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AUTOFLUORESCENT GRANULAR CELLS IN HUMAN GINGIVA

by

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INTRODUCTION

In a study of normal skin in man *Adams-Ray and collaborators* (1) found a paleness which increased peripherally and which they ascribed to an increase in vascular tonus. In a comparative determination of catechols from the skin of the abdomen and calf, *Adams-Ray* (2) found, also, that the latter skin contained seven times as much noradrenaline, a fact that was ascribed to an increase in the sympathetic activity of the lower extremities. Similar findings were made in respect of the adrenaline content of the skin of the same areas. These clinical and physiological findings suggest the presence, in the skin, of a factor that exerts a local effect on the peripheral circulation.

It has been suggested (3, 4) that the skin contains a chromaffin cell system. These cells are described as 2—4 times as numerous in the skin of the calf as in that of the abdomen. Morphologically, they are reported as unipolar or bipolar, and have cytoplasmic processes that contain granules which fulfill the requirements for chromaffinity (5, 6). The granules are oxidized by potassium dichromate, are argentaffin, reduce a solution of ferric ferricyanide and display a yellow fluorescence. Furthermore, according to *Nordenstam & Adams-Ray* (4), the cells are

positive to *Hillarp & Hökfelt's* test (7) for adrenaline and noradrenaline. The results of electron microscopic examination of the cells and their localization around myelin-free nerve fibres and smooth muscle cells in small arteries and veins, have been interpreted as structural manifestations of their vasomotormechanism (8, 9).

The presence of a system of chromaffin cells and granules in the human skin has been discussed in the litterature in the recent years. Their presence is acknowledged by some authors (10—14) while others (15, 16) were unable to confirm them.

The purpose of the study to be reported below was to determine whether there is, in the human gingiva, an autofluorescent granular cell that has the same morphological and histochemical properties as cells demonstrated in the skin.

MATERIAL AND METHOD

The material consisted of specimens of gingiva from 20 patients with ages ranging from 15 to 55 years. In 5 of the subjects the gingiva was clinically healthy whereas in the others the gingiva presented chronic marginal disease of varied degrees.

The gingival specimens were removed under regional anaesthesia (3 % Carbocain Dental) and immediately fixed in neutral buffered formalin or Orth's 2.5 % potassium dichromate solution according to the method of *Lillie* (17) for 24—48 hours. After embedding in paraffin, serial 5 micron sections were cut. Some of the formalin-fixed sections were mounted unstained on slides (Reichert's Urtrag 75 13) in a non-fluorescent medium such as glycerin or DePeX (Gurr). These were examined in a Zeiss fluorescence microscope equipped with a mercury lamp (Osram HBO 200) and 2 BG 12 filters for transmission and one OG 4 and one OG 5 filter for absorption of ultraviolet light. The wavelength band used is seen in Fig. 1. For preliminary and histologic examination, Mayer's haemalun-eosin was used. Sections were also stained by the method of Sevki as specified by *Adams-Ray & Nordenstam* (3, 4) and with ferric-ferricyanide as described by *Lillie & Burtner* (18).

