The Incorporation of H^3-Thymidine in the Nuclei of the Cells of Adrenal Medulla of Rats[1]

It is known that adrenal medulla cells of adult animals are considered to be in an irreversible postmitotic state, like the nerve cells of the central nervous system (COWDRY[2]). According to MITCHELL[3], in the adrenal medulla of adult rats no mitoses are present, while they are present in young animals up to the 30th day of life.

The investigations of REICHARD and ESTBORN[4] and of FRIEDKIN et al.[5] have shown that during DNA synthesis H^3-thymidine is incorporated in the nuclei, which can be revealed by autoradiography.

The present investigations have been performed with the aim of ascertaining whether synthesis of DNA takes place in adrenal medulla cells in any of the following situations: (a) in the young rat; (b) in the adult rat; (c) in rats exposed to strong, long-lasting stimulation of the adrenal medulla. The stimulation was performed by exposing the animals to low temperature. In a preceding investigation[6-7], it was found that in such a situation considerable changes in the DNA content of the adrenal medulla nuclei take place, which suggests the possibility of DNA synthesis.

Four groups of albino male rats of the Italico strain were used. The first group consisted of 10-day-old animals; the second of normal adult animals; the third of adult animals exposed to cold 15 h a day for a total period of 100 and 300 h; the fourth group consisted of animals kept at room temperature for various periods (up to 10 days) after 300 h of exposure to cold.

H^3-thymidine (1 μc/g body weight) was given to each animal in single or repeated injections (see Table I). The animals were killed 8 h after the last injection. The adrenal glands were dissected. One gland was fixed in 95% alcohol and acetic acid, dehydrated and embedded in paraffin wax, together with an adrenal and a fragment of kidney from a normal animal.

Table I. % of the mitosis and the marked nuclei in adrenal medullas of 10-day-old rats

Counted nuclei	Mitosis	Mitotic index (% of cells in division)	Counted nuclei	Labelled nuclei	% of labelled nuclei
8000	70	0.87	4109	57	1.39
8000	97	1.21	5000	51	1.02

Table II. % of the labelled nuclei in the rat adrenal medulla during recovery after 300 h of intermittent exposure to cold

Room temperature time in h	Smears			Sections		
	Counted nuclei	Labelled nuclei	% of labelled nuclei	Counted nuclei	Labelled nuclei	%
8	8000	56	0.70	371	4	1.08
9	3000	22	0.73	1300	10	0.77
10	3000	38	1.26	1000	19	1.90
32	8000	113	1.41	860	20	2.32
33	8000	198	2.47	1500	17	1.13
56	8000	607	7.60	546	31	5.67
56	3000	60	2.00	2000	40	2.00

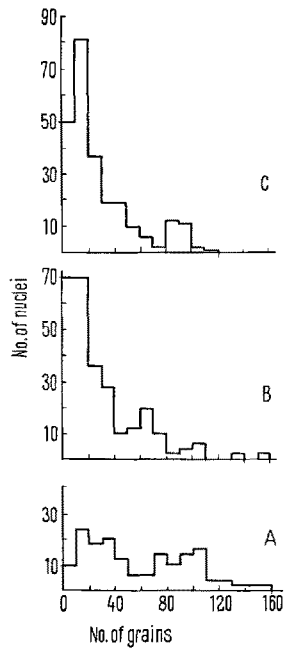
From the other gland, the adrenal medulla was dissected according to a technique previously described[7], and smeared on a portion of a slide on which also nuclei of the control adrenal medulla and kipney were smeared.

The smears and the sections (12 μ thick) were stained according to the method of FEULGEN[8]. They were thereafter covered with a thin film of photographic emulsion (G_5 Ilford) according to CARO[9] and exposed for 10 days in a lightproof box at + 4°C.

The slides were then developed with Kodak D.80 for 2 min, rinsed in a bath of 1% acetic acid, fixed in Kodak Rapid Fixer for 10 min, and washed in tap water for 1 h. The background was scarce and amounted to 1–1.5 grains/nucleus.

Labelled nuclei were found in the adrenal medulla of young rats, whereas no incorporation was observed in the normal adult rats.

Adult rats exposed to cold for 100 h and 300 h did not show labelled nuclei within the adrenal medulla. If, after the exposure to cold for 300 h, they were allowed a period at room temperature, clear-cut labelling of adrenal medulla nuclei was noticed.



Histograms on the basis of the number of silver grains of the labelled nuclei of adrenal medulla cells of rats exposed intermittently to cold for 300 h and kept at room temperature for 8–10 (A), 32–33 (B) and 56 (C) h.

[1] This investigation was supported by a grant to this Department from the Consiglio Nazionale delle Ricerche (No. 04/76/4/3482).

