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Fluorescamine Fluorescence Detection of Growth Hormone-Producing Cells in Human Pituitary Adenomas

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Summary. Formalin-fixed and paraplast-embedded tissue specimens of human pituitary, thyroid, and pancreas were investigated using fluorescamine fluorescence and immunohistochemical methods. Growth hormone-producing cells present in normal and neoplastic pituitary tissue exhibited fluorescamine fluorescence. The other tissues examined showed no fluorescamine binding.

Key words: Fluorescamine fluorescence — Immunohistochemistry — Growth hormone — Pituitary adenomas.

Introduction

Recently, fluorescamine has been introduced as a reagent in the fluorometric detection of amines, amino acids, peptides, and proteins in solutions (Udenfriend et al., 1972; Weigele et al., 1972) .In tissue specimens fixed in formaldehyde vapor, fluorescamine was found to give strong and selective fluorescence of certain cell types, many of which either proved to produce or store polypeptide hormones or have been assumed to belong to this category (Håkanson et al., 1974). Therefore, it has been suggested that fluorescamine fluorescence may be helpful in the histopathologic diagnosis of certain endocrine tumors (Sundler et al., 1974). In human pituitaries, growth hormone-producing cells were found to bind fluorescamine particularly (Larsson et al., 1975).

During the last decade, immunohistochemical methods, particularly the peroxidase-labeling technique, have proved to be valuable in the identification of peptide hormones in routinely formalin-fixed and paraffin-embedded tissue specimens. Growth hormone-producing cells in pituitary adenomas can be specifically identified using this technique (Nieuwenhuijzen Kruseman et al., 1976). The aim of the present investigation was to determine whether fluorescamine fluorescence could be used as a rapid screening method to detect polypeptide hormone-producing or -containing cells in human tumors. The results of this technique were compared with the immunohistochemical reactions.

Materials and Methods

Tissues. Surgically removed pituitary adenomas from five patients with acromegaly, one patient with Cushing's syndrome and two patients without clinically apparent endocrine disorders were examined. As control tissues, specimens of two normal pituitaries, one medullary

carcinoma of the thyroid, and one insuloma of the pancreas were added to this series. The control pituitaries were obtained at autopsy. One of these came from a woman who died of eclampsia and therefore could be expected to contain prolactin-producing cells.

Fixation and Embedding. The tissue specimens were fixed in 10% phosphate-buffered neutral formalin for about 6 h at room temperature. Afterward, the tissues were routinely processed in paraplast at 56 °C and cut at 4 μm.

Fluorescamine Staining. Deparaffinized and rehydrated sections were mounted in Tris HCl-buffered glycerin (1:9, pH 8.0) and were initially examined for autofluorescence. Subsequently, the coverslips were removed and the slides briefly washed in a 0.2M phosphate buffer, pH 8.0. Immediately after removal of the slides from the buffer, each section was drained and covered with a few drops of the fluorescamine solution (2 mg Fluram^R, Hoffman-La Roche, Basel, Switzerland, in 10 ml acetone) for about 15 s (Håkanson et al., 1974). The slides were then washed repeatedly in phosphate buffer, mounted in Tris HCl-buffered glycerin and examined with an Ortholux microscope (Leitz) equiped with a high-pressure mercury-arch HBO 100 light source and a standard Ploemopak vertical illuminator in position1 (peak exitation at 365 nm) and a K 490 (Leitz) as barrier filter.

Immunohistochemistry. The fluorescamine-stained sections were subjected to an indirect immunofluorescence method with TRITC-labeled antibodies (Nordic, Tilbury, The Netherlands). Adjacent, unstained sections were subjected to an indirect immunoenzyme method using peroxidase-labeled antibodies (Miles, Kankakee, Ill., USA). The first layer consisted either of antiserum against human growth hormone, porcine ACTH, human calcitonin, or bovine insulin. The growth hormone antibodies were purchased from Wellcome (Beckenham, Kent, England), the insulin antibodies from Miles. The ACTH and calcitonin antibodies were prepared by immunization of adult New Zealand rabbits with a zinc preparation of porcine ACTH (Cortrophine-Z, a generous gift from Organon, Oss, The Netherlands) and human synthetic calcitonin (a generous gift from Ciba-Geigy, Basel, Switzerland), respectively. The TRITC-treated sections were mounted in Tris HCl-buffered glycerin and examined with an Ortholux microscope as outlined before using the standard filter setting in position 4 (peak excitation at 560 nm). The peroxidase-treated sections were stained according to Graham and Karnovsky with 0.075% 3.3′-diaminobenzidine-tetra-HCl (Sigma Chemical Co., St. Louis, Mo., USA) and 0.01% hydrogen peroxide (Perhydrol, Merck, Darmstadt, Western Germany) in 0.05M Tris HCl buffer, pH 7.6. After passing through a graded alcohol series, the sections were mounted in malinol. Appropriate controls on the immunohistochemical preparations were performed (Nieuwenhuijzen Kruseman et al., 1976).

