proliferation, but greatly augmented lobule-alveolar differentiation.

1. Kirkham, W. R., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 123.

2. Lajtha, L. G., Phil, D., Oliver, R., *Lab. Invest.*, 1959, v8, 214.

3. Leblond, C. P., Messier, B., Kopriwa, B., *ibid.*, 1959, v8, 296.

4. Amano, M., Messier, B., Leblond, C. P., *J. Histochem. Cytochem.*, 1959, v7, 153.

5. Cowie, A. T., Folley, S. J., Malpress, F. H., Richardson, K. C., *J. Endocrinol.*, 1952, v6, 64.

Received December 30, 1963. P.S.E.B.M., 1964, v115.

Immunofluorescent Localization of LH and FSH in the Human Adenohypophysis.* (29121)

DAVID KOFFLER AND MARVIN FOGEL (Introduced by Hans Popper)

Departments of Pathology and Obstetrics and Gynecology, Mount Sinai Hospital, New York City

Antisera to human chorionic gonadotropin (HCG) and human menopausal gonadotropin (HMG) have previously been prepared(1,2). Anti-HCG serum contains contaminating antibodies to FSH and anti-HMG serum contains contaminating antibodies to LH. Purified antisera of strong potency may be obtained by absorption with appropriate antigen resulting in immunoelectrophoretically specific antisera to HCG and HMG(3). Anti-HCG serum inhibits biological LH activity and anti-HMG serum inhibits biological FSH activity(2).

We attempted to utilize purified antisera to localize LH and FSH in cells of the human anterior pituitary gland by immunocytochemistry. The sites of production of growth hormone(4) and ACTH(5) have been clarified by similar techniques.

Materials and methods. Preparation of antisera. Rabbits were immunized intramuscularly with 10.0 mg of HCG† and 3.5 mg of HMG‡ 4 times at weekly intervals. Aliquots of these anti-sera were absorbed with lyophilized human serum proteins. Anti-HCG serum was absorbed with HMG and anti-HMG serum was absorbed with HCG. Microimmunoelectrophoresis revealed that the antisera reacted with homologous antigen only, after allowing the plates to develop for 5 days. Globulin fractions were precipitated and aliquots of each antiserum fluoresceinated. Chromatographically purified 7s rabbit gamma globulin was injected into goats 10 mg at weekly intervals for 4 weeks. The globulin fraction was precipitated and fluoresceinated.

Human tissues. Eight pituitary glands and sections of liver, kidney and thyroid were obtained within 24 hours of autopsy and frozen in a mixture of dry ice and isopentane at −70°C.

Fluorescent antibody technique. A Leitz ortholux fluorescence microscope with HB-200 light source, 2 BG-12 exciter filters and an OG-4 barrier filter were used for observation. Cryostat sections were made at 6 μ and serum was incubated with a section for 30 minutes at room temperature. Pituitary sections were stained by the following procedures: (1) Normal rabbit serum (NRS) + fluoresceinated goat anti-rabbit gamma globulin (FGARGG); (2) Purified anti-HCG (RALH) + FGARGG; (3) Purified anti-HMG (RAFSH) + FGARGG; (4) FGARGG; (5) Fluoresceinated RALH (FRALH); (6) Fluoresceinated RAFSH (FRAFSH); (7) RALH + FRALH; (8) RAFSH + FRAFSH; (9) RALH absorbed with HCG + FGARGG; (10) RAFSH absorbed with HMG + FGARGG. Sections of

* Supported by U.S.P.H.S. research grant and Mount Sinai Hospital Obstetrics and Gynecology Fund.

† APL—brand of human chorionic gonadotropin generously supplied by Ayerst Laboratories.

‡ Pergonal (PR2015)—brand of human menopausal gonadotropin generously supplied by Cutter Laboratories.

TABLE I. Fluorescent Antibody Staining of Cells in Anterior Pituitary Gland.

Case	Age and sex	Cause of death	Interval between time of death and quick freezing (hr)	Indirect fluorescent staining		Direct fluorescent staining	
				RALH and FGARGG	RAFSH and FGARGG	FRALH	FRAFSH
A	76 ♂	Aortic stenosis	24	+	+	0	0
B	71 ♂	Bronchogenic carcinoma	18	++	++	+	0
C	92 ♂	ASHD with complete heart block	8	+++	+	±	0
D	72 ♂	CVA	16	+++	++	±	+
E	63 ♂	Bronchogenic carcinoma	6	+++	++	+	+
F	65 ♀	Congestive heart failure with mitral insufficiency	6	+	+++	±	±
G	50 ♀	Acute myelomonoblastic leukemia	7	++	+++	±	+
H	67 ♂	Hodgkin's disease	10	+	++	±	+

Number of fluorescent cells: 0 = None; ± = Rare cell; + = Few cells; ++ = Moderate number of cells; +++ = Many cells.

liver, kidney and thyroid were stained by procedures No. 2, 3, 5 and 6. Serial cryostat sections were fixed in Bouin's fixative and stained with hematoxylin and eosin, and the aldehyde-fuchsin technique.

