THE BIOSYNTHESIS, INTRACELLULAR TRANSPORT, AND PACKAGING OF MELANOCYTE-STIMULATING PEPTIDES IN THE AMPHIBIAN PARS INTERMEDIA

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ABSTRACT

Experiments in which glycine-^{3}H has been introduced into excised neurointermediate lobes of *Xenopus laevis* incubated in a modified Krebs-Ringer bicarbonate medium have shown that ~ 50% of the incorporated radioactivity is present in small peptides which have an electrophoretic mobility characteristic of the melanocyte-stimulating (MSH) peptides shown to be elaborated within the tissue. Based on these results and the demonstration that a discrete ~ 7 min pulse of the label can be introduced into the tissue, electron microscope radioautography has been employed to follow the subcellular events concerned with the synthesis, intracellular transport, and packaging of the labeled secretory product. Together, these studies indicate that the newly synthesized material arises in peptide form, rather than as part of a larger prohormone molecule, on the ribosomes of the rough endoplasmic reticulum within the parenchymal cells of the intermediate portion of the lobe. A proportion is then incorporated into and remains for an extended period within the intracisternal granules which are a feature of the rough endoplasmic reticulum within these cells in vitro Most (~ 60%) of the labeled secretory product, however, is transferred to the Golgi complex within 30 min and, within a further 10 min, becomes packaged into small (~ 200 mμ) electron-opaque secretory granules. It is probable that under the conditions employed these granules represent the final intracellular location of secretory product before it is released

INTRODUCTION

Although an extensive literature exists on the control of secretory activity in the trophic hormone cells of the pituitary (1) the only detailed information available concerning the secretory cycle of these cells is provided by the observations of Tixier-Vidal and her coworkers (2, 3) on the avian prolactin cell. The purpose of the present study is to extend these observations to another pituitary cell type and, in so doing, to investigate for the first time at the fine structural level the elaboration of a small-peptide pituitary hormone. For this work the melanocyte-stimulating hormone (MSH) cells within the neurointermediate lobe of the pituitary of *Xenopus laevis* were chosen because in vivo, their secretory activity can be readily stimulated and synchronized and because, in vitro, they have been shown to elaborate and secrete MSH in a controlled manner (4). A further advantage in using this cell type derives from the fact that within the excised neurointermediate lobes used in the in vitro system the MSH cells are the only intact, and thus can be expected to be the only fully functional, secretory elements. It is thus possible to relate quantitative data obtained from tissue extracts of

the whole lobe directly to subcellular events within the MSH cell. In this report the results are presented of experiments in which glycine-^{3}H has been used to follow the biosynthesis, intracellular transport, and packaging of secretory product in the MSH cell. Glycine was chosen because it is present within the heptapeptide "active center" of all known MSH peptides (5). Leucine, the more usual choice for this kind of study, has not been found to be a component of any MSH peptide although it is present in the structurally related adrenocorticotrophic hormone (6).

Quantitative data concerning the rate of incorporation of the label into newly synthesized secretory product and the character of that product have been obtained by submitting acid extracts of the tissue to gel filtration and electrophoresis, while the intracellular pathway taken by the labeled secretory product has been followed by means of electron microscope radioautography. Together, these approaches demonstrate that approximately 50% of the ^{3}H label introduced into the tissue is incorporated into low molecular weight material and that within this material there are predominantly three compounds, each with the electrophoretic mobility typical of the three MSH peptides present within the gland. Synthesized on the ribosomes of the endoplasmic reticulum, the labeled secretory product takes 30 min to reach the Golgi cisternae and, within a further 10 min, it is packaged into small electron-opaque secretory granules.

METHODS

Large (7–9 cm long, ~ 150 g weight), female *X. laevis* were used after they had been kept at least 7 days on a black, illuminated background. This treatment ensures that the cells of the pars intermedia are maximally stimulated and capable of elaborating and releasing MSH in vitro (4). For each experiment, batches of up to 15 individuals were used. Each animal was decapitated without anesthesia, the pituitary removed, and the neurointermediate lobe dissected away from the pars anterior. The neurointermediate lobes were pooled and placed in a siliconized Erlenmeyer flask containing a modified Krebs-Ringer bicarbonate (KRB) medium and incubated in a shaker water bath at 20°C. The KRB contained 77 mM NaCl, 154 mM KCl, 11 mM $CaCl_2$, 154 mM K_2HPO_4, 154 mM $MgSO_4 7H_2O$, 154 mM $NaHCO_3$ and 600 mg/liter glucose together with Krebs' cycle intermediates as outlined by Krebs (7) and a supplement of essential amino acids as in Eagle's medium (8). The gas phase was 95% O_2/5% CO_2.

Earlier work (4) showed that an incubation period of 2 hr was required for the rate of synthesis and release of MSH from the gland to approach a maximum, a preincubation period of this length was therefore used in all experiments in the present study.

