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Inhibition of Azo Dye Carcinogenesis by Adrenalectomy and Treatment With Desoxycorticosterone Trimethylacetate.*† (23564)

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Symeonidis, Mulay and Burgoyne(1) reported that adrenalectomy or overdosage of intact rats with desoxycorticosterone acetate (DCA) prevented azo dye carcinogenesis. On the other hand Griffin, Richardson, Robertson, O'Neal and Spain(2) got just the opposite results. The former authors employed 4-dimethylaminoazobenzene (DAB) and subcutaneous implants of pellets of DCA while the latter workers used 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB), and subcutaneous injections of DCA in oil. The amount of steroid given per month by either group was approximately the same, making it highly unlikely that differences in methods of administration of DCA accounted for the divergent findings. Also, it seems highly unlikely that the use of different carcinogens could account for the discrepancies since the effects produced by carcinogenic azo dyes are qualitatively the same(3); 3'-Me-DAB is a more rapidly acting hepatic carcinogen than is DAB(4). During the past 5 years, we have been attempting to determine the effects of adrenal steroids on the course of azo dye carcinogenesis and have followed the work of the above authors with considerable interest. We find, as did Symeonidis *et al.*(1), that treatment of totally adrenalectomized rats with 11-desoxycorticosterones prevents azo dye carcinogenesis, but unlike these workers, we find that overdosage of intact rats with 11-desoxycorticoids fails to prevent the carcinogenic process. Furthermore, we find that the presence of accessory or regenerated adrenal cortical tissues inhibits the protective effect of adrenalectomy and hormonal treatment. The basis of these conclusions is reported here.

Methods and materials. Male rats of the Long-Evans strain weighing approximately

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TABLE I. Effect of Adrenalectomy and DCT Treatment on Body and Liver Weights of Male Rats Fed 3'-Me-DAB for 4 Months.

Group	Treatment	Body wt gain, g	Liver wt, g/100 g body wt	Liver wt, g	
				Non-nodular†	Nodular
		Mean ± S.E.			
1 a	Intact, food *ad lib*	1 ± 19* (6)	9.2 ± 3.4 (5)	10.5 ± .3 (5)	11.2 ± 12.1 (4)
b	" 10 g food/day‡	18 ± 8 (6)	8.2 ± 1.4 (6)	14.9 ± 3.1 (6)	4.4 ± 2.9 (5)
2 a	" food *ad lib*, 250 mg DCT	61 ± 11 (16)	8.1 ± 1.0 (15)	15.1 ± 1.0 (15)	7.9 ± 3.9 (9)
b	" 10 g food/day, 250 mg DCT	41 ± 9 (5)	8.7 ± 1.7 (5)	12.9 ± 1.8 (5)	8.4 ± 4.2 (5)
3 a	Adrx., food *ad lib*, 250 mg DCT	58 ± 4 (8)	4.1 ± .1 (8)	10.8 ± .5 (8)	None
b	" 10 g food/day, 250 mg DCT	68 ± 5 (3)	4.2 ± .2 (3)	11.8 ± .6 (3)	"
4	Subtotal adrx., 250 mg DCT (2 from fed group) (3 from *ad lib* group)	56 ± 9 (5)	5.2 ± .8 (5)	14.5 ± .7 (5)	§

* S.E. $= \pm \sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

† Non-nodular refers to liver tissue other than dissectable, neoplastic nodules. In grossly tumorous livers, non-nodular tissue contained scattered lesions and tiny nodules.

‡ In groups 1 b, 2 b and 3 b amt of food was 15 g/day for 2 wk, thereafter 10 g/day.

§ Nodules too small to be dissected and weighed.

