These studies demonstrated the existence of chromaffin-reacting granules in the human skin. The chromaffin reaction was provided by bichromate fixation. Subsequent special staining by the modified Sevki procedure revealed these granules to be localized usually to characteristic long, eel-like cells with distinctive tintorial features. Although the true significance of these cells is not certain, they are believed to represent the local source of a vasopressor material for the peripheral tissues. Much indirect physiologic evidence supports this belief.

Adams-Ray and Nordenstam accepted the classic definition of the "chromaffin reaction" as the formation of dark brown-staining compounds by bichromate fixation. By using various histochemical techniques, but particularly Orth's solution (bichromate) fixation and a modified Sevki stain, they were able to demonstrate not only the presence of chromaffin granules in the skin but their apparent localization to characteristic cells. The Orth solution fixation provided the chromaffin reaction in the typical granules but, without subsequent staining, cellular characteristics were indistinct. For this reason the modified Sevki stain, which is known to stain chromaffin tissue but is not specific for it, was used after fixation. The characteristic cells thus identified were typically long, narrow, and eel- or snake-like, with elongated pale-staining nuclei surrounded by a narrow zone of cytoplasm. Cytoplasmic protrusions of pale-staining material were mainly bipolar and usually contained typical red to bluish-red granules under Sevki staining. Occasionally, the cytoplasmic protrusions gathered in clusters to produce the appearance of "flowering cowslips." The elongated nuclei were 1 to 2 micra wide and 6 to 10 micra long. The granules had diameters of about 0.2 to 0.3 micron. The cells were irregularly distributed among collagen fibers in the corium of the skin and of other organs as well, being more predominant in the vicinity of blood vessels, nerves, hair follicles, and sebaceous glands.

Initially, differentiation of the special cells from mast cells was difficult because they appeared to stain similarly. This, however, was clarified subsequently by the positive...
results obtained for the chromaffin cells and negative results for the mast cells to the following histochemical procedures: argentaffin reaction, azocoupling reaction, indophenol reaction, ferriferricyanide reduction tests, and autofluorescence on ultraviolet illumination (3660 Å). In addition, of course, the mast cells did not contain chromaffin-positive granules. Furthermore, on Sevki staining, the mast cell and its granules were larger (0.6 to 0.7 micron) than the chromaffin cell and its granules. Finally, the general configuration and nuclear structures of the cells differed somewhat.\(^\text{10, 11}\)

We have extended the studies of Adams-Ray and Nordenstam to include investigations on human digital skin designed to identify these cells even further by locating them photographically under successive processes of fixing in Orth’s solution, staining with Sevki solution, destaining, and then restaining. By this means, the chromaffin granules were shown to be in the special cells and not in the mast cells; this also served to differentiate artifacts and ectopic melanin.

**METHODS**

Specimens of skin were obtained by punch biopsies from the finger and toe tips and from the legs of patients in the Charity Hospital. Several additional samples were gathered from fresh postmortem material. The adrenal glands of dogs were studied as a positive source of chromaffin cells.

The tissue was immediately fixed in Orth’s solution (10:1 mixture of Muller’s fluid and neutral formalin)\(^\text{10, 11}\) for 24 to 48 hours. The tissue was then washed, dehydrated, embedded in paraffin, sectioned, mounted, and deparaffinated with xylene, after which a cover slip was placed over it. The unstained tissue was examined for cells with chromaffin-appearing granules, and these areas were photographed.

The tissue was then stained with modified Sevki stain\(^\text{10}\) in the following way. After the cover slip was removed, the tissue was washed consecutively with xylene, alcohol of graded strength, and distilled water. After application of a modified Sevki stain (2 drops of Giemsa’s solution per milliliter of distilled water for 2 hours at 50°C),
the tissue was rinsed in distilled water and differentiated, under microscopic guidance, first in a 70 per cent solution of ethyl alcohol, then successively in a 90 per cent solution, a 95 per cent solution, absolute alcohol, and finally xylene. The cover slip was then reapplied. The area with the chromaffin-appearing granules previously recorded photomicrographically (under Orth bichromate fixation) was again located in the Sevki-stained tissue. It was photographed again for comparison with the unstained tissue.

The coverslip was again removed, and the tissue was destained with acid alcohol, a process analogous to one described elsewhere with reference to toluidine blue. The same cells in the tissue, now returned to the Orth’s fixed, unstained state, were identified once more and photographed. Finally, the tissue was restained with the modified Sevki solution, and the same cells were rephotographed.

By studying the resultant series of photomicrographs, one could be certain that the chromaffin-appearing granules identified in the Orth’s fixed tissues were the same granules later noted in the special cells of that particular section on Sevki staining. As a control, the medulla of the adrenal gland of a dog was simultaneously processed by identical methods as the human skin.

