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Fluorescamine-Induced Fluorescence in C Cell Tumours of the Thyroid

F. Sundler, L.-I. Larsson, R. Håkanson, and O. Ljungberg

Departments of Histology and Pharmacology, University of Lund, Lund, Sweden and Department of Pathology, Malmö General Hospital, Malmö, Sweden

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Summary. Fluorescamine induces strong fluorescence in the cells of human thyroid medullary carcinoma and of canine C cell adenoma (formaldehyde-fixed specimens). Non-neoplastic human C cells do not exhibit fluorescamine-induced fluorescence whereas canine C cells do. The difference between neoplastic and non-neoplastic C cells in man may be helpful in the early histopathological diagnosis of thyroid medullary carcinoma.

Introduction

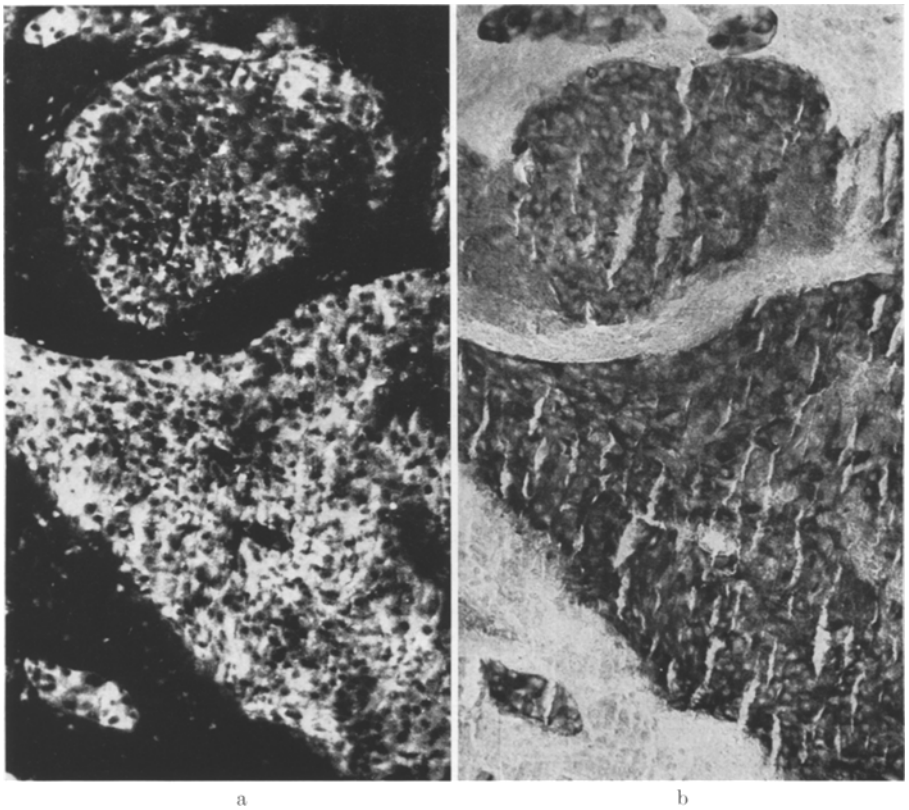
Fluorescamine is a highly sensitive reagent for the fluorometric determination of primary amino groups in amines, amino acids, peptides and proteins (Udenfriend, Stein, Böhlen, Dairman, Leimgruber and Weigele, 1972; Böhlen, Stein, Dairman and Udenfriend, 1973). Recently, fluorescamine was introduced as a reagent for the fluorescence histochemical demonstration of amino groups (Håkanson, Larsson and Sundler, 1974). It was observed, that fixation with gaseous formaldehyde, known to block amino groups, did not prevent fluorescamine from inducing intense fluorescence in certain cell systems, many of which had an established or anticipated endocrine function. Such cell systems included adenohypophyseal cells, thyroid C cells of certain species, a population of pancreatic islet cells and endocrine-like cells of the gastrointestinal mucosa. A possible explanation for this phenomenon is that certain amino groups in these cells are "hidden" in the sense that they are protected from reacting with formaldehyde, thus leaving them accessible for condensation with fluorescamine (Håkanson *et al.*, 1974). The present report describes fluorescamine-induced fluorescence in cells of human thyroid medullary carcinoma and canine C cell adenoma.

