

# Cellular changes of adrenal under the acute stress of O-chlorobenzylidene malononitrile (CS)

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**Summary.** This investigation describes histological and cytometrical changes of cortical and medullary tissue of adrenal in rats under the acute stress of O-chlorobenzylidene malononitrile (10 mg/kg and 20 mg/kg). It has been observed that after injection of CS, the adrenal gland showed histological changes both in the cortical and medullary region.

The lachrymator, orthochlorobenzylidene malononitrile (CS), was claimed as nontoxic, but recent investigations have brought new information on its toxicity<sup>2-6</sup>. The present communication indicates the histological and the cyto-dynamic changes in the adrenals of the rat under acute exposure to CS.

**Materials and methods.** CS was synthesized according to standard methods and its LD<sub>50</sub> as determined on laboratory bred female albino rats according to Miller & Tainter<sup>7</sup> was 40 mg/kg (i.p.).

30 female albino rats from the colony maintained at this establishment with an average body weight of  $180 \pm 5$  g

were divided into 3 groups, A, B and C, with 10 animals in each group. The animals of group B were given a daily i.p. injection of CS in olive oil at a dose of 10 mg/kg for 10 days, while those of group C received the same compound at a dose of 20 mg/kg for the same period; the animals of group A served as control and were given a daily injection of olive oil for 10 days.

The animals were sacrificed by cervical dislocation and the adrenals processed and stained by haematoxylin and eosin according to standard methods. Cytometry was carried out with an ocular microscope at 160 fold magnification. The results are recorded in the table.

Cytometric analysis of adrenal gland after i.p. benzylidene malononitrile injection in rats

Group	Control (A)	CS 10 mg/kg (B)	CS 20 mg/kg (C)
Nuclear diameter of different zone of adrenal gland ( $\mu$ m) (20)			
Zona glomerulosa	$0.682 \pm 0.026^*$	$0.850 \pm 0.029^a$	$0.900 \pm 0.040^a$
Zona fasciculata	$0.776 \pm 0.016$	$0.624 \pm 0.035^a$	$0.908 \pm 0.028^a$
Zona reticularis	$0.690 \pm 0.028$	$0.757 \pm 0.022^c$	$0.780 \pm 0.028^b$
Medulla	$0.793 \pm 0.019$	$1.256 \pm 0.032^a$	$1.212 \pm 0.027^a$
Zonal cellular diameter ( $\mu$ m) (10)			
Cortical cell diameter	$3.973 \pm 0.354$	$5.226 \pm 0.491^c$	$6.032 \pm 0.308^a$
Medullary cell diameter	$2.436 \pm 0.238$	$7.453 \pm 0.524^a$	$9.454 \pm 0.758^a$
Zonal cellular width ( $\mu$ m) (10)			
Capsule	$2.018 \pm 0.123$	$1.104 \pm 0.034^a$	$1.162 \pm 0.046^a$
Zona glomerulosa	$5.570 \pm 0.142$	$4.438 \pm 0.126^a$	$5.56 \pm 0.109^c$
Zona fasciculata	$22.880 \pm 0.116$	$15.370 \pm 0.304^a$	$18.56 \pm 0.912^a$
Zona reticularis	$3.480 \pm 0.243$	$2.438 \pm 0.055^a$	$2.90 \pm 0.159^c$
Medulla	$5.858 \pm 0.580$	$15.980 \pm 0.753^a$	$26.39 \pm 2.610^a$

Number of observations from slides in paranthesis.

\* Mean  $\pm$  SE; <sup>a</sup>  $p < 0.001$ ; <sup>b</sup>  $p < 0.05$ ; <sup>c</sup> Not significant.

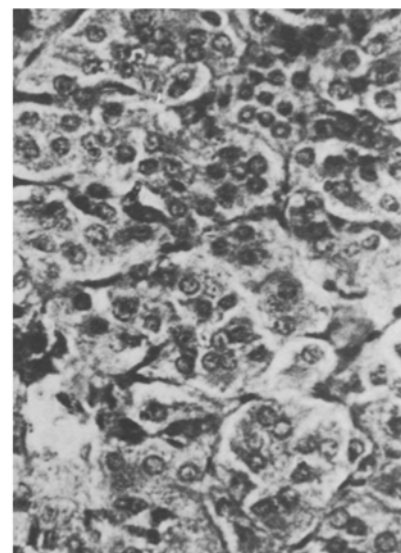
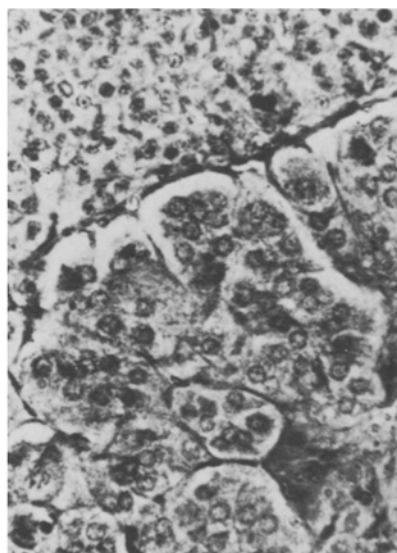
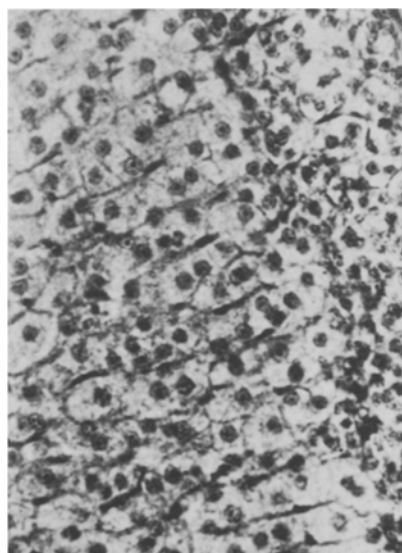


Fig. 1. Normal histological feature of adrenal gland of rat.  $\times 200$ . Fig. 2. 10 mg/kg CS-induced hypertrophy of the chromaffin cells throughout the gland.  $\times 200$ . Fig. 3. 20 mg/kg CS-treated chromaffin cell of medullary zones of adrenal.  $\times 200$ .

