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Immunofluorescent Localization of Parathyroid Hormone in Extracellular Spaces of the Bovine Parathyroid Gland* (32982)

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Various investigators have described intercellular spaces in the parathyroid glands of several species. A significant role for these spaces in the physiology of the gland has not previously been shown. Allara (1), using light microscopy, described spaces between parenchymal cells in human parathyroid glands. He pointed out that these spaces were surrounded by two thin membranes. Ham (2) noted that clumps of chief cells may form follicles in the parathyroid glands of older people and suggested that these follicles might represent locations for the storage of secretions. Trier (3), using light microscopy, demonstrated the presence of intercellular spaces in the parathyroid glands of the macaque monkey. He found that the membranes surrounding the spaces stained intensely with periodic acid Schiff, and that material within the spaces stained only faintly. In electron microscopic studies, Lever (4) identified subendothelial and parenchymal spaces and described the presence of an amorphous material within these membrane limited spaces. Munger and Roth (5) have also described connective tissue spaces in the parathyroid

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glands of Virginia deer. They proposed, on the basis of electron microscopy that a membrane-limited, electron-dense "secretory granule" is the stored form of the hormone in the cells. Hargis and Williams, attempting to visualize the hormone in the gland, found diffuse immunofluorescent staining in all the cells of the bovine parathyroid gland (6). However, the antisera used were prepared against an impure preparation of parathyroid hormone. The heterogeneity of the antigen raised doubts about the specificity of the localization of the hormone, and prompted us to reinvestigate the localization of parathyroid hormone in the bovine gland using the fluorescent antibody method.

Methods. Bovine parathyroid hormone was extracted and partially purified by the method of Aurbach and Potts (7). The final product of the purification scheme, although of high biological activity ($3800 \overset{\times}{\div} 1.25$ USP U/mg)[3], was not homogeneous when examined electrophoretically. Further purification was made using vertical starch gel electrophoresis (8). Following electrophoretic separation, a single, biologically active, protein band was eluted from the gel, and was used as the immunizing antigen in rabbits. The elution and immunization procedures have been described in detail by Tashjian *et al.* (8).

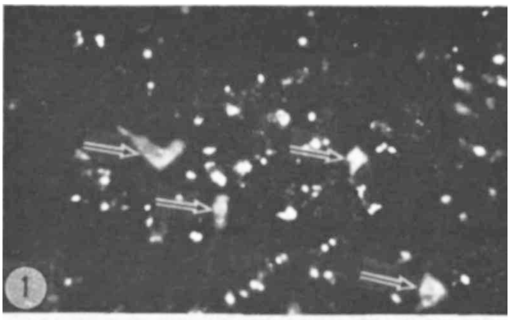
The antiserum used in these experiments was taken as a single bleeding from one rabbit. It was shown to contain antibodies to parathyroid hormone in neutralization experiments. The biological activity of parathyroid hormone was lost after incubation with this immune serum but not after incubation with normal rabbit serum (8). It was also shown previously that the biological activity could be recovered from the dissociated immune precipitate (9), ruling out nonspecific destruction of the hormone as the cause for the loss of activity. The particular methods used for isolating and preparing the immunizing antigen were chosen to increase the likelihood of obtaining immunochemically homogeneous antiserum. The antiserum was examined for heterogeneity (antibodies to nonparathyroid hormone contaminating substances) by several methods (10). No evidence of heterogeneity was detected. Four-times concentrated antiserum gave only one band of coincidence by double diffusion with the immunizing antigen, partially purified parathyroid hormone, and crude extracts of bovine parathyroid glands. Only a single smooth complement fixation curve was seen with the antiserum even in far antigen excess with crude extracts. If a contaminating antigen–antibody system had been present, a second complement-fixation curve would probably have been seen (11).

This antiserum was used as the first stage in the indirect fluorescent antibody method (12). Fluorescein-labeled sheep antirabbit globulin (Baltimore Biological Laboratories) was used to visualize the rabbit antibody. Rabbit antiserum against parathyroid hormone was absorbed with bovine thyroid powder. Fluorescein-labeled antirabbit globulin was treated with acetone powder of bovine parathyroid tissue (13). Both antisera were then absorbed with mouse liver powder.

Parathyroid glands, obtained from normal healthy steers, a bull, and nonlactating, nonpregnant cows, at slaughter, were immediately frozen in a dry ice–ethanol bath. Sections (6 μ) were cut on a cryostat, fixed for 15 min in 95% ethanol at 37°C, incubated with rabbit antiparathyroid hormone serum, and subsequently incubated with fluorescein-labeled antirabbit globulin as described by Coons (12), except that the incubation times were 3 hours with rabbit antiserum and 30 min with antirabbit globulin. In control experiments, sections were incubated with (i) saline alone; (ii) normal rabbit serum instead of antiparathyroid hormone serum; (iii) fluorescein-labeled antirabbit globulin applied directly to the sections without prior treatment with rabbit antiparathyroid hormone serum; and (iv) rabbit antiparathyroid hormone serum which had been previously incubated with parathyroid extract (Lilly, 100 U/ml) for 1 hour at 37°C, before incubation with the fluorescein-labeled antiserum.

As an additional control for the specificity of the staining, sections of bovine thyroid and

[3] The factor following the symbol, $\overset{\times}{\div}$, when multiplied by or divided into the potency estimate, gives the limits of the standard error.



