tion confirms the histological observation of Kuramitsu and Loeb(10) on guinea pig mammary gland. These workers reported numerous mitoses 6 to 12 hours after labor and established lactation 2 days after labor. In contrast, they observed rare mitoses 6-12 hours after labor in the rat with "active mitotic proliferation" 2 days after labor.

Summary. The growth of guinea pig mammary glands during pregnancy, parturition, lactation and involution was measured using total weight, total nitrogen content, total DNA and RNA/DNA ratios as parameters of cellular changes. Only slight changes were found to occur during pregnancy. The rapid increase in total weight after parturition, correlated with the increase in total nitrogen and DNA, indicates that the observed growth is primarily a hyperplastic process. Extensive hypertrophy of pre-existing cells as measured by increased RNA/DNA ratios occurs after the period of rapid proliferative growth, thus the greatest cellular changes in guinea pig mammary glands occur immediately after parturition.

1. Kirkham, W. R., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1954, v83, 123.
2. Shimizu, Hirokazu, *Tohoku J. Agr. Res.*, 1957, v7, 339.
3. Kuretani, Kazuo, *Kagaku Kenkyusho Hôkoku*, 1957, v33, 80.
4. Greenbaum, A. L., Slater, T. F., *Biochem. J.*, 1957, v66, 155.
5. Smith, T. C., Richterich, Britta, *Arch. Biochem. Biophys.*, 1958, v74, 398.
6. Hogeboom, G. H., Schneider, W. C., Palade, G. E., *J. Biol. Chem.*, 1948, v172, 619.
7. Conway, E. J., *Microdiffusion Analysis and Volumetric Error*, Van Nostrand, New York, 1950, 3rd Ed.
8. Ceriotti, G., *J. Biol. Chem.*, 1955, v214, 59.
9. Nelson, W. L., Kaye, A., Moore, M., Williams, H. H., Herrington, B. L., *J. Nutr.*, 1951, v44, 585.
10. Kuramitsu, C., Loeb, L., *Am. J. Physiol.*, 1921, v56, 40.

Received November 6, 1961. P.S.E.B.M., 1962, v109.

Investigations on Somatotropin Production of Human Anterior Pituitary Cells in Tissue Culture. (27208)

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The demonstration of species specific biological activity of growth hormones necessitates new sources for human growth hormone since only human and simian preparations are active in man(1,2). A logical approach to this problem is the study of *in vitro* cultivation of human or simian anterior pituitary cells.

The present communication describes isolation of anterior pituitary cell lines from human glands and investigations of somatotropin production using a highly sensitive and specific fluorescent antibody method.

Materials and methods. Isolation of cell lines. The cell lines used in these studies originated from human pituitary glands obtained at autopsy* which were designated Borg-11, Borg-12 (both from 7-month-old male fetuses) and Cook-3 (from 60-year-old male). Surrounding membranes and the posterior lobe of intact glands were removed aseptically. The anterior lobe was washed in sterile culture medium and minced with sterile scalpels. Tissue fragments were then treated for 15 minutes at 37°C in 0.01% collagenase solution(3). Single cells as well as the remaining tissue fragments were recovered by centrifugation and transferred into 100 × 20 mm plastic tissue culture petri dishes† containing 15 ml of culture medium. Prior to inoculation the bottom of each dish was lined with 10-13 sterile Corning 18 mm

* Courtesy of Drs. P. S. Rutherford and F. H. Cox, Borgess Hospital, Kalamazoo, Mich. and Dr. P. B. Santoz, Cook County Hospital, Chicago, Ill.
† Falcon Plastics Co., Los Angeles, Calif.

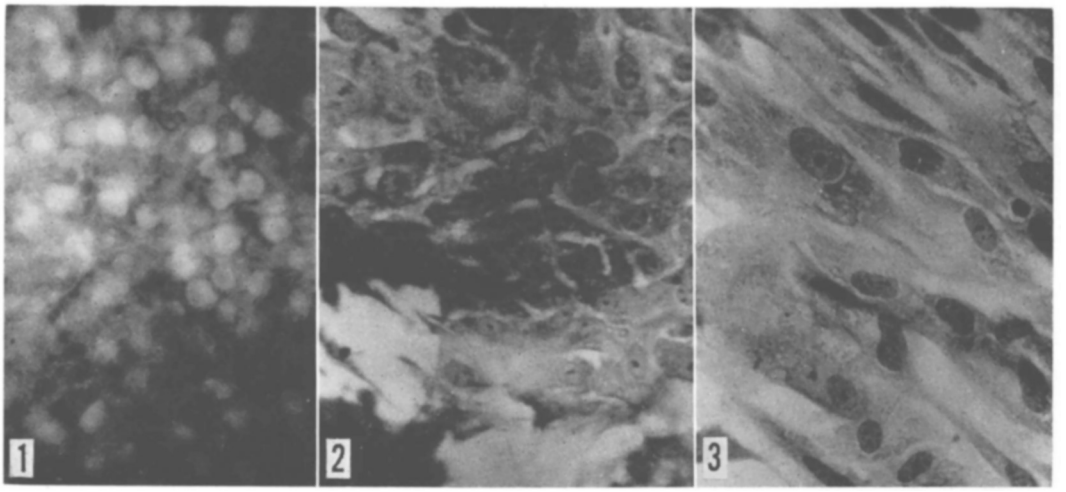

FIG. 1. Section of pituitary gland of 3-mo-old infant treated with fluorescent antibody specific for human growth hormone. 215×.