210 g initially were fed a semisynthetic diet, patterned after Price *et al.*(5), containing 0.058% 3'-Me-DAB for 126 days. The dietary ingredients were:

Dextrose	620	g/kg
Salt mixture (Wesson)	40	
Vit. test casein	240	
Corn oil	100	
Thiamin (Cl)	3	mg/kg
Riboflavin	1.5	
Pyridoxine (HCl)	2.5	
Ca pantothenate	7	
Choline (Cl)	30	
3'-Me-DAB	580	
Halibut liver oil	300	
Tetracycline-Vet	100	

The diet was similar to that used by others working on azo dye carcinogenesis except that it contained a higher percentage of casein. Also, an antibiotic, (Pfizer, Tetracycline-Vet), was added in hopes that it would reduce respiratory infections that commonly occur in rats fed purified diet containing azo dye. Sixty rats, all of which were fed the carcinogenic diet, were divided into 3 groups: group 1, 12 rats, served as intact controls; group 2, 24 intact rats, received desoxycorticosterone trimethylacetate (DCT); group 3, 24 adrenalectomized rats, received DCT. Each major group was subdivided into 2 subgroups as given in Table I. Desoxycorticosterone trimethylacetate (DCT), a long-acting adrenal-like steroid(6), was injected intramuscularly in 5 mg doses immediately following adrenalectomy. One week later, after the healing of incisions, the rats were injected with 70 mg DCT and placed on the carcinogenic diet. One month later, 75 mg of the steroid was given, followed with 50 mg at the beginning of the second and third months. Intact rats received DCT in a manner and time sequence similar to adrenalectomized ones; each treated rat received a total of 250 mg of the corticoid. The animals were sacrificed after they had been on the diet for approximately 4 months (120-126 days). At sacrifice, the animals were weighed and injected with a lethal dose of Nembutal. The blood vascular system was perfused with physiological saline; the liver was removed, examined, photographed and weighed. Small pieces of the left lateral lobe were removed for histological and enzyme studies. Nodules of neoplastic tissue, when present, were dissected, weighed and saved for nitrogen determinations. Histological, nitrogen, and enzyme studies will

TABLE II. Effect of DCT on Adrenal and Thymus Weights in Male Rats Fed 3'-Me-DAB for 4 Months.

Group	Treatment	Adrenal wt, mg/100 g body wt, mean ± S.E.	% change from control	Thymus wt, mg/100 g body wt, mean ± S.E.	% change from control
1	Intact control	15.5 ± 1.3 (11)		46.0 ± 3.4 (10)	
2	Intact, 250 mg DCT	11.0 ± .5 (20)	-29	42.4 ± 3.9 (20)	- 7.8
3	Adrx., 250 mg DCT			65.5 ± 8.8 (11)	+42.4
4	Subtotal adrx., 250 mg DCT	3.0 ± 1 (5)	-81	54.2 ± 5.4	+17.8

be reported later. The adrenals (when present) and thymi were weighed. In adrenalectomized rats the perirenal sites were examined for accessory cortical tissue grossly; when such tissue was found it was saved for histological study.

Results. Untreated intact rats feeding *ad lib.* showed the poorest weight gain. Treatment of rats, either intact or adrenalectomized, with DCT markedly improved weight gain and physical appearance. Hormonal treatment resulted in significant weight gain both in rats on a restricted and *ad lib.* diet.

None of the intact, non-treated rats died during the experimental period; two of the 24 intact animals treated with DCT, and eight of the 24 adrenalectomized rats treated with DCT, died.

Five of the 16 "adrenalectomized" survivors had adrenal cortical tissue. Such cases were classified as being subtotally adrenalectomized. As seen in Table II, the accessories were small, averaging 3 mg/100 g body weight. From previous work it is known that when rats are maintained for awhile on supportive therapy after adrenalectomy, appreciable numbers develop adrenal accessories and survive indefinitely(6).

Intact rats, including those on hormonal treatment had livers that were markedly enlarged (Table I) averaging about twice the weight of livers from adrenalectomized rats. Livers from intact rats fed the azo dye contained abnormal nodular growths, ranging in diameter from approximately 1 to 20 mm. The nodules were whitish in color and firm in texture. It was possible to dissect, by ordinary means, several nodules from most of these livers; such nodules, when examined histologically, were hepatomatous.

Adrenalectomized rats, without adrenal accessories, receiving hormonal treatment had normal appearing livers that were slightly larger than livers from intact rats fed a stock diet. Apparently the purified diet containing azo dye caused some liver enlargement, even in livers protected from the carcinogenic influence of the dye. The mean liver weight of adrenalectomized rats treated with DCT was significantly less than the mean liver weight of intact rats in either of groups 1b, 2a and 2b ($P < 0.05$ in each analysis).

