GENERAL AND COMPARATIVE ENDOCRINOLOGY 49, 277–285 (1983)

Isolation of the Biosynthetic Products of the PAS Positive Pars Intermedia Cells in the Cichlid Teleost *Sarotherodon mossambicus*

G. J. J. M. VAN EYS, C. W. G. M. LÖWIK, AND S. E. WENDELAAR BONGA[1]

Department of Zoology, University of Nijmegen, Toernooiveld 25, 6525 ED Nijmegen, The Netherlands

Accepted April 4, 1982

The pars intermedia of teleosts contains two types of granular cells with the predominant type being similar to the pars intermedia cells in other vertebrate groups and containing peptides derived from the pro-opiomelanocortin precursor molecule. The function and products of the second cell type, the PAS positive cells, are unknown. This study reports on the identification of biosynthetic products of the PAS positive cells of the cichlid teleost *Sarotherodon mossambicus*. The experimental regimen took advantage of earlier morphometric analyses which showed marked differences in metabolic activity of the PAS positive cells resulting from adaptation to different background colours and illumination. Autoradiography at the light microscopic level showed that both cell types of the pars intermedia incorporate labeled amino acids during *in vitro* incubation. To identify the products synthesized by the PAS positive cells, labeled products of the pars intermedia tissue were analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and high-pressure liquid chromatography. Comparison of pulse incubations of pars intermedia tissue of fish adapted to different backgrounds and conditions of illumination revealed that an increase in the number and metabolic activity of the PAS positive cells, as deduced from morphometric data, was paralleled by an increase of the amount of label incorporated into 27K and 25K molecules. Pulse–chase experiments with pars intermedia lobes of white and black background adapted fish showed that these two products, unlike the other newly synthesized products, were not involved in any precursor-product relationship. Our data, therefore, suggest that the 27K and 25K peptides were synthesized by the PAS positive cells.

The pars intermedia of the pituitary gland of teleosts is unusual in that it contains two different endocrine cell types. From physiological and immunohistochemical studies we have concluded that the predominant cell type in the pars intermedia of *Sarotherodon mossambicus* is comparable to the endocrine cells of the pars intermedia of other vertebrates which produce MSH (Van Eys, 1980a; Van Eys and Van den Oetelaar, 1981; Van Eys and Peters, 1981). The second cell type of the pars intermedia, the PAS positive cells, are unique to teleosts and have been implicated, inter alia, in the endocrine control of background adaptation, osmoregulation, and calcium metabolism (Baker and Ball, 1970; Ball and Batten, 1981; Malo-Michele, 1975, 1977; Olivereau, 1969; Olivereau *et al.*, 1980, 1981; Pang *et al.*, 1973; Van Eys, 1980b). However, the techniques used to study the function of the PAS positive cells were limited to light- and electron microscopy and measurements of cell and nuclear volumes, since nothing is known about the chemical nature of the cellular products. This situation hampers further investigations concerning the function of the PAS positive cells, so the main purpose of the present investigation was to identify the product(s) of these cells.

Isolation of the product(s) of the PAS positive cells is severely hindered by the fact that these cells are intermingled with MSH cells. Therefore, an attempt was made to separate the PAS positive cells

[1] To whom correspondence should be addressed.

0016-6480/83/020277-09$01.50/0

from the MSH cells using Percoll (Pharmacia) gradient centrifugation. However, the results were disappointing. Therefore, we adopted a different strategy based on the results of previous morphometrical studies, which showed that the MSH cells and PAS positive cells responded differently to changes in background color and illumination to which the fish were exposed (Van Eys, 1980a,b). These studies showed that fish adapted to a black background had both active MSH cells and PAS positive cells, whereas in fish kept in darkness the MSH cells were inactive. Further, in white background adapted fish the activity was low for both cell types and under these conditions the reduced activity of the PAS positive cells was accompanied by a dramatic reduction in the number of PAS positive cells. Accordingly, it was assumed that the PAS positive cells would show reduced synthesis in white background adapted fish compared with black background or darkness adapted fish. Further we hypothesized that such a difference might be correlated with differences in the biosynthetic capacity of the PAS positive cells, which would enable us to identify the products of these cells.

MATERIALS AND METHODS

Animals. For the adaptation experiments sexually mature males of the cichlid teleost *S. mossambicus* with a body length of 10 to 12 cm and a body weight of 9 to 12 g were used. Sexually mature females of the same length and weight were used for pulse and pulse–chase experiments. The fish were kept in fresh water; water temperature was about 25°. The fish were divided into groups, which were placed under one of the following regimes:

Group W: 14 days on a white background with a day–night rhythm of 12 hr light alternating with 12 hr darkness.

Group B: 14 days on a black nonreflecting background, with lighting conditions as described for group W.

Group D: 14 days on a nonreflecting black background in total darkness.

Animals were anesthetized with MS 222 (Sandoz) and killed by cutting the spinal cord.

Morphometric analysis. For light microscopic examination five pituitary glands were fixed in Bouin–Hollande fluid. Sections were stained in periodic acid–Schiff (PAS) followed by MacConaill's lead hematoxylin. The ratios of the volumes occupied by PAS positive cells and MSH cells were estimated by measuring the areas occupied by both cell types in sagittal sections of the pars intermedia at a final magnification of ×400 by means of a drawing prism. The drawings were morphometrically evaluated with a Kontron Digiplan. The data were tested for statistical significance with a Student's *t* test.

