# DNA SYNTHESIS IN THE RAT ANTERIOR PITUITARY

## An Electron Microscope Radioautographic Study

ANDREA MASTRO, EMMA SHELTON, and W. C. HYMER. From the Biology Department, The Pennsylvania State University, University Park, Pennsylvania 16802. Dr. Shelton's address is the Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

### INTRODUCTION

The anterior pituitary gland has been classified by Leblond and Walker (1) as an organ of low mitosis and no cell renewal. Although mitoses in the gland are rarely seen (2–4), recent radioautographic studies have shown that thymidine-$^3$H is incorporated into rat anterior pituitary nuclei in vivo (5, 6). We have shown by light microscope radioautography that thymidine-$^3$H is also incorporated into epithelial nuclei of rat anterior pituitaries in vitro (7).

The cell types of the anterior pituitary are characterized by the presence of cytoplasmic granules which are known to contain specific pituitary protein hormones (8, 9). Using the differential staining of these granules as a basis for identification of cell type, we determined at the light microscope level that acidophils, basophils, and chromophobes were synthesizing DNA (7). At the electron microscope level, the size of the granules is characteristic for a given cell type (10).

We used the technique of electron microscope radioautography to identify more precisely the pituitary cell types synthesizing DNA. The results of this electron microscope radioautographic study complement our previous work and confirm that DNA synthesis occurs in pituitary cells which contain cytoplasmic hormone granules.

### MATERIALS AND METHODS

Anterior pituitaries from 140–150-g male rats (Holtzman Co., Madison, Wisconsin) were incubated for 3 hr as described previously (7). This method involves incubation of single anterior pituitary glands in 1.5-ml Medium 199 containing thymidine-$^3$H (2 $\mu$c/ml; 6.7 ci/mmole; New England Nuclear Corp., Boston) at 37°C under 95% $O_2$–5% $CO_2$. After incubation, the glands were washed with nonradioactive medium and prepared for light microscope or electron microscope radioautography. In the former case, six pituitaries were fixed in phosphate-buffered 10% formalin, cleared, dehydrated, and embedded in paraffin. 4-$\mu$ sections taken from central and peripheral areas of the gland were coated with Kodak NTB-3 liquid emulsion (3 parts emulsion to 1 part water) and exposed for 3 days (7). The radioautographs were developed and stained by a Gomori trichrome method (13).

For electron microscope radioautography, five anterior pituitary glands were washed in cold cacodylate buffer (0.067 M, pH 7.4 in 1% sucrose). Peripheral slices of the gland approximately 1 mm thick were minced in cold 2% glutaraldehyde in cacodylate buffer at pH 7.4, and fixed in glutaraldehyde for 30 min. After being rinsed in buffer, the

glands were postfixed for 30 min in 1% osmium tetroxide in cacodylate buffer (pH 7.4), dehydrated through an alcohol series, placed in propylene oxide, and embedded in Epon. Pale gold sections were cut on an LKB Ultratome and mounted on parlodion-coated, 200-mesh copper grids. After being stained with 1% uranyl acetate in 50% methanol and lead citrate (11), they were coated with a thin layer of carbon.

Monolayers of Ilford L-4 emulsion (1 part emulsion to 3.3 parts water) were placed over the grids according to the technique of Caro and van Tubergen (12) except that loops of 1 cm in diameter were used to make films of the emulsion. Grids covered with emulsion were exposed for 4 wk at 4°C in a vacuum desiccator and developed in Microdol X for 3 min at 24°C. Grids without sections were treated in the same way and monitored for background grains. Sections were examined and photographed with a Siemens Elmiskop IA with a double condenser, 50-$\mu$ objective aperture and 80 kv.

## RESULTS AND DISCUSSION

### *Light Microscope Radioautography*

After examination of approximately 200,000 cells from six pituitaries, the percentage of labeled nuclei was calculated and the labeled cells were classified by cell type on the basis of their staining characteristics (Table I). These data are in good agreement with those of our previous experiments (7).

### *Electron Microscope Radioautography*

Electron microscope radioautography revealed that thymidine-$^3$H was incorporated into the nuclei of the three types of epithelial cells (Fig. 1 *a–c*) as well as into the nuclei of capillary endothelial cells and stromal fibroblasts. After 4 wk of exposure, each labeled epithelial nucleus contained an average of 46 grains; occasional grains over the cytoplasm were not above the background level of one grain/1000 $\mu^2$. A labeling index of 1.3% was calculated after counting 1500 nuclei.