FIG. 2. Human pituitary cells grown in tissue culture. Original explant present at lower left. Stain: Azure-Eosin. 215×.

FIG. 3. Human pituitary cells grown in tissue culture. Note unidirectional growth pattern. Stain: Azure-Eosin. 215×.

cover glasses. The dishes were incubated at 37°C in an atmosphere of 8% CO_2—92% air. After primary explants had developed outgrowth, culture medium was replaced every 2 days. *Culture medium.* The culture medium used throughout these experiments contained: Eagle's basal medium,‡ 400 ml; calf serum, 10%; glutamine, 2 μM ml; Bactopeptone,§ 2 mg/ml; insulin, 4.5 μg/ml; penicillin, 250 units/ml; streptomycin, 250 μg/ml. *Preparation of fluorescent antibody.* Crystalline human growth hormone was obtained from Dr. C. H. Li. Antisera and γ-globulin preparations containing specific antibodies for human growth hormone were prepared and tested for specificity as described by Li *et al.*‖(4). Batches of γ-globulin were coupled with fluorescein isothiocyanate as described by Marshall *et al.*(5). The conjugates were absorbed with mouse liver powder as recommended by Coons *et al.*(6) to remove "non-specific staining." The fluorescent conjugate was tested for specificity on tissue sections from intact pituitary glands and was found to be highly specific (Fig. 1). *Staining.* After satisfactory outgrowth was observed from the explants on cover glasses (4 to 6 weeks after planting) the glasses were removed from the culture medium and rinsed in buffered saline. For fluorescent antibody treatment the cover glasses were dipped successively for 30 sec in 100% and 95% ethanol and again in buffered saline for 2 minutes. Further treatment with fluorescent antibody conjugate was performed as described by Vazquez and Dixon(7). Control preparations were first treated with non-fluorescent γ-globulin followed by fluorescent antibody conjugate(7). The cover glasses were mounted on microscopic slides with a drop of glycerol-jelly and stored in the refrigerator. Preparations for morphological studies were stained by Lillies' Azure-Eosin and May-Grunwald-Giemsa. *Fluorescence microscopy.* Slides were examined under a Leitz fluorescence microscope with the blue light BG-12 filter, having a maximum transmittance at 410 mμ and the corresponding barrier filter OG-1. *Ouchterlony technic.* The supernatant medium at the time of harvest was tested for presence of human growth hormone by a modified Ouchterlony technic as described by Li *et al.*(4).

Results. Microscopic examination revealed that the cells growing out from the explants of all 3 cell lines under investigation

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§ Difco Laboratories, Detroit, Mich.

‖ Courtesy of Drs. C. W. DeWitt and S. Eppstein, Upjohn Company, Kalamazoo, Mich.

were fibroblastic. Each nucleus contained several nucleoli. The surrounding cytoplasm contained granules and cytoplasmic pseudopods could be observed. Some of the cells retained eosinophilic or basophilic characteristics. Cells in the immediate neighborhood of the original explant on the cover glasses usually did not show any preferential direction of growth while cells located farther from the original explants showed a well-defined unidirectional growth patern (Fig. 2,3).

Preparations treated with fluorescent antibody conjugate specific for growth hormone did not show any specific fluorescence when observed under the fluorescence microscope and even the residual tissue material from the original explant had lost the specific fluorescence of growth hormone. Cytoplasm of the outgrowing cells showed a very weak greenish autofluorescence but the nuclei remained completely dark. Frequently areas with green fluorescence, characteristic of growth hormone could be observed. This fluorescence was not present on control slides treated with non-labelled antibody prior to application of fluorescent antibody and was therefore material absorbed with unlabelled antibody. Upon inspection in visible light, these areas did not appear to be intact cells and are therefore presumed to be artifacts. No evidence for synthesis of growth hormone in the growing cells was obtained.

Immunochemical tests of the supernatant culture medium at the time of cell harvest by a modified Ouchterlony technic did not show any positive reaction for human growth hormone.

Discussion. The experiments indicate that growing cells from 3 human pituitary glands differ markedly from cells in the intact gland tissue. Some cells did retain their eosinophilic and basophilic characteristics and contained cytoplasmic granules. All cells showed fibroblastic growth when attached to a glass surface and contained well-defined nuclei of varying size. Human growth hormone could not be demonstrated within the cytoplasm of the cells by use of the highly sensitive fluorescent antibody technic nor was any detectable hormone activity secreted into the culture medium as evidenced by an immunochemical assay of the Ouchterlony type. This is in contrast to the findings of earlier investigators (8) indicating that human pituitary cell lines cultured *in vitro* might produce growth hormone. However, their results were based on the conventional test of measuring width of tibial epiphysis in the hypophysectomized rat. Studies reported herein with more sensitive and specific immunochemical methods indicate that freshly isolated human pituitary cells grown in tissue culture do not retain their functional properties for growth hormone production under the described culture conditions.