For the quantitative estimation of the incorporation of the ^{3}H label batches of 5 or 10 neurointermediate lobes were incubated in 1 ml KRB lacking glycine-^{1}H but containing 20 μCi glycine-^{3}H (SA, 1000 mCi/mmole). To dilute the glycine-^{3}H concentration in pulse-chase experiments and thus, after the pulse, to reduce effectively its incorporation to a minimal level the tissue was transferred from the glycine-^{3}H medium to KRB containing 2 mM glycine-^{1}H. For the quantitative assay of radioactivity the neurointermediate lobes were homogenized in a ground-glass Potter homogenizer in 0.5 ml 0.1 N HCl containing 0.5% deoxycholate and left to stand for 4 hr at 5°C. Carrier bovine serum albumin (BSA) was added to give a final concentration of 1 mg/ml and, after thorough mixing, any debris remaining was spun down at 1000 g. Preliminary experiments showed that the MSH peptides are not precipitable in trichloracetic acid, and so free ^{3}H activity and incorporated ^{3}H activity in the HCl extracts were separated by gel filtration on a 40 × 1.5 cm G25 Sephadex column (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) (Fig. 1). The effluent from the column was monitored at 230 nm and the BSA peak was used to indicate the position of the void volume, that is, of molecules too large (< MW 4500, see Fig. 1) to be included in the G25 gel. In preliminary experiments a mixture of glycine-^{3}H and unlabeled HCl extract was used to indicate the elution volume of free glycine-^{3}H. Also, since glycine-^{3}H can be expected to form glycyl-^{3}H–tRNA molecules which would, in addition to larger (< 4500 MW) proteins containing ^{3}H, also be excluded from the G25 Sephadex, the contribution of glycyl-^{3}H–tRNA to the void volume was estimated by incubating this material for 1 hr at 37°C at pH 10.5 (9). Rechromatographing the hydrolyzed material on G25 indicated the amount of glycine-^{3}H removed from the glycyl-^{3}H–tRNA and repeated analysis in this way demonstrated that approximately 20% of the void volume activity is due to glycyl-^{3}H–tRNA.

0.5 ml samples from the column elute were prepared for liquid scintillation counting by using the toluene-based scintillation fluid described by Hall and Cocking (10). They were counted in a Packard Tricarb 3000 counter (Packard Instrument Co., Inc., Downers Grove, Ill.) with an efficiency of 20%.

For experiments in which the labeled material was submitted to starch block electrophoresis the included volume (52.5–65.0 ml) (see Fig. 1) of the G25 effluent derived from tissue labeled for 15 min with glycine-^{3}H was lyophilized and then reconstituted in 0.5 ml 0.1 M pyridine-acetate buffer. Electrophoresis was then

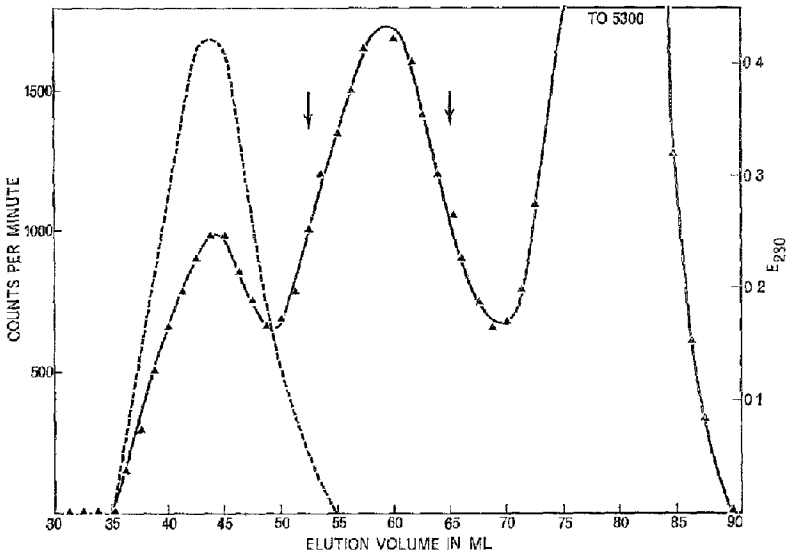


FIGURE 1 Eluate profile from G25 Sephadex when an HCl extract derived from 15 neurointermediate lobes labeled for 5 min with glycine-^{3}H is chromatographed in 2.5% acetic acid. — shows the void volume position indicated by bovine albumin monitored at E_{230}. Also in this 35–52.5 ml position is the first peak containing incorporated ^{3}H. The second peak, 52.5–65 ml, contains the small molecular weight ^{3}H material while the third, at 70–90 ml, contains free glycine-^{3}H The arrows indicate the fraction taken for estimating the rate of ^{3}H incorporation (Fig. 2 *a*) and for electrophoresis (Fig. 3 *c*). In this column ACTH, MW 4530 emerges in the void volume at 46 ml, and Bacitracin, MW 1450 at 65 ml. *Xenopus* MSH peptides as shown by bioassay are in the included volume between 57.5 and 67 ml.

carried out with 0.01 ml portions of this extract at 5°C on a starch block for 30 hr at 200 v and 14 ma in 0.1 M pyridine acetate buffer (pH 4.9) as described by Thody (11) When electrophoresis was complete, 0 5 cm segments were cut from the block, extracted in 2 ml 2.5% acetic acid, lyophilized, reconstituted in 0.5 ml 2 5% acetic acid, and assayed by liquid scintillation counting as above The distribution of MSH peptides in the gel filtration eluate and in the starch block electrophoresis separation was established by using the skin of *Anolis carolinensis* to bioassay for MSH (12).

Electron Microscope Radioautography

Preincubation and incubation in the glycine-^{3}H and chase media were carried out as for the preparation of HCl extracts except that the concentration of glycine-^{3}H was increased to 300 mCi/ml in the pulse medium. After incubation the neurointermediate lobes were fixed in a dilute Karnovsky fluid (13) containing 2% paraformaldehyde, 3% glutaraldehyde in 0.067 cacodylate buffer for 3 hr. They were postosmicated for 1 hr, dehydrated in alcohol, and embedded in Maraglas (Polysciences, Inc., Warrington, Pa) (14). Gold sections cut with a diamond knife on a Huxley ultramicrotome (LKB Instruments, Inc., Rockville, Md.) were mounted on collodionized glass slides and coated with Ilford L4 emulsion (Ilford Ltd., Ilford, England) as described by Salpeter and Bachmann (15). After an exposure of 1–4 wk the radioautographs were developed in Microdol X, fixed, floated off the slides, and mounted on 300-mesh grids. For staining, the collodion was removed from the surface of the preparation by immersing the grids in amyl acetate for 30 min. Staining with lead citrate (16) for a further 30 min then followed. Electron micrographs were taken in a Siemens Elmiskop I

The distribution of silver grains over the tissue was assessed by counting the grains present over the endoplasmic reticulum, intracisternal granules, Golgi cisternae, secretory granules, mitochondria, and nuclei. Grains were assigned to an organelle if they were superimposed upon it by more than 50%.