RESULTS

These studies not only confirmed, but also added further evidence in support of the observation of Adams-Ray and Nordenstam, who demonstrated the presence of chromaffin-reacting granules in characteristic cells of the human corium. If the cytochemical criterion of a chromaffin granule is that it stains brown with bichromate, then the identification of the same cells containing chromaffin granules in our series of photographs confirmed the presence of these granules within cells with the morphologic characteristics described (figs. 1-5). It should be strongly em-
phasized that identification of these special cells on the original histologic sections under the light microscope was much easier than is apparent from the photomicrographs because of the benefits of increased clarity and color differentiation.

Although the chromaffin cells were not numerous in the tissues examined, a few were found in almost all samples of human skin studied. They appeared to be concentrated more heavily in the skin of the toe tip and the region of the calf than in the finger tip and were more numerous around blood vessels, nerves, and sweat and sebaceous glands.

Sevki-stained cells with the morphologic characteristics of the chromaffin cells but without chromaffin granules were also found (fig. 3). Whether or not these same cells had secreted their chromaffin material before the tissue was collected is not known. The morphologically and tinctorially characteristic cells contained chromaffin granules of variable numbers and depths of staining. Furthermore, chromaffin granules were sometimes found dispersed in the extracellular spaces of the corium. Whether this represents a normal state of distribution, or is the result of preparation of the tissues, remains unknown. In addition, cells were found with atypical morphologic characteristics, but with typical granules and staining properties (fig. 4).

**DISCUSSION**

These studies showed the existence in human skin of the digits and legs of positively reacting chromaffin granules. Although the granules were localized principally in characteristic cells, some were extracellular. Many cells had morphologic and tinctorial characteristics of the special cell but did not contain chromaffin-reacting granules. Moreover, chromaffin-reacting granules were found in cells that were tinctorially, but not morphologically, characteristic of those described by Adams-Ray and Nordenstam. The number of chromaffin-reacting granules, the intensity of their staining, and the number of special cells varied from tissue to tissue. Conceivably, such variations might all be related to the phase of secretory function of the cells.

Even though cells with chromaffin-reacting granules exist in the corium of human skin, their physiologic significance still remains unknown. That the chromaffin-reacting results from the oxidation, especially by chromates, of epinephrine, norepinephrine, or related compounds is generally accepted. Recent work has indicated that the catechol amines in chromaffin cells are enclosed in microspheres (presecretory granules) and that interference with the integrity of membrane of these granules results in the release of the active material. That the chromaffin reaction is not specific is well known. Red blood cells and the entodermal argentaffin granules have a positive chromaffin reaction, as do hydroxytyramine, dioxyphenylalanine, 5-hydroxytryptamine and possibly other related compounds. Since all methods of demonstrating chromaffin tissue are to some degree nonspecific, the presence of cells that possibly secrete epinephrine, norepinephrine, or other vasoactive substances must be confirmed more directly by both his-
tochemical and pharmacologic approaches.\textsuperscript{13}

Widely distributed outside the adrenal medulla are cells that contain chromaffin material. Thus far, chromaffin tissue has been demonstrated in the paraganglia (widespread, small collections of cells in various retroperitoneal structures, including the organs of Zuckerkandl), in the connective tissue between the aorta and pulmonary artery, in the coronary sulci (especially the left) and in the superior cervical ganglia.\textsuperscript{18, 20} Also of interest are the enterochromaffin cells (argentaffine system) of the gastroenteral tract, which demonstrate the classical chromaffin reaction.\textsuperscript{13, 17, 18, 23, 24} Some authors consider the reacting material in these cells to be 5-hydroxytryptamine (enteramine, serotonin),\textsuperscript{20, 25} but others are not certain.\textsuperscript{24} That the reacting substance is not epinephrine or norepinephrine appears certain.\textsuperscript{13}

Thus, it is not possible to state definitely that the characteristic chromaffin cells found in the human skin are the local source of epinephrine, norepinephrine, or both. That these cells may represent atypical mast cells in completely different stages of development, or that the granules may represent the local source of 5-hydroxytryptamine or some other related compound, cannot be denied. However, it does appear that these cells may well represent a local source of a vasopressor substance.

Of the structures in the body that produce catechol amines (the adrenergic nerves, the suprarenal medulla, and the chromaffin cells in the tissue), the adrenal medulla is the major source of epinephrine, whereas adrenergic nerve terminals, as well as the adrenal medulla, are major sources of epinephrine.\textsuperscript{5-7, 15, 16, 26, 27} Possible precursors of norepinephrine include dihydroxyphenylserine and hydroxytyramine. Epinephrine is apparently formed by the methylation of norepinephrine, the methyl group being supplied by such donors as methionine.\textsuperscript{26}

The sympathomimetic substances derived from the adrenal medulla appear to originate from two specific types of chromaffin cells, one for the production and release of epinephrine and another for norepinephrine.\textsuperscript{7, 15, 16} Whether or not this also applies to extra-adrenal chromaffin cells is not certain.

