

Adrenal Cortical Accessory Tissue and Azo Dye Carcinogenesis

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Introduction

Adrenal cortical tissue in lower vertebrates is scattered along the dorsal body wall in close association with cardinal veins. In mammals, however, such tissue is generally considered to be organized into a discrete gland, forming a cortex which encloses the adrenal medulla. The presence of cortical tissue in laboratory rats, accessory to that found in the main glands, has been reported in experiments designed to study the influence of adrenalectomy and hormone treatment on azo dye carcinogenesis (1, 2). The present investigations were undertaken in attempts to determine the extent to which adrenal cortical accessory tissue is present in Long-Evans laboratory rats, and the influence that such tissue may have upon the course of azo dye carcinogenesis.

Materials and Methods

The procedure for producing liver carcinogenesis was similar to that previously reported (1, 3). Rats were fed a semisynthetic diet containing 0.058% 3'-methylparadimethylaminoazobenzene (3'-Me-DAB) for four months. Controls were unoperated, whereas experimental rats were adrenalectomized and treated with either 50-75 mg/month or 10 mg/month of desoxycorticosterone trimethylacetate (DCT). In the first series of experiments (Table 1) the perirenal areas were searched macroscopically for adrenal cortical accessory tissue. Animals in which such tissue was found were considered separately (Group 4, Table I) and comparisons were made on the degree of liver involvement in this group with that in other groups in which no accessory tissue was found. Upon autopsy, livers were excised, weighed, and examined for the presence and size of lesions. Diseased livers without obvious lesions showed rough granulated-appearing surfaces and were classified as "lobulated." Livers with lesions were separated into two categories: those with small lesions (<5.0 mm in diameter) and those with large lesions (>5.0 mm in diameter). Paraffin and frozen sections of representative areas of the livers were prepared and stained respectively with hematoxylin and eosin and Scharlach R. Tissue from livers was classified as carcinomatous only when neoplastic areas were epitheloid in appearance.

In another series of experiments 41 rats were adrenalectomized, treated with low doses of DCT and fed the carcinogen for four months. At autopsy the perirenal areas were examined grossly for accessories and when such bodies were found they were preserved, sectioned, and studied to determine whether or not they were truly cortical tissue. In these animals the liver and perirenal areas were stripped from the carcass and prepared for histological study. Adrenal cortical tissue found in perirenal areas was then classified as either macroscopic or microscopic accessories. Livers were studied histologically and classified as carcinomatous or non-carcinomatous. Correlations were then made between sizes of accessory bodies and cancer incidence.

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TABLE I
Liver Appearance and Cancer Incidence in Rats Fed
3'-ME-DAB for 4 Months

Group	Treatment	No. Rats	Liver wt. g/100gBW mean±S.E.	Liver Appearance	No. Carcinomatous		
					Livers	No.	%
1	none	36	7.7±0.2	Lobulated	3	0	86
				Small lesions	13	11	
				Large lesions	20	20	
					36	31	
2	Adrenalectomy 50-75 mg DCT/month (no macroaccessories)	15	4.3±0.1	Normal	13	0	0
				Lobulated	1	0	
				Small lesions	1	0	
				Large lesions	0	0	
					15	0	
3	Adrenalectomy 10 mg DCT/month (no macroaccessories)	38	4.8±0.2	Normal	19	0	13
				Lobulated	11	1	
				Small lesions	7	3	
				Large lesions	1	1	
					38	5	
4	Adrenalectomy DCT (macroaccessories)	15	5.9±0.4	Normal	1	0	53
				Lobulated	7	2	
				Small lesions	4	3	
				Large lesions	3	3	
					15	8	

Results

All control rats fed azo dye, but otherwise receiving no treatment, had enlarged and abnormal appearing livers; 86% of these were found to be carcinomatous (Table I, Group 1). The five non-cancerous livers in this group were either grossly lobulated or contained small lesions. Microscopic examination revealed areas that appeared swollen, inflamed, and hemorrhagic; there was an increase in stainable fat and connective tissue.