MATERIALS

MSH was the gift of Ferring A. B., Malmö, Sweden, and ox posterior pituitary powder the gift of Armour Laboratories, London. ACTH was obtained from Sigma Chemical Co., St Louis; Bacitracin from Mann Research Labs. Inc., New York; and glycine-2-^{3}H (SA, 1000 mCi/mmole) from the Radiochemical Centre, Amersham, England.

RESULTS

Previous fine structural studies on the pars intermedia of *X. laevis* under conditions in which its secretory activity was carefully controlled have been made both in vivo and in vitro (4, 17). Typically in vitro the parenchymal cells of the pars intermedia derived from black background–adapted individuals appear as in Fig. 4. The subcellular organization is characteristic of an actively secreting adenohypophyseal cell with abundant rough endoplasmic reticulum, a well-developed Golgi complex and few secretory granules. The most abundant secretory granules are found within the Golgi area; they are 150–250 mμ in diameter and have a homogeneous electron-opaque content. Less abundant are larger, 250–275 mμ granules with a finely fibrous content (Fig. 5). In vivo the small electron-opaque granules are always found predominantly in the Golgi area whereas the larger (250–270 mμ) granules are abundant only in the glands of animals maintained on a white, illuminated background, a condition which induces the storage of MSH (17).

Preliminary experiments evaluated the kinetics of the incorporation of ^{3}H label into the cells of the pars intermedia. The tissue was incubated for 5–60 min in KRB containing 20 μCi/ml glycine-^{3}H, homogenized, extracted with 0.1 N HCl, and chromatographed on G25 Sephadex. Three distinct peaks (Fig 1) were obtained: (*a*) the void volume material, which by mild hydrolysis at pH 10.5 could be shown to contain ~ 20% glycyl-^{3}H–tRNA, (*b*) the fractionated or partially included material with a molecular weight of between 1000 and 5000 (see Fig. 1), and (*c*) the included volume containing free glycine-^{3}H. By assuming that the activity contained within peak (*b*) was directly related to the amount of ^{3}H label incorporated into small molecular weight material, it can be shown that during the first 60 min the amount of ^{3}H label incorporated into this material increases in an approximately linear fashion (Fig. 2 *a*).

Preliminary experiments indicated than an incubation of at least 5 min in the glycine-^{3}H medium was required to produce an acceptable EM radioautograph after 4 wk exposure. Thus using a 5 min incubation period to introduce a pulse label into the tissue, experiments were carried out to find out if at the end of this time the intracellular pool of unincorporated glycine-^{3}H could be effectively diluted by incubation in KRB containing 2 mM glycine-^{1}H. G25 Sephadex filtration was again used to separate the free and incorporated ^{3}H material and, as shown in Fig 2 *b*, it was found that more than 85% of the free glycine-^{3}H within the tissue at the end of a 5 min incubation in glycine-^{3}H KRB is displaced by a 5 min wash in 2 mM glycine-^{1}H medium Since significant incorporation is not observed until after the first 2 min of incubation in the glycine-^{3}H medium (when it is half maximal), this result demonstrates that a discrete pulse of glycine-^{3}H, ~7 min in length, can be introduced into the pars intermedia. It will be shown below that labeled secretory product spends at least 10 min in each cellular compartment and, thus, within the limits of the radioautographic technique, a 7 min pulse allows the intracellular pathway followed by the secretory product to be identified unequivocally.

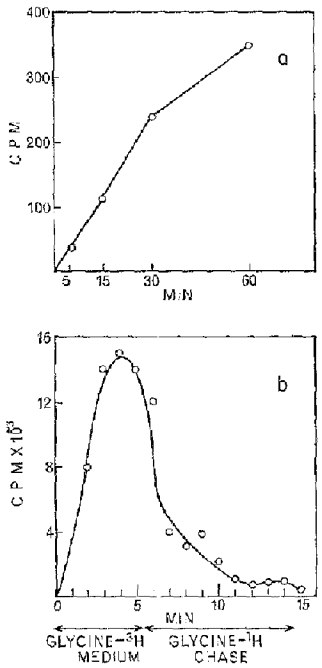


FIGURE 2 (*a*) Rate of incorporation of ^{3}H label with time into the material contained within the 52 5–65 ml G25 Sephadex peak shown in Fig. 1 Batches of five neurointermediate lobes were incubated for 5, 15, 30, and 60 min, homogenized, HCl extracted, and chromatographed (*b*) Availability of free glycine-^{3}H within the tissue during a 5 min incubation in the glycine-^{3}H medium and a 10 min wash with 2 mM glycine-^{1}H. Batches of five neurointermediate lobes were used at each minute time point. They were blotted dry, homogenized, extracted with HCl, and chromatographed on G25 Sephadex. The free glycine-^{3}H obtained from the tissue was present in the 70–90 ml free glycine-^{3}H peak.

The observation that more than 50% of the incorporated ^3H material present within the fractionation range of G25 Sephadex is of interest since this is to be expected if it is incorporated into one or more of the MSH peptides (see Fig 1). To investigate this point further a starch block electrophoretic method previously employed to separate MSH peptides in the ox and other mammals (11, 12) was used to separate those present in acid extracts of the *Xenopus* pars intermedia. As shown in Figs. 3 *a* and 3 *b*, three of the four components shown to be present in the ox pituitary are present in the pars intermedia of *Xenopus* In the ox pituitary these common components have been identified as β-glutamyl MSH, α-MSH, and an as yet uncategorized MSH (11). When the second peak (52 5–65 ml) of the G25 Sephadex eluate (derived from tissue labeled for 15 min in vitro with glycine-^3H) is electrophoresed in this system (Fig. 3 *c*), three peaks, each corresponding in electrophoretic mobility to an MSH peptide shown to be present within the *Xenopus* pars intermedia, can be clearly identified It should be added than an essentially similar, although for illustrative purposes less satisfactory, profile is obtained from tissue incubated for only 5 min with glycine-^3H.