Material and Methods

Tissues. Specimens from three cases of medullary carcinoma and from three cases of toxic goiter were obtained at surgery. The tissue material also included one C cell adenoma, found in a dog thyroid, as well as specimens from thyroids of three normal dogs.

Histochemical Procedures. Small tissue blocks were frozen to the temperature of liquid nitrogen in a mixture of propane and propylene, freeze-dried, and exposed to gaseous *formaldehyde* for 1 hr at 80°C according to Falck and Hillarp (for details, see Björklund, Falck and Owman, 1972). The specimens were then embedded in paraffin *in vacuo* and sectioned at 6 μ. The sections were deparaffinized and mounted in xylene and examined in a fluorescence microscope for formaldehyde-induced fluorescence. After removal of the cover-slip, the dried sections were briefly immersed in 0.2 M sodium phosphate buffer, pH 8.5, drained and covered with a few drops of *fluorescamine* solution (2 mg Fluram®[1] in 10 ml of acetone) for 30 sec. The sections were blotted with a filter paper, mounted in the buffer and examined

1 Generous gift from Hoffman-La Roche, Basel, Switzerland.



a b

Fig. 1. a) Fluorescamine-induced fluorescence in tumour cells of medullary carcinoma. b) The fluorescent tumour cells are argyrophil. × 150

in the fluorescence microscope. *Filter settings.* Schott BG 12 was used as lamp filter and Schott OG4 as barrier filter. *Light microscopy.* Sections were stained with silver according to Grimelius (1968) (*argyrophil staining*). With this procedure thyroid C cells are stained while the follicle cells are not (Pearse, 1968).

Results and Comments

Parts of the human thyroid medullary carcinomas, particularly at the periphery of the tumours, contained scattered cells emitting moderate to intense yellow, formaldehyde-induced fluorescence characteristic of 5-hydroxytryptamine. The bulk of tumour cells were non-fluorescent. No cells in non-tumourous human or canine thyroids or in the canine C cell adenoma emitted formaldehyde-induced fluorescence. After exposure to fluorescamine, an intense yellow-green fluorescence developed in the cytoplasm of all tumour cells in the human medullary carcinomas (Fig. 1a) as well as in the canine adenoma (Fig. 2). All fluorescent cells were argyrophil (Fig. 1b). In the non-afflicted parts of the dog thyroid and in the thyroid of normal dogs, scattered clusters of argyrophil cells with a parafollicular localization emitted strong fluorescence (Fig. 3). The thyroid follicle cells and the follicle content gave no or very weak fluorescence. In the non-tumourous human

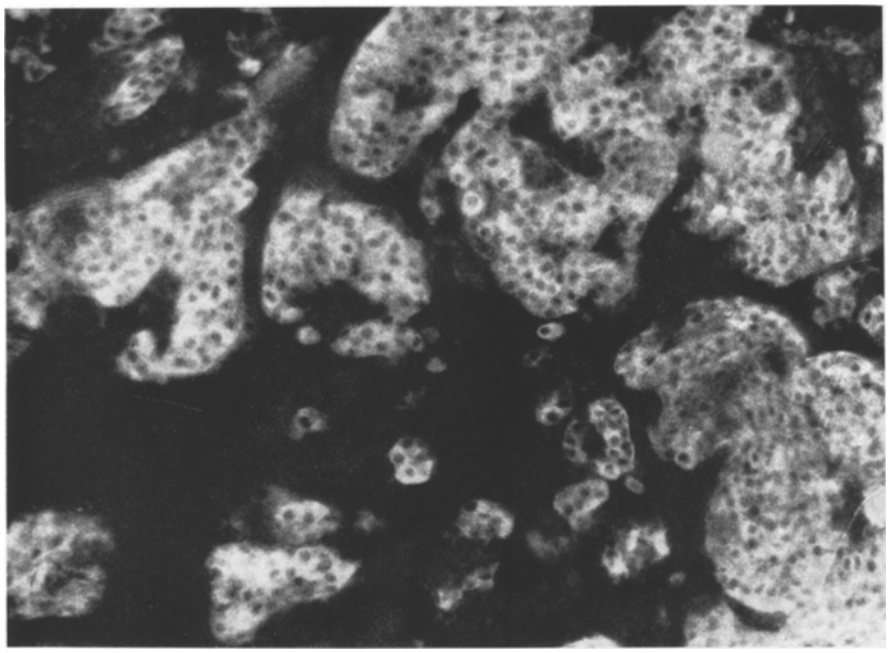


Fig. 2. Canine C cell adenoma. Large nodules of cells showing fluorescamine-induced fluorescence. × 170