Pulse and pulse–chase incubations. After the fish were killed, pituitary glands were quickly dissected and the pars distalis was removed. The pars intermedia lobes were transferred into slightly modified Dulbecco's modified Eagle's medium (MDM). This medium differs from normal Dulbecco's modified Eagle's medium by the absence of L-valine and L-cysteine, by the presence of 212 mg/liter $CaCl_2$ instead of the prescribed 265 mg/liter, and by replacement of Na_2HCO_3 by 20 mmol Hepes[2] (Sigma). The final osmotic value of the medium was 310 mOsm and the Ca^{2+} concentration was about 2.5 meq/liter. These values are similar to the osmolality and nonprotein bound Ca^{2+} concentration found in the blood plasma of *S. mossambicus*.

Pars intermedia tissues were placed in 100 μl MDM and preincubated for 90 min in a metabolic shaker at 24°. After the preincubation period the tissues were transferred to 100 μl MDM containing 40 μCi [^{3}H]lysine (New England Nuclear, spec. act. 90 Ci/mmol). In pulse–chase experiments, 30 min of pulse labeling was followed by chase period of increasing duration in MDM containing 5 m*M* L-lysine at 24°. At the end of the pulse or chase incubation pars intermedia tissues were homogenized in 500 μl 0.1 *M* acetic acid in an all glass homogenizer. The homogenate was centrifuged at 10,000*g* for 5 min in a Beckman microfuge and the supernatant was stored at −20° for later high-pressure liquid chromatography (HPLC) or freeze dried for later gel electrophoretic analysis.

High-pressure liquid chromatography. The 500-μl samples were analyzed with a Spectra Physics SP 8000 high-pressure liquid chromatograph (Spectra Physics, Eindhoven, The Netherlands) equipped with a stainless-steel column packed with Spherisorb 10 ODS (Chrompack BV, Middelburg, The Netherlands). The linear gradient consisted of a 0.5 *M* formic acid–0.14 *M* pyridine mixture (pH 3.0) and 1-propanol. The flow rate over the column was 2 ml/min, and 1-ml fractions were collected with a fraction collector (LKB Redirac, Model 2112). Four milliliters of Aqua Luma (Baker

[2] Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Chemicals) were then added and the fractions were counted in a Philips liquid scintillation analyzer (Model PW 4540). Synthetic α-MSH was used as a marker.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis. For estimation of relative molecular weight of pars intermedia products, the homogenates were analyzed by SDS–polyacrylamide gel electrophoresis. Analysis was performed according to Laemmli (1970) with the exception that a slab gel was used instead of gel rods. The separating gel contained 15% acrylamide (Serva) 0.4% methylene bisacrylamide (Biorad), and 0.1% SDS (Serva). A stacking gel was applied. On SDS polyacrylamide gels, labeled molecules with known molecular weights (92K, 69K, 46K, 30K, and 14K; New England Nuclear) and labeled $ACTH_{1-39}$ and human β-endorphin (generous gifts of Dr. Rigter, Organon BV, Oss, The Netherlands) were used as markers. Staining was performed in an aqueous solution of methanol (25 ml/liter) and acetic acid (40 ml/liter), containing 2.5 g/liter Coomassie brilliant blue (Serva). The gels were destained in the same aqueous solution of methanol and acetic acid, processed for autoradiography according to Bonner and Laskey (1974), and dried after the procedure described by Berns and Bloemendal (1974).

Pars intermedia homogenates and peak P of the HPLC analysis were further analyzed by two-dimensional gel electrophoresis, according to the method of O'Farrell (1975), using isoelectric focusing (IEF) in the first dimension and a 13% SDS gel in the second dimension. The electrophoretic procedure and visualisation of the radioactive spots by scintillation autoradiography were identical to the method described for SDS gel electrophoresis.

Autoradiography. For autoradiography pars intermedia lobes were incubated for 2 hr in MDM containing 20 μCi/100 μl [^{3}H]lysine (New England Nuclear, spec. act. 90 Ci/mmol). After pulse labeling the lobes were fixed in Carnoy's fixative for 24 hr and embedded in JB-4 (Meyvis, The Netherlands). Autoradiography was performed with an Ilford K-5 emulsion. Exposure time was 7 days.

RESULTS

Morphometrical analysis of sections of the pars intermedia by light microscopy revealed statistically significant differences between the relative total volume of the pars intermedia occupied by the PAS positive cells of fish adapted to white background, black background, and to darkness (Table 1; Fig. 1). The percentage of volume occupied by the PAS positive cells of fish adapted to a white background (group W) was significantly lower than that in fish adapted to either a black background (group B) or to darkness (group D). Such differences were less pronounced between fish of groups B and D.

Autoradiography at the light microscopic level showed that during *in vitro* incubations of pars intermedia of black background adapted fish the MSH cells as well as the PAS positive cells incorporated comparable amounts of labeled lysine (Fig. 2). Almost no incorporation of label was observed in the neurohypophysial processes penetrating the pars intermedia.

For the analysis of newly synthesized products from the pars intermedia of *S.*

TABLE 1

VOLUME OF THE PARS INTERMEDIA OCCUPIED BY THE PAS POSITIVE CELLS, AND RELATIVE METABOLIC ACTIVITY OF PAS POSITIVE CELLS AND MSH CELLS AS DEDUCED FROM ANALYSIS BY MORPHOMETRIC TECHNIQUES

Group	PAS positive cells as % of pars intermedia tissue	Metabolic activity* of PAS positive cells	Metabolic activity* of MSH cells
W	7.55 ± 2.62	−	−
B	22.71 ± 7.93[a]	+	+
D	30.12 ± 6.34[b]	++	−

Note. Significantly different from group W (Student's t test). [a] $P < 0.01$; [b] $P < 0.001$. (−, low activity; +, high activity; ++, very high activity).

