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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It is possible to find some mitoses in the chromaffin cells. In the entire adrenal medulla, there are 12–16 in 60–80 days old animals. This number is reduced to 6–7 in 350-day-old animals.

The mitoses appear frequently condensed in small zones, sometimes they are located near the cortex, sometimes in the center of adrenal medulla; however they do not show a particular localization.

Most of the dividing cells observed are in prometaphase, the remaining in anatelophase. Only 2–4 mitoses of connective cells can be found in the entire adrenal medulla. No mitoses of the ganglion cells, which can be found in small clusters between the adrenal medullary cells, have been observed.

Circadian rhythm of mitoses in albino rats treated with colchicine 3 h before sacrifice

h of day	No. of animals	No. of mitoses in Wistar rats	No. of mitoses in Italico rats
03.00	1	12	13
	2	14	16
	3	12	12
09.00	1	20	27
	2	19	37
	3	18	32
12.00	1	32	33
	2	35	36
	3	34	35
18.00	1	29	36
	2	21	25
	3	18	27

In the 60–80-day-old animals treated for 3 h with colchicine, the number of mitoses varies depending upon the hour of the day at which the animal is killed. In animals killed at 03.00 the number of mitoses ranges from 12–16 and increases throughout the day. A maximum is reached at noon. The mitoses (almost all metaphases) present the same distribution as that found in non-treated rats. The medullary cells in cariocinesis show the chromaffin reaction, although to a lesser degree than the quiescent ones.

These observations show that mitoses are present in adrenal medullary cells, although in a very small number.

Their number varies at different time of the day, showing a peak at noon. This finding can be interpreted as evidence of circadian rhythm of mitotic activity, as observed in other tissues.

The presence of the chromaffin reaction shows that the cells in mitoses are differentiated elements of the adrenal medulla[11,12].

Riassunto. Nella presente ricerca si dimostra che nella midollare surrenale di ratto adulto, esaminata completamente con sezioni in serie, esistono mitosi delle cellule cromaffini. Se ne stabilisce il numero ed i rapporti con il ritmo nictemerale.

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[11] We wish to thank Prof. E. PUCCINELLI for stimulating discussion during the work and the preparation of the manuscript. We are also indebted to Miss G. VALLINI for valuable technical assistance.

[12] This investigation was supported by grant to this department from the Consiglio Nazionale delle Ricerche (No. 115/487/375).

The Generation Time of Two Day Chick Neuroepithelial Cells

Neuroepithelial cells in the chick embryo synthesize DNA in the depth of the neural tube wall, following which they round up to the lumen to divide and then elongate back into the wall (MARTIN and LANGMAN[1]). The duration of the above pattern has been investigated recently, however the results obtained have varied widely (FUJITA[2], ATLAS and BOND[3] and KAUFFMAN[4]). The present study was undertaken therefore to investigate the duration of the cell cycle in neuroepithelial cells of the 2 day chick embryo.

Materials and methods. White leghorn chick eggs, incubated at 101 °F for 48 h, were removed from the incubator, and an opening made in the shell over the embryo. Three drops of thymidine H^3, concentration 10 μc/ml, were dropped onto the embryo, the opening in the shell closed and the eggs returned to the incubator. At half hour intervals following treatment, embryos were removed, fixed, dehydrated and embedded in Paraplast, serially sectioned at 5 μ, prepared for radioautography by the coating method of KOPRIWA and LEBLOND[5], and exposed for 5–7 days.

Results. The percentage of labeled mitotic figures, plotted against time between treatment with thymidine H^3 and fixation, is represented in Figure 1. The following values were found: G2 = 1.5 h, M = 1.0 h and a generation time of 10.5 h which agreed with a 10 h cell cycle found in an earlier study using colchicine (MARTIN[6]). By extrapolating the descending curve, assuming the rate of descent equals the rate of ascent, the time between the midpoints of the curves, equal to 8.5 h, gave the duration of the 'S' stage. When the values of G2 + M + S were compiled however the value of 11 h was found to be greater than the generation time of 10.5 h. Furthermore, according to the above method the G1 stage of the cell cycle was completely non-existent.

To investigate the above problems grain counts were made on 50 labeled mitotic figures at each time interval used to produce the preceeding curve. The average grain count/mitosis is represented in Figure 2. Assuming that cells incorporate thymidine H^3 at a constant rate through-

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