Adrenalectomized, azo dye-fed rats receiving large doses of DCT had carcinoma-free livers but two livers in the group (Table I, Group 2) appeared to be mildly diseased. Also, in most all livers of this group histological study revealed signs of hypertrophy, inflammation, and fatty infiltration, although mean liver weight was approximately that expected for stock Long-Evans rats.

Most of the adrenalectomized animals given small doses of DCT had livers that were cancer-free, but again histological evidence of swelling, fatty infiltration, and hemorrhage was present. Mean liver weight, while slightly greater than in the previous groups, was about normal (Table I, Group 3).

In the group of rats that was adrenalectomized but exhibited macroscopically visible accessories upon autopsy 14 of 15 had abnormal appearing livers, and eight of these were carcinomatous (Table I, Group 4).

In the second series of experiments where a search was made for both macroscopic and microscopic accessories the results showed that 20 of the 41 adrenalectomized rats had adrenal tissue present; twelve macroscopic and eight microscopic accessories were found. In this group the only rats

TABLE II

Correlation of Adrenal Cortical Accessories with Liver Carcinomas
in DCT-Treated Adrenalectomized Rats Fed 3'-ME-DAB

		No. with carcinomas
Number rats studied	41	8
Number with acces. tissue	20	8
Number with macro acces.	12	8
Number with micro. acces.	8	0

with cancerous livers were those that had adrenal cortical accessories of macroscopic dimensions. Rats with microscopic accessories did not have liver cancer but evidence of liver pathology was present as evidenced by marked eosinophilia of liver parenchyma cells and hemorrhagic areas. Also, those livers from rats in which no accessory tissue was found showed evidence of histological change since liver cords appeared swollen and cells were strongly eosinophilic.

Accessory adrenal cortical tissue did not exhibit the typical histological appearance as seen in adrenal glands. The adrenal cortical cells were large and well differentiated but arranged in irregular nests and columns. The connective tissue capsule around the accessories was thin and indistinct, unlike the well differentiated capsule of regular glands.

Discussion

The experiments reported here again confirm the observations made previously (1, 2, 3) that adrenalectomy and desoxycorticosterone treatment inhibits azo dye carcinogenesis. However, they also indicate that such procedures may not completely suppress the carcinogenic process. The finding that protected livers show histologic deviations, confirms the report of Symeonides *et al* (2). Whether the signs of hypertrophy and inflammation in otherwise normal appearing livers are reflections of resistance to carcinogenic dye, to DCT treatment, or to some other factor, is unknown.

The results reported here clearly demonstrate that azo dye cancer incidence is higher in adrenalectomized desoxycorticosterone treated animals with macroscopic adrenal cortical accessory tissue than it is in similarly treated animals where no such tissue could be found. Such findings imply that adrenalectomy and desoxycorticosterone treatment is an effective means of inhibiting azo dye carcinogenesis, providing functional accessory adrenal cortical tissue is absent. Credence is given to this implication by previous studies which showed that azo dye carcinogenesis could not be inhibited by desoxycorticosterone treatment in unoperated rats (3).

Summary

Adrenal cortical accessory tissue was found to be present in large numbers of long-term adrenalectomized Long-Evans rats. In a detailed study of the perirenal areas from 41 adrenalectomized rats, 12 animals were found to have accessories that were macroscopically detectable and 8 others had accessories of microscopic dimensions.

Adrenalectomy and desoxycorticosterone treatment effectively inhibited azo dye carcinogenesis, especially in animals lacking macroscopically detectable adrenal cortical tissue. The presence of small accessories appeared to have little, if any, influence upon the protective effects of adrenalectomy and desoxycorticosterone treatment.

The protective effects of adrenalectomy and desoxycorticosterone treatment in azo dye carcinogenesis were somewhat limited since microscopic studies revealed that normal-appearing livers contained areas of inflammation and cellular hypertrophy.

Literature Cited

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