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The search for mitosis on the sections of the adrenal medulla of young rats treated with colchicine demonstrates the occurrence of mitotic figures in a percentage that agrees with that of labelled nuclei (Table I). In adult rats no mitosis was ever found, either in normal or experimental conditions, i.e. during cold exposure and recovery period.

These experiments show that the adrenal medulla cells in the adult animal have irreversibly lost the ability to multiply, but are still capable of synthesizing DNA if subjected to appropriate stimuli.

After the marked decrease induced by exposure to cold[7], synthesis of DNA ex novo takes place when the animals are kept at room temperature (Table II). This is in full agreement with the results of histophotometric determination[10]. After 8–10 h of recovery, the nuclei generally show numerous grains and only a few are weakly marked. In the following hours (up to 56 h), the nuclei are weakly marked (Figure). This means that a rapid synthesis takes place in the first hours of exposure to room temperature and slow synthesis in the following hours. No labelled nuclei are found when H^3-thymidine is given after 5 or 10 days at room temperature.

It can be concluded that the decrease of the content of DNA/nucleus, observed during exposure to low temperature, is real and that during the recovery period a synthesis ex novo of DNA takes place in the nuclei in the absence of any mitotic process[11].

Riassunto. In questa ricerca si dimostra sintesi di DNA con l'uso di timidina H^3 non accompagnata a mitosi nella midollare surrenale di ratto adulto in seguito ad esposizione a bassa temperatura.

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[10] M. P. VIOLA-MAGNI, submitted to J. Cell Biol.

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Effetto della ouabaina sul tessuto nervoso in coltura in vitro

Dalla letteratura recente (BELKIN et al.[1], YANG et al.[2]) sono noti vari casi di vacuolizzazione di cellule in coltura (fibroblasti di pollo, cellule ascitiche, cellule HeLa) provocata da sostanze di natura diversa, quali gli anestetici del gruppo della procaina, l'atropina, l'efedrina, la pilocarpina ed i sali di basi deboli quale il cloruro d'ammonio. Vacuolizzazioni naturali sono state riscontrate in alcune forme morbose quali la *malattia di Jakob-Creutzfeldt*, il *Kuru* (umane) e lo *Scrapie* (degli ovini). Recentissime ricerche eseguite nel nostro Istituto (BIGNAMI e PALLADINI[3–5]) hanno permesso di constatare l'identità delle alterazioni cerebrali morfologiche ed elettroencefalografiche (EEG) della malattia di Jakob-Creutzfeldt con quelle ottenute trattando animali (ratti, cavie) con iniezioni intracraniche di un glucoside, la *ouabaina* (g-strofantina). In entrambi i casi, associata alla comparsa di un *ritmo periodico* EEG, si ha uno *stato spongioso* di zone corticali. Microscopicamente lo stato spongioso, sia naturale che artificialmente provocato, consiste nella vacuolizzazione della cosidetta «sostanza fondamentale» della sostanza grigia corticale.

Recentissime osservazioni di GONATAS et al.[6] studiando tale tessuto di casi di malattia umana al microscopio elettronico, hanno accertato che lo stato spongioso è dovuto a rigonfiamento di processi cellulari di astrociti e di neuroni. A questo quadro si accompagna la sparizione del reticolo endoplasmico e dei ribosomi; non vi sarebbero alterazioni a carico dei mitocondri; gli astrociti colpiti sono ipertrofici. Vi sarebbero inoltre varie alterazioni della mielina, del pericarion e dei bottoni sinaptici. Va ricordato che questi dati sono stati ricavati da materiale bioptico e quindi inevitabilmente alterato.

È ormai chiaramente dimostrato (SKOU[7,8], BECKER et al.[9], GARDOS[10], BONTING[11], POST e SEN[12] e molti altri) che la g-strofantina (ouabaina) è un potente inibitore di un complesso enzimatico di membrana, l'ATP-asi Na^+-K^+ dipendente, deputato al trasporto attivo di acqua e cationi, studiato nel sistema nervoso da TORACK e BARNETT[13,14]. Dalle osservazioni di JUDAH e AHMED[15] risulta che questo farmaco ha una azione elettiva sulla ATP-asi della membrana degli elementi del tessuto nervoso. A seguito degli incoraggianti risultati ottenuti da BIGNAMI e PALLADINI in vivo ed in toto, mentre proseguono le ricerche tendenti a chiarire nella spongiosi indotta a quali elementi della 'sostanza fondamentale' vada riferita la vacuolizzazione, essendo ancora non molto probatorie le osservazioni succitate su materiale bioptico umano, si è ritenuto importante accertare l'effetto del glucoside sugli elementi di tessuto nervoso coltivato in vitro, sia per la

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