Virchows Arch. B Cell Path. 26, 195–214 (1978)

Virchows Archiv B
© by Springer-Verlag 1978

An Ultrastructural, Morphometric and Autoradiographic Study of the Effects of 7,12-Dimethylbenzanthracene on the Rat Adrenal Cortex

Anna S. Belloni, Giuseppina Mazzocchi, Claudia Robba, Anna M. Gambino and Gastone G. Nussdorfer*

Department of Anatomy, Laboratory of Electron Microscopy, University of Padua, I-35100 Padua, Italy

Summary. The effects of chronic treatment (up to 9 consecutive days) with 7,12-dimethylbenzanthracene (DMBA) on the adrenal glands of adult male Wistar rats were investigated. Morphometry showed that DMBA provokes atrophy of the zona reticularis which was due to the decrease in both cell volume and number. The zona fasciculata showed only a decrease in the cell volume, whereas the zona glomerulosa did not display any significant changes. Autoradiography demonstrated that DMBA induces a significant increase in the number of mitoses and "S" phase cells in the zona glomerulosa and outer zona fasciculata, which may be interpreted as a repair mechanism of the DMBA-provoked slight necrosis in the inner adrenocortical layers. The mechanism(s) underlying the cytotoxic effect of DMBA is discussed in the light of our ultrastructural observations showing that the chemical causes a decrease in the volume of the mitochondrial and nuclear compartments and in the surface of smooth endoplasmic reticulum as well as an increase in the volume of the lipid compartment.

Key words: Adrenal cortex — 7,12-dimethylbenzanthracene — Electron microscopy — Stereology — Rat.

Introduction

Several years ago Huggins and Morii (1961) reported that the intravenous administration of 7,12-dimethylbenzanthracene (DMBA) induced massive necrosis of the inner adrenocortical zones in adult female Sprague-Dawley rats. Similar results were not obtained in young animals (Morii and Higgins, 1962).

* The authors would like to thank Miss A. Coi and Mr. G. Gottardo for their technical and photographic assistance. Special thanks are also due to Prof. R. Mitchell for reading the manuscript. This work was partly supported by a contract with CNR (Italy) (CT.74.00226/115.3439)

For offprints contact: G.G. Nussdorfer, Istituto di Anatomia Umana Normale, Via Gabelli, 65, I-35100 Padova, Italy

0340-6075/78/0026/0195/$04.00

Although many investigators considered this phenomenon to be sex and strain specific (Cefis and Goodall, 1965; Marchant, 1967), Horvath et al. (1969) more recently described DMBA-induced adrenal necrosis in adult Wistar rats.

The DMBA-provoked ultrastructural changes in rat adrenocortical cells have also been investigated. Horvath et al. (1969) described the short-term effects of a single oral administration of 50 mg of DMBA in sunflower oil, whereas Murad et al. (1973) studied the long-term effects of 3 intravenous injections of 2 mg of DMBA. Horvath and co-workers (Horvath et al., 1969) claimed that the adrenolytic effect of DMBA must be caused by a primary damage to the endothelial wall of the adrenal sinusoids; Murad et al. (1973), on the contrary, in agreement with the earlier hypothesis (Higgins and Morii, 1961), suggested a direct cytotoxic effect of the chemical on adrenocortical cells. Contrasting findings were also reported for the mitogenic activity of DMBA on the rat adrenal cortex: Murad et al. (1973) demonstrated mitoses in all 3 zones of the gland, while Wheatley (1967) and Danz et al. (1973) found mitoses only in the outer adrenocortical layers.

Therefore, it seemed worthwhile to re-investigate the effects of DMBA on the ultrastructure and mitotic activity of adrenocortical cells of adult male Wistar rats.

Materials and Methods

Male adult albino rats (Wistar-derived) of about 200 g were used.

Experiment I. Forty-eight animals were divided into 8 groups. Six groups received i.p. injections of 25 mg/Kg of DMBA (Sigma Chemical Company, St. Louis, Mo, USA) dissolved, according to Amlacher et al. (1974), in 0.5 ml of dimethylsulphoxide (DMSO) (Sigma) for 1, 3, 4, 5, 6 and 9 consecutive days, respectively. The other 2 groups served as controls, of which the first received i.p. injections of 0.5 ml of DMSO for 9 consecutive days, and the second i.p. injections of 0.5 ml of normal saline for the same period.

The animals were sacrified by cervical dislocation. Each rat's right adrenal was fixed in Bouin's fluid for 12 h and embedded in paraffin. For each gland 7 µm-thick sections were cut serially and stained with hematoxylin-eosin. Fragments of the left adrenal of each animal were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (Sabatini et al., 1963), post-fixed in 1% OsO_4 in 0.1 M phosphate buffer (Millonig, 1961) and embedded in an epoxy resin (Lockwood, 1964). One µm-thick sections were made with an LKB III ultramicrotome, stained with toluidine blue and observed with the light microscope to select the 3 adrenocortical zones. Thin sections were counterstained with lead-hydroxide (Karnovsky, 1961) and examined in a Hitachi HS-9 electron microscope.

For morphometric assessments the sampling procedure used was that described elsewhere (Nussdorfer et al., 1973, 1974). On the light micrographs of the largest paraffin sections the volume of the adrenal gland and of the zona glomerulosa were calculated as described previously (Nussdorfer et al., 1973). On the same light micrographs the percent of the gland volume occupied by the zonae fasciculata, reticularis and medullaris was estimated by the method of "differential point counting" (Weibel, 1969). By multiplying these relative values by the adrenal volume, the volume of the 3 zones were then obtained. The volume of the cells from the 3 adrenocortical zones and also the percentile volume occupied in each zone by the extracellular space were calculated on light micrographs of the 1 µm-thick sections at a final magnification of 1,250 (Nussdorfer, 1970). The number of parenchymal cells in each adrenocortical zone was then determined by dividing the volume of the zone (minus that occupied by the extracellular space) by the cell volume.

On electron micrographs at a final magnification of 18,000 the cell volume percent occupied by the nuclei, mitochondria, lipid droplets and "membrane space" (i.e., the cellular space occupied by

the membranes of the smooth endoplasmic reticulum, SER, including Golgi apparatus) (Loud, 1962) was determined by the method of "differential point counting" (Weibel, 1969) and the numerical density of the mitochondria (i.e., the number of mitochondria per μm^3 of cell) was calculated according to Nussdorfer et al. (1974) and Mazzocchi et al. (1977). On electron micrographs at a final magnification of 40,000, the surface concentration of the SER tubules and mitochondrial cristae (i.e., μm^2 of SER membranes or mitochondrial cristae per μm^3 of cell) were evaluated by the "crossing method" (Loud, 1962). By multiplying these relative stereologic parameters by the mean cell volume, the absolute amount of the various subcellular parameters were then obtained. The average volume of single mitochondria was calculated by dividing the volume of the mitochondrial compartment by the number of mitochondria per cell.

Experiment II. Thirty-two rats were divided into 8 groups and treated as in Experiment I. Two h before sacrifice the animals were given 4 µCi/g of 3H-thymidine (Specific activity, 15Ci/mM, New England Nuclear Corporation, Frankfurt/M, W-Germany), intraperitoneally.

The adrenal glands of each animal were processed for optical microscopy as detailed in Experiment I. Seven µm-thick sections were processed for autoradiography by dipping the slides into Ilford K5 nuclear emulsion (1:4 diluted with double distilled water and melted at 50°C). The specimens were allowed to dry for 2 h on metal grids inclined at 45° and stored at 4°C in a light-proof desiccator for 21 days. The autoradiographs were developed in Kodak D-19 and stained with hematoxylin-eosin.

The number of 3H-thymidine positive parenchymal cells ("S" phase cells) and of mitoses in each adrenal zone were counted under the light microscope in 7 randomly chosen specimens from each rat. The specimens were then photographed and the percentage of the total area of the micrograph occupied by the various adrenal zones was obtained by the method of "differential point counting" (Weibel, 1969). The number of mitoses or "S" phase cells per μm^2 of surface area of the various adrenal zones was thus determined.

Statistical Treatment of Results. The data obtained from each rat were averaged per experimental group and the standard error was calculated. Student's t-test was used for the statistical comparison of the data: the difference between two mean values was considered to be significant if the probability of error (p) was found to be less than 0.05. The fitting of the curves (morphometric or autoradiographic parameters versus time) was performed by the least square analysis. A two-sided t-test (Armitage, 1971) was run to determine whether the slopes differed significantly from each other. All the statistical procedures were done with an electronic microcomputer (Olivetti P652/ROM01 – MLU600).

Results

Macroscopic Observations

Autopsy (1) of the control rats treated with either DMSO or normal saline did not reveal any obvious alteration, and (2) of animals treated with DMBA for 1 or 3 days showed marked peritoneal hyperemia and atrophy of the adrenal glands. After the 4th day of treatment the liver was pale and hyperemic and the intra- and retroperitoneal tissues showed miliary fat necroses, as described earlier by Danz et al. (1973); the adrenals were enlarged. The longer the treatment continued, the more severe the lesions became; in those animals which had received DMBA for 9 consecutive days, ascites was conspicuous.

