7. Cowie, A. T., Tindal, J. S., Benson, G. K., *J. Endocrinol.*, 1960, v21, 115.

8. Haun, C. K., Sawyer, C. H., *Endocrinology*, 1960, v67, 270.

9. McCann, S. M., Friedman, H. M., *ibid.*, 1960, v67, 597.

10. Pasteels, J. L., *Compt. rend. Acad. Sci.*, 1961, v253, 3074.

11. ———, *ibid.*, 1962, v254, 2664.

12. Talwalker, P. K., Ratner, A., Meites, J., *Am. J. Physiol.*, 1963, v205, 213.

13. Gala, R. R., Reece, R. P., PROC. SOC EXP. BIOL. AND MED., 1963, v114, 422.

14. ———, *ibid.*, 1964, v115, 232.

15. Dixon, W. J., Massey, F. J., Jr., *Introduction to Statistical Analysis*, 2nd Ed., McGraw-Hill Book Co., New York, 1958.

Received June 29, 1964. P.S.E.B.M., 1964, v117.

Cytological Detection of Parathyroid Hormone by Immunofluorescence. (29712)

GARY K. HARGIS, VINCENT J. YAKULIS, GERALD A. WILLIAMS, ARTHUR A. WHITE
(Introduced by Paul Heller)

Research Laboratory and Medical Service, Veterans Administration West Side Hospital, and Department of Medicine, University of Illinois College of Medicine, Chicago

This laboratory(1) previously reported the production of a specific antiserum to bovine parathyroid hormone (PTH). This antiserum was used for immunochemical detection of PTH in crude and purified extracts of bovine parathyroid glands (PTG). The limited quantities of human and rat parathyroid tissue available for extraction prevented satisfactory comparative immunochemical study of the PTH of these species.

The fluorescent antibody technic(2) allows the immunochemical study of cellular substances in tissue section and therefore requires only a small quantity of tissue. This technic has been used by others(3-8) to detect and localize several of the polypeptide hormones in cells of endocrine glands.

The present study, utilizing the fluorescent antibody technic, was undertaken to determine 1) the feasibility of this technic for detecting PTH in tissue, 2) the species-specificity of PTH, 3) the cellular site of PTH synthesis and/or storage in the PTG.

Materials and methods. Rabbit anti-bovine parathyroid hormone serum(1) was conjugated with fluorescein isothiocyanate by the method of Rinderknecht(9). The gamma globulin fraction, containing the fluorescent antibody, was isolated with DEAE-cellulose by the method of Baumstark(10). This fluorescent anti-PTH globulin was absorbed with bovine serum and thyroid tissue as previously described(1).

Specimens of bovine and rat parathyroid, thyroid and lymph node were obtained immediately after death. Similar tissue specimens were obtained from 2 human subjects (55- and 60-year-old males with no evidence of abnormal parathyroid function) at autopsy 4 hours after death. Each specimen was quick-frozen in absolute alcohol precooled in an acetone-dry ice bath. The specimens were subsequently embedded in paraffin, sectioned at 3-5 μ thickness, prepared, stained and mounted by the technic of Saint-Marie(11), with slight modifications. The tissue sections were reacted with fluorescent anti-PTH globulin in a moist chamber on a rotating table at room temperature for 4 hours. In control studies, the sections were allowed to react with a) non-conjugated anti-PTH serum prior to application of the fluorescent anti-PTH globulin, or b) fluorescent anti-PTH globulin which had been previously absorbed with bovine PTH. In initial studies the fluorescent anti-PTH globulin was absorbed with activated charcoal(12) or liver homogenate(13). However, this absorption procedure did not alter the staining characteristics with any of the tissues, and was therefore discontinued.