Livers from subtotally adrenalectomized rats weighed, on the average, more than livers from totally adrenalectomized animals, but less than livers from intact rats fed azo dye. The carcinogenic process appeared to be delayed in livers from subtotally adrenalectomized rats.

As seen in Table II, DCT treatment in intact animals resulted in a significant decrease in mean adrenal weight, but had little or no effect on thymus weight. The mean thymus weight of treated adrenalectomized rats was significantly greater than that of treated or untreated intact rats ($P < 0.05$). Thymus gland weights of treated, subtotally adrenalectomized rats were essentially the same as those taken from intact controls. In other words, DCT treatment resulted in an expected atrophy (presumably due to ACTH suppression) of adrenal glands when present, but probably had little or no effect upon thymus weight.

Discussion. Results of these experiments indicate that 3'-Me-DAB carcinogenesis is inhibited by a combination of total adrenalectomy and treatment with desoxycorticosterone trimethylacetate. The presence of small amounts of adrenal cortical tissue allows the carcinogenic process to proceed, even when animals are treated with large doses of 11-desoxycorticoid. Preliminary data from our laboratory offer fragmentary evidence in

support of the report by Symeonidis *et al.* (1) that total adrenalectomy, without steroid treatment, prevents azo dye carcinogenesis. It is extremely difficult to keep the carcinogen-fed, untreated, adrenalectomized rat alive for several months; mortality is extremely high, even when saline is given as drinking fluid. Also, many of the "adrenalectomized" animals that survive the experimental period have adrenal cortical nodules with concomitant hepatomas; such factors complicate experiments and make it difficult to gather sufficient numbers to prove unequivocally that total adrenalectomy alone will prevent azo dye carcinogenesis. It may be, in our experiments, that the function of DCT was to keep the animal alive in the absence of other types of adrenal hormones, whereas, total adrenalectomy was the important factor in preventing carcinogenesis.

Controlling the daily intake of food was of little importance in these experiments since restrictive feeding or *ad lib.* feeding gave essentially the same results. During the first 4-6 weeks on the diet several adrenalectomized, DCT-treated animals died, but the survivors ate well, gained weight and were in good physical condition when sacrificed. Apparently DCT-treatment in either intact or adrenalectomized rats stimulated appetite; animals on the restrictive feeding regimen would have eaten more of the diet than was given. It is likely, in the *ad lib.* feeding experiments, that treated adrenalectomized rats ate more of the azo dye than did the intact controls. In other words, the amount of azo dye consumed by the rats with normal appearing livers was as great as in animals not protected from the carcinogenic process. Adrenalectomy (and DCT treatment?) apparently made it possible for the liver to reject the carcinogenic influence of 3'-Me-DAB.

Treatment of intact rats with DCT caused significant atrophy of the adrenal cortex but had little or no effect on thymus weight. Totally adrenalectomized rats treated with DCT had large thymuses, but this was probably due to lack of atrophy in the absence of the adrenals rather than to hormonal treatment. Such results are consistent with the findings of others who have studied adrenal and thymus weights in adrenalectomized rats and in intact and adrenalectomized rats treated with 11-desoxycorticoids(7). The finding that 29% reduction in adrenal weight induced by DCT had no influence upon the carcinogenic process is consistent with the finding that even small amounts of adrenal tissue support carcinogenesis. Total adrenalectomy is apparently the key factor in inhibiting azo dye carcinogenesis.

Summary. 1) Feeding of a semi-synthetic diet containing 0.058 percent 3'-methyl-para-dimethylaminoazobenzene (3'-Me-DAB) to intact rats for four months resulted in liver enlargement and development of large neoplastic nodules in 100 percent of the cases. 2) *Ad lib.* or restrictive feeding of azo dye were equally effective in inducing liver carcinogenesis. 3) Treatment of dye-fed, intact rats with large doses of desoxycorticosterone trimethylacetate (DCT), over a 4-month period, had no effect on liver carcinogenesis. 4) Treatment of dye-fed, totally adrenalectomized rats for 4 months with large doses of DCT prevented marked liver enlargement and inhibited development of macroscopic lesions and neoplastic nodules. 5) The presence of small amounts of adrenal cortical tissue allowed the carcinogenic process to proceed in rats fed azo dye and treated with large doses of DCT.

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