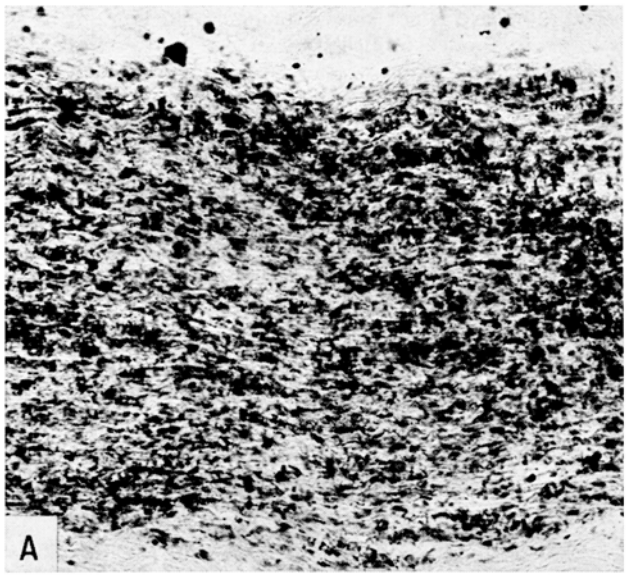
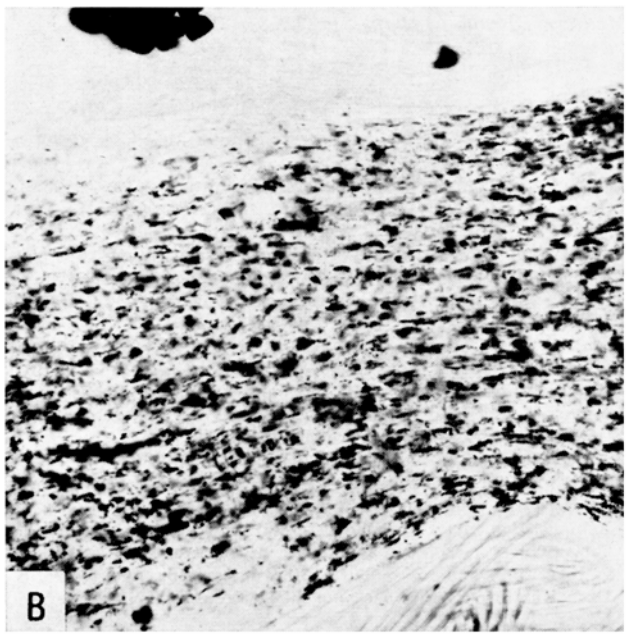
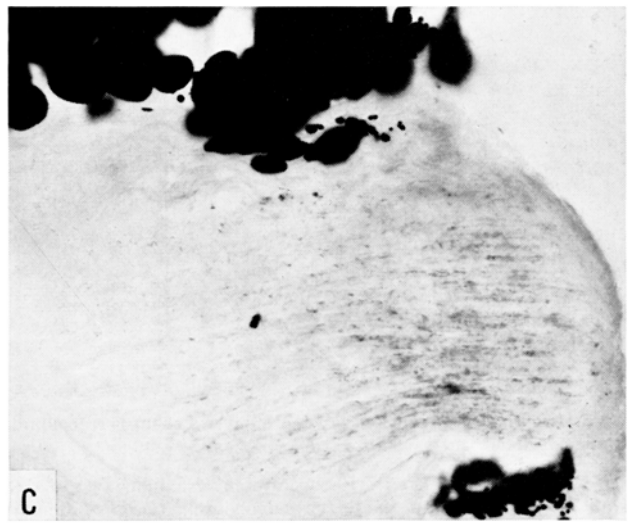
A Note on the Mechanism of the MARCHI Reaction in Degenerating Myelin

Since its conception[1], the MARCHI method has been extensively used to investigate the organizational anatomy of nerve tracts within the central and peripheral nervous systems. In spite of the specificity of the MARCHI reaction in degenerating myelin, the frequent occurrence of MARCHI artifact has often introduced a degree of uncertainty into findings based upon the use of this staining procedure[2,3]. It is surprising, therefore, that there have been only a few reports which deal with the MARCHI reaction in nervous tissue[4–8] and which thus provide data necessary to achieve a reduction in this artifact. Several of these reports[6–8] have been concerned with the reaction of the constituents of normal and degenerating myelin with osmium tetroxide and potassium chlorate as utilized in the SWANK-DAVENPORT modification of the MARCHI method[9]. Osmium tetroxide and potassium chlorate comprise the essential components of the SWANK-DAVENPORT solution, the staining reagent used in this modification. With this solution the amount of artifact associated with the traditional MARCHI method employing osmium tetroxide and potassium dichromate is substantially reduced. From these studies[6–8] it has been concluded that both osmium tetroxide and potassium chlorate are soluble in normal, hydrophilic myelin, whereas osmium tetroxide alone is soluble in degenerating, hydrophobic myelin; that the specificity of the MARCHI reaction within nervous tissue is due to the reduction of osmium (VIII) to osmium (IV) preferentially by unsaturated bonds in degenerating myelin; and that within normal myelin, the reduction of osmium (VIII) to osmium (IV) is blocked by chlorate. In the present report the validity of these conclusions is tested by introducing Triton X100 (alkyl phenoxy polyethoxy ethanol)[10] into the SWANK-DAVENPORT solution. Triton X100 is a nonionic wetting agent which, when added to this solution, will allow normally insoluble potassium chlorate free access to the constituents of degenerating myelin.