The tissue sections stained with fluorescent

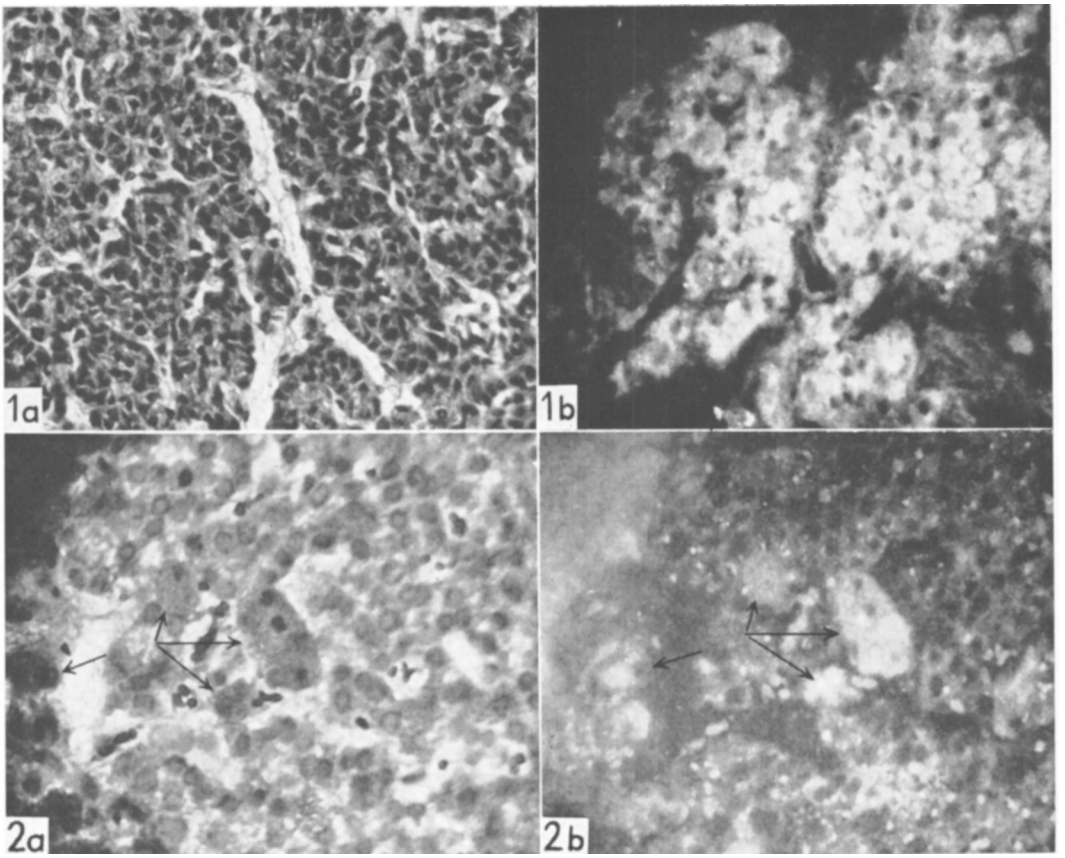

FIG. 1. a. Bovine PTG, H & E stain, showing single cell type. × 216. b. Bovine PTG, fluorescent anti-PTH globulin stain, showing specific fluorescence confined to cytoplasm of parathyroid cells. × 258.

FIG. 2. a. Human PTG, H & E stain, showing predominant chief cells, and occasional oxyphil cells (indicated by arrows). × 360. b. Human PTG, fluorescent anti-PTH globulin stain, same microscopic field as Fig. 2a. Section portrayed specific fluorescence of chief cell cytoplasm, and autofluorescence of several unstained cells (indicated by arrows). These unstained cells are identical with the oxyphil cells of Fig. 2a. The small bright spots are artifacts. × 360.

antibody were examined and photographed with 90-120 seconds exposure, using a Zeiss fluorescence microscope unit with OSRAM HBO-200 light source, BG12 exciter filter and OG47 barrier filter. Anscochrome Daylight film was used and developed by forced processing to achieve ASA 200. Some of the human PTG sections were studied as follows: The section was initially stained with fluorescent anti-PTH globulin, examined microscopically and photographed. The cover slip and mounting material were then removed with 95% alcohol, the section stained with hematoxylin and eosin (H&E), and the previously-photographed areas were identified and re-photographed using the same microscope converted to light microscopy conditions. Comparison of the photographs allowed identification and examination of the same cells, stained first with fluorescent antibody and then with H&E.