* Metabolic activity was estimated by morphometric analysis of cellular and nuclear volumes (light microscopical level) and of relative cytoplasmic volumes of mitochondria, rough endoplasmic reticulum, Golgi apparatus, and secretory granules (for details see Van Eys, 1980a,b).

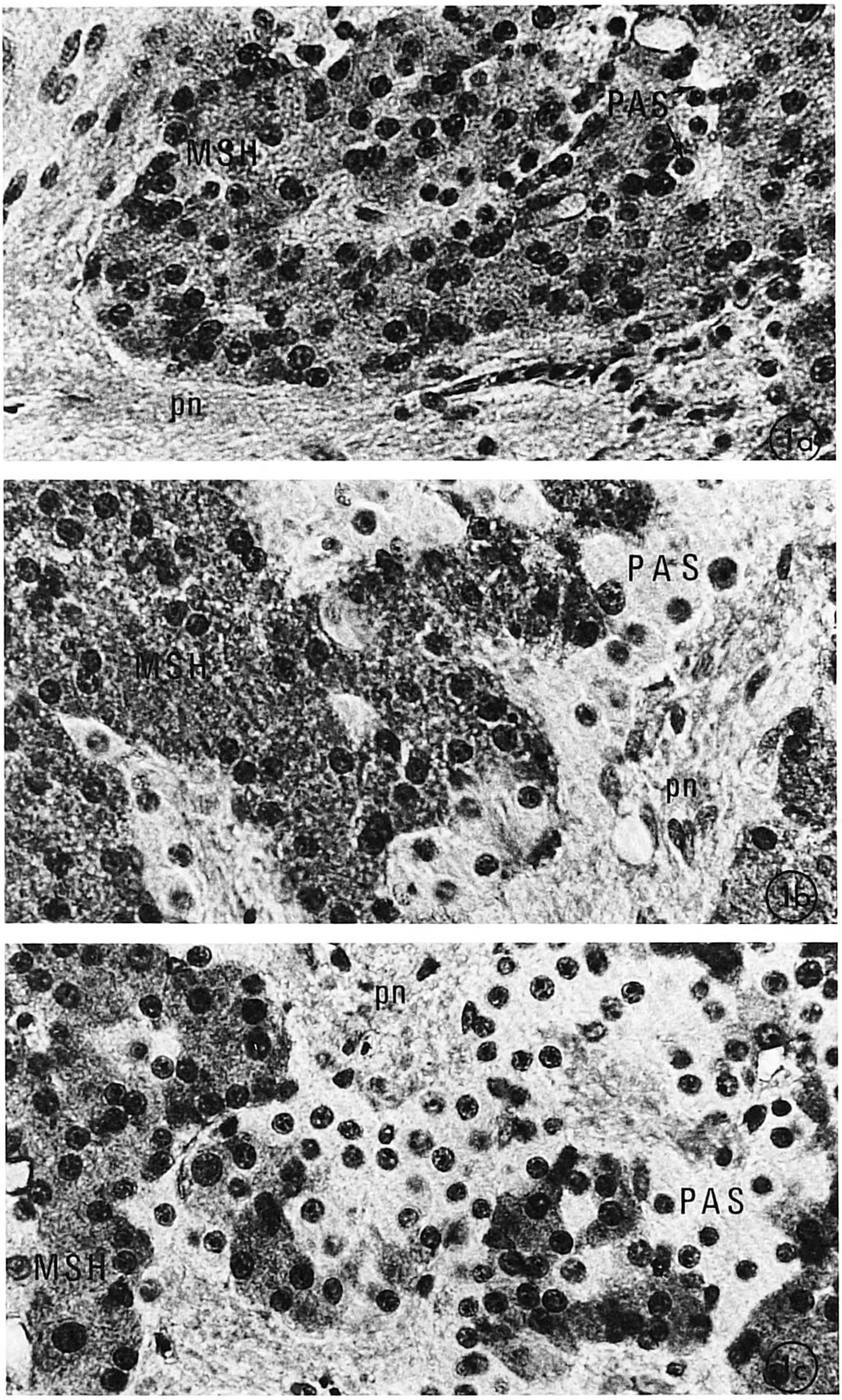

FIG. 1. (a) Light microscopic section of pars intermedia of fish adapted to a white background under normal day–night conditions. Notice the small number of PAS positive cells (PAS). MSH, MSH cells; pn, pars nervosa. Periodic acid–Schiff–lead haematoxylin staining. ×400. (b) Light microscopic sections of pars intermedia of fish adapted to black background under normal lighting conditions. ×400. (c) Light microscopic section of pars intermedia of fish adapted to darkness. PAS positive cells are abundant. ×400.