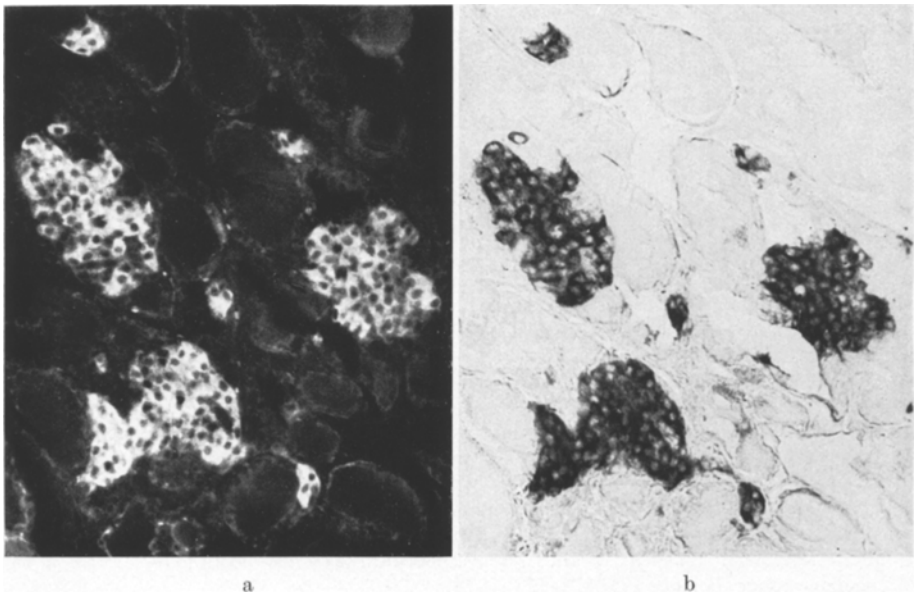


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Fig. 3a and b. Dog thyroid. Section from area adjacent to C cell adenoma. a) Clusters of parafollicular cells with fluorescamine-induced fluorescence. b) The cells are identified as C cells by their argyrophilia. The C cell clusters are enlarged compared to those seen in the thyroids of the normal dogs. × 160

thyroids there were no cells exhibiting fluorescamine-induced fluorescence, whereas argyrophil cells were present.

A characteristic feature of the fluorescamine-induced fluorescence was its rapid fading upon exposure to UV light. After 6 to 8 hrs in the dark, however, fluorescence was restored. This is in agreement with earlier observations (Håkanson *et al.*, 1974).

Discussion

In solution, fluorescamine reacts readily with primary amino groups to yield intensely fluorescent products (Udenfriend *et al.*, 1972). It is probable that the same reaction takes place in tissue sections. In unfixed tissues, fluorescamine treatment induces an intense, uniform fluorescence presumably due to the abundancy of free amino groups. In theory, amino groups in tissues fixed with formaldehyde should be blocked and thus inaccessible for reaction with fluorescamine. In practice, fluorescamine was found to be useful for demonstrating certain endocrine-like cell types in formaldehyde-fixed specimens; as an explanation it was proposed that certain amino groups in these cells are "protected" from condensation with formaldehyde (cf. Håkanson *et al.*, 1974). It is probable that the amino groups demonstrated with fluorescamine derive from peptides or proteins or glucoseaminoglycans, since it is to be expected that amines and amino acids should be extracted during the processing of the tissue sections, which involves the use of aqueous media. The fluorescamine reaction, applied to sections from freeze-dried, formaldehyde-fixed specimens, seems to be a simple and convenient way of demonstrating neoplastic C cells in the fluorescence microscope. It should be noted that the non-neoplastic human C cell (as opposed to the canine C cell) did not exhibit fluorescamine-induced fluorescence. This difference may be helpful in the early diagnosis of thyroid medullary carcinoma.

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Dr. F. Sundler
Department of Histology
University of Lund
Biskopsgatan 5
S-223 62 Lund/Sweden