Evidence Against Presence of Chromaffin Cells in Human Skin

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Adams-Ray and Nordenstam1,2 claim to have demonstrated “chromaffin cells” in human skin and have thereby created a fundamentally new concept in the control of the peripheral circulation.3,4 Their findings have been confirmed by a group of independent investigators,5 and electron microscopic studies have supported the suggestion that these cells are distinct from mast cells.6,7 These reports have initiated a comparative study between the “chromaffin cells” in normal skin and skin from patients with peripheral vascular disease without histological differences being detected.8

Mercantini9 seems to be the only investigator who has encountered difficulty in demonstrating chromaffin granules in human skin. He followed the methods of Adams-Ray and Nordenstam but found it impossible to differentiate mast cell granules staining purple or bluish-red from other granules staining clear-red or bluish-red presumed to be chromaffin granules. We have also been unable to demonstrate chromaffin cells in human skin but for different reasons. These reasons have been mentioned briefly in a preliminary report.10

Adams-Ray and Nordenstam demonstrated the presence of chromaffin granules in the corium of human skin by approved tinctorial methods, but only the chromaffin reaction, autofluorescence in ultraviolet light, and the modified Sevki stain gave satisfactory results. The tinctorial superiority of the modified Sevki method was emphasized because it disclosed chromaffin substance to a much greater extent than other staining procedures and because it produced excellent contrast between the red chromaffin structures and the surrounding tissues.

In support of their statement that a red color indicates chromaffinity, they refer to Pearce,11 but have in addition demonstrated the same reaction in adrenal medulla treated identically. However, when discussing the histochemistry of epinephrine and norepinephrine and specifically the modified Giemsa stain used by Sevki for identification of chromaffin granules, Pearce12 stated: “This method originally designed for use with non-chromated tissue, gives a pink colour with chromaffin cells under such conditions. In the case of chromated tissue, however, the colour is green.” Coupland13 also stated that when Giemsa stain was used following formol-di-chromate fixation, the cytoplasm of adrenal medullary cells stained a blue-green color. Since Adams-Ray and Nordenstam1,2 and Burch and Phillips5 used the Sevki stain on chromated tissue, chromaffin granules should have been colored green.

It seemed important to investigate these inconsistent reports in chromaffin staining, especially because the presence of chromaffin cells in tissues could be of fundamental importance in autonomic transmission following the hypothesis of Burn and Rand14 which involves the presence of a tissue store of norepinephrine, possibly in chromaffin cells.

Two human adrenal glands, removed at operation for breast cancer, were collected within 10 minutes of the vessels being ligated. The organs were immediately placed in Orth’s solution and treated as described by Burch and Phillips.5 Four mouse adrenals, removed immediately after death, were treated similarly. All unstained sections displayed a diffuse golden-brown chromaffin reaction. Sections stained with the modified Sevki method showed the color reaction of the adrenal.
medulla to be dark green. The nuclei of medullary cells also took part in the reaction, becoming dark green to greenish-black. It was noted that, whereas red blood cells are chromaffin positive and could be seen as a brown disc in unstained Orth-fixed adrenal, they did not turn green with the Sevki stain, suggesting that catecholamine was necessary for this color reaction. Mouse adrenal medulla was compact, while human medulla contained many cells of the zona reticularis which separated medullary cells into irregular cords or groups. These cortical cells did contain fine red to reddish-purple granules with the modified Sevki method of staining.

To confirm that the green color seen in true medullary cells was due to catecholamine, the adrenal from a mouse which had received reserpine (5 mg./Kg.) 24 hours previously was compared with that from a litter mate which received reserpine vehicle at the same time. Both glands were fixed in Orth's solution and examined unstained and stained by the modified Sevki technique. Little brown chromaffin material or green medullary staining occurred in the former, but a marked chromaffin reaction and the usual dense green Sevki reaction were seen in the latter.