Histologic Observations

No significant qualitative nor quantitative changes were observed in the adrenal glands of the control rats (Tables 1 and 2). In the DMBA-treated animals no

Table 1. Effects of DMBA on the volume of the rat adrenal gland

Treatment	Volume of gland (mm^3)	Volume of zona glomerulosa (mm^3)	Volume of zona fasciculata (mm^3)	Volume of zona reticularis (mm^3)	Volume of zona medullaris (mm^3)
None (6)	19.12±2.03	2.16±0.39	13.06±1.40	2.84±0.39	0.96±0.22
DMSO (6)	18.87±1.99	2.14±0.38	12.94±1.54	2.78±0.41	1.01±0.26
P_0	NS	NS	NS	NS	NS
1-Day (6)	15.86±1.61	1.92±0.30	10.67±1.01	2.34±0.32	0.89±0.20
P_0	<0.01	NS	<0.01	<0.02	NS
3-Days (6)	15.70±1.58	1.90±0.29	10.51±1.03	2.37±0.29	0.96±0.21
P_0	<0.01	NS	<0.01	<0.05	NS
4-Days (6)	15.82±1.70	2.01±0.43	10.70±1.11	2.05±0.31	1.06±0.23
P_0	<0.01	NS	<0.01	<0.02	NS
P_3	NS		NS		
5-Days (5)	16.54±1.71	1.94±0.36	11.74±1.09	1.85±0.23	1.01±0.22
P_0	<0.02	NS	<0.05	<0.01	NS
P_3	NS		<0.05		
6-Days (6)	16.72±1.69	2.04±0.41	12.12±1.23	1.41±0.18	0.95±0.23
P_0	<0.02	NS	NS	<0.01	NS
P_3	NS		<0.01		
9-Days (5)	17.75±1.82	2.17±0.47	13.51±1.42	1.09±0.15	0.98±0.21
P_0	NS	NS	NS	<0.01	NS
P_3	<0.05		<0.01		

The number of animals in each group is indicated in parentheses. Each value represents the group mean ±SE. P_0, level of significance of the difference from the control normal saline-treated group; P_3, level of significance of the difference from the 3-days DMBA-administered rats. NS, not significant

evidence of massive necrosis of the inner portion of the gland was found, although numerous pyknotic nuclei were present in the zona reticularis particularly after 6 or 9 days of treatment.

Quantitative evaluations (Table 1) showed that the volume of the gland decreased significantly up to the 3rd day of treatment and thereafter increased linearly from the 4th to the 9th day of DMBA-administration. The volume of the zona glomerulosa and zona medullaris did not display significant changes, and that of the zona fasciculata closely followed the variations in the volume of the entire gland. The volume of the zona reticularis decreased linearly with the number of days of DMBA-administration (Fig. 1A). The effects of DMBA on the cell volume varied according to the adenocortical zone (Table 2): the volume of the cells in the zonae fasciculata and reticularis decreased linearly. However the rate of decrease in this parameter in the zona reticularis was significantly higher ($P < 0.01$) than in the zona fasciculata (Fig. 1B). The number of cells (Table 2) in the zona glomerulosa did not show significant variation, while that in the zona fasciculata after a significant decrease up to the 3rd-4th day of treatment, increased linearly. In contrast, this parameter in the zona reticularis decreased linearly as a function of the duration of DMBA-administration (Fig. 1C).

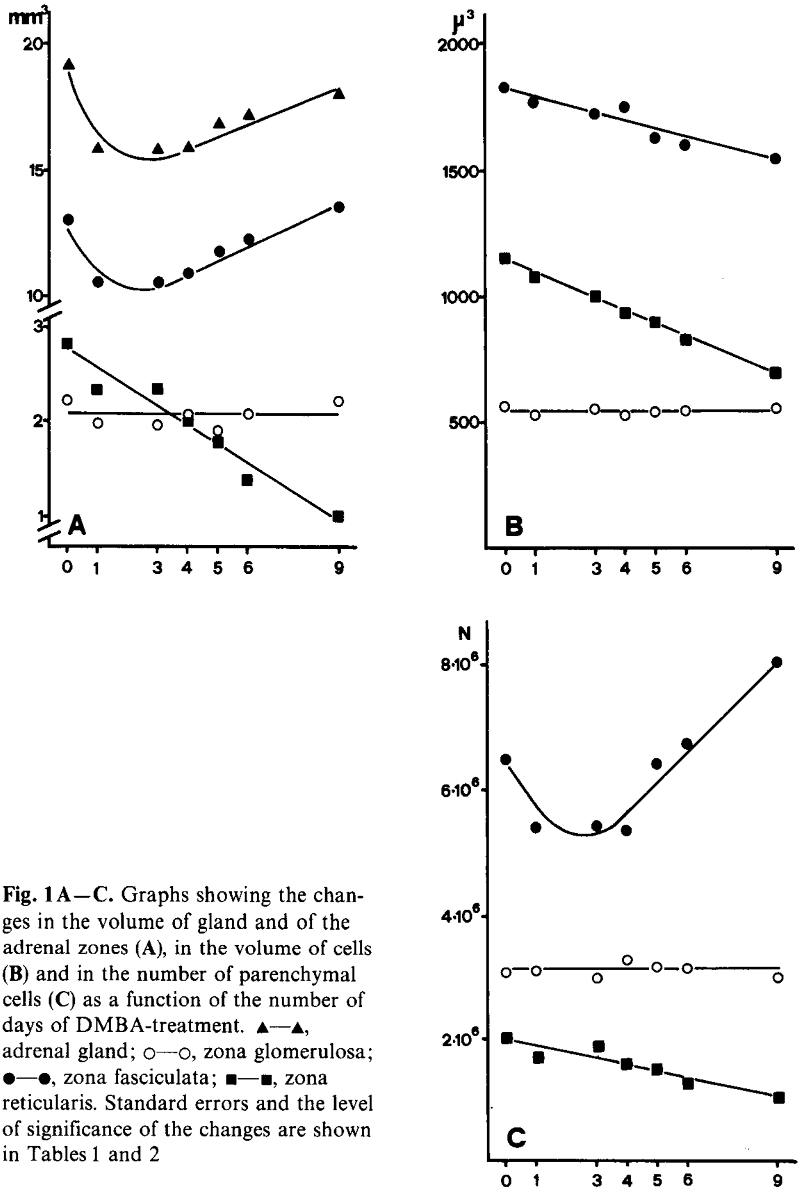


Fig. 1 A—C. Graphs showing the changes in the volume of gland and of the adrenal zones (**A**), in the volume of cells (**B**) and in the number of parenchymal cells (**C**) as a function of the number of days of DMBA-treatment. ▲—▲, adrenal gland; o—o, zona glomerulosa; ●—●, zona fasciculata; ■—■, zona reticularis. Standard errors and the level of significance of the changes are shown in Tables 1 and 2

Autoradiographic Observations

Neither mitoses nor "S" phase cells were found in the zona reticularis and zona medullaris. Mitoses and "S" phase cells were observed only in the zona glomerulosa and outer zona fasciculata (Fig. 2) of both control and treated rats. There were no significant differences between DMSO- and normal saline-treated animals. The number of mitoses and thymidine-positive cells increased linearly

Table 2. Effects of DMBA on the volume and number of rat adrenocortical cells

Treatment	Volume of cells (μm^3)			Number of cells		
	Zona glomerulosa	Zona fasciculata	Zona reticularis	Zona glomerulosa	Zona fasciculata	Zona reticularis
None (6)	567.5 ± 66.0	1,817.6 ± 176.5	1,151.6 ± 101.6	3,124,757 ± 346,127	6,466,887 ± 670,423	2,022,229 ± 206,221
DMSO (6)	542.8 ± 70.5	1,856.3 ± 175.4	1,149.5 ± 107.2	3,351,142 ± 357,834	6,273,770 ± 668,112	1,983,123 ± 207,136
P_0	NS	NS	NS	NS	NS	NS
1-Day (6)	538.8 ± 54.0	1,771.6 ± 169.6	1,087.5 ± 99.8	3,136,001 ± 323,121	5,420,338 ± 500,195	1,764,413 ± 161,097
P_0	NS	NS	NS	NS	<0.01	<0.01
3-Days (6)	550.2 ± 60.2	1,726.7 ± 171.4	1,000.8 ± 98.7	3,040,007 ± 361,243	5,478,034 ± 561,274	1,894,448 ± 176,195
P_0	NS	NS	<0.01	NS	<0.01	NS
4-Days (6)	536.9 ± 56.7	1,760.2 ± 168.3	953.4 ± 96.1	3,318,452 ± 351,180	5,355,002 ± 507,821	1,677,155 ± 158,842
P_0	NS	NS	<0.01	NS	<0.01	<0.01
5-Days (5)	540.3 ± 52.3	1,638.8 ± 159.9	921.0 ± 90.7	3,212,701 ± 312,197	6,447,273 ± 650,707	1,566,775 ± 148,072
P_0	NS	<0.05	<0.01	NS	NS	<0.01
P_4					<0.01	
6-Days (6)	544.9 ± 57.6	1,611.6 ± 150.7	831.7 ± 82.5	3,219,569 ± 318,623	6,768,315 ± 690,804	1,322,351 ± 127,778
P_0	NS	<0.02	<0.01	NS	NS	<0.01
P_4					<0.01	
9-Days (5)	568.2 ± 61.2	1,552.3 ± 151.4	712.6 ± 80.4	3,083,636 ± 307,961	8,088,041 ± 901,620	1,177,799 ± 107,264
P_0	NS	<0.01	<0.01	NS	<0.01	<0.01
P_4					<0.01	