TABLE I

*Labeling Indices and Distribution of Label among Cell Types in Rat Anterior Pituitary Glands*

| | Labeling index (labeled nuclei) | Total labeled cells classified | Distribution of label among cell types | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Acidophils | | Basophils | | Chromophobes | | Unknowns | |
| | % | | *total* | % | *total* | % | *total* | % | *total* | % |
| Light microscope radioautography | 1.8 ±.1 | 910 | 149 | 16.4 ±1.4 | 83 | 9.1 ±.8 | 498 | 54.7 ±1.4 | 80 | 19.8 ±1.2 |
| Electron microscope radioautography | 1.3 | 43 | 20 | 46.5 | 10* | 23.2 | 13 | 30.2 | | |

For light microscopy, the labeling index was obtained by examining an average of 33,000 cells for each of six glands; distribution of label was based on approx. 150 labeled cells/gland. The standard errors are of the counts among six pituitaries. The electron microscope labeling index was obtained by counting 1500 nuclei. The cell types are based on 43 cells photographed from sections of five pituitaries.

* Of the labeled basophils, seven were thyrotrophs and three were gonadotrophs.

Using a method similar to that described by Farquhar and Rinehart (10), we identified acidophils, basophils, and chromophobes on the basis of cytoplasmic granule size. Cells in which the granules had a diameter of 250–400 m$\mu$ were classified as somatotrophic acidophils; those with granules of a diameter of 100–200 m$\mu$ were designated as thyrotrophic basophils; and those with granules of 200–250 m$\mu$ were classified as gonadotrophic basophils. The degree of cytoplasmic granulation was variable, but granules were present in sufficient number to make identification of cell type possible. We found, as previously reported (10), that cells completely devoid of granules are seldom seen at the electron microscope level. In this study, cells containing less than five cytoplasmic granules were classified as chromophobes. The distribution of label among