It thus became clear, both from examination of the literature and from personal experience, that with the modified Sevki staining technique on chromated tissue, the criterion of chromaffinity was a green color reaction and not red. This point being established, the presence of chromaffin cells in human skin was re-examined.

Methods

Specimens of skin were taken with a 3-mm. punch biopsy, as described by Phillips and Burch, from the medial side of the terminal phalanx of the little finger after ulnar nerve block at the elbow and from the anterior aspect of the forearm with local injection of 2 per cent lignocaine. Skin was also taken from the lower abdomen at operation and from autopsy material (finger, abdomen, and calf). The tissues were immediately placed in Orth's solution. Portions of the specimen taken from the abdomen at operation and from autopsy material were fixed in formol-saline. Processing of tissues followed the methods described by Burch and Phillips, and serial sections were examined unstained, stained with hematoxylin and eosin, and stained by the Sevki technique. In addition, some sections were stained with toluidine blue.

Results and Discussion

In unstained, Orth-fixed sections, a brown color could only be identified with certainty in the epidermis (melanin granules), subepidermal layer (dermal chromatophores), and red blood cells. The brown pigment granules were observed under the maximum light intensity that would give clear definition of red cells. Dermal chromatophores were readily seen in skin from all areas except the finger tip. Usually they occurred close beneath the epidermis, but some were seen deep in the dermis. Stained with hematoxylin and eosin, these cells had bipolar cytoplasmic projections containing dark to golden brown granules irrespective of whether the tissue had been fixed in Orth's solution or formol-saline, confirming that this color was due to pigment. The granules varied considerably in size, and their natural color could not be distinguished from the true chromaffin reaction of the adrenal medulla. With the Sevki method on chromated or nonchromated tissue, epidermal melanin and dermal chromatophores, but not red blood cells, frequently had a greenish tinge added to their natural brown color.

The Sevki method on chromated tissues also revealed many elongated cells around blood vessels, nerves, and hair follicles. These snake-like bipolar or unipolar cells, containing fine red granules, differed morphologically from the mast cells seen in the same sections and were undoubtedly the "chromaffin cells" of Adams-Ray and Nordenstam. Tintorially, however, they could not be distinguished from mast cells since their granules produced the same red color with Sevki staining and, in addition, both stained metachromatically with toluidine blue. Many cells were seen that could be accepted morphologically as either mast cells or "chromaffin cells." The granules of elongated "chromaffin cells" that had been carefully marked could
not be located when the section was returned to the Orth-fixed state, by destaining with acid-alcohol, but were readily visible again when sections were restained with Sevki. We were therefore unable to confirm the findings of Burch and Phillips* that brown granules seen in unstained Orth-fixed skin occur as red granules in "chromaffin cells" when stained with the Sevki method and revert to the brown chromaffin reaction on destaining.

In formal-saline fixed, Sevki-stained skin, typical mast cells and elongated cells with red granules were seen, which were in no way different from the cells and granules in Orth-fixed, Sevki-stained sections.

Cells giving the deep green staining reactions of the adrenal medulla could not be found in any section.

Our search for chromaffin cells was initiated during a study of the peripheral dilator action of reserpine in human skin.10 Indirect evidence suggested that this was due to an interference with a tonic influence quite apart from the sympathetic constrictor fibers. Chromaffin cells seemed a likely source of this tonic influence.17 Since in the present report we were unable to find true chromaffin cells in human skin, this suggested action of reserpine must be modified. Nevertheless, we have proceeded to examine these elongated cells after treatment with reserpine and in chronically sympathectomized skin because changes have been described in "chromaffin cells" of the cat's skin and nictitating membrane following sympathectomy and reserpine treatment that would indicate their association with the sympathetic innervation.18

Reserpine (50 µg./min. for 10 minutes) was infused into the left brachial artery of two male subjects following methods described elsewhere.10,17 Two hours later, when the hand and forearm were flushed, skin was taken by punch biopsy from the little finger of one subject after ulnar nerve block and from the forearm of the other after local injection of 2 per cent lignocaine. Skin was also taken at the same time from an identical situation on the right side as a control. The control limb was not flushed and is not affected by this dose given intra-arterially into the opposite side.16 These specimens were fixed in Orth's solution and treated in the manner described. Serial sections of reserpine-treated skin were compared with control skin after staining with the Sevki method. In sections of both treated and control skin, the elongated cells were readily found. There was no obvious reduction in granular content in treated skin. Similarly, typical mast cells were unaffected by this treatment.