Radioautographical Results

Based on the results obtained with HCl-extracted homogenates, electron microscope radioautography was used to follow the intracellular pathway taken by the labeled secretory product Initially, this approach also served to confirm the view that in the neurointermediate lobe all and only the parenchymal cells of the pars intermedia incorporated ^3H label, as neither neuronal elements of the pars nervosa nor any contaminating portions of the anterior lobe became labeled significantly above background levels.

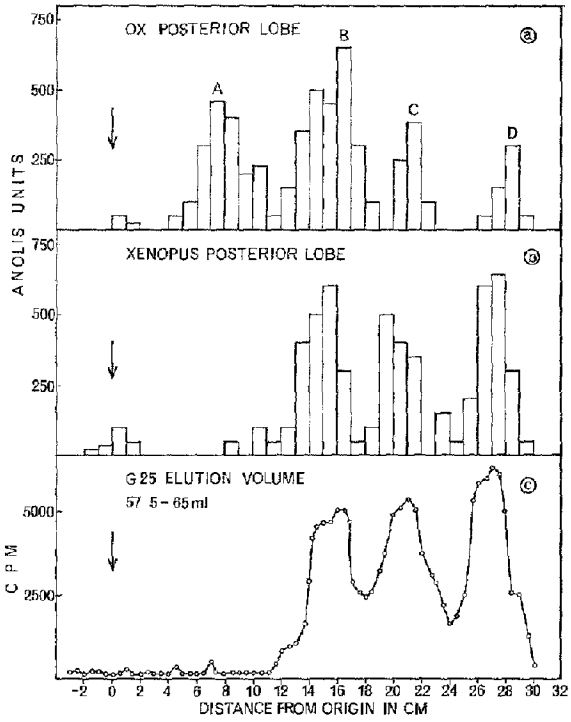


FIGURE 3 Profiles (*a*) and (*b*) were obtained by electrophoresing ox posterior lobe and *Xenopus* posterior lobe extracts as described by Thody (11) and bioassaying extracted 1.0 cm segments for MSH. Profile (*c*) was obtained by electrophoresing the 52 5–65 ml G25 Sephadex fraction derived from the acid extract of 15 neurointermediate lobes incubated for 15 min in the glycine-^3H medium and assaying the extract from 0.5 cm segments by liquid scintillation counting. The arrows mark the point of origin. *ABCD* refers to the MSH peptides present in the ox posterior pituitary which correspond to β-seryl MSH, β-glutamyl MSH, α-MSH, and an as yet uncategorized MSH, respectively.

At the end of a 5 min pulse in the glycine-^{3}H medium 67% of the silver grains are over the rough endoplasmic reticulum (Table I, Fig 5) while almost all of the remainder are over either mitochondria or nuclei. However, since it is known that small peptides such as the decapeptide gramicidin S can be synthesized in ribosome-free systems (18) it was necessary to confirm that the label observed over the endoplasmic reticulum in the MSH cell represented peptides which were being synthesized or had been synthesized on the attached ribosomes Control experiments were therefore carried out in which 10^{-4} M cycloheximide, an inhibitor of ribosomal-linked protein synthesis (19), was added to the preincubation medium 5 min before the pulse and to the pulse medium. Under these conditions some labeling of the nuclei was still observed, but, more significantly, labeling of the endoplasmic reticulum was reduced to background levels In passing, it should be added that this reduction in the amount of label over the endoplasmic reticulum induced by cycloheximide also excludes the possibility that a significant number of the grains over the tissue fixed in the presence of free glycine-^{3}H are the result of an artifactual, nonspecific binding of unincorporated label.

A further noteworthy feature of the label found over the endoplasmic reticulum when ribosomal incorporation of ^{3}H label is allowed to proceed without inhibition concerns that found over intracisternal granules. These granules are spherical, electron-opaque masses (Figs. 4 and 6) which are rarely observed in vivo and which in vitro are found most frequently within the endoplasmic reticulum cisternae of the MSH cells that lie immediately adjacent to the pars nervosa. They are not significantly labeled after a 5 min glycine pulse, but grains are observed either over them or close to them after the pulse has been chased for 20 min The association of the label with these granules is, however, most impressive after longer postpulse intervals, when less label is associated with the rough endoplasmic reticulum Thus, after a 40 min chase (Fig 6), when most of the label is found in the Golgi area, almost 60% of the grains found over the endoplasmic reticulum are over the intracisternal granules. It is also worth noting that even after a chase of 100 min, which is the longest interval studied, some labeled intracisternal granules have been observed.

As shown in Table I, silver grains predominate over the rough endoplasmic reticulum for up to 20 min postpulse After a 30 min chase, however, label appears over the Golgi complex and an abrupt change in the general distribution of silver grains occurs. After a 20 min chase, fewer than 20% of the silver grains are over the Golgi complex, whereas 45% are over the endoplasmic reticulum (Table I) After a 30 min chase the label over the endoplasmic reticulum is reduced to less than 30% of the total, and the Golgi label has increased threefold. Within the Golgi complex it is usually possible to distinguish between silver grains lying over the cisternae and those distributed over or close to the electron-opaque secretory granules, and thus it is possible to provide an indication of the rate of passage of secretory product through the cisternae. Thus, after 30 min almost 50% of the label is over the cisternae whereas only 25% is associated with the secretory granules (Fig. 7) 10 min later, the situation is reversed, much of the label having left the cisternae and having been packaged into granules After a 60 min chase the proportion of label over the secretory granules has increased and that over the cisternae further diminished (Fig 8) After chases of up to 100 min this distribution of grains remains essentially unchanged except that at this time electron-opaque secretory granules can also be found some distance away from the Golgi area (Fig 9). As has been mentioned earlier, in addition to the electron-opaque (150–250 mμ) granules a number of larger (250–275 mμ) secretory granules with a finely fibrous content are present in the parenchymal cell At no time in the present study has this granule population become significantly labeled.