The whole pituitary glands used to prepare sections for specificity tests with the labeled antibody conjugates originated from 5-day and 3-month-old infants and 58, 60 and 75-year-old adults. Localization of growth hormone in pituitary sections from adults proved somewhat difficult due to excessive fluorescence of portions of the stroma surrounding the cell groups. The observed fluorescence was in color brightness and shade almost identical with fluorescence from the antibody-antigen reaction. This might lead to the conclusion that the localization of growth hormone in pituitaries from adults is not exclusively cytoplasmic but also in the stroma. However, this fluorescence was also present on control preparations and was therefore material not absorbed by unlabelled γ-globulin. Autofluorescence of the stromal elements, particularly elastic fibers may account for the intensity of this non-specific fluorescence. A marked relative abundance of growth hormone containing cells in infant anterior pituitary sections was observed as compared to adult pituitaries. This would indicate that infant pituitaries contain and probably secrete more growth hormone than those of adults.

Summary. *In vitro* cell lines freshly isolated from human anterior pituitary glands showed fibroblastic growth. Some cells did retain eosinophilic and basophilic characteristics. No human growth hormone was detected within the cells by use of fluorescein labelled antibody specific for human growth hormone. Also, no growth hormone was

present in the supernatant medium. These findings suggest that human pituitary cells grown in tissue culture do not retain their functional properties for growth hormone production under the described culture conditions.

The authors gratefully acknowledge the helpful consultations with Dr. C. H. Li, Univ. of California, Berkeley, and his generous gift of human growth hormone.

1. Knobil, E., Greep, R. O., *Rec. Prog. Horm. Res.*, 1959, v15, 1.

2. Li, C. H., *Postgraduate Med.*, 1961, v29, 13.

3. Hinz, R. W., Syverton, J. T., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 19.

4. Li, C. H., Moudgal, N. R., Papkoff, H., *J. Biol. Chem.*, 1960, v235, 1038.

5. Marshall, J. D., Eveland, W. C., Smith, C. W., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 898.

6. Coons, A. H., Leduc, E. G., Connolly, J. M., *J. Exp. Med.*, 1955, v102, 49.

7. Vazquez, J. J., Dixon, F. J., *ibid.*, 1956, v104, 727.

8. Thompson, J. W., Vincent, M. N., Jensen, F. C., Price, R. T., Schapiro, E., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 403.

Received November 8, 1961. P.S.E.B.M., 1962, v109.

Acid Phosphatase and β-Glucuronidase Activities of Thymus and Spleen of Rats after Whole-Body X-Irradiation.* (27209)

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On the basis of differential centrifugation, de Duve and co-workers(1,2) proposed the name "lysosome" for a special group of intracellular particles which show a sedimentation distribution between the mitochondrial and the microsomal fractions; this group contains a number of easily soluble hydrolases (*i.e.*, acid phosphatase, β-glucuronidase, acid ribonuclease, deoxyribonuclease II, cathepsin, etc.) having in common an acid pH optimum (3).

To study the effect of x-irradiation on this particular group of particles, we have taken the thymus and the spleen of rats as our study object, since lymphoid tissue is far more radiosensitive than other tissues in which lysosomes have been studied. Among the lysosomal group of enzymes, so far we have tested only acid phosphatase and β-glucuronidase.

Methods. X-irradiation procedure. Two sets of experiments were carried out with female Holtzmann rats. A total of 32 animals, 45 to 50 days old and weighing between 130 and 150 g, were studied for enzyme activities in Experiment 1; 24 of them were given a dose of 200 r, and the remaining 8 were used as controls. In Experiment 2, 48 animals were studied; 36 of them were given a single dose of 1000 r, and the remaining 12 were used as controls. The average dose rate was 50 r/min in Exp. 1, and 190 r/min in Exp. 2, delivered by a 250 kv machine with 0.5 mm Cu and 3.0 mm bakelite filters; the target distance was 29 cm, and the rats were rotated during exposure.

Since we found that the control values (especially for acid phosphatase) varied between different shipments of rats, we have used a single shipment of rats for each set of experiments. The acid phosphatase and β-glucuronidase specific activities were found to be constant among individual rats in the same shipment. For the thymus (rats from 42 to 130 days of age), values for acid phosphatase range from 0.26 to 0.28 μg P/mg N/min and from 5.8 to 6.3 μM phenolphthalein/g N/min for β-glucuronidase. As for the spleen (rats from 45 to 70 days of age), values range from 0.39 to 0.42 μg P/mg N/min and 1.52 to 1.70 μM phenolphthalein/g N/min respectively.

Tissue preparations and enzymes assays. The animals were killed by decapitation, and thymus and spleen were removed and chilled at once in 0.25 M sucrose at 0°C; the tissue was then cut into a few small pieces and ho-

* This work was performed under the auspices of U. S. Atomic Energy Commission.