Further Observations on the Role of the Pituitary and the Adrenal Gland in Azo Dye Carcinogenesis

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Several investigators have observed that the pituitary exerts an effect on carcinogenesis and also on the growth of tumors. Ferguson and Visscher (1) found that hypophysectomy prevented the development of postcastration adrenal cortical nodular hyperplasia in C3H mice. Moon and associates (7–9) reported that the administration of growth hormone to female rats (Long-Evans strain) resulted in tumors of the lymphatic tissues, reproductive organs, and adrenals. Hypophysectomized rats of the same strain similarly treated with growth hormone exhibited a decreased incidence of tumors (5). Subsequently, these same workers found that the implantation of methylcholanthrene failed to induce tumors in hypophysectomized rats (6). Korteweg and Thomas (4) observed fewer carcinomas in hypophysectomized rats treated with 3,4-benzpyrene than in treated controls. Recently, Zamurowitch (13) has reported that there was no difference in the latent period of tumor formation in normal and hypophysectomized rats. It was found in this laboratory that hypophysectomy effectively inhibited the formation of liver tumors in male rats fed diets containing 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) (3, 10, 11). These studies were extended to determine whether the pituitary hormones or hormones from target glands of the pituitary could influence the carcinogenicity of the azo dye in the hypophysectomized rats. The hypophysectomized azo dye-fed male rats injected with adrenocorticotrophins showed liver tumors after 21 weeks while similarly fed, hypophysectomized rats given cortisone, DOCA, crude gonadotrophin, or testosterone and the saline injected controls showed no tumors after 21 weeks (10, 11).

This paper reports on further studies to determine if other pituitary hormones restore the carcinogenic action of 3′-Me-DAB in hypophysectomized rats. Hypophysectomized female rats were also included in the present study.

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METHODS

Groups of hypophysectomized male or female albino rats[1] were maintained on a synthetic basal diet containing 0.06 per cent 3′-Me-DAB (2). The animals were maintained in wire-bottomed cages. Food and water were given ad libitum, and body weights and food intakes were recorded weekly. Groups of ten rats were injected subcutaneously every 48 hours with hormone preparations as follows.

GROUPS I–VI, MALE RATS

Group I.—Two units ACTHAR gel (Armour & Co., Chicago).

Group II.—Two units HP ACTHAR gel (Armour & Co., Chicago). The HP ACTHAR is purified over twenty-fold from the older types of preparations. Its main contaminants are melanophore hormone and traces of posterior pituitary hormones. The strengths used were 20 units/ml in gelatin. From previous investigations there was some question as to whether these two ACTH preparations produced the same response in restoring the carcinogenic action of the azo dyes, so both preparations were included.

Group III.—0.5 mg. of beef growth hormone in aqueous suspension (Armour Laboratories, Chicago). The growth hormone obtained from Armour Laboratories, according to their assay, had an activity of 75–100 per cent of Armour Standard. Its main contaminant was 0.1 U.S.P. units thyrotrophin/mg.

Group IV.—0.5 mg. of thyrotrophin in aqueous suspension (Armour Laboratories, Chicago). The Armour thyrotrophin (TSH), according to their assay (chick iodine depletion method), contained 0.8 U.S.P. units/mg. It is relatively free of other pituitary hormones.

[1] The rats were of the Sprague-Dawley strain and were purchased from Hormone Assay Laboratories, Inc., Chicago, Ill.

Group V.—0.5 Units of Synapoidin aqueous solution (Parke, Davis & Co.). This preparation is a combination of chorionic gonadotrophin and a pituitary FSH fraction. According to Parke, Davis & Co., it is unlikely that enough, if any, TSH or ACTH is present in the chorionic fraction to exert any physiological activity. The pituitary FSH is free of growth hormone, prolactin, ACTH, and LH, but there is a possibility of contamination with TSH, although no data on actual amounts present was given.

Group VI.—0.5 mg. Pranturon (chorionic gonadotrophin, Schering Corp.). No information is available on the purity of this drug.

GROUPS VII-XII, FEMALE RATS

Group VII.—Controls.

Group VIII.—Two units ACTHAR gel.

Group IX.—Two units HP ACTHAR gel.

Group X.—Three mg. testosterone cyclopentyl propionate in sesame oil.

Group XI.—Two mg. of desoxycorticosterone acetate, aqueous suspension.

Group XII.—0.05 mg. Pituitrin.

Control groups of intact rats were identically treated. A few hypophysectomized rats maintained on Purina Laboratory Chow with no azo dye were also treated with the above hormone preparations.

Animals from each of these groups were sacrificed at approximately 8, 14, and 21 weeks after the start of the dye feeding. The rats were killed by ether anesthetization. A complete autopsy was performed, and the tissues were fixed in 10 per cent formalin for further histological studies.

RESULTS

Considerable difficulty was encountered in maintaining the hypophysectomized animals for the long periods of time required in the present study. There was a higher mortality of animals than previously observed in experiments of this type. The number of animals per group was smaller than desired because of the high costs of these studies. A sufficient number of the animals did survive, so that we can report some of the effects of the pituitary and the adrenal in azo dye carcinogenesis.