Histologic Staining. The pituitary cells were characterized in adjacent sections using the trichrome methods of Cason (1950) and Brookes (1968). Other sections were stained with hematoxylin and eosin.

Results

After fluorescamine staining a number of cells in pituitary adenomas of acromegalic patients as well as in the normal control pituitaries revealed a distinct granular fluorescence. Immunohistochemical examination of these cells proved that they contained growth hormone. In the pituitary of a woman who died of eclampsia these cells could easily be differentiated from prolactin-producing cells which showed no fluorescamine fluorescence but were carmoisinophilic in adjacent Brookes' stained sections. The intensity of fluorescamine fluorescence correlated well with the degree of immunohistochemical granulation. No fluorescamine fluorescence was noticed in the other pituitary adenomas, the medullary thyroid carcinoma, and the pancreatic insuloma (Table 1).

No differences in immunostaining intensity were observed between sections that had been previously exposed to fluorescamine and unexposed controls. Similarly, immunostaining did not appear to affect the intensity of subsequent fluorescamine fluorescence. Therefore, both fluorescamine fluorescence and im-

Table 1. Fluorescamine fluorescence and immunohistochemical staining of hormone-producing tumors

	Secretion product	Immunohistochemical granulation	Fluorescamine fluorescence
1. Acidophilic adenoma of the pituitary	growth hormone	+++	+++
2. Acidophilic adenoma of the pituitary	growth hormone	++	++
3. Acidophilic adenoma of the pituitary	growth hormone	++	++
4. Acidophilic adenoma of the pituitary	growth hormone	+	+
5. Acidophilic adenoma of the pituitary	growth hormone	+	+
6. Acidophilic adenoma of the pituitary	none	—	—
7. Chromophobe adenoma of the pituitary	none	—	—
8. Basophilic adenoma of the pituitary	ACTH	++	—
9. Medullary carcinoma of the thyroid	calcitonin	+++	—
10. Insuloma of the pancreas	insulin	++	—

munofluorescence can be carried out on one section, simultaneously. The preparations can be examined easily by switching the filter setting of the fluorescence microscope.

In agreement with earlier observations (Håkanson et al., 1974; Sundler et al., 1974), fluorescamine fluorescence was found to fade rapidly upon exposure to UV light. After storage of the sections for some hours protected from daylight fluorescence was restored. The fluorescamine-stained sections could be stored at −20 °C without serious decrease of fluorescence intensity.

Discussion

In solution, fluorescamine reacts with primary amino groups (Udenfriend et al., 1972). The same reaction has been ascribed to formaldehyde. Fluorescamine fluorescence of certain cell types after fixation in formaldehyde vapor could therefore be explained by the presence in certain proteins of hidden amino groups which are not condensed after formaldehyde fixation and hence are still capable of binding fluorescamine (Håkanson et al., 1974). In human tissues, these cell types include cells containing growth hormone, gastrin, insulin, and calcitonin (Sundler et al., 1974; Larsson et al., 1975). In our hands, pituitary cells containing growth hormone from normal as well as neoplastic tissues still exhibited fluorescamine fluorescence when routine formalin fixation and paraplast embedding was used instead of fixation in formaldehyde vapor. The other cell types examined were found to be devoid of fluorescamine affinity after routine histologic tissue processing. From these observations, it may be expected that fluorescamine binding is a simple and convenient way to determine rapidly whether or not acidophilic granulation of pituitary adenomas is correlated with the presence of growth hormone. It remains to be determined whether this is so, and under what specific conditions fluorescamine staining of other polypeptide hormone-producing cells in routinely processed tissues can be achieved.