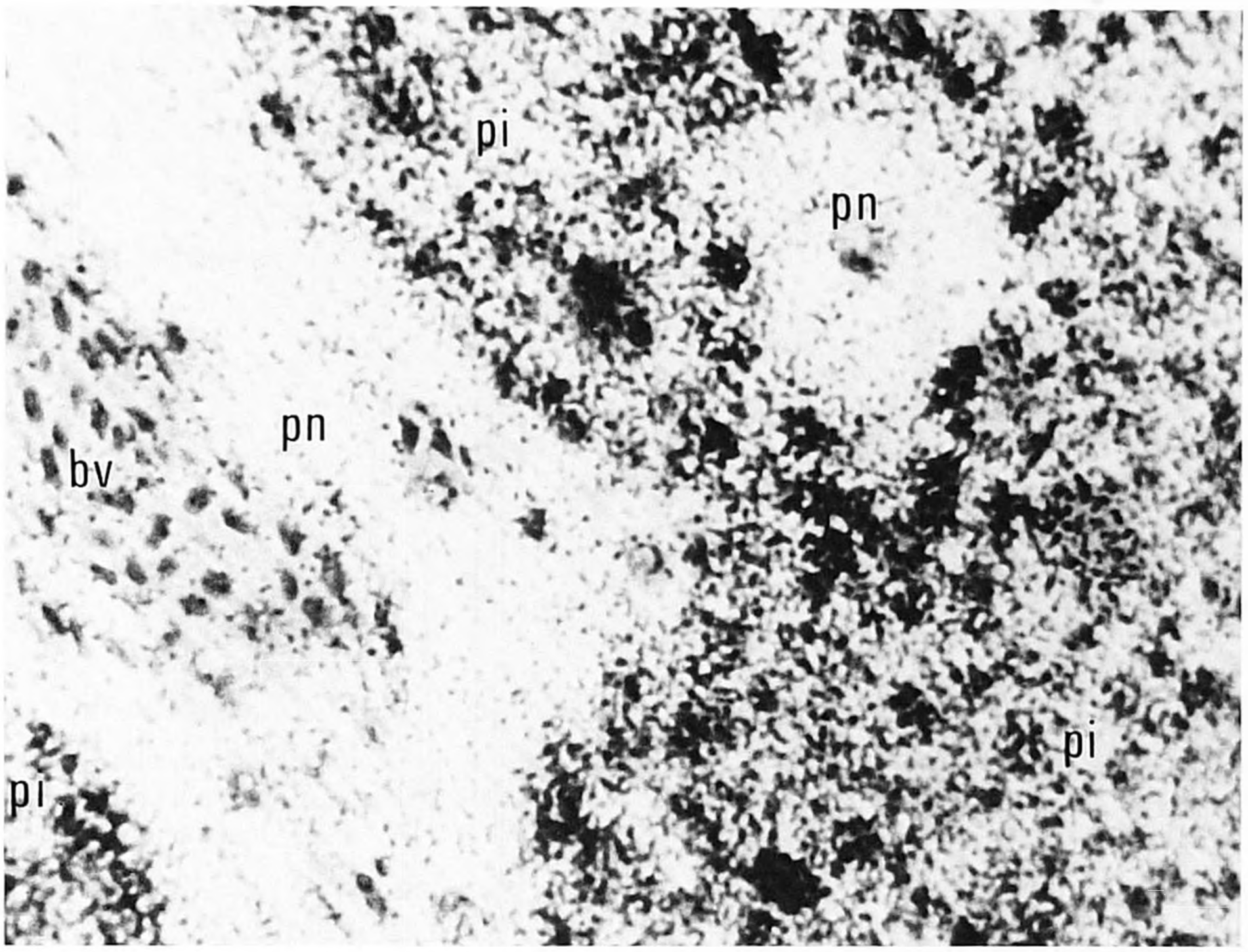

FIG. 2. Autoradiograph of light microscopic section of pars intermedia of black background adapted fish. There is a clear difference in the number of grains between the pars intermedia (pi) and pars nervosa (pn) tissue. There are no indications for differences in incorporation between the PAS positive cells and the MSH cells. bv, blood vessel. ×500.

mossambicus, SDS–polyacrylamide gel electrophoresis and high-pressure liquid chromatography were applied. The former technique has advantage over HPLC for the separation of large molecules, whereas HPLC can give excellent separation of relatively small molecules. An additional advantage of the HPLC method is that all molecules in the sample are recovered, whereas in SDS–gel electrophoresis molecules below a certain molecular weight are lost.

SDS–gel electrophoresis of pars intermedia homogenates from the various experimental groups showed differences with regard to newly synthesized products (Fig. 3). Two compounds, with an apparent molecular weight of 25K and 27K, were found in much smaller amounts in the pars intermedia tissue of white background adapted fish than in fish of the other experimental groups. HPLC analysis revealed marked differences in only fractions 95–99 (Peak P; Fig. 4). On SDS gels, these fractions appeared to contain the 25K and 27K products. Further analysis of these fractions by two-dimensional gel electrophoresis revealed that the 25K and 27K products both consist of a mixture of two molecules with different p*K* values (Fig. 5). The estimated p*K* values of the 25K product were 6.3 and 6.7, whereas those of the 27K product were 5.8 and 6.1.

When pars intermedia lobes of black background adapted fish were incubated with [^{3}H]lysine for different periods of time, SDS–gel electrophoresis showed that the appearance of the newly synthesized products followed a definite temporal order (Fig. 6). The 25K and 27K products appeared simultaneously. A pulse labeling followed by chases of increased duration showed that the 30K product disappeared as a number of smaller products appeared.