**Results and discussion.** Autopsy revealed hypertrophy of the adrenals in the treated animals. Microscopic examination indicated hypertrophic changes in the cells of both the cortical and the medullary regions of the adrenals of the treated animals; in addition, the cells of the zona fasciculata of the treated groups were distorted with hyalinated cytoplasm, the nuclei being in a condition of pyknosis; the nuclei of the cells of the medulla were vesicular, enlarged and less stainable (figures 1-3).

Cytometrical study indicated that in group C receiving 20 mg CS/kg, there was a highly significant rise in the cell nuclear diameter and cell diameter of both the cortex and the medulla, whereas in group B receiving 10 mg CS/kg, the same effect was observed only in the cells of the zona glomerulosa, zona fasciculata and the medulla. As regards zonal cellular width, while a highly significant fall was found in cells of the zona glomerulosa, zona fasciculata and zona reticularis in group B, the same effect was observed in group C only in cells of the zona fasciculata; a consistent feature was the sharp rise in the cellular width in the medulla of both groups B and C.

It is well known that the biochemical response of the adrenals to any stress is the increased secretion of catecholamines and glucocorticoids. Histological changes of the adrenals under both acute and chronic stress have also been recorded<sup>8-10</sup>. In the present investigation, the very sharp rise of the cell nuclear diameter, cell diameter and cellular

width in the medulla recorded under the action of CS may be closely associated with hypersecretion of the catecholamines. The increase in the cell nuclear diameter and cell diameter of the cortex also may be connected with increased secretion of glucocorticoids; however, the consistent fall in the cellular width in the cortex is difficult to explain at this stage<sup>11</sup>.

- 1 The authors are thankful to Dr P.K. Ramachandran, Director, Defence Research and Development Establishment, Gwalior, for his sustained interest and critical suggestions in the course of this study.
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### A preliminary study on the behaviour and biochemical responses subsequent to the injection of 5,6-dihydroxytryptamine into the substantia nigra of the rat

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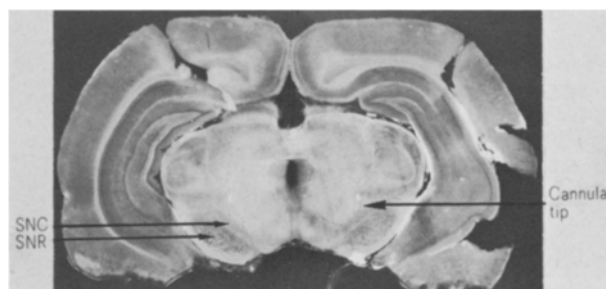
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**Summary.** Injection of 5,7-dihydroxytryptamine into the substantia nigra of rats produces an increase of dopamine in the ipsilateral striatum, and when these animals are injected with amphetamine they do not exhibit any rotation. The mode of action of this neurotoxin is compared with that of 6-hydroxydopamine.

Recent studies suggest that ascending 5-hydroxytryptamine (5-HT) neurones may modulate the output of the nigrostriatal dopamine (DA) mechanism. The use of the rotating rat model<sup>2</sup> demonstrated that circling responses to both direct and indirect DA agonists are potentiated by drugs attenuating whole brain 5-HT transmission, while treatment enhancing 5-HT transmission decreases such rotations<sup>3</sup>. It is possible, therefore, that 5-HT neurones directly influence the DA neurones in the substantia nigra (SN), which would mean that neurones in the striatonigral and nigrostriatal loop are all directly or indirectly affected by the 5-HT neurone endings in the SN. To test this idea, the neurotoxins 5,7-dihydroxytryptamine (5,7-DHT) and 6-hydroxydopamine (6-OHDA) were injected unilaterally into the rat's SN and the animals observed for evidence of rotational locomotor activity characteristic of nigrostriatal activity<sup>4</sup>. The DA concentration and choline acetyltransferase (ChAc) activity in the corresponding corpora striata were determined as a biochemical measure of DA and acetylcholine (ACh) cell activity. It is known that 6-OHDA specifically degenerates catecholamine neurones<sup>5</sup>, while 5,7-DHT specifically degenerates 5-HT and noradrenaline (NA) neurones<sup>6</sup>.

**Methods.** Wistar albino rats, 200-260 g, were anaesthetized with nembutal. Either 5,7-DHT or 6-OHDA at a concentration of 0.016 M (in 1% ascorbic acid) was injected

stereotactically in a volume of 2 µl Hamilton syringe. The left SN received an equivalent volume of physiological saline containing 1% ascorbic acid. On recovery, the animals were placed immediately and 14 h later in rectangular grill boxes (20 × 20 × 20 cm), and observed for rotating and general behaviour; 2 weeks after the operations, the animals were injected i.p. with amphetamine (5 mg/kg) and placed once again in the boxes to observe their rotations and behaviour. The animals were then killed 3 weeks after chemical lesion with 6-OHDA or 5,7-DHT and the striata carefully dissected and assayed radiochemically for DA<sup>8</sup>



Frontal section of rat brain. SNC, substantia nigra pars compacta; SNR, substantia nigra pars reticularis.