The number of animals in each group is indicated in parentheses. Each value represents the group mean ±SE. P_0, level of significance of the difference from the control normal saline-treated group; P_4, level of significance of the difference from the 4-days DMBA-administered rats. NS, not significant

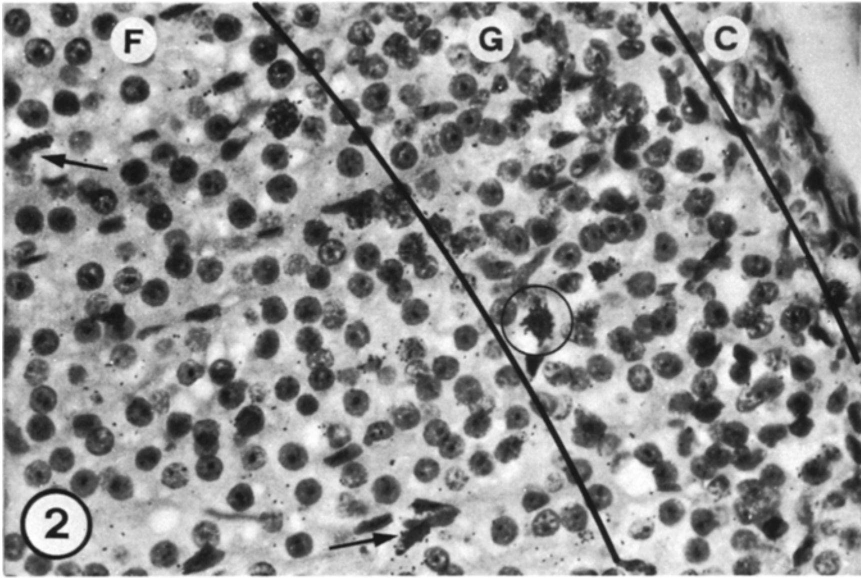


Fig. 2. Autoradiograph of the outer adrenocortical layers: *C*, capsule; *G*, zona glomerulosa; *F*, zona fasciculata. Many "S" phase cells are present in the zona glomerulosa and outer zona fasciculata. A mitosis is encircled. In the inner zona fasciculata thymidine-positive cells are mainly of the mesenchymal type (*arrows*). ×480

in the zona glomerulosa of the DMBA-treated rats (Fig. 3). Similar findings were obtained in the zona fasciculata, but the increase was significantly less conspicuous ($P<0.01$) than in the zona glomerulosa.

Ultrastructural Observations

The fine structure of the normal rat adrenocortical cells has been described in numerous previous papers (for review see Idelman, 1970), and will be considered here only briefly.

The cells of the zona glomerulosa contained elongated mitochondria with tubular cristae, few SER tubules, numerous free ribosomes and abundant lipid droplets. The cells of the zona fasciculata showed round mitochondria with vesicular cristae, and an abundant meshwork of anastomosed SER tubules intermingled with many free ribosomes. Lipid droplets and primary lysosomes were also quite numerous. In the zona reticularis the cells displayed round mitochondria containing both vesicular and tubulo-convolute cristae, abundant SER profiles and free ribosomes. The lipid droplets were scanty, whereas a fair number of primary lysosomes as well as some lipofuscinic pigment inclusions were found, especially in the cells located in the inner juxtamedullary portion of this zone.

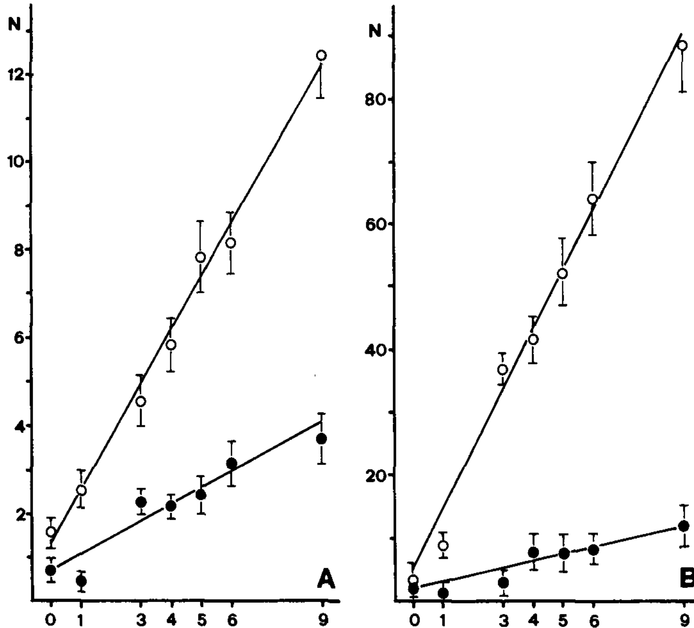


Fig. 3A and B. Graphs showing the increase in the number of mitoses (A) and "S" phase cells (B) per mm^2 of section area of the zona fasciculata (●—●) and zona reticularis (○—○) as a function of the duration of DMBA-treatment. Standard arrors are indicated

No qualitative nor quantitative changes were observed either in the DMSO- or in the 1 day DMBA-treated animals (Tables 3–6). The prolonged DMBA-treatment did not induce ultrastructural alterations in the cells of the zona glomerulosa (Table 3), except for occasional mitoses. After 3 days of DMBA-administration, qualitative ultrastructural changes were found both in the zona fasciculata and in the zona reticularis: lipid droplets seemed more abundant (Fig. 4) and giant mitochondria, some bizarre (Fig. 5), other regular in shape (Fig. 6) were observed. Often mitochondria contained electron-opaque intramatrical inclusions (Figs. 7 and 8). The Golgi apparatus was small and fragmented. These changes were increased by prolonged treatment and were most apparent in the zona reticularis cells. After the 4th day of treatment numerous mitochondria of degenerate appearance and an unusual number of autophagic vacuoles containing mitochondrial debris were found in both zones (Figs. 9 and 10). In the zona reticularis of the treated animals images suggesting cell degeneration and destruction were frequently seen. The affected cells showed shrinkage and condensation of nucleus and cytoplasm, resulting in a severe accumulation of lipid droplets and irregularly-shaped membrane-bound residual bodies (Fig. 11). These cells seemed to be deleted just into the subendothelial space, where they were ingested by pericytes (Fig. 12). No similar degenerate cells were observed within the sinusoids.

Stereologic data for the zona fasciculata and zona reticularis cells are shown in Tables 4, 5 and 6. The cells showed a significant linear decrease in the volume

Table 3. Morphometric parameters per single cell of rat adrenal zona glomerulosa after DMBA-treatment

Treatment	Volume of nuclei (μm³)	Volume of mitochondrial compartment (μm³)	Volume of lipid compartment (μm³)	Membrane space (μm³)	Surface of mitochondrial cristae (μm²)	Surface of smooth endoplasmic reticulum (μm²)
None (6)	86.4±9.3	99.6±10.1	60.8±7.0	322.5±39.7	806.1± 98.5	3,512.6±400.1
DMSO (6)	85.7±8.9	100.5±11.4	70.2±8.4	286.4±34.8	819.5± 96.0	3,330.9±381.3
P_0	NS	NS	NS	NS	NS	NS
1-Day (6)	83.2±9.0	101.0±11.3	56.5±5.3	298.1±30.6	901.3±103.5	3,129.7±405.8
P_0	NS	NS	NS	NS	NS	NS
3-Days (6)	79.8±8.7	95.4±10.2	57.2±6.6	317.8±32.2	859.4± 96.7	3,457.8±451.6
P_0	NS	NS	NS	NS	NS	NS
4-Days (6)	78.6±8.2	103.6±11.2	59.2±6.1	296.7±29.7	900.6±121.4	3,292.6±398.5
P_0	NS	NS	NS	NS	NS	NS
5-Days (5)	80.9±8.5	97.4± 9.5	61.8±5.8	309.2±30.7	861.5± 98.9	3,450.7±406.8
P_0	NS	NS	NS	NS	NS	NS
6-Days (6)	88.7±9.3	100.8± 9.8	60.4±5.6	295.1±30.2	898.4±100.1	3,310.1±407.9
P_0	NS	NS	NS	NS	NS	NS
9-Days (5)	86.5±8.7	97.4±10.2	56.5±6.1	327.8±39.4	872.5± 95.6	3,498.5±451.0
P_0	NS	NS	NS	NS	NS	NS

The number of animals in each group is indicated in parentheses. Each value represents the group mean ±SE. P_0, level of significance of the difference from the control normal saline-administered group. NS, not significant

Table 4. Morphometric parameters per single cell of rat adrenal zona fasciculata after DMBA-treatment