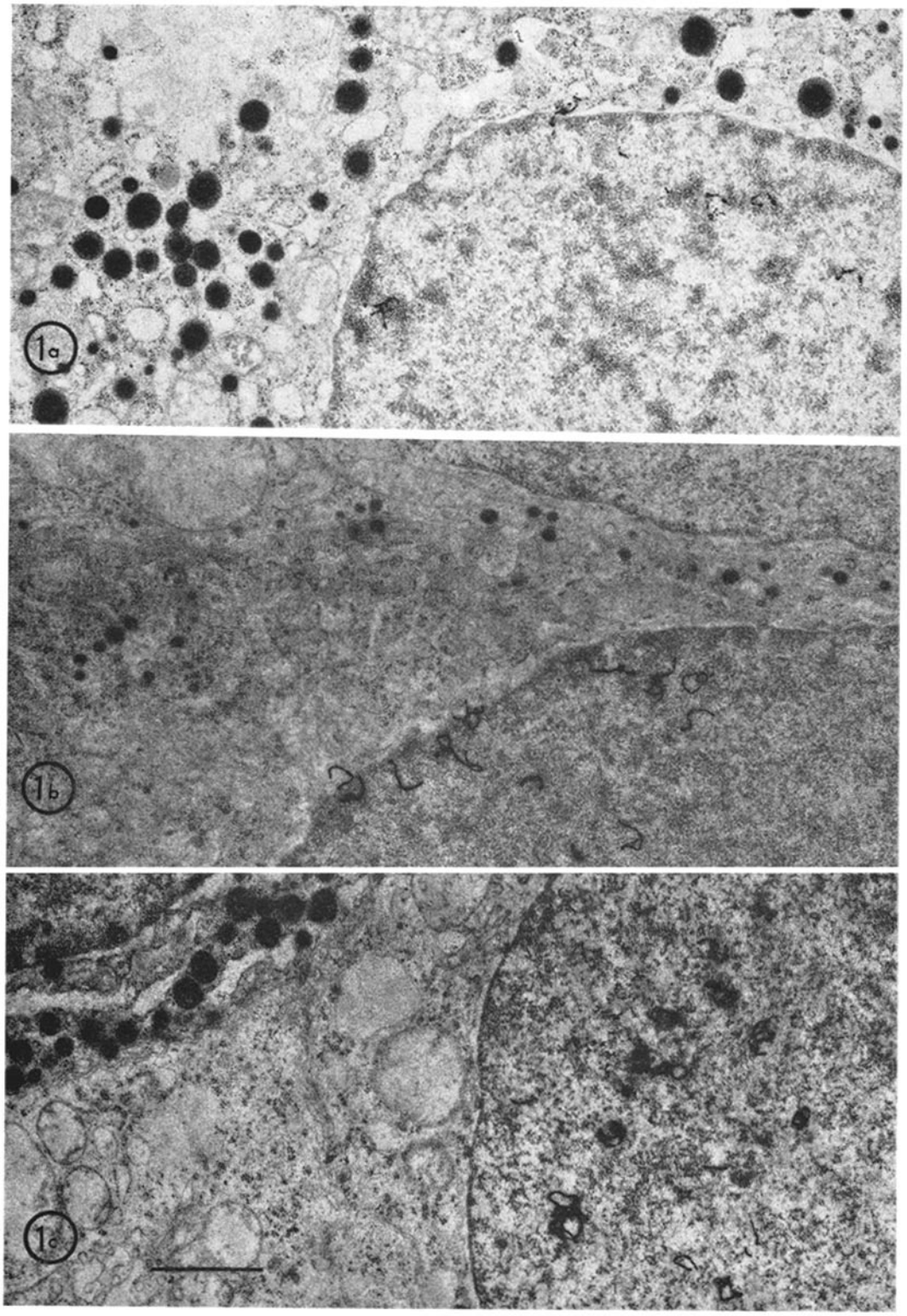


FIGURE 1 *a–c* Electron microscope radioautographs of rat anterior pituitary glands after 3-hr incubation in Medium 199 containing 2 $\mu$c/ml thymidine -$^3$H. *a*, Portion of a somatotrophic cell showing the labeled nucleus. The largest cytoplasmic granules are approximately 350 m$\mu$ in diameter. *b*, Portions of two thyrotrophic basophil cells, one with a labeled nucleus. Granules are approximately 140 m$\mu$ in diameter. *c*, Portion of a labeled chromophobe cell bordered by an acidophil. Magnification, 17,500. Line represents 1 $\mu$.

these cell types and the labeling index are shown in Table I. The labeling index (1.3%) correlates well with that obtained by light microscopy (1.8%).

Chromophils may often appear as chromophobes at the light microscope level because differential staining depends upon an adequate number of granules in the cytoplasm. Furthermore, cells which are heavily granulated but which have little cytoplasm also may appear as chromophobes (14). These factors probably account for the finding that more labeled chromophils were identified with electron microscopy than with light microscopy (Table I).

It is well documented that pituitary glands in short-term cultures retain their functional activity and, with proper stimuli, are able to synthesize and release trophic hormone (15). The present results confirm that DNA synthesis also occurs in this system and demonstrate that cells of the rat anterior pituitary committed to the synthesis of specific protein hormones can also synthesize DNA.

Miss Mastro is a National Science Foundation predoctoral fellow. This study was supported in part by an Atomic Energy Commission Grant, AT-(30-1)-3827-8, and by a Public Health Service Research Career Development Award (No. 1-K04-AM 15808-01 AMK) from the Institute of Arthritis and Metabolic Diseases to Dr. Hymer.

We thank Mr. Charles Mock and Miss Rosalyn Hemmer for their fine technical assistance.

*Received for publication 3 June 1969.*

## REFERENCES

1. LEBLOND, C. P., and B. E. WALKER. 1956. Renewal of cell populations. *Physiol. Rev.* **36**:255.
2. FAND, S. B., L. MESSINEO, C. W. EHMANN, and A. J. BUSCAGLIA. 1967. *Proc. Soc. Exp. Biol. Med.* **125**:192.
3. MILLHOUSE, E., JR. 1961. *J. Histochem. Cytochem.* **9**:661.
4. SEVERINGHAUS, A. E. 1937. *Physiol. Rev.* **17**:556.
5. CRANE, W. A. J., and R. S LOOMES. 1967. *Brit. J. Cancer.* **21**:787.
6. DHOM, G., and E. STÖCKER. 1964. *Experientia.* **20**:384.
7. MASTRO, A., W. C. HYMER, and C. D. THERRIEN. 1969. *Exp. Cell Res.* **54**:407.
8. HYMER, W. C., and W. H. MCSHAN. 1963. *J. Cell Biol.* **67**:86.
9. MCSHAN, W. H. 1964. *Excerpta Med. Int. Congr. Ser.* **83**:382.
10. FARQUHAR, M G., and J. F. RINEHART. 1954. *Endocrinology.* **54**:516.
11. VENABLE, J., and R. COGGESHALL. 1965. *J. Cell Biol.* **24**:107.
12. CARO, L. G., and R. P. VAN TUBERGEN. 1962. *J. Cell Biol.* **15**:173.
13. HUMASON, G. L. 1967. Animal Tissue Technique. W. H. Freeman and Company, San Francisco.
14. PURVES, H. D. 1961. Morphology of the hypophysis related to its function. *In* Sex and Internal Secretions. W. C. Young and G. W. Corner, editors. The Williams & Wilkins Co., Baltimore. 1.
15. MCCANN, S. M., and J. C. PORTER. 1969. *Physiol. Rev.* **49**:240.