Endocrinol.* 38, 172–182.