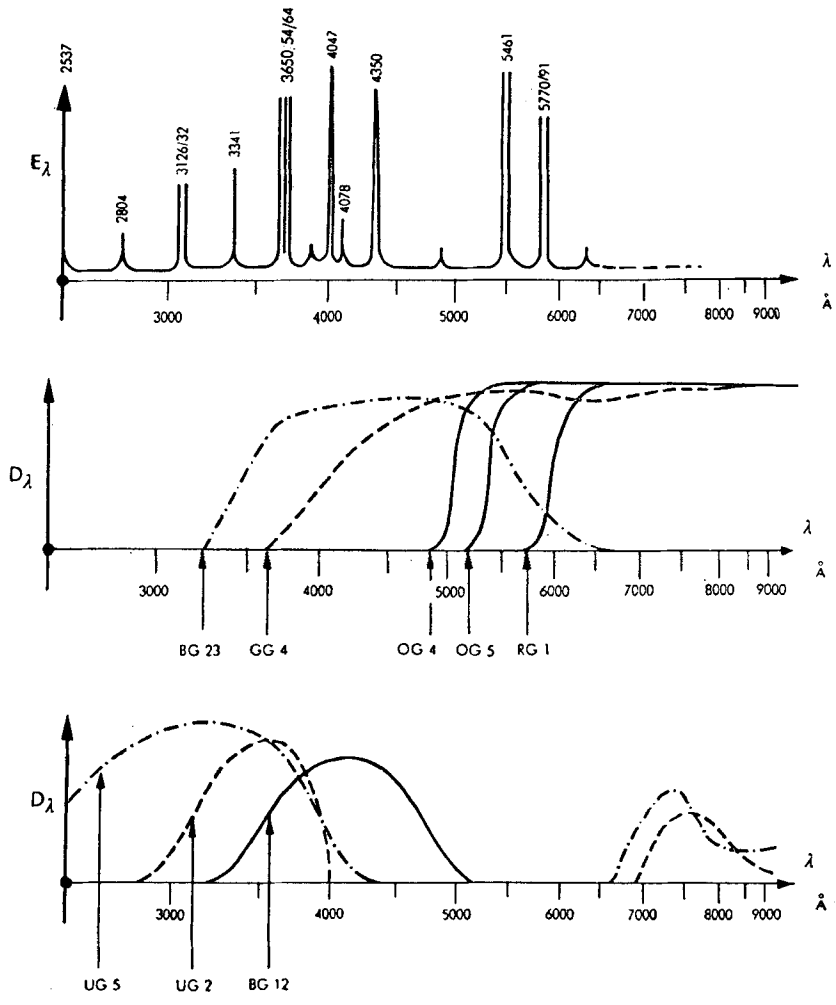

Fig. 1. 2 BG 12 filters for transmission and one OG 4 and one OG 5 filter for absorption of ultraviolet light were used.

RESULTS

On examination of the formalin fixed, unstained specimens in the fluorescence microscope, granular structures with a yellow fluorescence were noted in the subepithelial connective tissue (Fig. 2). These granules were associated with cells of which the structure and localization were similar to those described by

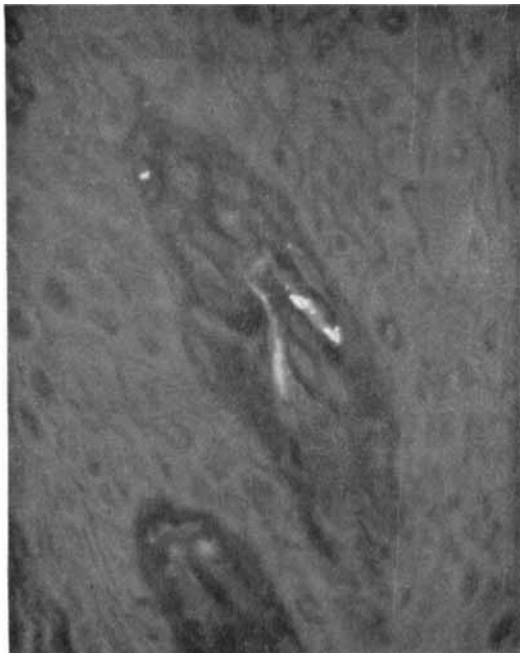

Fig. 2. In an island of connective tissue surrounded by epithelial cells, a bipolar cell containing auto-fluorescent granules can be observed. Note the occurrence of extracellular granules.

Zeiss fluorescence microscope X 950.

Adams-Ray & Nordenstam (3, 4). It was also found that the fluorescent granules reacted positively with the ferric-ferricyanide test and were stained by the method of Sevki.

Accumulations of extracellular granules with a yellow fluorescence were often encountered. Corroborating findings were made on subsequently stained sections.

In sections stained by the method of Sevki, elongated cells were observed which often occurred near vessels in the subepithelial connective tissue. The cytoplasm of these cells contained granules that stained bluish red or green. In general, the cells appeared to be bipolar but some were unipolar or branched (Fig. 3). Granules were found also outside the cells, often in large groups. The cells were similar to those observed in the fluorescence microscope.