Skin was also taken from the little finger and forearm of three patients suffering from idiopathic autonomic degeneration who were known to have complete postganglionic degeneration of the sympathetic nerves to these areas. Two of these subjects have been discussed elsewhere (subjects one and three, Parks et al.19); the third was shown to have sympathectomized hands by the same criterion (i.e., absence of response to large doses of intra-arterially administered ephedrine). Again, the elongated cells and typical mast cells were seen but were in no way different from the normal.

It has therefore not been possible either on histological or pharmacological grounds to differentiate the elongated cells in skin from typical mast cells, and despite an apparent difference in morphology, the metachromatic nature of the cytoplasmic granules of these elongated cells suggests that they are mast cells. The importance of morphology is questionable. Cells tend to be elongated when situated among parallel bands of fibers. In serial sections of skin, some cells were found which conformed to the description of elongated "chromaffin cells" in one section but appeared as mast cells in the next. Adams-Ray and Nordenstam suggested that granule size was important in distinguishing between mast cells and "chromaffin cells." They found that the "chromaffin cell" granule had a diameter of about 0.2 to 0.3 µ, whereas the mast cell granule was larger (0.6 to 0.7 µ). Asboe-Hansen,20 however, reviewed the morphology of the mast cell granule and stated
that all workers except one had described the granules to be about 0.3 μ in diameter.

Although these findings and conclusions suggest that the elongated cells are mast cells, there is some evidence to the contrary. Electron microscopic studies have reported consistent differences between the granules of "chromaffin cells" and mast cells, while Burn et al. have found that the "chromaffin cells" of cat's skin and nictitating membrane are depleted of their granular content by reserpine and sympathectomy. In the present study in man, however, neither intra-arterial reserpine nor sympathectomy had an obvious effect on these cells. It could be argued that the doses of reserpine used were too small to have a depleting action on catecholamine stores, so that the appearance of "chromaffin cells" would be unchanged by such treatment. There is no evidence in man available on this point, but these doses have a marked effect on skin blood vessels, an effect which seems to depend on the release of some vasoactive substance in skin. It could also be argued that normal "chromaffin cells" were found in sympathectomized skin because of incomplete denervation. However, the skin blood vessels of these patients were unresponsive to high doses of intra-arterially injected ephedrine and methedrine which indicates absence of the peripheral store of norepinephrine and, therefore, complete postganglionic sympathetic degeneration.

The important conclusion to be drawn from these findings is that, if the postganglionic sympathetic transmission theory of Burn and Rand applies to human skin, then the stores of norepinephrine are unlikely to exist in the "chromaffin cells" of Adams-Ray and Nordenstam.

Summary
"Chromaffin cells" in human digital skin, described by previous workers, are readily recognized, but their granules do not produce the true chromaffin reaction of catecholamine, nor do they become green when subsequently stained with the Sevki technique. Although "chromaffin cells" differ morphologically from mast cells, they have similar tinctorial characteristics. Many cells were seen that could be accepted as either mast cells or "chromaffin cells." These cells in human skin are unaffected by intra-arterial reserpine and had the normal appearance in three patients with chronic degeneration of the sympathetic nerves. It is suggested that the "chromaffin cells" are mast cell variants and are not involved in the autonomic effector mechanism.

Addendum
After this paper was submitted for publication, it was brought to the authors' notice that essentially the same conclusions had been reached by Coupland, R. E., and Heath, I. D.: J. Endocrinol. 22: 59, 1961.

References
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