Treatment	Volume of nuclei (μm³)	Volume of mitochondrial compartment (μm³)	Volume of lipid compartment (μm³)	Membrane space (μm³)	Surface of mitochondrial cristae (μm²)	Surface of smooth endoplasmic reticulum (μm²)
None (6)	136.6±14.0	650.2±64.3	134.3±12.7	896.5±90.4	8,582.6±901.0	12,371.2±1,342.8
DMSO (6)	138.2±13.9	649.8±60.6	135.7±13.1	932.8±94.3	8,459.5±898.5	12,768.4±1,409.2
P_0	NS	NS	NS	NS	NS	NS
1-Day (6)	125.2±11.8	635.1±60.2	142.1±13.2	869.2±85.3	8,382.0±856.7	11,908.5±1,252.6
P_0	NS	NS	NS	NS	NS	NS
3-Days (6)	117.4±11.2	612.5±59.4	142.5±12.8	854.3±80.6	7,956.6±809.4	11,703.9±1,269.8
P_0	<0.01	NS	NS	NS	NS	NS
4-Days (6)	116.5±12.0	625.3±60.0	141.6±14.1	876.8±86.2	8,122.5±849.0	11,924.6±1,308.5
P_0	<0.01	NS	NS	NS	NS	NS
5-Days (5)	119.7±12.6	560.8±50.2	148.2±14.5	810.1±79.9	7,234.3±720.9	10,935.2±1,095.4
P_0	<0.02	<0.01	<0.05	<0.05	<0.05	<0.01
6-Days (6)	108.0± 8.6	545.1±51.8	150.5±16.2	807.3±79.8	6,977.2±706.2	10,622.4± 995.4
P_0	<0.01	<0.01	<0.01	<0.05	<0.01	<0.01
9-Days (5)	109.5± 9.0	506.4±52.4	169.7±17.4	757.6±68.3	6,380.6±678.2	10,070.2± 993.6
P_0	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

The number of animals in each group is indicated in parentheses. Each value represents the group mean ±SE. P_0, level of significance of the difference from the control normal saline-treated group. NS, not significant

Table 5. Morphometric parameters per single cell of rat adrenal zona reticularis after DMBA-treatment

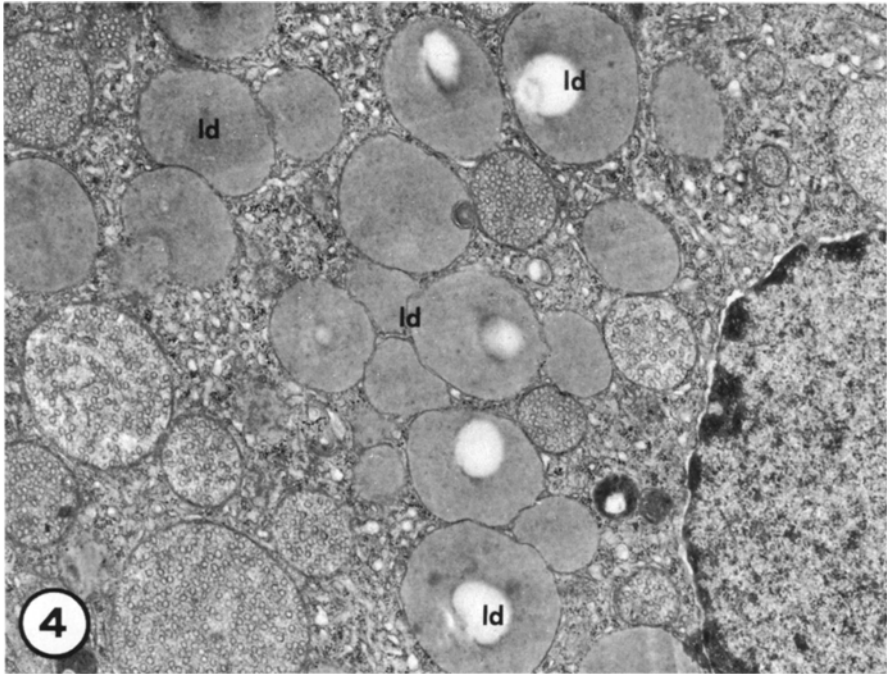
Treatment	Volume of nuclei (μm^3)	Volume of mitochondrial compartment (μm^3)	Volume of lipid compartment (μm^3)	Membrane space (μm^3)	Surface of mitochondrial cristae (μm^2)	Surface of smooth endoplasmic reticulum (μm^2)
None (6)	95.4 ± 9.0	500.2 ± 50.1	69.8 ± 6.5	486.2 ± 45.3	6,750.0 ± 700.8	6,806.8 ± 706.2
DMSO (6)	96.1 ± 10.5	510.4 ± 58.2	68.3 ± 7.2	474.7 ± 46.9	6,824.6 ± 734.3	6,790.1 ± 698.2
P_0	NS	NS	NS	NS	NS	NS
1-Day (6)	95.3 ± 9.8	462.3 ± 42.6	70.3 ± 7.1	459.6 ± 42.4	6,194.8 ± 599.0	6,388.4 ± 657.2
P_0	NS	NS	NS	NS	NS	NS
3-Days (6)	90.1 ± 10.6	385.2 ± 38.2	78.2 ± 7.4	447.3 ± 45.3	4,990.6 ± 507.6	6,123.9 ± 618.3
P_0	NS	<0.01	<0.05	NS	<0.05	<0.01
4-Days (6)	80.4 ± 9.1	362.4 ± 39.2	85.3 ± 8.6	425.3 ± 39.8	4,856.2 ± 497.6	5,784.1 ± 602.8
P_0	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
5-Days (5)	85.3 ± 8.4	320.1 ± 30.4	90.4 ± 8.4	425.2 ± 43.1	4,225.3 ± 432.4	5,800.5 ± 595.0
P_0	<0.05	<0.01	<0.01	<0.02	<0.01	<0.01
6-Days (6)	79.4 ± 7.6	300.8 ± 31.1	99.2 ± 9.0	352.4 ± 36.2	3,910.6 ± 382.7	4,757.4 ± 495.1
P_0	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
9-Days (5)	70.1 ± 6.9	240.3 ± 28.9	117.1 ± 12.5	285.1 ± 29.4	3,123.9 ± 322.5	3,734.7 ± 394.1
P_0	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

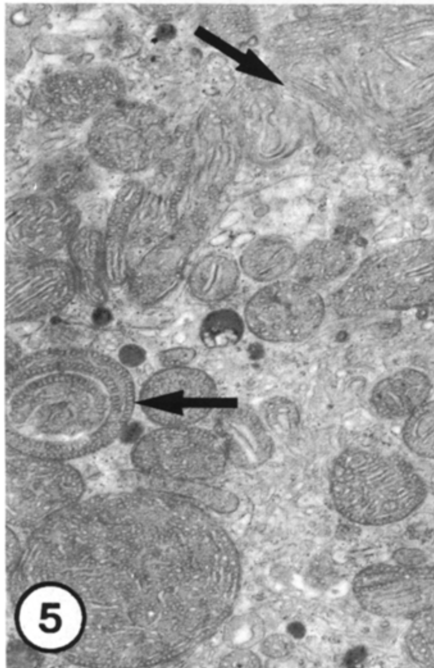
The number of animals in each group is indicated in parentheses. Each value represents the group mean ±SE. P_0, level of significance of the difference from the control normal saline-treated control group. NS, not significant

Table 6. Effects of DMBA on the volume and number of rat adrenocortical mitochondria

Treatment	Average volume of single mitochondria (μm^3)			Number of mitochondria per cell		
	Zona glomerulosa	Zona fasciculata	Zona reticularis	Zona glomerulosa	Zona fasciculata	Zona reticularis
None (6)	0.191 ± 0.020	0.548 ± 0.051	0.560 ± 0.057	521.5 ± 58.3	1,186.5 ± 120.6	893.2 ± 88.6
DMSO (6)	0.194 ± 0.019	0.551 ± 0.052	0.562 ± 0.063	518.0 ± 50.4	1,178.3 ± 119.5	908.1 ± 94.2
P_0	NS	NS	NS	NS	NS	NS
1-Day (6)	0.200 ± 0.022	0.552 ± 0.054	0.544 ± 0.055	505.2 ± 54.6	1,150.5 ± 112.4	849.8 ± 80.7
P_0	NS	NS	NS	NS	NS	NS
3-Days (6)	0.189 ± 0.019	0.550 ± 0.049	0.562 ± 0.052	504.7 ± 60.2	1,113.6 ± 109.5	667.5 ± 70.4
P_0	NS	NS	NS	NS	NS	<0.01
4-Days (6)	0.192 ± 0.023	0.531 ± 0.051	0.542 ± 0.051	539.6 ± 60.1	1,177.1 ± 120.6	668.6 ± 70.1
P_0	NS	NS	NS	NS	NS	<0.01
5-Days (5)	0.189 ± 0.020	0.500 ± 0.048	0.525 ± 0.050	515.3 ± 54.2	1,121.2 ± 107.4	609.7 ± 63.4
P_0	NS	<0.05	NS	NS	NS	<0.01
6-Days (6)	0.201 ± 0.026	0.505 ± 0.049	0.500 ± 0.048	501.5 ± 56.0	1,078.8 ± 98.8	601.6 ± 59.4
P_0	NS	<0.05	<0.05	NS	<0.05	<0.01
9-Days (5)	0.195 ± 0.021	0.482 ± 0.047	0.461 ± 0.047	499.4 ± 53.7	1,050.6 ± 99.4	521.7 ± 55.7
P_0	NS	<0.02	<0.02	NS	<0.05	<0.01