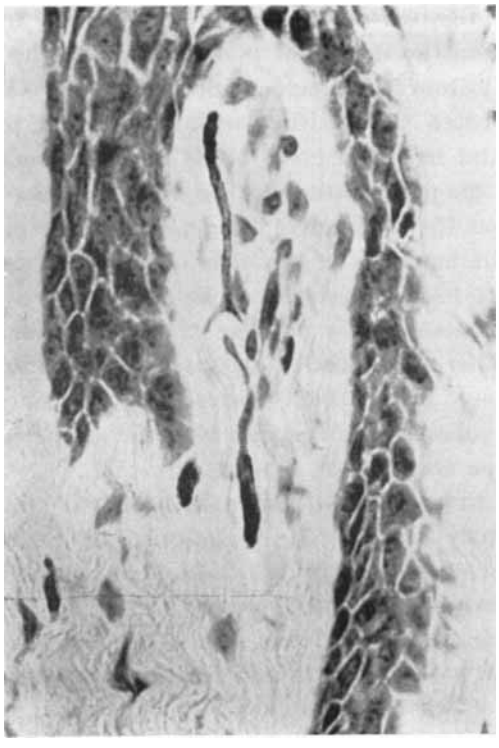

Fig. 3. Two elongated unipolar cells containing accumulations of granules can be seen in one of the papillae of lamina propria. Sevki X 950.

Sevki staining also revealed cells with granules that stained bluish red but were round or oval in shape and had no cytoplasmic processes.

In sections stained with ferric-ferricyanide, cells were observed which contained blue granular structures. In their localization and appearance, the cells were similar to some of those observed in the Sevki-stained sections.

DISCUSSION

According to *Orban* (19) the basal cell layer in the gingival epithelium of man contains pigment granules (melanin). While this pigmentation is common among negroes, it is relatively rare among whites, and when present, is chiefly among persons with

a dark skin. *Adams-Ray, Bloom & Ritzén* (20) found no correlation between the degree of pigmentation of the skin and its content of autofluorescent cells. Since *Hamperl's* (21) investigation has indicated that neither melanin nor its pre-stages are fluorescent, and in the present study no fluorescent cells were found in the gingival epithelium or at the border between the epithelium and the connective tissue, it may be presumed that the fluorescent material in the cells observed is not melanin.

Fluorescence has been shown to be a property of certain substances which also give a positive chromaffin reaction (5, 6). However, neither the chromaffin reaction nor autofluorescence can be considered as specific for these reactions may be given by such varying substances as certain biogenic amines and pigments of varying type (lipofuscin, lipochrom etc.).

As regards intensity and contrast, the Sevki staining method gave satisfactory results. The technique has certain disadvantages, however, for the staining solution contains different staining substances. Consequently, some of the red granular cells might belong to mastcells. The risk of confusion has been pointed out by several workers. In an early paper by *Adams-Ray & Nordenstam* (3) it was considered possible to distinguish between chromaffin cells and mastcells by virtue of structural and tinctorial differences. In a later paper (4), however, these workers stress the risk of confusion and state that the fluorescence of the cell, its argentaffin properties and its capacity for reducing ferric-ferricyanide are more objective means of distinguishing it from the mast cell.

The ferric-ferricyanide test may be used to demonstrate the presence of lipofuscin granules and SH-groups. It is, however, not specific for the catecholamines that are possibly present in the chromaffin cells. The test is of no use in elucidating the exact chemical nature of the granules, since it only indicates the presence of substances capable of reducing ferric-ferricyanide to ferricyanide. The chromaffin substances share this property with tissues containing, e. g. ascorbic acid, oxalic acid and inorganic sulphides (22).

It is realized that the methods used in the present investigation are mostly non-specific. For the time being therefore, it must be considered that the physiologic significance of the ob-

served autofluorescent cell and the chemical nature of its granules still remain to be established. Further research on this type of cell is indicated to better understand the physiology of the gingiva.

SUMMARY

A study has been performed in which the gingiva has been shown to contain a granular type of cell that has an appearance and tinctorial properties similar to those described in chromaffin cells. The specificity of the methods employed is discussed as is the possible physiologic significance of the cells and the chemical nature of their granules.