Results. Yellow auto-fluorescent granules within pituitary cells were easily differentiated from specific apple-green fluorescence, which was strongest when the indirect method was employed in fresh frozen pituitary glands (Table I). Staining with either purified anti-HCG or anti-FSH resulted in fluorescence localized within isolated cells, or clumps of cells, in each case studied (Fig. 1a, b). In contrast to granular auto-fluorescence, cytoplasmic fluorescence was homogeneous in appearance. Multiple sections from a given pituitary gland demonstrated differences in the number of cells stained and intensity of staining by anti-HCG as contrasted with anti-FSH, although a comparison of all pituitary glands studied revealed no consistent staining pattern characteristic for HCG or FSH. FSH fluorescence generally appeared to be of greater intensity in the 2 postmenopausal females studied. Serial sections treated by the aldehyde fuchsin technique exhibited a distribution of basophils similar to the fluorescent cells.

Control staining procedures No. 1 and No. 4 revealed no staining of pituitary sections, and absorption of antisera (No. 9 and No. 10) extinguished most of the specific fluorescence. Immunological blocking (No. 7 and No. 8) was achieved with non-fluoresceinated antisera in the direct staining procedure. Specific fluorescence was not encountered in liver, kidney or thyroid tissue.

Discussion. The feasibility of preparing immunoelectrophoretically specific antisera reacting with HCG and FSH has been confirmed. Although the presence of contaminating non-precipitating antibodies has not been completely eliminated it appears that the purified antisera have significant biological and immunocytochemical differences. Isersky *et al*(2) have shown that antisera to HMG neutralizes only the FSH activity of an HCG preparation as determined by augmentation assay of ovarian weight in mice. Antisera to FSH do not react with extracts of trophoblastic tissue containing HCG(3). Our results indicate differences in the staining patterns of anti-HCG and anti-FSH serum in a given pituitary gland.

No consistent pattern for LH or FSH localization in cells of the various pituitary glands was found, other than correlation with basophil-type cells as visualized by aldehyde-fuchsin staining. Histochemical characteriza-

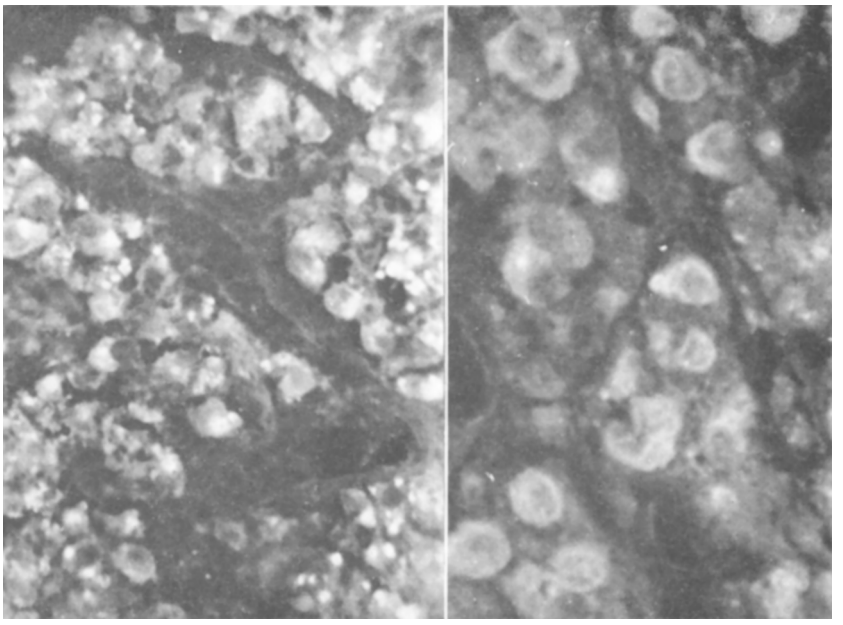

FIG. 1. Human anterior pituitary gland stained with: (a) (left) rabbit anti-FSH and fluoresceinated goat anti-rabbit gamma globulin. Note fluorescent cells arranged in clumps. ×250. (b) (right) rabbit anti-LH and fluoresceinated goat anti-rabbit gamma globulin. Note isolated fluorescent cells. ×400.

tion of the nature and distribution of the fluorescent cells is currently being attempted. Immunofluorescent localization of antisera to HCG in the anterior pituitary further substantiates the immunologic similarity of LH and HCG.

Conclusions. Purified antisera to HCG and HMG, specific for LH and FSH, have been demonstrated to localize in cells of the human adenohypophysis by the fluorescent antibody technique.

We are gratefully indebted to Dr. Hans Popper and Dr. Saul B. Gusberg for helpful advice and criticism, and to Patricia Saigo and Joan Horwitz for excellent technical assistance.

1. Lunenfeld, B., Isersky, C., Shelenyak, M., *J. Clin. Endocrin. Metab.* 1962, v22, 555.

2. Isersky, C., Lumenfeld, B., Shelenyak, M., *ibid.*, 1963, v23, 54.

3. Goss, D., Lewis, J., *ibid.*, 1963, v23, 986.

4. Leznoff, A., Fishman, V., Goodfriend, L., McGarry, E. E., Beck, V. C., Rose, B., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 232.

5. Leznoff, A., Fishman, V., Talbot, M., McGarry, E. E., Beck, V. C,. Rose, B., *J. Clin. Invest.*, 1962, v41, 1720.

Received December 3, 1963. P.S.E.B.M., 1964, v115.