The number of animals in each group is indicated in parentheses. Each value represents the group mean ±SE. P_0, level of significance of the difference from the control normal saline-treated group. NS, not significant





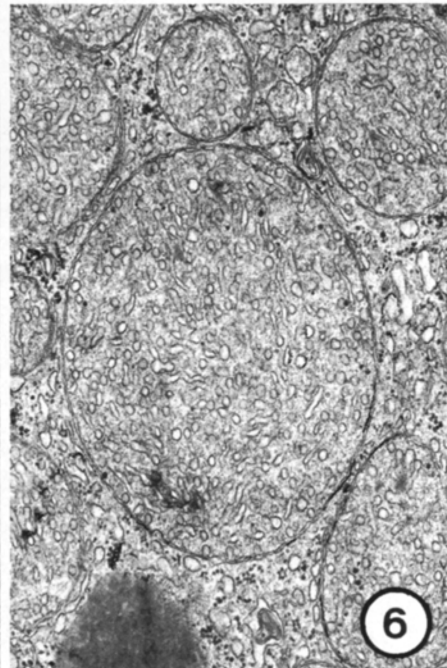
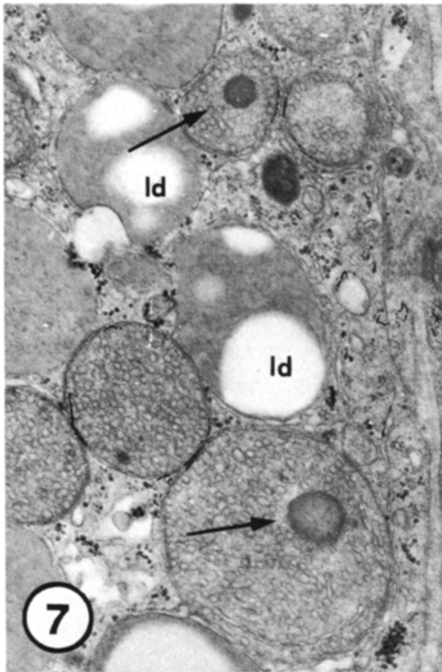
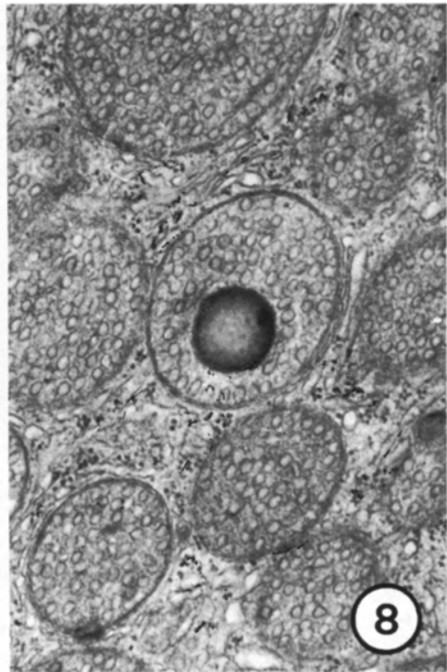


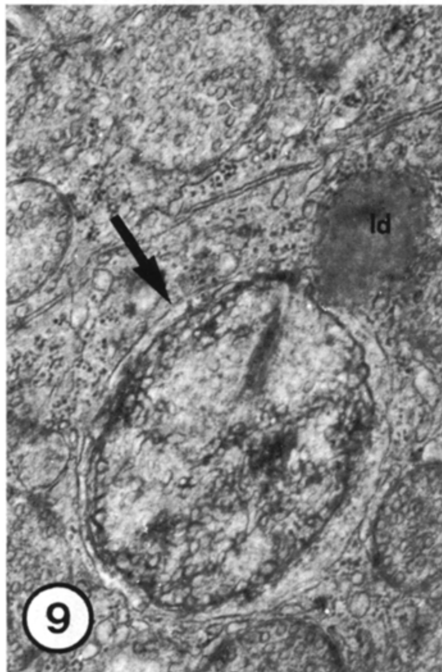
Fig. 4. Cell of the inner portion of the zona fasciculata of a 5 days-DMBA-treated rat. Lipid droplets (*ld*) are very numerous. ×15,000

Fig. 5. Zona reticularis of a 9 days DMBA-treated rat. Frequently pleomorphic giant mitochondria containing cristae arranged in bizarre patterns (*arrows*) are observed. ×12,000

Fig. 6. A regularly-shaped giant mitochondria (maximum diameter about 3 μm) in a cell of the inner portion of the zona fasciculata of a rat after 6 days of DMBA-administration. ×19,000







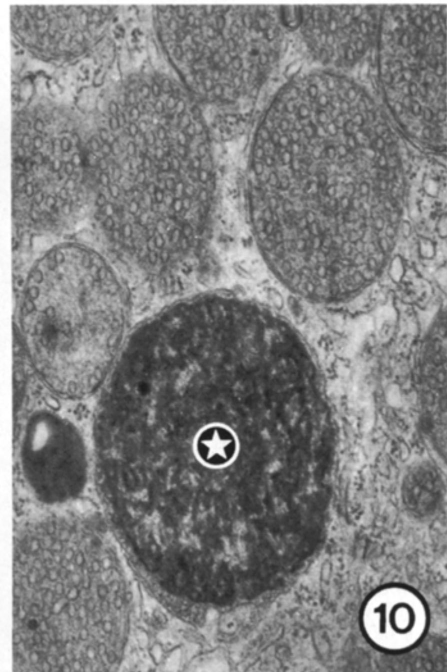
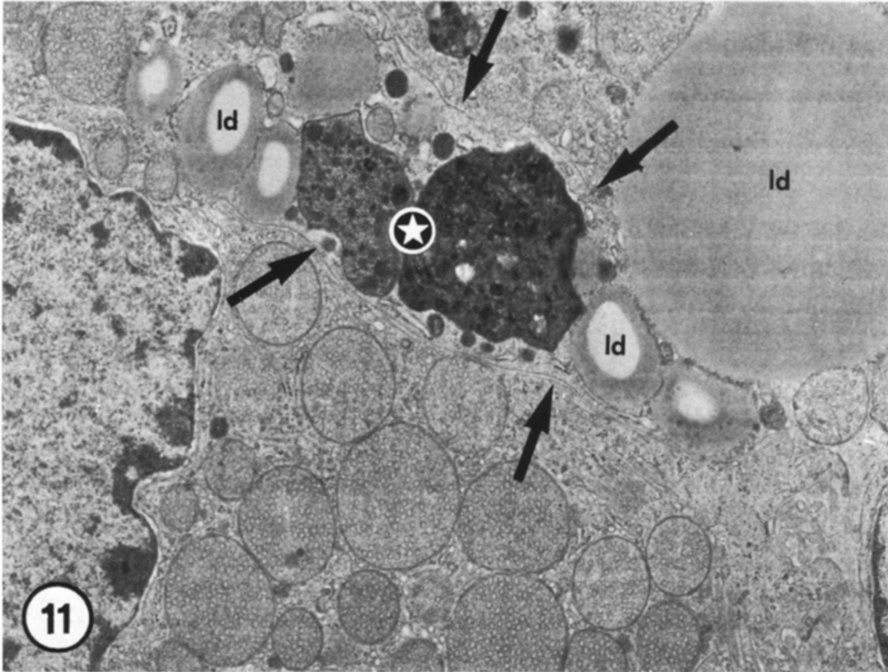


Fig. 7. Zona reticularis of a 4 days DMBA-treated rat. Mitochondria frequently contain intramatrical osmiophilic inclusions (arrows). *ld*, lipid droplets. ×21,000

Fig. 8. Mitochondrion with an osmiophilic inclusion in a zona fasciculata cell from a 3 day DMBA-treated rat. ×25,000

Fig. 9. The arrow indicates an autophagic vacuole containing mitochondrial debris in a zona reticularis cells of a 5 days DMBA-treated rat. ×26,000

Fig. 10. Zona fasciculata cell of a 6 days DMBA-treated rat. The asterisk marks a degenerate mitochondrion. *ld*, lipid droplet. ×26,000