Results. Though Levine(14) reported the presence of both chief and oxyphil cells in bovine PTG, all bovine parathyroid tissue observed in this study portrayed only a single cell type, characteristic of the chief cell (Fig. 1a). Specific fluorescence was present in the cytoplasm of all discernible parathyroid cells, with minimal variation in the intensity of fluorescence among the cells (Fig. 1b). The nuclei of these cells and all non-parathyroid tissue were non-fluorescent.

The characteristics of fluorescent antibody staining of rat parathyroid tissue were the same as those of bovine parathyroid tissue, except that the fluorescence was less intense.

The human parathyroid tissue consisted predominantly of chief cells. However, approximately 1% of the parathyroid cells (irregularly distributed singly and in small clumps throughout the tissue) had characteristics of oxyphil or transitional oxyphil cells: They were approximately 1½ times the size of the chief cells; had a more distinct cellular outline, a denser nucleus, and cytoplasm with rather dense eosinophilic granulation (Fig. 2a). When fluorescent anti-PTH globulin was applied to human parathyroid tissue, specific fluorescence was confined to the cytoplasm of the parathyroid cells, this fluorescence being less intense than that observed with bovine parathyroid tissue. Approximately 1% of the parathyroid cells failed to show the characteristic specific green fluorescence, but instead showed the bright blue autofluorescence characteristic of unstained cells (Fig. 2b). Examination of those tissue sections in which the same microscopic fields were photographed, first after fluorescent antibody staining and then after H&E staining, revealed that these unstained cells were identical with the eosinophilic cells described above (Figs. 2a and b).

Absorbing the fluorescent anti-PTH globulin with bovine PTH before applying it to bovine parathyroid tissue sections resulted in absence of specific fluorescence. Reacting the tissue sections with non-conjugated anti-PTH serum before applying fluorescent anti-PTH globulin also resulted in absence of specific fluorescence. The same results were observed when these tests of specificity were carried out with tissue sections of human and rat PTG. Specific fluorescence was not observed when fluorescent anti-PTH globulin was applied to lymph node or thyroid tissue of any of the 3 species studied.

Discussion. These studies indicate that anti-PTH globulin reacts, not only with extracted preparations of bovine PTH(1,15, 16), but also with endogenous PTH located in parathyroid tissue. The specificity of this reaction is demonstrated by a) the localization of fluorescent staining to the cytoplasm of parathyroid cells, b) inhibition of the fluorescent staining by prior reaction of the tissue with non-conjugated anti-PTH serum, c) failure of fluorescent anti-PTH globulin to produce specific staining after it had been incubated with bovine PTH.

These experiments indicate that PTH is produced and/or stored in the cytoplasm of the parenchymal cells of the PTG. In the bovine PTG, which was composed of only chief cells, the fluorescent staining was present in all discernible cells, suggesting that all cells were functioning simultaneously. The observed variation in intensity of the fluorescence among the cells suggests variation in degree of PTH synthesis and/or storage, though further attempts at quantification are needed before this can be stated with assurance. Electron microscopic study of the PTG of other species has revealed variation in the number of secretory granules in the chief cells, leading to the conclusion of variation in cellular secretory activity(17).

Fluorescent staining which resulted from reacting human parathyroid tissue with fluorescent anti-bovine PTH globulin indicates that there is immunochemical cross-reactivity between the PTH of these two species. The somewhat less intense fluorescence with human parathyroid tissue suggests that the hormones are not, however, antigenically identical. Tashjian *et al*(15) and Berson *et al*(16) have reported significant cross-reactivity between bovine and human PTH by different immunochemical technics. The present study suggests that there is immunochemical cross-reactivity, but not identity, between bovine and rat PTH also.

Human parathyroid tissue predominates in chief cells, but also contains a variable number of oxyphil cells and transitional forms, and may have water-clear cells(18). Electron microscopic(17) and histochemical(19) studies indicate that the oxyphil cells contain many mitochondria and have high levels of several enzymes. However, Munger and Roth (17) have concluded that, because of the lack of secretory granules, the oxyphil cells do not secrete significant amounts of PTH, at least under normal conditions. The pres-

ent study demonstrated an absence of PTH in the oxyphil cells, as manifested by their absence of specific immunochemical staining. This study therefore presents further evidence that the oxyphil cells do not synthesize or store PTH.

