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CHOLINESTERASE ACTIVITY IN PIGMENTED NAEVI

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Introduction

Many studies have been devoted to the histogenesis of pigmented naevi. Especially the behaviour of dendritic cells under physiological and pathological conditions and so also in cellular naevi has been investigated. The number of authors accepting the theory of the neural origin of naevus cells is increasing. Special methods of staining are necessary to prove this theory: beside silver stains and supravital methylene blue, the determination of cholinesterase activity has recently come into the foreground in connection with the problem.

Soldan [9] was the first to suggest the possible interconnections between naevus tissue and nervous system. In 1926 and later in 1951 Masson [6] discussed this subject very thoroughly and claimed that naevus cells originate partly from epidermal melanoblasts and partly from Schwann's cells of the cutaneous nerves. He described the dense network of nerve fibres in the naevus-cell nests, and discussed the formation of the foliated lamina and naevic corpuscle by the differentiation of naevic cymplasm. Feyrter and John [1, 3] — though coming to other conclusions than Masson — also regard naevi to be of neural origin. Later this view was confirmed by the investigations of Roth [7] and Szodoray [10]. According to Kawamura [4], all kinds of pigmented naevi are derivatives of neural crest cells. Of recent, Shelley and Arthur [8], with supravital methylene blue staining demonstrated a dense network of delicate nerve fibres in intradermal naevi and the dermal part of compound naevi. The fibres — some myelinated, others without myelin sheath — were shown to issue from large, compound nerve trunks. These authors subscribe to Masson's view that naevi are due to a dysplasia of neural elements.

Material and method

Serial sections of 17 pigmented naevi were studied. The histological distribution was as follows: two junctional and fourteen compound or intradermal naevi. One of the changes clinically diagnosed as naevus was histologically proved to be melanoma. The age of the patients was between 6 and 50 years. One of our patients 20 years of age stated that 60 to 80 pigmented naevi had appeared on his trunk within a few months. After preparing frozen sections from all the substances, we determined their cholinesterase activity with the method of Koelle—Friedenwald as modified by Gomori [2]. Acetylcholine-iodide served as the substrate.

The procedure was as follows:
(1) Preparation of 30 to 50 μ frozen sections from fresh unfixed material.
(2) Incubation of sections (floating or mounted on cover slip) at 37° C for 60 minutes.

Incubating solution

A. *Stock solution :*

$CuSO_4$ $5H_2O$	0.300 g
Glycine	0.375 g
$MgCl_2$ $6H_2O$	1.000 g
Malleic acid	1.750 g
nNaOH	30 ml
Na_2SO_4 (saturated hot, about 40 per cent	170 ml)

The solution was adjusted to pH 6 by means of NaOH. This stock solution can be stored for a few weeks.

B. *Incubation.* After dissolving 15 to 20 mg of acetylcholine-iodide in a few drops of distilled water, 10 ml of the stock solution was added.
(3) Rinsing 3 to 4 times, each time in a fresh saturated solution of Na_2SO_4.
(4) Washing for 1 to 2 minutes in dilute (yellow) ammonium-sulphide solution.
(5) Washing in distilled water.
(6) Contrast staining with 0.5 per cent aqueous cresyl violet.
(7) Dehydration with 70—80—96 per cent abs. alcohol.
(8) Clarification in xylene.
(9) Mounting in Canada balsam.

Material incubated without substrate or sections treated with prostigmine (1 γ/ml) served as controls. One specimen of all material being fixed and embedded in paraffin, were stained with haematoxylin-eosin in order to determine the type of naevus.

Results

Except the usual cutaneous nerves, we found no nerve fibres in either malignant melanomas or junctional naevi. While containing exceedingly large amounts of pigment, neither the naevus cells nor the tumour cells displayed cholinesterase activity. Interpreting this phenomenon, SZODORAY [11] supposes that both the epidermal dendric cells and their derivative naevus cells have lost the cholinesterase activity characteristic of nerve cells.