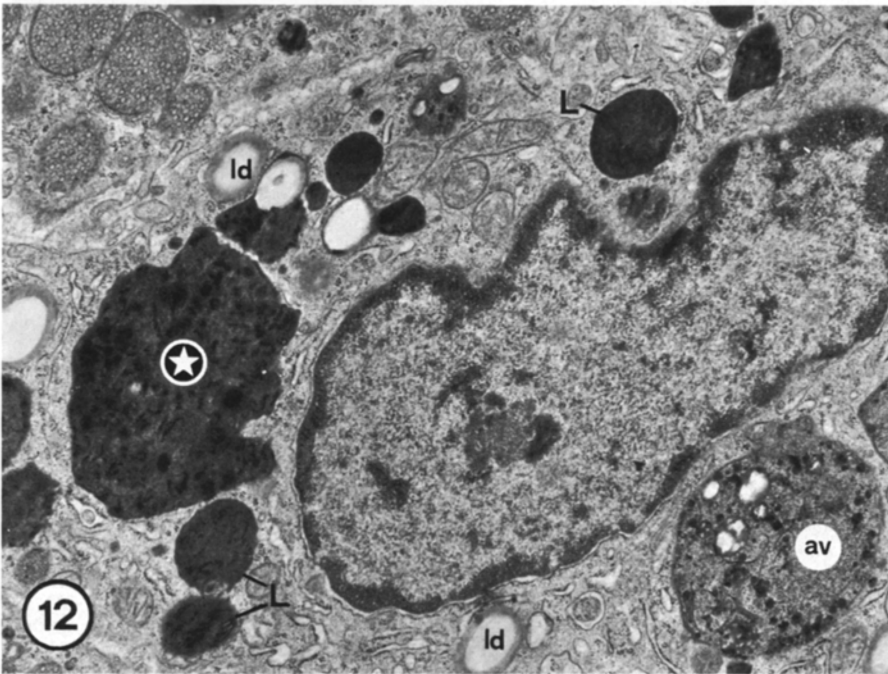


Fig. 11. Zona reticularis of a 6 days DMBA-treated rat. The arrow indicates a degenerate cell containing many lipid droplets (*ld*) and irregularly-shaped residual bodies (*asterisk*). The adjacent cell displays well preserved ultrastructural features. × 17,000

Fig. 12. Zona reticularis of a 9 days DMBA-treated rat: a macrophage containing fragments of adrenocortical cells. *ld*, liquid droplets; *L*, lysosomes; *asterisk*, residual body; *av*, autophagic vacuole. × 17,500

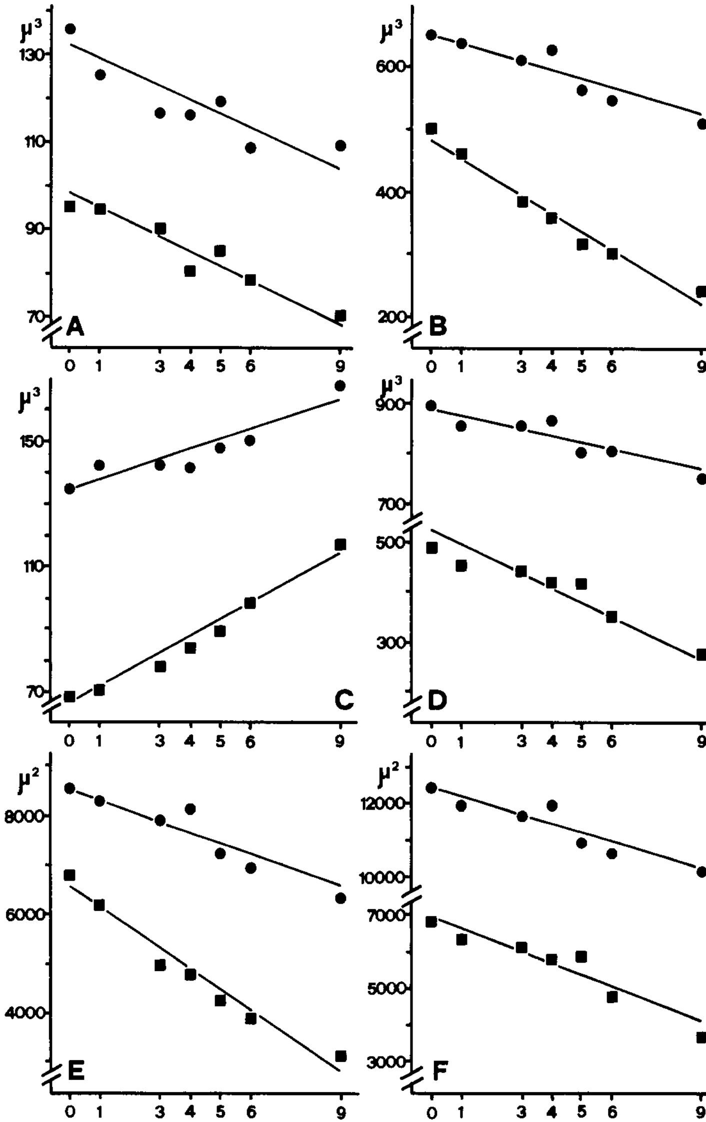


Fig. 13A–F. Graphs showing the changes in the volume of the nuclear compartment (**A**), the mitochondrial compartment (**B**), the lipid compartment (**C**), the "membrane space" (**D**), and in the surface of the mitochondrial cristae (**E**) and SER membranes (**F**) in the zona fasciculata (●—●) and zona reticularis cells (■—■) as a function of the number of days of DMBA-treatment. Standard errors and the levels of significance of the changes are shown in Tables 4 and 5

of the nuclei, mitochondrial compartment and "membrane space" as well as in the surface of SER tubules and mitochondrial cristae (Fig. 13A, B, D, E, F). In contrast, the volume of the lipid compartment increased linearly as a function of the number of days of DMBA-administration (Fig. 13C). These changes, except those concerning nuclei and SER, were found to be significantly more

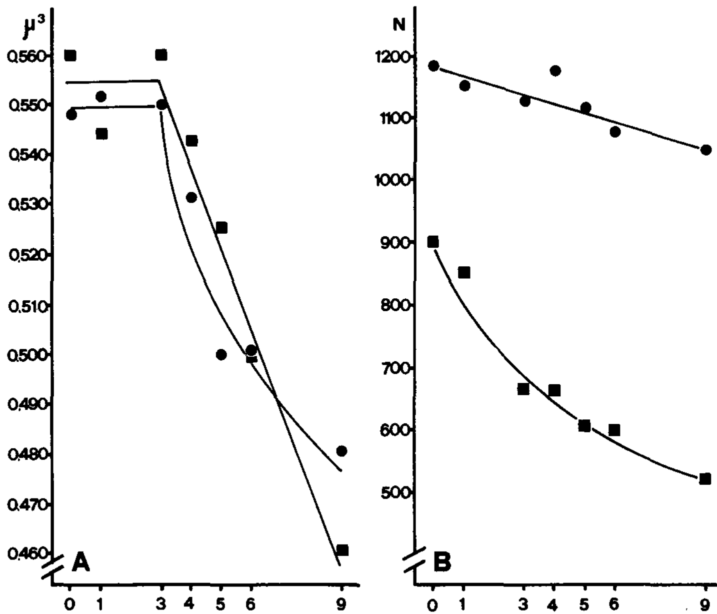


Fig. 14A and B. Graphs showing the changes in the average volume of single mitochondria (**A**) and in the number of mitochondria per cell (**B**) in the zona fasciculata (●—●) and zona reticularis (■—■) as a function of the number of days of DMBA-treatment. Standard errors and the levels of significance are shown in Table 6

conspicuous in the zona reticularis cells than in the zona fasciculata elements ($P<0.05$) (Fig. 13). The average volume of single mitochondria showed no significant changes up to the 3rd day of treatment, and thereafter it decreased significantly (Fig. 14A). The number of mitochondria per cell decreased linearly in both zones as a function of the duration of DMBA-treatment, the rate of decrease being significantly higher in the zona reticularis than in the zona fasciculata ($P<0.01$) (Fig. 14B).

Discussion

Like Danz et al. (1973), we found that chronic DMBA treatment was unable to induce massive hemorragic necrosis in the inner adrenal zones of adult male Wistar rats. However, morphometry indicates that DMBA induced a conspicuous decrease in the volume of the zona reticularis because of the decrease in both cell number and volume. The DMBA-provoked atrophy of the zona reticularis cells is likely to have caused the death and, consequently, the deletion of the degenerate elements from this zone. This view is confirmed by the demonstration that cell degeneration processes, similar to those described by Willie et al. (1973a, b), occurred in the zona reticularis of DMBA-treated animals.

The volume of the zona fasciculata showed a transient decrease up to the 3rd

day of treatment, which was due to a decrease in both cell number and volume. The presence of images indicating cell degeneration in the inner juxtareticular portion of the zona fasciculata suggests that a mechanism similar to that described for the zona reticularis is also operative in the zona fasciculata of DMBA-treated rats. Nevertheless, from the 4th to the 9th day of treatment the volume of the zona fasciculata displayed a significant increase, which was obviously due solely to the increase in the cell number, since the average cell volume showed a slight but significant decrease during the entire experimental period. These findings together with the observation, that proliferative processes are present only in the zona glomerulosa and to a lesser extent in the outer zona fasciculata (which differs from the results of Murad et al., 1973), and that the number of cells in the zona glomerulosa does not vary during DMBA-treatment, suggest that a continuous flow of new-formed elements from the zona glomerulosa to the zona fasciculata could mask the DMBA-induced cell death occurring in the inner portion of the zona fasciculata. Hence, the decrease in the average volume of the cells in the zona fasciculata could be due not only to the DMBA-provoked cell atrophy in the inner portion of this zone, but also to the fact that the newly formed cells migrating to the outer zona fasciculata have very probably less volume than the mature elements. The lack of significant variations in the average volume of the zona glomerulosa cells during DMBA-treatment might be the result of a balance between the number of growing cells in the premitotic phase and the rate of cell division.