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RÉSUMÉ

CELLULES GRANULEUSES AUTOFLUORESCENTES DANS LA GENCIVE HUMAINE

Au cours de cette étude, il a été démontré que la gencive contient un type granuleux de cellules dont l'apparence et les propriétés tinctoriales sont semblables à celles qui ont été décrites pour les cellules chromaffines. Discussion sur la spécificité de la méthode employée et sur la signification physiologique possible de ces cellules et la nature chimique de leurs granules.

ZUSAMMENFASSUNG

AUTOFLUORESZIERENDE GRANULIERTE ZELLEN IM MENSCHLICHEN ZAHNFLEISCH

Das Vorkommen eines granulierten Zelltyps, welcher unter anderem die morphologischen und histochemischen Eigenschaften des in der Haut vorkommenden chromaffinen Zellen hat, ist in der Gingiva des Menschen nachgewiesen worden.

Sowohl die Spezifitäten der angewendeten Methoden als auch die physiologische Signifikans der Zelle und die chemische Natur der Zellengranula wurden diskutiert.

REFERENCES

1. *Adams-Ray J., S. Hagberg & A. Reuterskiöld* 1955: High tone in the cutaneous venules of the lower extremities. A factor in the postural reactions. Minerva Cardioangiol. europ. *1*: 185.
2. *Adams-Ray J.*, 1956: L'hypertonie des veinules de la peau dans les cas d'état pathologique et chez les individus bien portants. Acta neuroveg. *14*: 283.
3. *Adams-Ray J. & H. Nordenstam*, 1956: Une système de cellules chromaffines dans la peau humaine. Lyon chir. *52*: 125.
4. *Nordenstam H. & J. Adams-Ray*, 1957: Chromaffin granules and their cellular location in human skin. Z. Zellforsch. *45*: 435.
5. *Lison L.*: *Histochimie et cytochimie animal. Principes et méthodes.* 2e edit. Paris, Gauthier-Villars, Édit.
6. *Gomori G.*, 1954: Histochemistry of the enterochromaffin substance. J. Histochem. Cytochem. *2*: 50.
7. *Hillarp N. A. & B. Hökfelt*, 1955: Histochemical demonstration of noradrenaline and adrenaline in the adrenal medulla. J. Histochem. Cytochem. *3*: 1.
8. *Adams-Ray J., H. Nordenstam & J. Rhodin*, 1958: Über die chromaffinen Zellen der menschlichen Haut. Acta neuroveg. *18:* 304.
9. *Rhodin J., J. Adams-Ray & H. Nordenstam*, 1959: Electron microscopy of human skin cells containing chromaffin granules. Z. Zellforsch. *49*: 275.
10. *Burch G. E. & J. H. Phillips*, 1958: Chromaffin reacting cells in human digital skin. Circulat. Res. *6*: 416.
11. *Burn J. & M. Rand*, 1958: Noradrenaline in artery walls and its dispersal by reserpin. Brit. med. J. *1*: 903.
12. *Niebauer G. & A. Wiedman*, 1958: Thema II b: Histochemie des neurovegetativen Systems der Haut. Acta neuroveg. *17*: 175.
13. *Hibbs R., G. Burch & J. Phillips*, 1960: Electronmicroscopic observations on the human mast cell. Amer. Heart J. *60*: 121.
14. *Phillips J., G. Burch & R. Hibbs*, 1960: Significance of tissue chromaffin cells and mast cells in man. Circulat. Res. *8*: 692.
15. *Mercantini E.*, 1960: Failure to show presence of a chromaffin system of cells in human skin. J. invest. Derm. *34*: 317.
16. *Coupland R. E. & I. D. Heath*, 1961: Chromaffin cells, melanophores and mast cells. J. Endocr. 59.
17. *Lillie R. D.*, 1954: *Histopatologic technic and practical histochemistry.* The Blakinston Company, N. Y.
18. *Lillie R. D. & H. Burtner*, 1953: The ferric ferricyanide reduction test in histochemistry. J. Histochem. Cytochem. *1*: 87.
19. *Orban B.*, 1962: *Oral Histology and Embryology.* The C. V. Mosby Company, Saint Louis.