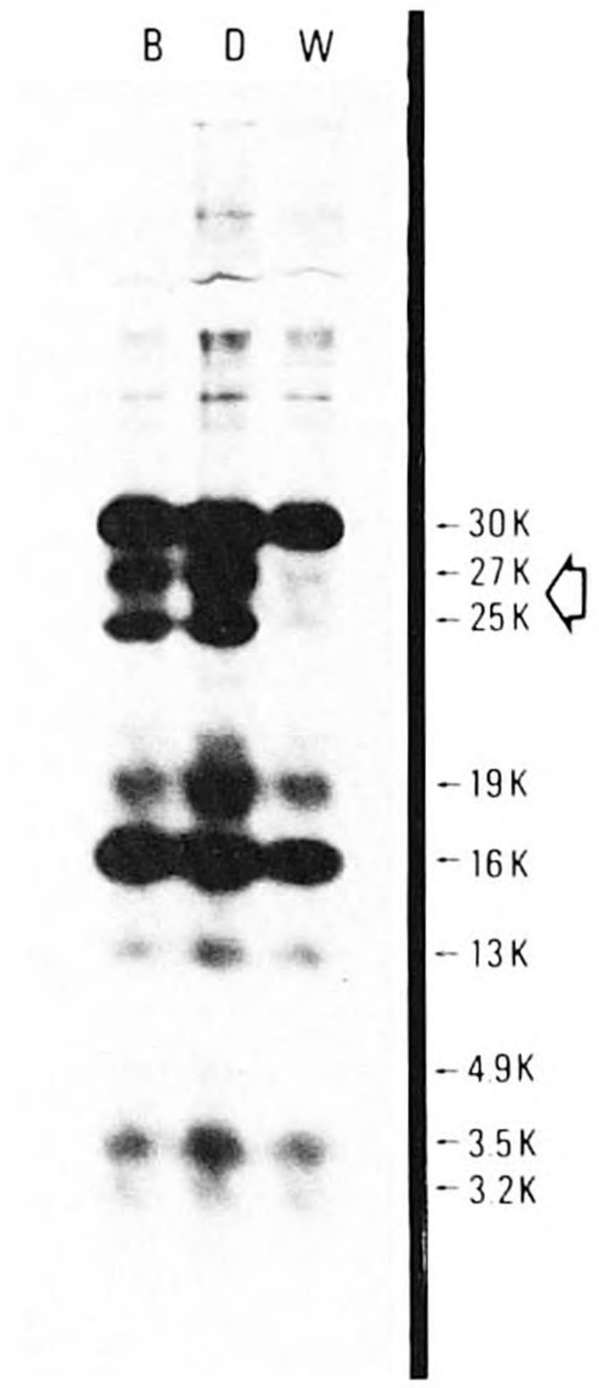

FIG. 3. Autoradiograph of SDS–gel electrophoretic analysis of pars intermedia tissue of black background adapted fish (B), white background adapted fish (W), and darkness adapted fish (D). Note that virtually no newly synthesized 25K and 27K products (arrow) are present in the pars intermedia tissue of white background adapted fish. To get comparable amounts of radioactivity in each lane, 4 pars intermedia lobes were used for lane W, 3 for lane B, and 4 for lane D. Lobes were incubated in 40 μCi/100 μl [^{3}H]lysine for 4 hr at 22°.