DISCUSSION

In previous studies on the pars intermedia of *X laevis* it was demonstrated that in vitro this tissue elaborated and released melanocyte-stimulating hormone in readily bioassayable quantities (4, 17) It was also shown that for up to 4 hr in vitro secretory activity continued in a controlled and predictable fashion and that at the end of this time the fine structural organization showed no overt evidence of deterioration. In the present study the linear rate of amino acid incorporation over the experimental period employed supports these results while the radioautographic evidence illustrates that for the purposes of the present study the neurointermediate lobe can be regarded functionally as a homogeneous population of well synchronized pars intermedia secretory cells.

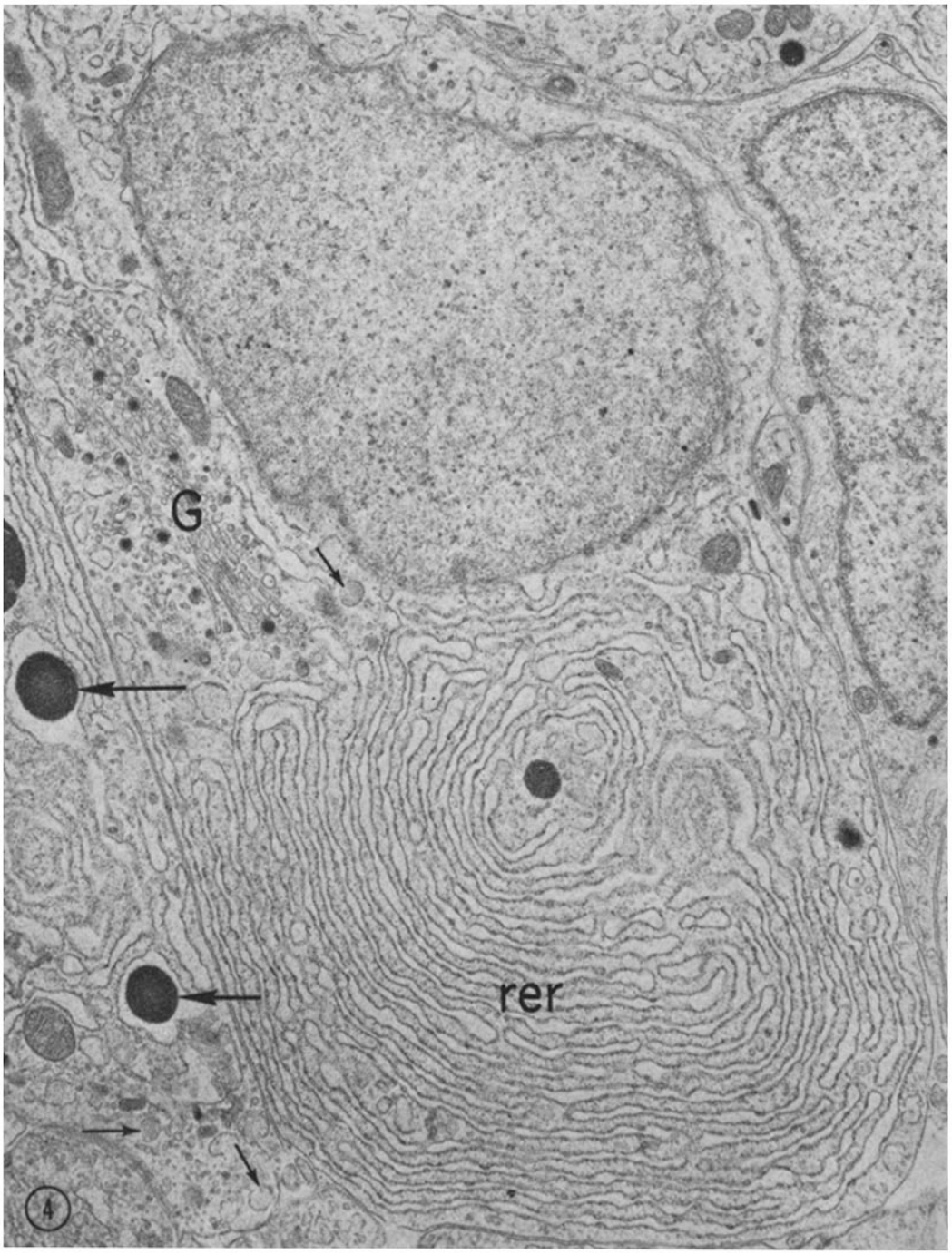
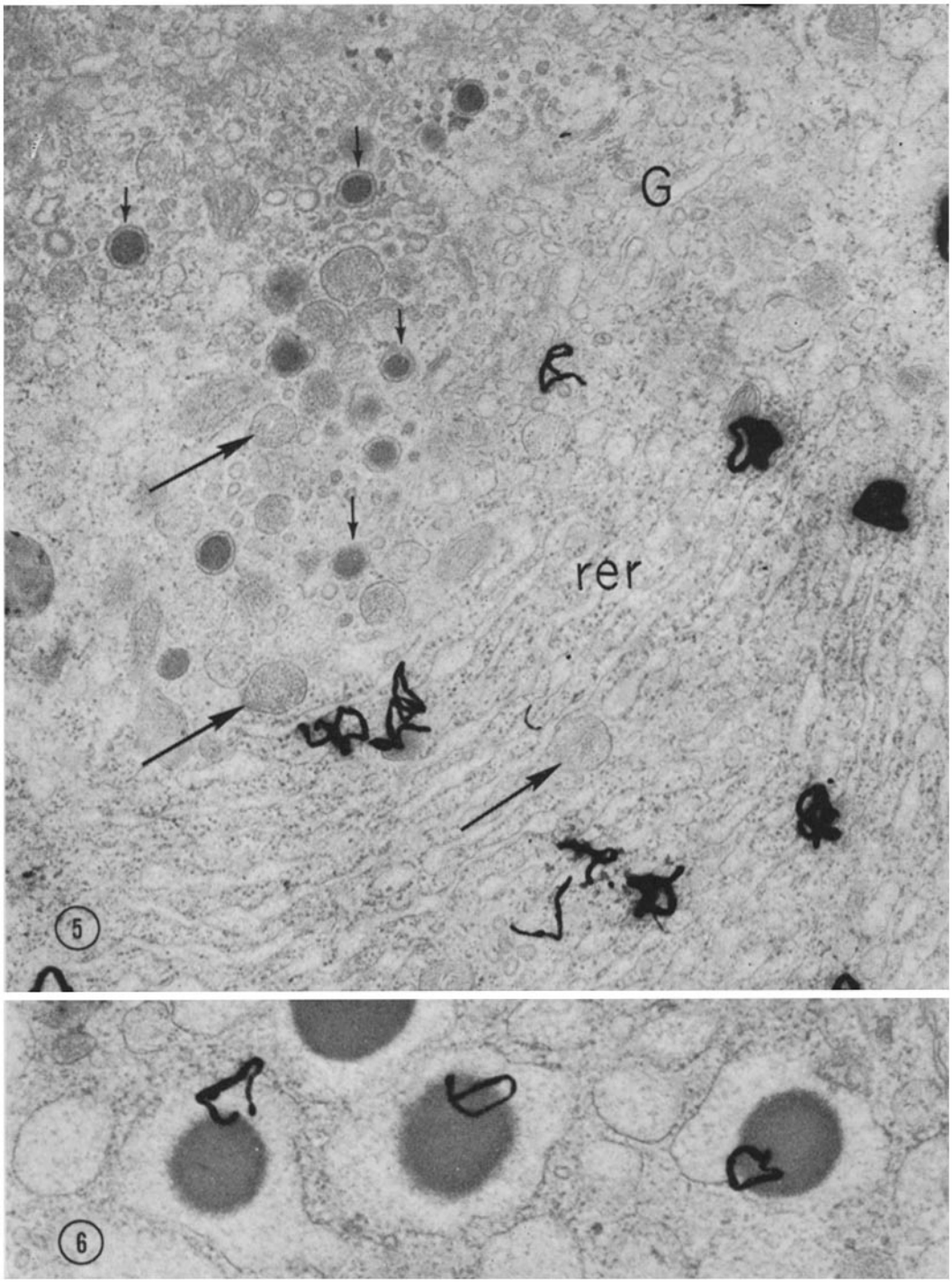