There were profuse networks of delicate fibres with cholinesterase activity in the intradermal naevi and in the cell nests situated deep in the dermis (Fig. 1); their connection with the nerve trunks in the deep strata of the corium or with the nervous network of the esodermal structures was striking in most instances. No nerve fibres were found in the papillary layer and among the naevus-cell nests in the superficial layers of the corium. We failed to demonstrate acetylcholinesterase in the naevus cells. Apart from the fibre plexus, in some naevi other neural elements also have drawn our attention, in four cases of the last group (Figs. 2, 3). In some of the naevus-cell nests of the middle layer of the corium we found *several dendron-like structures with long undulating extensions the size of which was many times that of the naevus cells ;* these structures appeared to be connected by means of their extensions. They exhibited vigorous cholinesterase activity which could be inhibited by prostigmine.

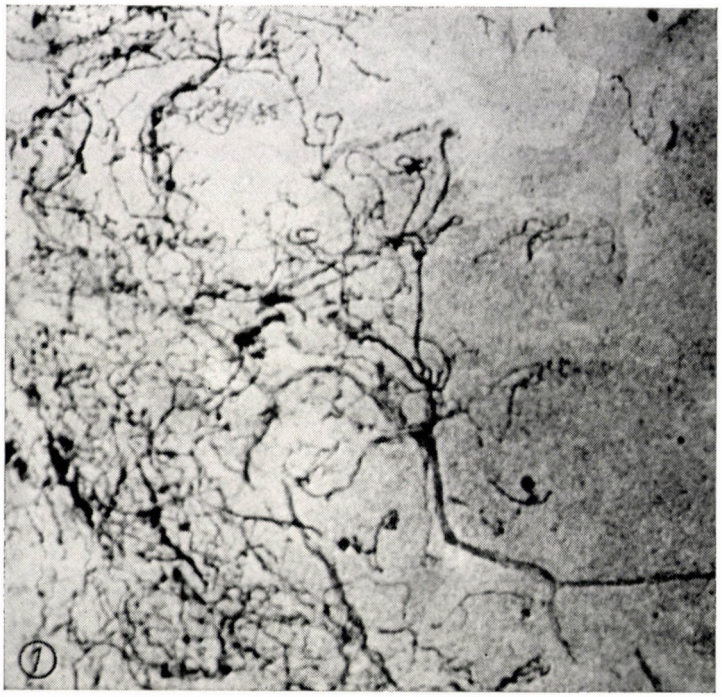

Fig. 1. Dense plexus of nerve fibres in the naevus region. One ascending fibre terminates on a cell of the subdermal naevus-cell meat displaying cholinesterase activity. 400× magnification

Discussion

Our results have confirmed MASSON's view in that the presence and multiplication of neural elements are decisive factors in the formation of naevus tissue. The presence of the network of nerve fibres in the naevus tissue has long been known. WINKELMANN [12] succeeded in demonstrating strong non-specific cholinesterase activity in the cells of dermal naevi (Figs. 4, 5). The non-specific cholinesterase activity of the Schwann's cells has been known since KOELLE's experiments [5]. If, therefore, MASSON's view concerning the origin of naevus cells is adopted there is no difficulty in interpreting WINKELMANN's and our own results: originating from Schwann's cells, the cells of dermal naevi have retained these characteristics. Cholinesterase activity cannot be expected in junctional naevi whose cells derive from epidermal melanoblasts seeing that in the epidermal dendritic cells such an activity has not been demonstrated. Likewise the cells of malignant melanomas do not contain cholinesterase, nor do the blue naevi according to the findings of WINKELMANN. It is suggested by this author that non-specific cholinesterase activity and malignancy are inversely proportional, and that it is necessary to differ-