Male hypophysectomized rats.—Administration of ACTH partially restored the carcinogenic activity of azo dyes as was observed in our previous report (11). From Table 1 it may be observed that male rats given ACTH exhibited liver cirrhosis after 14 or 22 weeks of feeding of the azo dye diet. There were no differences between the two corticotrophins, ACTH and ACTH HP, in this respect, as far as could be ascertained from the current studies. The adrenals of these animals showed regeneration, and the livers at 14 weeks showed marked cirrhosis. The survivors at the 21-week period showed cirrhosis and bile duct adenomas. Somatotrophin also restored the carcinogenic activity of 3'-Me-DAB; however, this effect was not so great as we have consistently observed with corticotrophic preparations. Cirrhosis was evident after 13–14 weeks, and liver tumors could be observed after 22 weeks. The histological findings were variable in this group; two of the growth hormone-treated animals had bile duct adenocarcinomas, and benign adenomas were present in the other two rats.

The livers of all groups of hypophysectomized male rats injected with other pituitary hormone preparations indicated some return of the carcinogenic process (Table 1). However, the thyrotrophin and the gonadotrophin preparations (Groups IV, V, VI) were not so active as ACTH or growth hormone. Livers from animals injected with thyrotrophin were cirrhotic after 22 weeks of dye feeding. Histologically, the livers were normal after 8–14 weeks. Synapoidin induced some cirrhosis and bile duct adenomas at the 14- and 22-week periods. After 22 weeks there were benign bile duct adenomas and cirrhosis in some animals treated with all these preparations.

Female hypophysectomized rats.—Studies on the effect of hormones on azo dye carcinogenesis in hypophysectomized female rats are reported in Table 2. Many of these animals (Groups VII, Table 2) injected with saline and fed diets containing the azo dye diet did not survive the treatment. The livers of these animals after 8 and 14 weeks of dye feeding were normal, both grossly and microscopically. Two animals were sacrificed at 22 weeks. One rat had a normal liver, while the other one showed evidence of mild cirrhosis. There was some gross evidence of a pituitary tag in this latter animal which may account for its partial susceptibility to the action of the carcinogen. It should be mentioned that we have had a better survival rate in hypophysectomized males fed the dye. Livers of these male animals were normal even after 28 weeks of dye feeding.

Administration of ACTH preparations to female rats did restore the azo dye activity to a certain extent (Groups VIII and IX, Table 2). Hypophysectomized rats fed diets containing 3'-Me-DAB did not exhibit any liver damage after 8 weeks. There was evidence of mild cirrhosis in some of the animals after 13 or 14 weeks, and cirrhosis and liver tumors were evident at 20 weeks. The microscopic findings substantiated these

gross observations in the stromal thickening, and minimal cirrhosis was present at 13 weeks. Mild cirrhosis, bile duct adenomas, and adenocarcinomas were present at 20–22 weeks.

Hypophysectomized female rats injected with testosterone or Pituitrin had normal livers throughout the 22-week dye feeding period. Livers from rats given DOCA were normal up through the 14-week period; however, cirrhosis was observed in animals sacrificed after 22 weeks. Microscopically, cirrhosis and bile duct adenomas were present in these DOCA-treated animals. Administration of Pituitrin did not produce any gross liver changes in these rats; however, slight cirrhosis was observed microscopically.

DISCUSSION

From the results obtained thus far it appears reasonably certain that both hypophysectomized male and female rats are protected against the

TABLE 1

EFFECT OF HORMONES ON AZO DYE CARCINOGENESIS IN HYPOPHYSECTOMIZED RATS*

Gross observations on livers at times of sacrifice

			8 WEEKS			13–14 WEEKS			20–22 WEEKS		
TREATMENT	INITIAL WT. (GM.)	FOOD (GM/ RAT/DAY)	No. rats	Av. wt.	Liver	No. rats	Av. wt.	Liver	No. rats	Av. wt.	Liver
I. Adrenocorticotrophin (Armour ACTH)	166	10	2	137	Normal	2	144	Mild cirrhosis	1	144	Cirrhosis
II. Adrenocorticotrophin (Armour ACTH[HP])	164	9	2	135	Minimal cirrhosis	2	136	Moderate cirrhosis			
III. Somatotrophin	175	10	2	184	Mild cirrhosis	3	176	Two normal, one mild cirrhosis	4	160	One normal, three enlarged, cirrhotic with tumors
IV. Thyrotrophin	176	9	2	158	Normal	3	150	Normal	3	152	Two normal, one mild cirrhosis
V. Synapoidin†	158	8	2	148	Normal	3	142	Two normal, one mild cirrhosis	3	138	All mild cirrhosis, one tumor
VI. Pranturon‡	172	9	2	150	Normal	2	146	One normal, one cirrhotic	2	135	Mild cirrhosis

* Male rats of the Sprague-Dawley strain maintained on purified diets containing 0.06 per cent 3'-Methyl-4-dimethylaminoazobenzene.
† Synapoidin is a combination of chorionic gonadotrophin and follicle stimulating hormone, Parke, Davis & Company.
‡ Pranturon is chorionic gonadotrophic hormone, Schering Corporation.