FIGURE 4 Subcellular organization of the pars intermedia tissue incubated for 2 hr in KRB. Within the rough endoplasmic reticulum (*rer*) of some cells electron-opaque intracisternal granules are present (large arrows). The Golgi area (*G*) is typically perinuclear and includes secretory granules with an electron-opaque content. The small arrows indicate the few larger secretory granules present at this time × 4000.



FIGURES 5 and 6 Both radioautographs exposed 6 wk.

FIGURE 5 Electron microscope radioautograph of tissue labeled for 5 min with glycine-^{3}H. Most of the silver grains lie over the rough endoplasmic reticulum (*rer*). The Golgi cisternae (*G*), small electron-opaque secretory granules (small arrows), and the larger secretory granules (large arrows) are almost free of grains. × 17,000

FIGURE 6 Labeling of rough endoplasmic reticulum intracisternal granules after a 40 min chase. × 25,000.

TABLE I

Counts from Radioautographs Showing the Grain Distribution over the Cells of the Whole Pars Intermedia after a 5-min pulse of Glycine-^3H

Pulse duration	Chase duration	Total grains counted	Endoplasmic reticulum	Golgi cisternae	Secretory granules	Mitochondria	Nucleus
			%	%	%	%	%
5	0	980	67	5	0	16	12
5	20	900	45	13	10	22	10
5	30	600	27	43	26	4	5
5	40	1600	15	21	50	7	9
5	60	900	15	14	63	1	7

Grain counts over intracisternal granules are not included because it is only possible to identify grains associated with them with certainty after longer chase intervals (>30 min) when most of the labeled secretory product has left the endoplasmic reticulum.

The finding that after a 5 min incubation in the glycine-^3H medium the incorporated label is found predominantly in material contained within the included volume of the G25 Sephadex and the observation that incorporation is sensitive to cycloheximide are good indications that the pars intermedia tissue synthesizes and releases small peptides at the ribosome and argue against the possibility that the MSH peptides known to be elaborated by the tissue are initially synthesized as part of a larger precursor or prohormone molecule. Since the material obtained from the second peak of the G25 eluate can be further separated electrophoretically into three components which have the same mobility as the three MSH peptides shown to be present in the gland, it is reasonable to assume that all three of the peptides are being synthesized simultaneously.

The evidence obtained concerning the intracellular pathway followed by newly synthesized secretory product in the MSH cell is derived solely by radioautography, as at the present time there is little possibility of obtaining subcellular fractions from the neurointermediate lobe. Nevertheless, it is clear that this pathway is essentially the same as that which was originally described and evaluated in detail in the acinar cells of the exocrine pancreas (20–27) and which more recently has also been outlined in the secretory cells of a number of endocrine tissues (2, 28–30). In the pancreas, labeled secretory product reaches the Golgi area after a chase interval of 7–17 min (22) whereas in those endocrine cells which have been studied, as in the MSH cell, labeled material is observed in this area only after a chase interval of 30 min (2, 28). Since in the present study the length of time the labeled secretory product remains in the endoplasmic reticulum is much longer than the length of the glycine-^3H pulse, it seemed possible that a localized redistribution of label representing intracisternal transport within this intracellular compartment may occur. In particular, after the longer (15–20 min) chase intervals, labeling of the endoplasmic reticulum cisternae proximal to the Golgi area might be anticipated, however, no distribution of silver grains indicating such a relocation was observed.