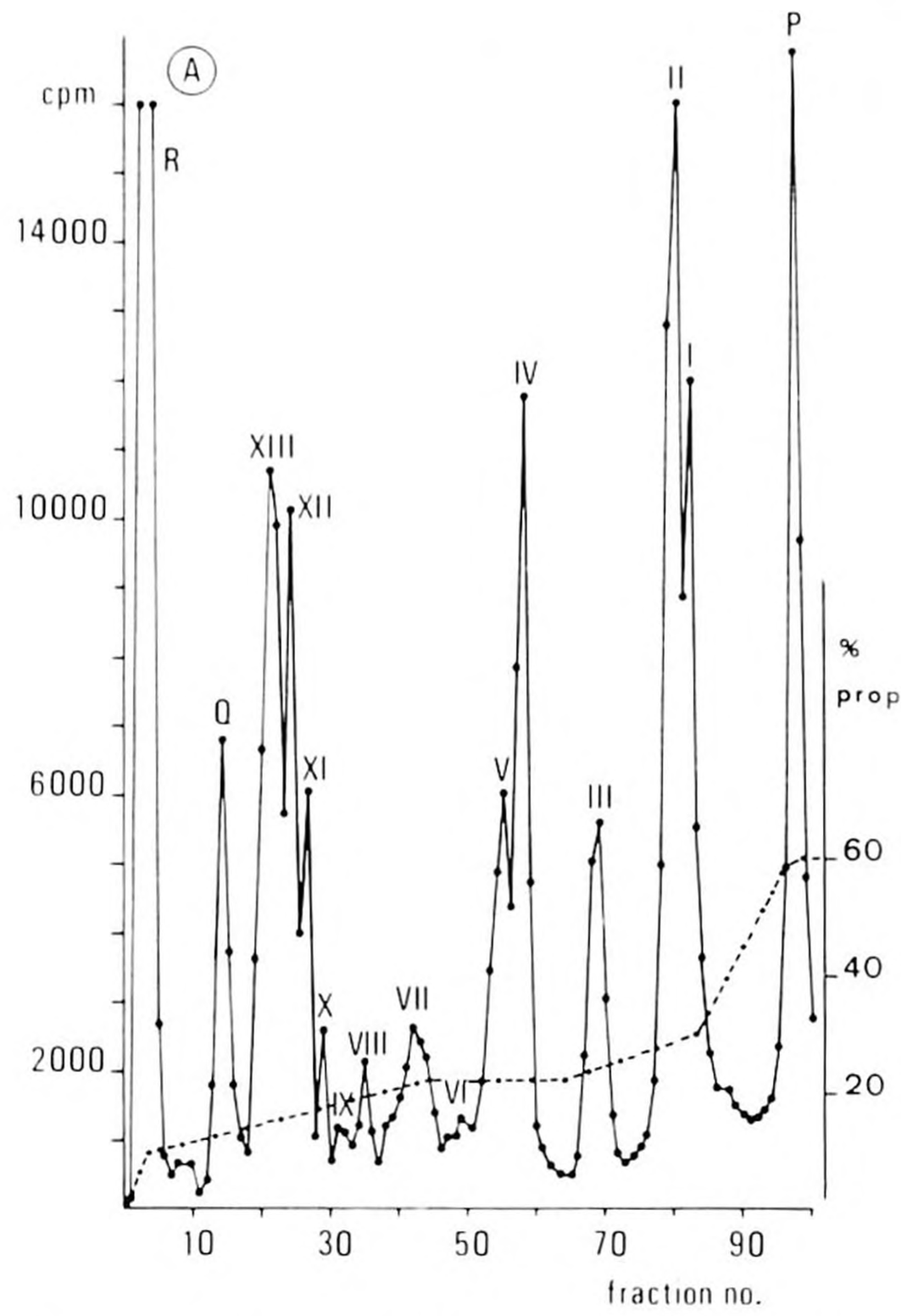

FIG. 4. High-pressure liquid radiochromatogram of pars intermedia products of black background adapted fish. The tissue was pulse incubated for 4 hr with [^{3}H]lysine. Flow rate was 2 ml/min. Fractions were collected every 30 sec. Primary solvent: 0.5 *M* formic acid–0.14 *M* pyridine (pH 3.0), secondary solvent 1-propanol. (Peaks Q and R represent freeze-dry contamination and [^{3}H]lysine, respectively.)

The 25K and 27K products, however, were present in all samples taken during the chase period from pars intermedia tissue of black background adapted fish (Fig. 7). In an identical experiment with pars intermedia lobes of white background adapted fish the 25K and 27K products were absent during the pulse labeling and the chase period (Fig. 8). The minor variations in the amount of incorporated label, as for instance found for the 27K and 25K products in the different chase groups (Fig. 7), are most likely the result of small variations in the volume of the pars intermedia lobes used in the experiments, since these variations were not reproducible. Another phenomenon is the apparent increase of label in the 30K, 27K, and 25K products during the first hour of the chase period. This increase in primary products, observed all three times the experiment was performed, may be attributed to the free labeled amino acids present in the tissue after the pulse period.

Most of the other newly synthesized products of the pars intermedia have been positively identified as originating from the MSH cells. A report on the analysis of these products is in preparation (for a preliminary report refer to Van Eys, 1981).

DISCUSSION

Autoradiography of the pars intermedia showed that both the MSH cells and the PAS positive cells incorporated labeled

FIG. 5. Autoradiograph of a two-dimensional gel electrophoretic analysis (using isoelectric focusing (IEF) in the first dimension and a 13% SDS gel in the second dimension) of peak P of HPLC analysis of black background adapted fish.

lysine under *in vitro* conditions. Pars nervosa tissue did not. Therefore, it seems reasonable to conclude that both endocrine cell types of the pars intermedia were synthetically active during incubation, and that the labeled products present in the homogenates used for SDS–gel electrophoresis and HPLC analysis are synthesized by the MSH cells and PAS positive cells.

To differentiate between the products of MSH cells and PAS positive cells, we took advantage of the differences in the number and activity of the PAS positive cells in fish adapted to different backgrounds and illumination periods. The sharp decline of the metabolic activity (Van Eys, 1980b), in addition to the dramatic reduction of the PAS positive cells in the pars intermedia in white background adapted fish was, on SDS gels, parallelled by a marked reduction of newly synthesized 25K and 27K products. This suggests that these molecules are produced by the PAS positive cells.

The results of pulse and pulse–chase experiments with pars intermedia lobes of black and white background adapted fish clearly demonstrate that the 25K and 27K products were not involved in a precursor–product processing system similar to that demonstrated for the MSH cells of mammals (Mains *et al.*, 1977; Mains and Eipper, 1979, 1980; Gianoulakis *et al.*, 1979), amphibians (Loh and Gainer, 1977; Loh, 1979), and the cichlid teleost *S. mossambicus* (Van Eys, 1981). The 25K and 27K products cannot be derived from the 30K product since disappearance of the labeled 30K or any other labeled product in the pars intermedia of white background adapted fish is not accompanied by an appearance of the 25K and 27K products. Furthermore, these two products are not processed into smaller molecules, as considerable amounts of labeled 25K and 27K product are present in the pars intermedia tissue of black background adapted fish even after prolonged chase periods. This supports the allegation that these two products are synthesized by the PAS positive cells, whereas the other products, all derived from the 30K product (Van Eys, 1981), seem to be synthesized by the MSH cells.

The occurrence of four different spots on two-dimensional gel does not imply the synthesis of four different substances by