In conclusion, the present morphometric and autoradiographic data can be interpreted by assuming that DMBA-induced cell atrophy and death into the inner fasciculata and reticularis zones causes a continuous flow of newly formed elements from the zona glomerulosa into the outer zona fasciculata. It seems reasonable, therefore, to advance the hypothesis that the mitogenic effect of DMBA may be the expresion of a repair mechanism triggered by its only morphometrically appreciable adrenolytic effect. Furthermore, we wish to stress that our data are consistent with those obtained in the adrenal enucleation experiments (for review see Long, 1975).

The mechanism underlying the DMBA-produced atrophy and death of the cells in the inner layers of the adrenal cortex requires further comment.

Obvious alterations were not seen in the endothelial wall in the adrenal cortex of DMBA-treated animals, so that cell death caused by ischaemic cellular alterations, as suggested by Horvath et al. (1969), can be excluded here. Instead, our findings seem to be consistent with the view that DMBA, or its metabolites produced in the liver (Boyland and Sims, 1967; Sydnor and Flesher, 1969) exerts a direct cytotoxic effect on adrenocortical cells (Boyland et al., 1965; Marchant, 1967; Murad et al., 1973). According to Dale and Scutchfield (1969), because of the steric resemblance of the molecules of DMBA and hydrocortisone, DMBA can exert a competitive inhibition of the 11β-hydroxylase system, which is located on the mitochondrial cristae (Satre et al., 1969; Dodge et al., 1970; Tamaoki, 1973). The fact that the subcellular alterations induced by DMBA mainly affect the mitochondrial compartment would seem to support this view. The impairment of the 11β-hydroxylating activity may explain the unusual number of mitochondrial intramatrical inclusions, which, according to Friend

and Brassil (1970), are to be interpreted as intramitochondrial accumulation of steroid-hormone precursors which cannot undergo further elaboration. The DMBA-induced alteration of mitochondrial activity might have provoked morphologic alterations in these organelles and subsequent degeneration ending in atrophy of the mitochondrial compartment. The increase in the volume of the lipid compartment also fits well with this hypothesis, since the lipid droplets contain cholesterol or cholesterol-esters (Moses et al., 1969; Sand et al., 1972) which are the most important precursor of steroid hormones.

However, our data are not quite comparable with those obtained by treating rats with metopirone, an inhibitor of the mitochondrial 11β-hydroxylase (Magalhães and Magalhães, 1969). Indeed, these investigators, although finding atrophy of the mitochondria compartment in the zona fasciculata cells, reported a significant hypertrophy of SER. Obviously the metopirone-induced block in the synthesis and secretion of definitive hormonal steroids would increase ACTH-secretion, due to the lack of the negative feed-back mechanism regulating the hypothalamo-hypophysial-adrenal axis (for review see Motta et al., 1969) and ACTH is known to induce the proliferation of SER (Idelman, 1970; Nussdorfer et al., 1971; Malamed, 1975). However, our results showed an evident SER decrease in the atrophic cells of DMBA-treated rats. This finding, although not excluding the possibility that the mechanism of DMBA action may involve the inhibition of the 11β-hydroxylase system, suggests that additional mechanism(s) may underlie the DMBA-provoked adrenocortical cell atrophy.

The ACTH-induced maintenance or stimulation of adrenocortical cell growth seems to require continuous cytoplasmic and mitochondrial protein synthesis (Farese, 1968; Ichii et al., 1970; Nussdorfer and Mazzocchi, 1972, 1973). Moreover, corticosterone was shown to inhibit directly the trophism of the rat adrenocortical cells both in vivo (Nussdorfer and Mazzocchi, 1970) and in vitro (Kahri, 1973) and we have demonstrated that this effect of the hormone could be due to the inhibition of mitochondrial and nuclear DNA-dependent protein synthesis (Nussdorfer and Mazzocchi, 1971). At present we are investigating whether DMBA could interfere with the translation or transcription processes of adrenocortical cells, in addition to exerting competitive inhibition of 11β-hydroxylase. This working hypothesis is supported also by the fact that DMBA-provoked cell atrophy is coupled with an evident decrease in the nuclear volume, which is known to be a parameter for the crude evaluation of nuclear function (Palkovitz and Fischer, 1968).

This mechanism of the action of DMBA would also explain the decrease in the surface of mitochondrial cristae and in the average volume and number of mitochondria in the atrophic cells, since a similar decrease was found to be induced in the rat zona fasciculata cells by chronic treatment with chloramphenicol, a quite specific inhibitor of mitochondrial protein synthesis (Mazzocchi et al., 1977b). Furthermore, we should recall that giant mitochondria originating from the fusion of pre-existing organelles were constantly observed in adrenocortical cells of hypophysectomized (Volk and Scarpelli, 1966; Canick and Purvis, 1972) or dexamethasone-treated rats (Nussdorfer et al., 1975) as well as in the hepatocytes of protein-starved (Svoboda et al., 1966) or ethidium

bromide-treated rats (Albring et al., 1973) and that mitochondrial fusion was interpreted as a mechanism for repairing the deficit in the synthesis of the proteins of the outer mitochondrial membranes (Tandler et al., 1968).

Whatever the mechanism of DMBA action may be, it is quite difficult to explain why cell atrophy and death are found only in the inner adrenal zones. In fact, active 11β-hydroxylase systems are contained also in the zona glomerulosa mitochondria (for review see Tamaoki, 1973) and continuous protein synthesis is necessary for the maintenance of growth of this adrenal zone (Mazzocchi and Nussdorfer, 1974; Mazzocchi et al., 1977a).

If the "migration theory" (for review see Long, 1975) is accepted, the cells in the inner zona fasciculata and the zona reticularis are the oldest, and it is reasonable to suppose that these exhausted cells are more susceptible to the action(s) of DMBA than the younger elements contained in the outer adrenocortical layers.

References

Albring, M., Radsak, K., Thoenes, W.: Giant mitochondria. II. Induction of matrix enriched megamitochondria in mouse liver parenchymal cells by ethidium bromide. Virchows Arch. Abt. B, Zellpath. **14**, 373–377 (1973)

Amlacher, E., Danz, M., Urban, H., Stiller, K.J.: Dimethyl sulphoxide as a solvent in carcinogen application with regard to the proliferative activity of the adrenal cortex. Exp. Path. **9**, 302–306 (1974)

Armitage, P.: Statistical methods in medical research. 1st ed., p. 281–282. Oxford-Edinburgh: Blackwell Scientific Publ. 1971

Boyland, E., Sims, P.: The effect of pretreatment with adrenal protecting compounds on the metabolism of 7,12-dimethylbenz(a)anthracene and related compounds by rat liver homogenates. Biochem. J. **104**, 394–403 (1967)

Boyland, E., Sims, P., Huggins, C.: Induction of adrenal damage and cancer with metabolites of 7,12-dimethylbenz(a)anthracene. Nature (London) **207**, 816–817 (1965)

Canick, J.A., Purvis, J.L.: The maintenance of mitochondrial size in the rat adrenal cortex zona fasciculata by ACTH. Exp. molec. Path. **16**, 79–93 (1973)

Cefis, F., Goodall, C.M.: Distribution and species limitation of the adrenal lesions induced by 7,12-dimethylbenz(a)anthracene. Amer. J. Pathol. **46**, 227–243 (1965)

Dale, E., Scutchfield, F.D.: Progesterone metabolism by adrenal homogenates of rats treated with 7,12-dimethylbenz(a)anthracene. Brit. J. Pathol. **50**, 165–171 (1969)

Danz, M., Amlacher, E., Urban, H., Bolck, F.: Different action of carcinogenic and noncarcinogenic substances on the proliferative activity of adrenal cortex cells. Exp. Path. **8**, 122–127 (1973)

Dodge, A.H., Christensen, A.K., Clayton, R.D.: Localization of a steroid 11-hydroxylase in the inner membrane subfraction of rat adrenal mitochondria. Endocrinology **87**, 254–261 (1970)

Farese, R.V.: Regulation of the adrenal growth and steroidogenesis by ACTH. In: Functions of the adrenal cortex (K.W. McKerns, ed.), 1st ed., p. 539–581. Amsterdam: North Holland Publ. Co. 1968

Friends, D.S., Brassil, G.E.: Osmium staining of endoplasmic reticulum and mitochondria in the rat adrenal cortex. J. Cell Biol. **46**, 252–266 (1970)

Horvath, E., Kovacs, K., Szabo, D.: An electron microscopic study of the adrenocortical lesion induced by 7,12-dimethylbenz(a)anthracene. J. Pathol. **97**, 277–282 (1969)