Summary. The fluorescent antibody technic has been utilized to detect and to localize parathyroid hormone in the chief cells of bovine, human and rat parathyroid tissue. Immunochemical cross-reaction has been found among these species. The oxyphil cells of human parathyroid tissue did not reveal specific fluorescence. This observation is regarded as further evidence that the oxyphil cells do not normally synthesize or store parathyroid hormone.

The authors are indebted to Mr. Clarence Sidwell and Mr. Lemuel Hall of the Research Laboratory for technical assistance; to Mrs. Patricia Boulware and Dr. K. Hsing Wu of the Pathology Service for assistance in tissue preparation and for histologic consultation; and to the Medical Illustration Service for preparing the photographs.

1. Williams, G. A., Hargis, G. K., Sidwell C. G., Yakulis, V. J., PROC. SOC. EXP. BIOL. AND MED., 1964, v115, 61.

2. Coons, A. H., Kaplan, M. H., *J. Exp. Med.*, 1950, v91, 1.

3. Lacy, P. E., Davies, J., *Diabetes*, 1957, v6, 354.

4. Baum, J., Simons, B. E., Unger, R. H., *ibid.*, 1962, v11, 371.

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

6. Leznoff, A., Fishman, J., Talbot, M., McGarry, E. E., Beck, J. C., Rose, B., *J. Clin. Invest.*, 1962, v41, 1920.

7. Emmart, E. W., Spicer, S. S., Bates, R. W., *J. Histochem. Cytochem.*, 1963, v11, 365.

8. Koffler, D., Fogel, M., PROC. SOC. EXP. BIOL. AND MED., 1964, v115, 1080.

9. Rinderknecht, H., *Nature*, 1962, v193, 167.

10. Baumstark, J. S., *Fed. Proc.*, 1964, v23, 547.

11. Sainte-Marie, G., *J. Histochem. Cytochem.*, 1962, v10, 250.

12. Chadwick, C. S., Nairn, R. C., McEntegart, M. G., *Biochem. J.*, 1959, v73, 41P.

13. Nairn, R. C., in *Fluorescent Protein Tracing*, R. C. Nairn, ed., E. & S. Livingstone Ltd., Edinburgh and London, 1962, 118.

14. Levine, M., *Anat. Rec.*, 1928, v39, 293.

15. Tashjian, A. H., Jr., Levine, L., Munson, P. L., *J. Exp. Med.*, 1964, v119, 467.

16. Berson, S. A., Yalow, R. S., Aurbach, G. D., Potts, J. T., Jr., *Proc. Nat. Acad. Sci.*, 1963, v49, 613.

17. Munger, B. L., Roth, S. I., *J. Cell Biol.*, 1963, v36, 379.

18. Roth, S. I., *Arch. Path.*, 1962, v73, 495.

19. Tremblay, G., Cartier, G. E., *Endocrinology*, 1961, v69, 658.

Received July 7, 1964. P.S.E.B.M., 1964, v117.

Erythropoietic Effect of Testosterone in the Polycythemic Mouse. (29713)

W. FRIED, R. DE GOWIN AND C. W. GURNEY (Introduced by L. O. Jacobson)
Department of Medicine, University of Chicago, and Argonne Cancer Research Hospital, Chicago, Ill.*

Recent clinical application of testosterone to the treatment of various refractory anemias(1-3), has led us to investigate the mechanism by which testosterone effects erythropoiesis. Several investigators have demonstrated increased red blood cell counts in rats after long-term treatment with testosterone. These effects were more striking in animals with hormone levels previously altered by castration or hypophysectomy(4-6), and were at best, very slight in normal animals(7). Most early studies failed to demonstrate significant increases in hemoglobin levels or in total red cell volumes(4,5,8).

Because of the marked decrease in erythropoiesis observed in the polycythemic mouse, this animal has proved valuable in studying the mechanism of erythropoietic stimulants (9,10). In this report, we shall describe the

* Operated by Univ. of Chicago for U. S. Atomic Energy Commission.