Intracisternal granules similar to those observed in the MSH cell have also been observed within the endoplasmic reticulum cisternae of thyrotrophic hormone cells after thyroidectomy (31, 32). In these cells conditions are similar to those in MSH cells removed from hypothalamic control (4, 33, 34), as after thyroidectomy there is also a dramatic increase in the rate of hormone release (35). On the basis of a fine structural study of the thyroidectomy cell, Farquhar (32) has suggested that as a secondary feature of the increased secretory activity the removal of secretory product from the endoplasmic reticulum cisternae fails to keep pace with the production of it and, as a result, it accumulates and eventually prematurely condenses in the form of intracisternal granules. This interpretation of the events which accompany increased secretory activity within thyroidectomy cells also satisfactorily accounts for the formation of intracisternal granules within the endoplasmic reticulum of the MSH cells in vitro and is supported by the finding that there is a short delay before these granules become labeled and by the observation that they then remain labeled for an extended period.

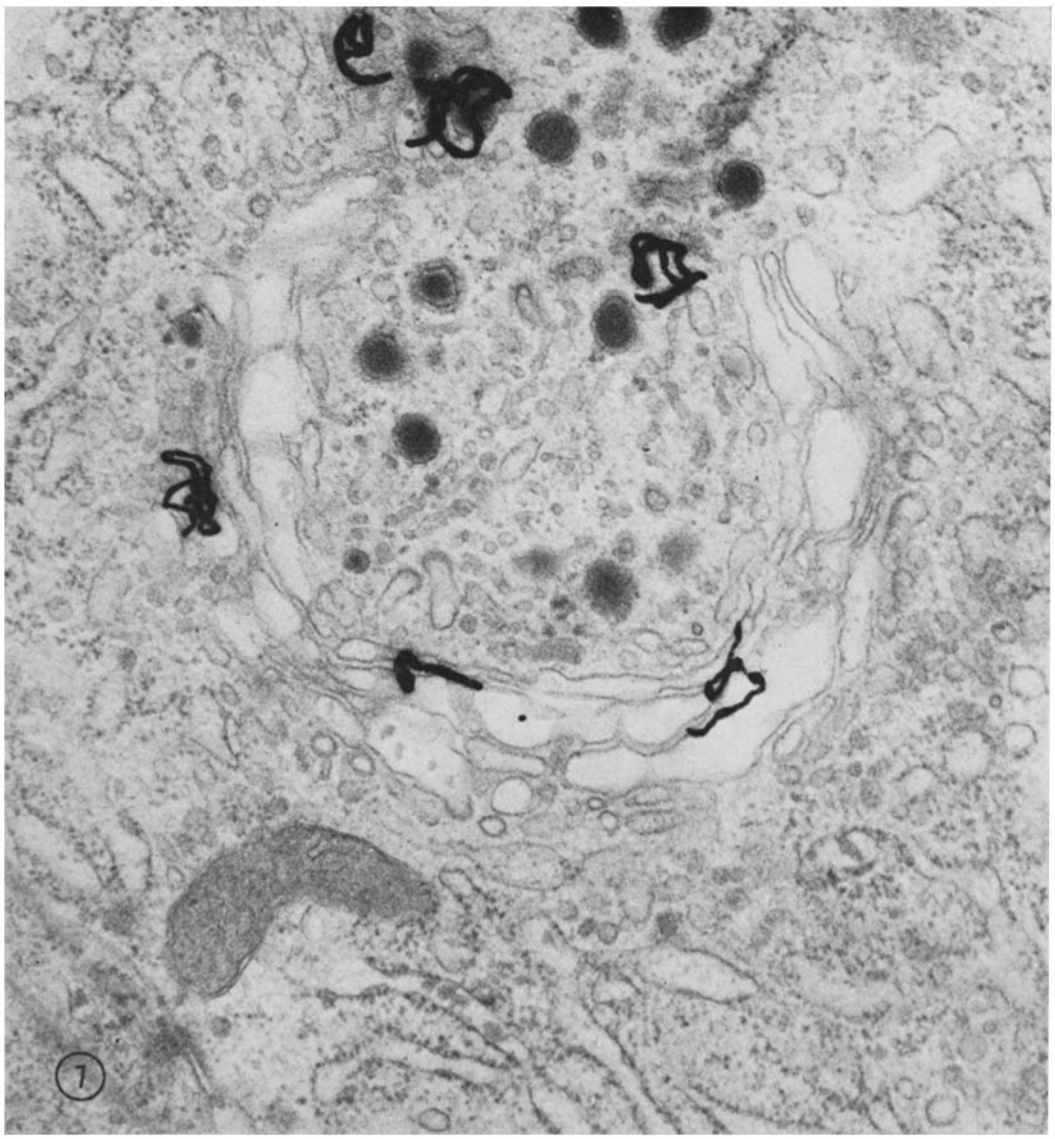
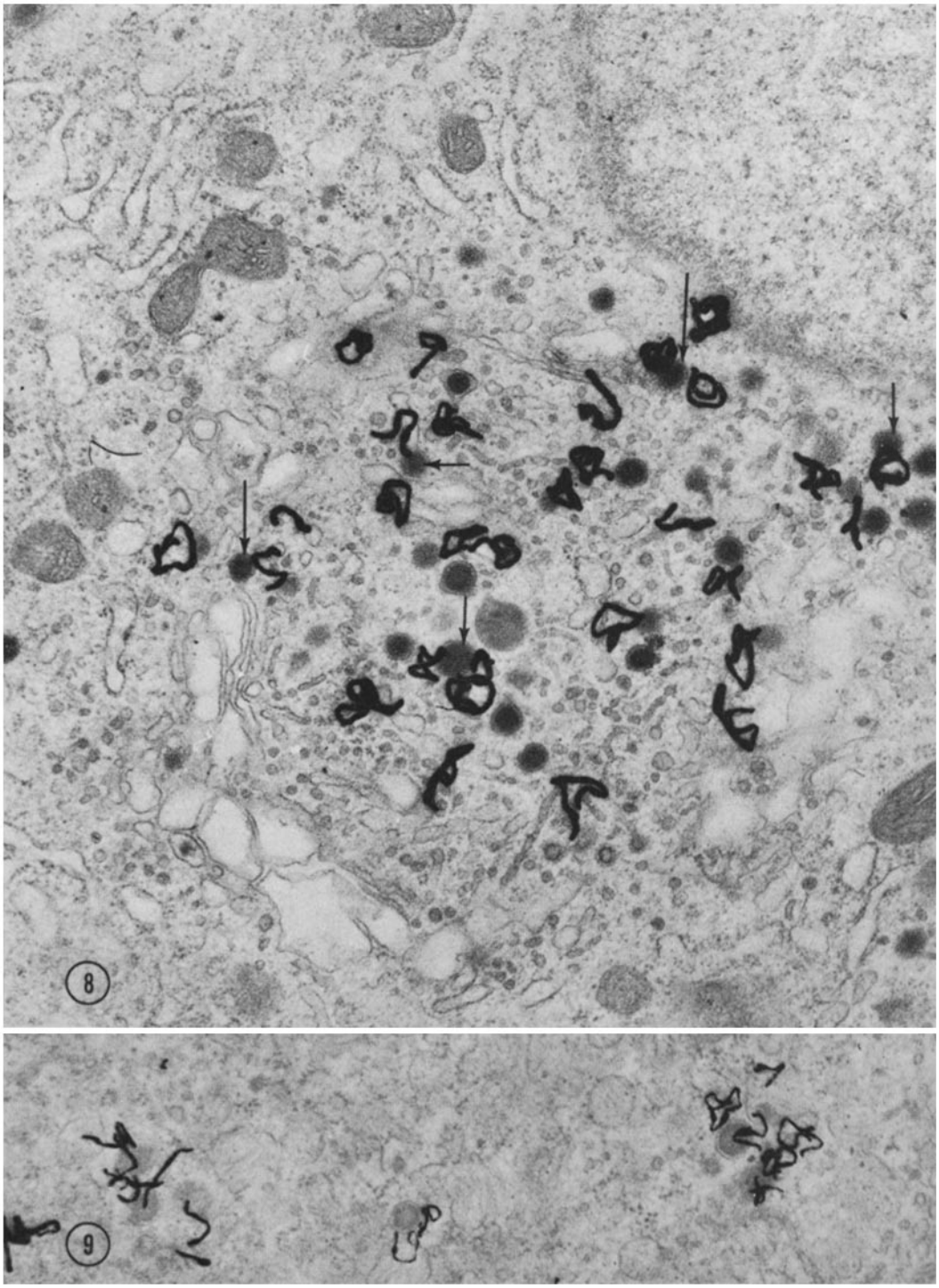