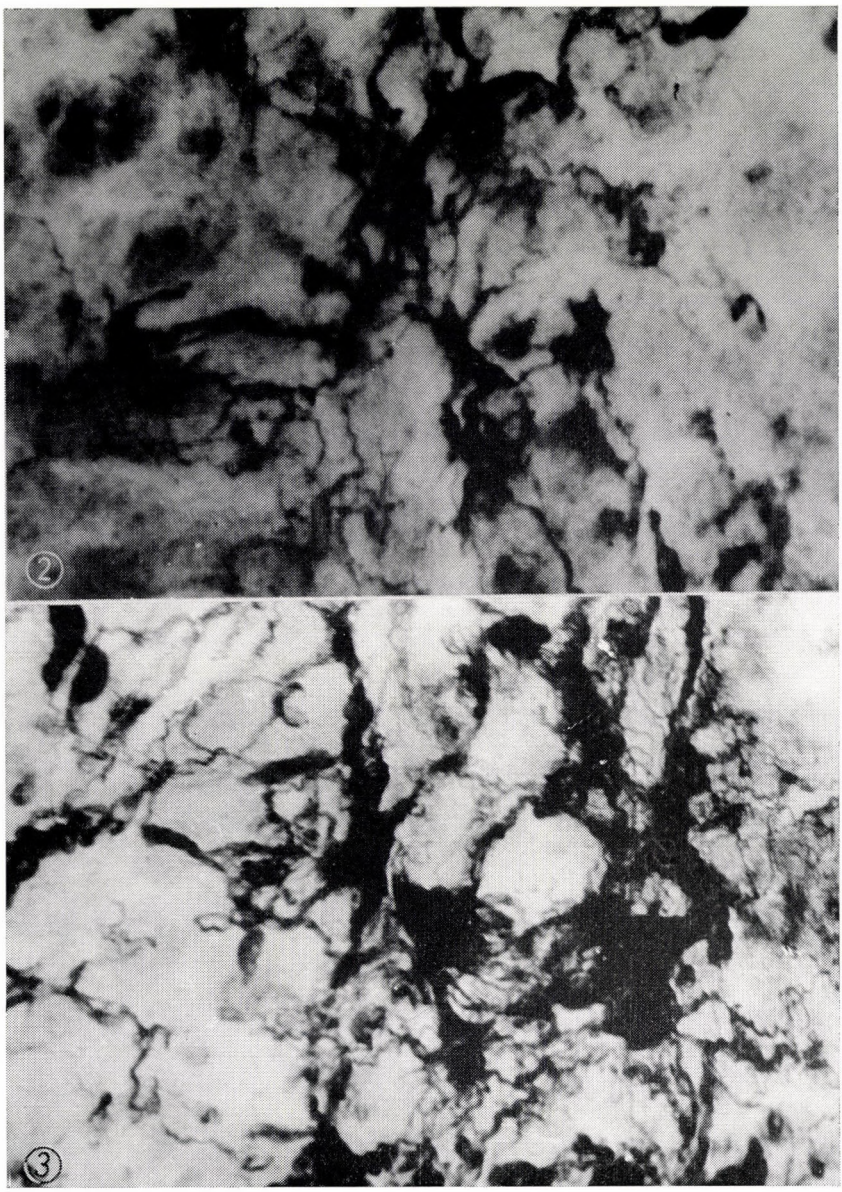

Fig. 2, 3. Dendron-like, intensively cholinesterase-positive cell elements and fibres with undulating extensions in the region of some naevus-cell nests. 400× magnification