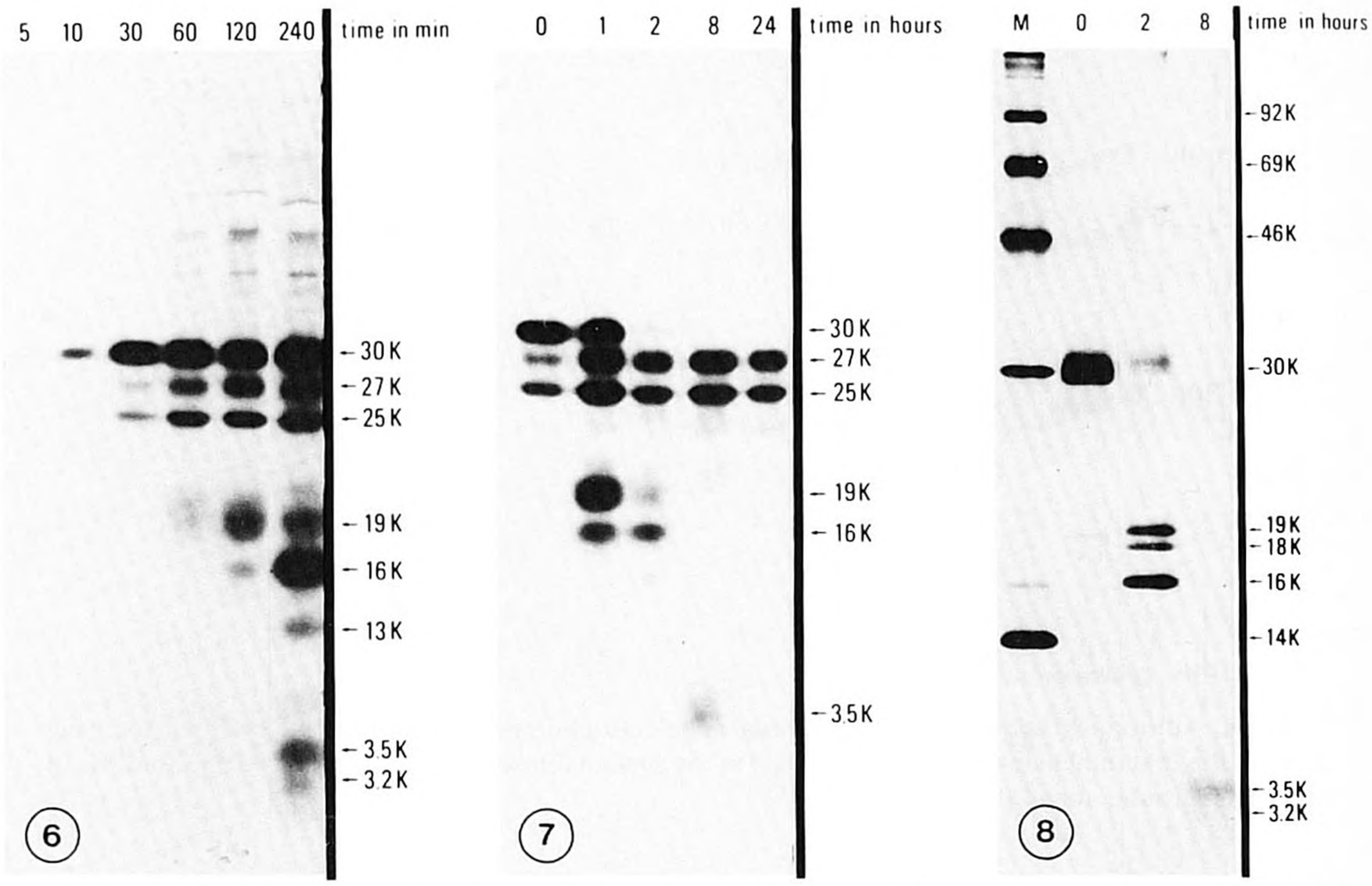

FIG. 6. Autoradiograph of SDS–gel electrophoretic analysis of pars intermedia tissue of black background adapted fish, incubated in MDM containing [^{3}H]lysine, for periods of 5, 10, 30, 60, 120, and 240 min (40 μCi/100 μl MDM, 22°). Each group contained 3 lobes.

FIG. 7. Autoradiograph of SDS–gel electrophoretic analysis of pars intermedia lobes of black background adapted fish, pulse labeled for 30 min in MDM containing 40 μCi/100 μl [^{3}H]lysine and subsequently chased for 1, 2, 8, and 24 hr in MDM containing 5 m*M* L-lysine. Note the presence of 25K and 27K products in the samples of all chase periods (3 lobes per group).

FIG. 8. Autoradiograph of SDS–gel electrophoretic analysis of pars intermedia lobes of white background adapted fish, treated as described under Fig. 7. Notice the absence of the 25K and 27K products in all samples (3 lobes per group). M, markers of known molecular weight.

the PAS positive cells. Investigations on biosynthesis of MSH and other cells have demonstrated that peptides may be acetylated or deaminated (Smyth *et al.*, 1979; Rudman *et al.*, 1979), which can result in differences in p*K* value; differences in apparent molecular weight may reflect differences in glycosylation (Crine *et al.*, 1979; Loh, 1979) and/or differences in nuclear RNA processing or coding by two different genes (Kawauchi *et al.*, 1980; Herbert, 1981; Drouin and Goodman, 1980). The distribution of the biosynthetic material of the PAS positive cells over two pairs of spots by two-dimensional gel electrophoresis may be attributed to such phenomena.

This first and preliminary characterization of the biosynthetic products of the PAS positive cells will give new opportunities to investigate the physiological function of these cells.

ACKNOWLEDGMENTS

The authors thank Dr. F. Ramaekers and Dr. H. Driessens (Department of Biochemistry) for their cooperation and valuable suggestions, and Professor J. C. Fenwick for critically reading the manuscript.

REFERENCES

Baker, B. I., and Ball, J. N. (1970). Background adaptation and pituitary in teleost fishes. *J. Endocrinol.* **48**, 26–27.

Ball, J. N., and Batten, T. F. C. (1981). Pituitary and melanophore responses to background in *Poecilia latipinna* (Teleostei): Role of the pars intermedia PAS cell. *Gen. Comp. Endocrinol.* **44**, 233–248.

Berns, A. J. M., and Bloemendal, H. (1974). Translation of mRNA from vertebrate eye lenses. *In* "Methods in Enzymology" (K. Moldave and L.

Grossman, eds.), Vol. 30, pp. 675–694. Academic Press, New York and London.

Bonner, W. J., and Laskey, R. A. (1974). A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* **46**, 83–88.