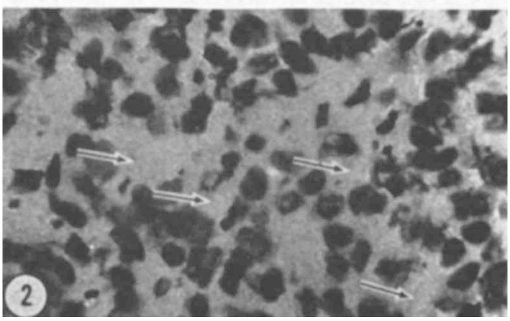


FIG. 1. Section of bovine parathyroid gland (6 μ) incubated with rabbit antiparathyroid hormone serum and fluorescein-labeled sheep antirabbit globulin showing pools of specific yellow-green fluorescent staining for parathyroid hormone (arrows) and smaller punctate areas of orange autofluorescence. Orig. mag. 400×.

FIG. 2. Identical area as shown in Fig. 1, stained with toluidine blue. Intercellular spaces which correspond exactly to areas of yellow-green fluorescence (Fig. 1) are shown by the arrows. Darkly stained areas are nuclei of parathyroid cells. Orig. mag. 400×.

pancreas were treated with antiparathyroid hormone serum and fluorescein-labeled antiserum under the same conditions used for sections of parathyroid glands.

Sections were examined with a Sylvania light source and the dark field condenser, and an OG 53 barrier filter. Sections, which had originally been stained with antiparathyroid hormone serum and fluorescein-labeled antirabbit globulin, after having been examined and photographed under ultraviolet illumination, were counterstained with toluidine blue directly on the microscope stage. The original fluorescent area was rephotographed under brightfield illumination without movement of the section. By this method, it was possible to compare and identify the areas of fluorescent staining, and the corresponding structures revealed by toluidine blue. Sections were photographed using High Speed Ektachrome (ASA 160) daylight film.

Results. Sections of bovine parathyroid gland incubated with rabbit antiparathyroid hormone serum and fluorescein-labeled antirabbit globulin showed two distinct types of fluorescence: (a) large, irregularly shaped pools of bright *yellow-green* fluorescence in numerous areas throughout the sections, and (b) small, punctate foci of bright *orange* fluorescence (Fig. 1). The orange fluorescence was also present to the same degree in control sections which had been incubated only in saline. Orange autofluorescence was first described by Hamperl (14) and is probably due to lipid material in the gland.

Bright yellow-green fluorescence was not observed when sections of parathyroid gland were incubated with normal rabbit serum instead of rabbit antiparathyroid hormone serum in the indirect technique, or with fluorescein-labeled antirabbit globulin applied directly to the sections without prior treatment with antiparathyroid hormone serum. Treatment of sections with antiparathyroid serum which had been incubated with parathyroid extract prevented the typical yellow-green fluorescent staining ordinarily obtained by incubating with unabsorbed antiparathyroid hormone serum. Nonspecific fluorescent staining of all bovine tissues was ruled out by the experiments with thyroid and pancreas. No yellow-green fluorescence was seen when sections of bovine thyroid or pancreas were treated with antiparathyroid hormone serum and fluorescein-labeled antiserum in the indirect technique. These control experiments lead us to conclude that the yellow-green fluorescent staining was specific for parathyroid hormone, and that the fluorescent areas represent local concentrations of hormone in the parathyroid gland.

In each section examined the areas of bright yellow-green fluorescence (Fig. 1) corresponded to *inter*cellular spaces between cords and within acini made up primarily of chief cells (Fig. 2). These spaces, often stellate in configuration, appeared to be surrounded by a fibrillar membrane. Yellow-green fluorescence was not seen within any of the cells of the gland.

Discussion. Local concentrations of parathyroid hormone have been visualized for the first time in intercellular spaces lying between groups of chief cells. The validity of this finding rests on the specificity of the antiparathyroid hormone serum used in the fluorescent antibody studies. No evidence for immunochemical heterogeneity was detected by three independent criteria: double diffusion, complement fixation, and immunofluorescence (using bovine tissues other than parathyroid glands). Furthermore, the specificity of the immunofluorescent studies was shown by the lack of staining when (i) normal rabbit serum was employed instead of antiparathyroid hormone serum, when (ii) fluorescein-labeled antirabbit globulin was applied directly to the sections without prior treatment with rabbit antiparathyroid hormone serum, and when (iii) the antiparathyroid hormone serum had been absorbed with parathyroid extract.

Since no reaction with antiparathyroid hormone serum could be demonstrated within any of the cells of the parathyroid gland, it is possible that hormone in the cells is not in a form which can be demonstrated by the indirect fluorescent antibody technique, or that the technique may not be sensitive enough to visualize the quantities of hormone present in each cell.

Summary. The indirect fluorescent antibody technique revealed local concentrations of parathyroid hormone in intercellular tissue spaces between cords and within acini formed primarily of chief cells in bovine parathyroid glands. In this study, specific immunofluorescence was not seen within parenchymal cells of the parathyroid gland.

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Cholesterol Solubilization by Solutions of Bile Salts and Bile Salts Plus Lecithin* (32983)

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Cholesterol, the principal component of most gallstones, is essentially insoluble in water. Bile salts alone will not solubilize the quantities of cholesterol present in bile (1), but, the addition of lecithin to bile salt solutions increases cholesterol solubilization to values observed in human gallbladder bile (2,3). It is generally accepted that the bile

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