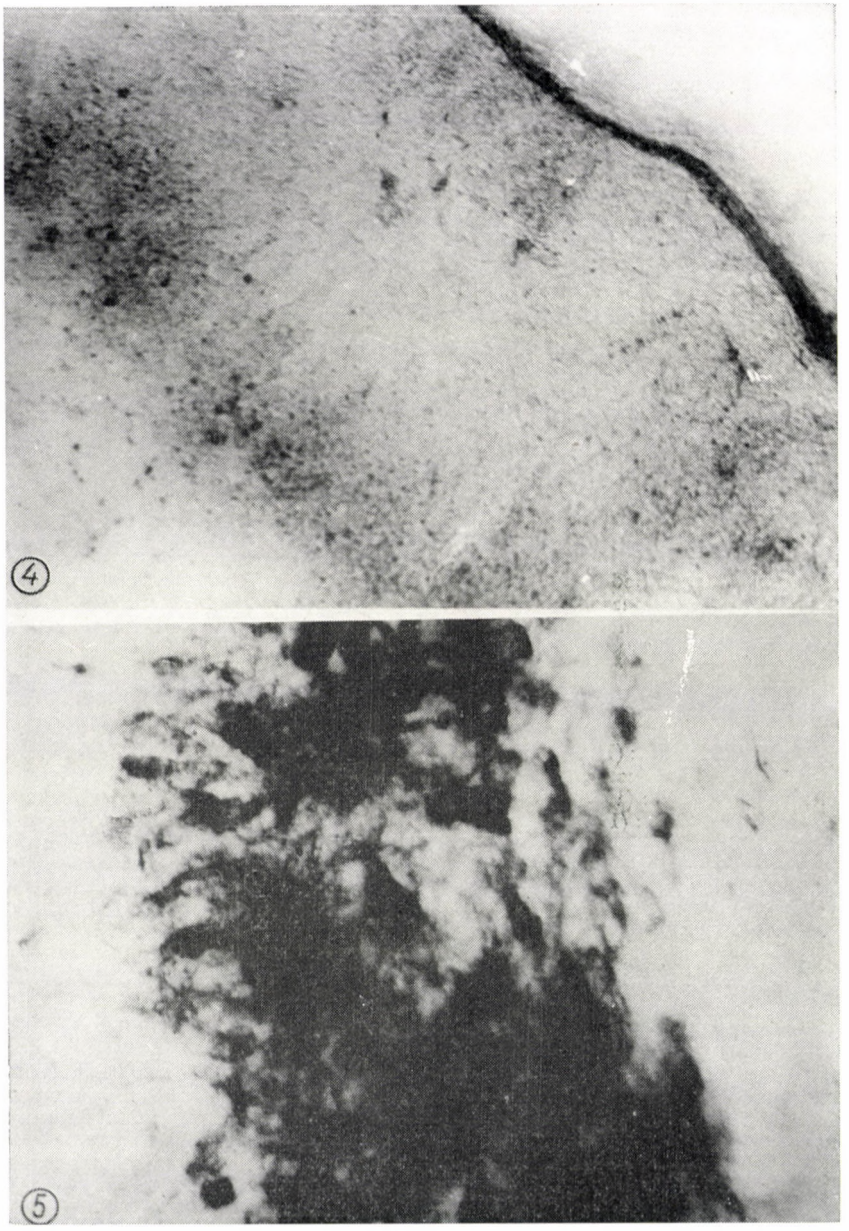

Fig. 4. Cholinesterase activity in the dermal portion of the naevus. 200× magnification
Fig. 5. Cholinesterase activity is absent after pretreatment with prostigmine (1γ/ml); the discolouration is due to pigmentation alone. 200× magnification

entiate the category of benign naevi containing non-specific cholinesterase. WINKELMANN [13] described the proliferation of nerve fibres with specific cholinesterase in persons of advanced age which appears mostly on the face in papillomatous, slightly pigmented and sometimes hairy neural naevi of the Masson-type. We found similar fibres also in naevi of other types and location which have been excised from young individuals.

The cholinesterase activity as well as the shape of the dendron-like structures we observed in the cell nests of some naevi seem to reveal their neural origin. So far we could not decide whether we were dealing with transformed melanoblasts (those of the basal layer do not contain cholinesterase) or some other neural elements. It is worthy of note that we found these cell elements in subjects of prepubertal age and in patients with eruptive naevi.

Acknowledgement

We are indebted to Prof. B. TANKÓ for preparing the acetylcholine-iodide.

Summary

Cholinesterase activity has been studied in 17 pigmented naevi with the Koelle—Friedenwald method as modified by Gomori. A profuse network of nerve fibres among the cells of the dermal portion of the naevi, and — in some naevi — dendronlike structures with cholinesterase activity have been demonstrated. Investigations are in progress with a view to elucidating their origin.

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АКТИВНОСТЬ ХОЛИНЭСТЕРАЗЫ В ПИГМЕНТНЫХ ПЯТНАХ

К. ВЕЗЕКЕНЬИ и К. ТУЗА

Для выявления активности холинэстеразы авторы исследовали 17 пигментных пятен методом Кэлле—Фриденвальда, модифицированным Гёмёри. Между клетками дермальной части пигментных пятен удалось выявить богатую сеть нервных волокон, и в некоторых пигментных пятнах клеточные элементы, обладающие холинэстеразной активностью, напоминающие дендритные клетки. Авторы желают дальнейшими исследованиями выяснить происхождение этих элементов.

CHOLINESTERASEAKTIVITÄT IN PIGMENTNÄVI

KLÁRA VEZEKÉNYI und KLÁRA TUZA

Es wurden 17 Pigmentnävi nach der von GOMORI modifizierten KOELLE—FRIEDENWALDschen Methode zum Nachweis der Cholinesteraseaktivität untersucht. Im dermalen Abschnitt der Nävi konnte ein reiches Nervenfasernetz zwischen den Zellen nachgewiesen werden, und in einzelnen Nävi waren an Dendritzellen erinnernde, Cholinesteraseaktivität aufweisende Zellelemente anzutreffen, deren Ursprung in weiteren Untersuchungen geklärt werden soll.

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