Crine, P., Gossard, F., Seidah, N. G., Blanchette, L., Lis, M., and Chrétien, M. (1979). Concomitant synthesis of β-endorphin and α-melanotropin from two forms of pro-opiomelanocortin in the rat pars intermedia. *Proc. Natl. Acad. Sci. USA* **76**, 5085–5089.

Drouin, J., and Goodman, H. M. (1980). Most of the coding region of rat ACTH-β-LPH precursor gene lacks intervening sequences. *Nature (London)* **288**, 610–613.

Gianoulakis, C., Seidah, N. G., Routhier, R., and Chrétien, M. (1979). Biosynthesis and characterisation of adrenocorticotropic hormone, α-melanocyte-stimulating hormone, and an NH_2-terminal fragment of the adrenocorticotropic hormone/β-lipotropin precursor from rat pars intermedia. *J. Biol. Chem.* **254**, 11903–11906.

Herbert, E. (1981). Discovery of pro-opiomelanocortin—a cellular polyprotein. *Trends Biochem. Sci.* **6**, 184–188.

Kawauchi, H., Adachi, Y., and Tsubokawa, M. (1980). Occurrence of a new melanocyte stimulating hormone in the salmon pituitary gland. *Biochem. Biophys. Res. Commun.* **96**, 1508–1517.

Laemmli, U. K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature (London)* **227**, 680–685.

Loh, Y. P., and Gainer, H. (1977). Biosynthesis, processing, and control of release of melanotropic peptides in the neurointermediate lobe of *Xenopus laevis*. *J. Gen. Physiol.* **70**, 37–58.

Loh, Y. P. (1979). Immunological evidence for two common precursors to corticotropins, endorphins, and melanotropin in the neurointermediate lobe of the toad pituitary. *Proc. Natl. Acad. Sci. USA* **76**, 796–800.

Mains, R. E., Eipper, B. A., and Ling, N. (1977). Common precursor to corticotropins and endorphins. *Proc. Natl. Acad. Sci. USA* **74**, 3014–3018.

Mains, R. E., and Eipper, B. A. (1979). Synthesis and secretion of corticotropins, melanotropins and endorphins by rat intermedia pituitary cells. *J. Biol. Chem.* **254**, 7885–7894.

Mains, R. E., and Eipper, B. A. (1980). Biosynthetic studies on ACTH, β-endorphin, and α-melanotropin in the rat. *Ann. NY Acad. Sci* **343**, 94–110.

Malo-Michele, M. (1975). Réactions cytologiques de la pars intermedia de l'hypophyse de la saupe, *Boops salpa* L. (poisson téléostéen marin. Sparidae) aux variation de la salinité. *Gen. Comp. Endocrinol.* **27**, 1–8.

Malo-Michele, M. (1977). Réaction cytologiques de la pars intermedia de la saupe, *Boops salpa* L. (poisson téléostéen Sparidae) aux variation de la couleur du fond et de l'illumination. *Gen. Comp. Endocrinol.* **31**, 343–349.

O'Farrell, P. H. (1975). High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250**, 4007–4021.

Olivereau, M. (1969). Complexe neuro-intermédiaire et osmorégulation: Comparaison chez l'anguille européenne et chez l'anguille japonaise d'élevage au course du transfert en eau de mer. *Z. Zellforsch.* **99**, 389–410.

Olivereau, M., Aimar, C., and Olivereau, J. M. (1980). PAS-positive cells of the pars intermedia are calcium-sensitive in the goldfish maintained in hyposmotic milieu. *Cell Tissue Res.* **212**, 29–38.

Olivereau, M., Olivereau, J. M., and Aimar, C. (1981) Specific effect of calcium ions on the calcium-sensitive cells of the pars intermedia in the goldfish. *Cell Tissue Res.* **214**, 23–31.

Pang, P. T. K., Griffith, R. W., and Schreibman, M. P. (1973) The pituitary gland and calcium metabolism in *Fundulus diaphanus* (Teleostei). *Gen. Comp. Endocrinol.* **20**, 358–361.

Rudman, D., Chawla, R. K., and Hollins, B. M. (1979). *N,O*-diacetylserine alpha-melanocyte-stimulating hormone: A naturally occurring melanotropic peptide. *J. Biol. Chem.* **254**, 102–108.

Smyth, D. G., Massey, D. E., Zakarian, S., and Finnie, M. D. A. (1979). Endorphins are stored in biological active and inactive forms: isolation of α-*N*-acetyl peptides. *Nature (London)* **279**, 252–254.

Van Eys, G. J. J. M. (1980a). Structural changes in the pars intermedia of the cichlid teleost *Sarotherodon mossambicus* as a result of background adaptation and illumination. I. The MSH-producing cells. *Cell Tissue Res.* **208**, 99–110.

Van Eys, G. J. J. M. (1980b). Structural changes in the pars intermedia of the cichlid teleost *Sarotherodon mossambicus* as a result of background adaptation and illumination. II. The PAS positive cells. *Cell Tissue Res.* **210**, 171–179.

Van Eys, G. J. J. M. (1981). Physiological and Biochemical aspects of the teleostean pars intermedia. Doctoral dissertation. University of Nijmegen, The Netherlands.

Van Eys, G. J. J. M., and Van den Oetelaar, P. (1981). Cytological localization of α-MSH, ACTH and β-endorphin in the pars intermedia of the cichlid teleost *Sarotherodon mossambicus*. *Cell Tissue Res.* **215**, 625–633.

Van Eys, G. J. J. M., and Peters, P. T. W. (1981) Evidence for a direct role of α-MSH in morphological background adaptation of the skin in *Sarotherodon mossambicus*. *Cell Tissue Res.* **217**, 361–372.