TABLE 2

EFFECT OF HORMONES ON AZO DYE CARCINOGENESIS IN HYPOPHYSECTOMIZED RATS*

Gross observations on livers at times of sacrifice

			8 WEEKS			13–14 WEEKS			20–22 WEEKS		
TREATMENT	INITIAL WT. (GM)	FOOD (GM/ RAT/DAY)	No. rats	Av. wt.	Liver	No. rats	Av. wt.	Liver	No. rats	Av. wt.	Liver
VII. Controls	177	7	2	150	Normal	1	153	Normal	2	141	One normal and one mild cirrhosis
VIII. Adrenocorticotrophin (Armour ACTH)	183	7	2	131	Normal	3	125	Two normal, small nodule in third	1	134	Cirrhosis (less than similarly treated males)
IX. Adrenocroticotrophin (Armour ACTH[HP])	178	7	2	150	One normal, one minimal cirrhosis	2	144	One normal, one mild cirrhosis	3	135	Two moderate cirrhosis, one cirrhotic (24 wks.) (less than similarly treated males)
X. Testosterone	175	6	1	159	Normal	2	148	Normal	1	155	(16 weeks) normal
XI. Desoxycorticosterone acetate	180	7	2	157	Normal	2	148	Normal	2	149	Moderate cirrhosis
XII. Pituitrin	177	6	2	143	Normal	1	139	Normal	1	137	Normal

* Female rats of the Sprague-Dawley strain maintained on purified diets containing 0.06 per cent 3'-Methyl-4-dimethylaminoazobenzene.

carcinogenic action of the azo dye compound, 3'-Me-DAB. It also appears certain that the administration of corticotrophin preparations will partially restore this activity in hypophysectomized animals. This hormone is not the only pituitary fraction involved, since approximately 20 weeks are required to induce tumors in the hypophysectomized rats, while 8–10 weeks of dye feeding are usually sufficient in intact animals. Both male and female rats responded to the ACTH. However, we feel that the females were more resistant than the males. At any given time of sacrifice the corticotrophin-treated males had more liver damage than the corresponding females. This finding is in general agreement with that of Rumsfeld, Miller, and Baumann (12), who found a higher incidence of liver tumors in male than in female rats given diets containing azo dyes. Since corticotrophin preparations have some effect in restoring the carcinogenic sequence in azo dye-fed hypophysectomized rats, it would appear probable that adrenal cortical function is involved in this process. In a previous study with male rats, administration of cortical hormones did not restore carcinogenic activity in hypophysectomized rats. Cortisone, DOCA, and corticosterone were all tried without success. In studies involving hypophysectomized females, administration of DOCA resulted in the appearance of mild cirrhosis and bile duct adenomas after 22 weeks of dye feeding. In future experiments we plan to investigate carefully the probable role of cortical hormones on liver carcinogenesis in hypophysectomized animals. Originally, it was believed that only corticotrophic preparations of the pituitary were responsible for restoring the carcinogenic activity of the azo compounds. In the current investigations, it was found that all the pituitary hormones were effective to a greater or lesser degree in the above respect. Growth hormone at the dosages employed was only slightly less active than corticotrophin, as the hypophysectomized rats fed the azo dyes and injected with the growth hormone for 20 and 22 weeks showed extensive cirrhosis and liver tumors. Adrenals of these animals were atrophied, and the reticularis and fasiculata zones were void of lipoid-staining materials. Thyrotrophin and the gonadotrophin preparations, while less active than growth hormone in restoring azo dye carcinogenesis in hypophysectomized rats, nevertheless showed some activity in this respect. Perhaps the optimal amounts of these hormone preparations were not administered, and it is also possible that they contained trace amounts of ACTH or even an unknown common factor to all that produced the effect. It should also be mentioned that no hepatic tumor other than bile duct adenocarcinomas was produced by the hormonal reactivation of azo dye carcinogenesis in the hypophysectomized rats.

SUMMARY

1. Male and female hypophysectomized rats were maintained on synthetic diets containing 3'-Me-DAB. Groups of these animals were treated with ACTH, growth hormone, thyrotrophin, pituitary gonadotrophins, and steroid hormones.

2. Hypophysectomized rats are protected against the carcinogenic action of the azo compounds. ACTH partially restored this activity in both sexes. However, the effect appeared less pronounced in females.

3. Growth hormone also restored the activity of the azo dyes in these hypophysectomized animals. Thyrotrophin and gonadotrophin were also active but to a lesser degree than ACTH or growth hormone in restoring this effect. Administration of DOCA to hypophysectomized female rats fed the dye resulted in cirrhosis and bile duct adenomas after 21 weeks in contrast to previous findings with males. Testosterone and Pituitrin were ineffective in restoring this activity.

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