FIGURE 7 Radioautograph after 30 min chase. Most silver grains are located either directly over the parallel Golgi cisternae or close to them although some may be related to secretory granules Grain counts made at this time point indicate that 26% of the grains are related to secretory granules, 43% to the Golgi cisternae, and 27% to the rough endoplasmic reticulum Exposure: 3 wk. × 20,000

The temporal resolution of the EM radioautographs in the present system allows the pulse label to be identified within the Golgi cisternae, and the evidence suggests that the transport of secretory product through these elements takes approximately 10 min. This is not true of all Golgi components, however, because some labeled material is present within elements residing close to the flattened cisternae after a 30 min chase, when it is first seen there, until after a 100 min chase, the longest interval studied The reason for this prolonged labeling is not clear, but presumably it means that some secretory product, if not some of the newly formed, electron-opaque secretory granules themselves, must be remaining in the Golgi area for extended periods A similar prolonged Golgi label



FIGURES 8 and 9 Both radioautographs exposed 6 wk.

FIGURE 8 Radioautograph after a 50 min chase. Most of the grains are now distributed at the releasing face of the Golgi stack. Many of them lie directly over secretory granules (arrows) although in such a complex area the possibility that some of the label resides within the abundant tubular and vesicular elements cannot be excluded. × 15,000.

FIGURE 9 Radioautograph after a 100 min chase. Granules some distance away from the Golgi area are now labeled. × 12,000.

has been found in the β cells of pancreatic islets (28).

Two types of secretory granules, differing in size and content, have been consistently described in the MSH cells of mammals (36–39) and other vertebrates (40, 41). The variation in the numbers of the larger (250–275 mμ) granules is directly related to the content of MSH in the neurointermediate lobe (17, 42), and thus there is good reason to believe that they contain the hormone. The significance of the smaller electron-opaque granules has remained obscure, but in view of the evidence obtained in the present study it is clear that they also contain MSH peptides. The relationship between the two types of granules, nevertheless, still remains unresolved because although much of the available evidence suggests that the smaller granules arising in the Golgi area are the immature form of the larger ones, the absence of label over the latter does not allow this maturation sequence to be demonstrated. The lack of label over these granules may be related to the increased rate of MSH release, which occurs when the gland is removed from its endogenous inhibitory control (33, 34). Thus it is possible that in vitro these granules are no longer produced or released, since they remain, and that the smaller, electron-opaque form represents the final intracellular location of hormone before release.

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