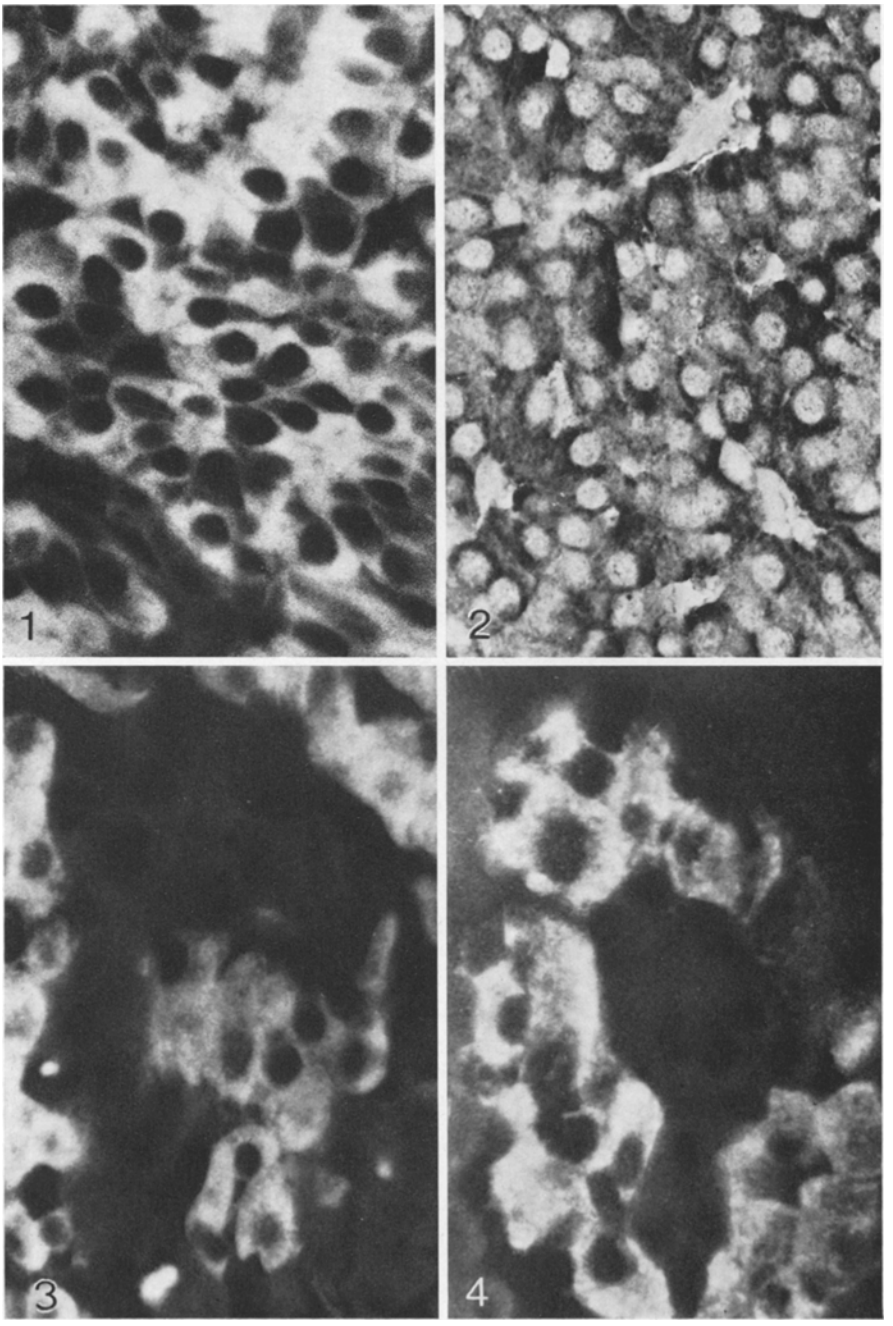

Fig. 1. Densely granulated growth hormone-secreting pituitary adenoma showing fluorescamine fluorescence. ×1,250

Fig. 2. Same adenoma as depicted in Figure 1, after immunostaining for growth hormone activity with peroxidase-labeled antibodies. ×1,250

Fig. 3. Normal pituitary showing fluorescamine fluorescence of growth hormone cells. ×1,250

Fig. 4. Same section as depicted in Figure 3, after immunostaining for ACTH activity with TRITC-labeled antibodies. Note that immunoreactive cells lack fluorescamine fluorescence. ×1,250

According to our immunohistochemical studies, the intensity of fluorescamine fluorescence appeared to correspond to the degree of granulation of the growth hormone cells. This finding is in agreement with observations of Larsson et al. (1975) who suggested that the fluorophore binds to secretory granules. These authors also noticed that treatment with fluorescamine greatly decreased immunoreactivity. In contrast, we found that immunostaining and fluorescamine fluorescence were not mutually antagonistic. This observation suggests that after formalin fixation fluorescamine binding does not take place at the same molecular site as that at which antibodies are bound. Consequently, this implies that fluorescamine is not necessarily bound by the hormone itself. As immunohistochemical demonstration of hormones is related to the immunologic activity of the identified hormone, the latter method is still preferable for definitive confirmation of diagnosis of growth hormone-producing pituitary adenomas.

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