Each of 20 rats was anesthetized with sodium pentobarbital and its left sciatic nerve transected at the midfemur level. The rats were sacrificed 9–14 days postoperatively with overdoses of pentobarbital and perfused







Distal segments of sciatic nerves 14 days after nerve transections. The nerve segments were treated by (A) the SWANK-DAVENPORT solution alone, (B) the SWANK-DAVENPORT solution preceded by Triton X100, and (C) the SWANK-DAVENPORT solution together with Triton X100. The MARCHI reaction is present in nerves (A) and (B), but is essentially blocked in nerve (C). Note that perineurial adipose cells have been stained. × 70.

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intra-aortically with physiological saline followed by 10% formalin. A degenerating distal segment from each transected nerve as well as a segment from the normal sciatic nerve of each of 3 experimental rats were fixed for 3–5 days in 10% formalin and subsequently treated by one of the following methods: (1) Eight of the degenerating nerves and the 3 normal nerves were immersed in the SWANK-DAVENPORT solution[9] for 11 days; (2) 4 of the degenerating nerves were treated with 0.1% Triton X100 for 5 days. These segments were washed for 48 h in the running tap and then immersed in the SWANK-DAVENPORT solution for 11 days; and (3) 8 of the degenerating nerves were immersed in a reagent consisting of 0.1% Triton X100 and the SWANK-DAVENPORT solution for 11 days. All of these specimens were then embedded in celloidin[11] and sectioned longitudinally at 50 μ.

With the SWANK-DAVENPORT modification of the MARCHI method[9] the normal, undegenerated sciatic nerves contained a small quantity of MARCHI artifact; whereas 9–14 days after sciatic nerve transection the fragments and beads of degenerating myelin in the distal segment of peripheral nerve were, as described by other workers[12,13], highly MARCHI reactive (Figure A). When treated simultaneously with Triton X100 and the SWANK-DAVENPORT solution[9], the MARCHI reaction in fibers of the degenerating segments was essentially blocked (Figure C). The pretreatment of degenerating segments with Triton X100 failed to prevent the MARCHI reaction as subsequently produced by the SWANK-DAVENPORT solution (Figure B), and thus it may be concluded that the suppressive action of Triton X100 (Figure C) is not primarily due to the removal of MARCHI reactive constituents from degenerating myelin.

The finding from this report supports other conclusions concerning the mechanism of the MARCHI reaction in degenerating myelin[6–8]. This finding suggests that the demonstrated suppression of the MARCHI reaction by Triton X100 (compare Figures A and C) results from the solubility within the fragments and beads of degenerating myelin of normally insoluble potassium chlorate[14].

Zusammenfassung. Behandelt man degenerierendes Myelin gleichzeitig mit der SWANK-DAVENPORT-Lösung und Triton X100, so wird die MARCHI-Reaktion, die gewöhnlich durch die SWANK-DAVENPORT-Lösung ausgelöst wird, im wesentlichen verhindert. Dies stimmt mit früheren Befunden überein. Das Ergebnis deutet darauf hin, dass diese Verhinderung durch Triton X100 aus der Löslichkeit des normalerweise unlöslichen Kaliumchlorats in degenerierendem Myelin resultiert.

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Mitoses in the Adrenal Medullary Cells

The adrenal medullary cells have been considered postmitotic irreversible cells (LEBLOND and WALKER[1]) on the basis of their origin from sympatogonic cells (POLL[2]).

Mitoses have been demonstrated during the first period of life in the rat (JACKSON[3], MITCHELL[4]), in the hamster (ITO[5]), in the rabbit (COUPLAND[6]) whereas in adult animals no mitotic activity has been shown.

In the rat mitoses are absent after 60 days of life (JACKSON[3], MITCHELL[4]), in the adult cat mitoses are rare (BENNETT[7]) and in the hamster 'occur in nearly all stages until the age of 200 days' (ITO[5]). MACKAY and MACKAY[8] do not report any increase of the volumes of adrenal medulla of rats after monoepinephrectomy. In homo- and etero transplants of adrenal tissues in the anterior chamber of the eye, COUPLAND[6] observed secretory activity, but no mitotic figures. Mitoses of the granule containing cells can rarely be found in phaeocromocytomas.

MESSIER and LEBLOND[9] and VIOLA-MAGNI[10] demonstrated an incorporation of H^3-thymidine in the adrenal medullary cells of adult rat, wich might suggest a premitotic synthesis of DNA.

The aim of the present investigation is to clarify the problem of the presence of mitotic activity in adrenal medullary cells of the adult rat. The results obtained seem to be conclusive.

Albino rats of Italico and Wistar strains have been used. Three Italico and Wistar rats 60–80 days old and 3 Italico 1 year old were killed by stunning at 18.00; 12 Italico and 12 Wistar rats 60–80 days old were injected i.p. with colchicine [0.1 ml/100 g body weight of a solution containing 10 mg of colchicine (Merck) in 1 ml of ethanol 80% and 9 ml of distilled water] 3 h before sacrifice. The animals were killed in groups of 3 at 03.00, 09.00, 12.00 and 18.00. The adrenal glands were quickly fixed either in ethanol-cloroform-acetic acid solution (6/3/1 V/V) for 3 h or in potassium bichromate 5% and formol 10%, in acetate buffer pH 5.8 for 12 h, in order to identify the chromaffin cells. Serial sections were cut at 5 μ thickness and stained with hematoxilin and eosin. Each section was accurately scanned under the microscope at a magnification of X 1000.

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