It has been suspected for some time that chromaffin cells exist outside the adrenal medulla and paraganglia, in various organs and peripheral tissues, and that they are capable of producing epinephrine, norepinephrine, or both. According to von Euler,\textsuperscript{7} almost all organs that have been examined have been shown to contain small amounts of epinephrine in addition to norepinephrine. The source of the epinephrine has been debatable. In support of the existence of chromaffin cells in peripheral tissues is the fact that after sympathetic denervation to some organs, the norepinephrine content drops sharply, whereas the epinephrine content falls only moderately.\textsuperscript{5, 7} This suggests that epinephrine is derived from chromaffin cells located in the tissues, which continue to elaborate their specific product even after sympathetic denervation. In addition, it has been shown repeatedly in the past that the peripheral vasomotor apparatus can re-establish essentially normal blood pressure after total body sympathectomy.\textsuperscript{28-30}

The presence of cells in human skin capable of producing a vasoconstrictive effect was predicted a long time ago. This prediction has been based on observations of the physiologic mechanism of tone and contractility of the minute vessels of the skin.\textsuperscript{1-31, 33} Although not conclusive, physiologic evidence has been presented to support the concept of local production of vasopressor substances by cells of peripheral tissue. For example, despite the importance of the sympathetic nerve fibers in the control of the dermal blood vessels, complete severance of these fibers is followed by only a temporary period of vasodilatation.\textsuperscript{34} This would imply compensation by some local phenomenon. The vessels may be demonstrating increased sensitivity to circulating vasopressor agents, but this is not definite.\textsuperscript{35-37} Vasopressor substances of local origin not only may exist but may actually be considerably more important. Furthermore, responses of
the peripheral circulation that are attributed
to direct action of various agents upon smooth
muscle of the blood vessels could be mediated
with such a special cell as an intermediary
step.

Probably some of the strongest evidence in
support of locally produced vasoconstrictor
substances is that offered by Sir Thomas
Lewis1 in his discussion of Bier’s spots. He
observed these red and white spots in the
skin of the lower arm a few minutes after the
brachial artery had been obstructed by a
pneumatic cuff. As noted by Lewis, the ves-
sels participating in this phenomenon were
those responsible for the color of the skin,
capillaries, and in particular, minute venules.
The white spots are of particular interest in
these studies related to the special cell be-
cause they are produced by intense con-
traction of dermal capillaries and venules.1 38

The presence of innervation to these vessels
is unsettled.1 34 38 The unequivocal statement
that the capillaries and venules are not in-
nervated34 appears to be incorrect. Through
a careful series of experiments, Lewis1 ex-
cluded cold temperature as well as central
nervous and local nervous factors as primary
causes of Bier’s white spots, which were noted
to form in denervated areas. He concluded
that in cutaneous areas in which the circula-
tion had been sufficiently reduced, vasocon-
stricor as well as vasodilator substances
are formed. Through evidence too exten-
sive to be presented here, he concluded
that vasoconstrictor substances are released
locally in the tissue spaces and are not
derived from the blood. These substances
must act against potent vasodilators, which
are known to be released when the circulation
is arrested. As noted by Lewis, Bier’s white
spots enlarge and coalesce progressively as
the skin is deprived of its circulation. If
this is continued (as in death), the whole cu-
taneous surface becomes involved. Further-
more, responses comparable to Bier’s spots
have been described for organs other than the
skin.39

Extremely high tone has been demonstrated
in the cutaneous venules of the lower extrem-
ity,31 the degree apparently being propor-
tional to the magnitude of the hydrostatic pres-
sure acting locally within the vessels. The
high tone present in the small cutaneous blood
vessels in some disease states, such as conges-
tive heart failure,82 33 may be in part due to
local mechanisms, perhaps in response to im-
pairment of the circulation to the tissues.
Thus, physiologic phenomena are known
which could depend, at least in part, upon
chromaffin cells located within the tissues,
such as skin.

SUMMARY

Confirming the recent original work of
Adams-Ray and Nordenstam, we have dem-
onstrated the presence of chromaffin-reacting
granules localized in characteristic cells of
the human digital corium. On the basis of
both anatomic and physiologic considera-
tions, these cells are presumably a local source
of some vasoconstrictor substance, probably epi-

nephrine, norepinephrine, or both, but their
true function remains to be demonstrated by
direct evidence.

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