Huggins, C., Morri, S.: Selective adrenal necrosis and apoplexy induced by 7,12-dimethylbenz(a)anthracene. J. exp. Med. **114**, 741–760 (1961)

Ichii, S., Ikeda, A., Izawa, M.: Short and long-term effect of ACTH in vivo on the incorporation of 3H-leucine into submitochondrial fractions from rat adrenal glands. Endocr. jap. **17**, 365–368 (1970)

Idelman, S.: The mammalian adrenal cortex. Int. Rev. Cytol. **27**, 181–282 (1970)

Kahri, A.I.: Inhibition of ACTH-induced differentiation of cortical cells and their mitochondria by corticosterone in tissue culture of fetal rat adrenals. Anat. Rec. **176**, 253–272 (1973)

Karnovsky, M.J.: Simple method for staining with lead at high pH in electron microscopy. J. biophys. biochem. Cytol. **11**, 729–732 (1961)

Lockwood, W.R.: A reliable and easily sectioned epoxy embedding medium. Anat. Rec. **50**, 129–140 (1964)

Long, J.A.: Zonation of the mammalian adrenal cortex. In: Handbook of Physiology, Section 7: Endocrinology, Vol. 6: Adrenal gland (H. Blaschko, G. Sayers and D. Smith, eds.), 1st ed., p. 13–24. Washington D.C.: American Physiological Society 1975

Loud, A.V.: A method for the quantitative estimation of cytoplasmic structures. J. Cell Biol. **15**, 481–487 (1962)

Magalhaes, M.C., Magalhaes, M.M.: A stereologic study of the effects of metopiron on the rat adrenal. Lab. Invest. **21**, 491–496 (1969)

Malamed, S.: Ultrastructure of the mammalian adrenal cortex in relation to secretory function. In: Handbook of physiology, Section 7: Endocrinology, Vol. 6: Adrenal gland (H. Blaschko, G. Sayers and D. Smith, eds.), 1st ed., p. 23–39. Washington D.C.: American Physiological Society 1975

Marchant, J.: Development of adrenal cortical carcinoma in C3H mice following castration and administration of 7,12-dimethylbenz(a)anthracene. Brit. J. Cancer **21**, 750–754 (1967)

Mazzocchi, G., Nussdorfer, G.G.: Effect of adrenocorticotrophin on 3H-uridine incorporation into glomerulosal cells of hypophysectomized rat adrenals. J. Endocr. **63**, 581–582 (1974)

Mazzocchi, G., Robba, C., Belloni, A.S., Gambino, A.M., Nussdorfer, G.G.: Investigations on the turnover of adrenocortical mitochondria. VIII. A stereologic study of the effect of chronic treatment with ACTH and dexamethasone on the morphology of mitochondria in zona glomerulosa cells of the rat. Cell Tiss. Res. (1977a) (in press)

Mazzocchi, G., Robba, C., Nussdorfer, G.G.: Ultrastructural morphometric studies on the effects of chloramphenicol on rat adrenal zona fasciculata. Biologie Cellulaire **29**, 3a (1977b)

Millonig, G.: Advantage of a phosphate buffer for OsO4 solutions in fixation. J. appl. Physiol. **32**, 1637 (1961)

Morii, S., Huggins, C.: Adrenal apoplexy induced by 7,12-dimethylbenz(a)anthracene related to corticosterone content of adrenal gland. Endocrinology **71**, 972–976 (1962)

Moses, H.L., Davis, W.W., Rosenthal, A.S., Garren, L.D.: Adrenal cholesterol: localization by electron-microscope autoradiography. Science **163**, 1203–1205 (1969)

Motta, M., Fraschini, F., Martini, L.: "Short" feedback mechanism in the control of anterior pituitary function. In: Frontiers of neuroendocrinology (W.F. Ganong and L. Martini, eds), 1st ed., p. 211–254. New York-London-Toronto: Oxford University Press 1969

Murad, T.M., Leibach, J., von Haam, E.: Latent effect of DMBA on adrenal glands of Sprague-Dawley rats: an ultrastructural study. Exp. molec. Pathol. **18**, 305–315 (1973)

Nussdorfer, G.G.: Effects of corticosteroid-hormones on the smooth endoplasmic reticulum of rat adrenocortical cells. Z. Zellforsch. **106**, 143–154 (1970)

Nussdorfer, G.G., Mazzocchi, G.: Correlated morphometric and autoradiographic studies of the effects of corticosterone on adrenocortical cells of intact and hypophysectomized ACTH-treated rats. Z. Zellforsch. **111**, 90–105 (1970)

Nussdorfer, G.G., Mazzocchi, G.: Effects of corticosterone on nuclear and mitochondrial DNA-dependent protein synthesis of adrenocortical cells of hypophysectomized ACTH-treated rats. Steroidologia **2**, 244–256 (1971)

Nussdorfer, G.G., Mazzocchi, G.: Effect of 3′:5′-cyclic AMP on nuclear and mitochondrial RNA synthesis of rat adrenocortical cells. Acta endocr. (Kbh) **70**, 81–88 (1972)

Nussdorfer, G.G., Mazzocchi, G.: Effects of 3′,5′-cyclic nucleotides on adrenocortical cells of hypophysectomized rats. A stereologic and autoradiographic study. Lab. Invest. **28**, 332-342 (1973)

Nussdorfer, G.G., Mazzocchi, G., Rebonato, L.: Long-term trophic effect of ACTH on rat adrenocortical cells: an ultrastructural, morphometric and autoradiographic study. Z. Zellforsch. **115**, 30–45 (1971)

Nussdorfer, G.G., Mazzocchi, G., Rebuffat, P.: An ultrastructural stereologic study of the effects of ACTH and adenosine 3′,5′-cyclic monophosphate on the zona glomerulosa of rat adrenal cortex. Endocrinology **92**, 141–151 (1973)

Nussdorfer, G.G., Mazzocchi, G., Rebuffat, P., Belloni, A.S., Gottardo, G., Gambino, A.M.: Investigations on the turnover of adrenocortical mitochondria. III. Stereologic and autoradiographic observations on the effect of chronic treatment with dexamethasone on the rat zona fasciculata mitochondria. Cell Tiss. Res. **163**, 273–290 (1975)

Nussdorfer, G.G., Rebuffat, P., Mazzocchi, G., Belloni, A.S., Meneghelli, V.: Investigations on the turnover of adrenocortical mitochondria. I. Effect of chronic treatment with ACTH on the size and number of rat zona fasciculata mitochondria. Cell Tiss. Res. **150**, 79–94 (1974)

Palkovits, M., Fischer, J.: Karyometric investigations. 1st ed. Budapest: Acadèmiai Kiadò 1968

Sabatini, D.D., Bensch, K., Barrnett, R.J.: Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J. Cell Biol. **17**, 19–58 (1963)

Sand, G., Frühling, J., Penasse, W., Claude, A.: Distribution du cholestérol dans la corticosurrénale du rat: analyse morphologique and chimique des fractions subcellulaires, isolées par centrifugation différentielle. J. Microscopie **15**, 41–66 (1972)

Satre, M., Vignais, P.V., Idelman, S.: Distribution of the steroid 11-hydroxylase and the cytochrome P-450 in membranes of beef adrenal cortex mitochondria. FEBS letters **5**, 135–140 (1969)

Svoboda, D., Grady, H., Higginson, J.: The effects of chronic protein deficiency in rats. II. Biochemical and ultrastructural changes. Lab. Invest. **15**, 731–749 (1966)

Sydnor, K.L., Flesher, J.W.: Comparative biologic activities of 7,12-dimethylbenz(a)anthracene, 7-hydroxymethyl-12-methylbenz(a)anthracene, 7,12-dihydroxymethylbenz(a)anthracene, and 4-methoxy-7,12-dimethylbenz(a)-anthracene in the Sprague-Dawley female rat. Cancer Res. **29**, 403–408 (1969)

Tamaoki, B.I.: Steroidogenesis and cell structure. Biochemical pursuit of sites of steroid biosynthesis. J. Steroid Biochem. **4**, 89–118 (1973)

Tandler, B., Erlandson, R.A., Wynder, E.L.: Riboflavin and mouse hepatic cell structure and function. I. Ultrastructural alteration in simple deficiency. Amer. J. Pathol. **52**, 69–78 (1968)

Volk, T.L., Scarpelli, D.G.: Mitochondria gigantism in the adrenal cortex following hypophysectomy. Lab. Invest. **15**, 707–714 (1966)

Weibel, E.R.: Priciples for morphometry in electron microscopic cytology. Int. Rev. Cytol. **26**, 235–302 (1969)

Wheatley, D.N.: Cilia and centrioles of the adrenal cortex. J. Anat. **101**, 223–237 (1967)

Wyllie, A.H., Kerr, J.F.R., Currie, A.R.: Cell death in the normal neonatal rat adrenal cortex. J. Pathol. **111**, 255–261 (1973a)

Wyllie, A.H., Kerr, J.F.R., Macaskill, I.A.M., Currie, A.R.: Adrenocortical cell deletion: the role of ACTH. J. Pathol. **111**, 85–94 (1973b)

Received June 28, 1977